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Genetic Improvement of Solanaceous Crops

Volume 2: Tomato
Tomato is one of the most consumed vegetables in the world and is the dietary source of vitamins, minerals and fiber, which are important for human nutrition and health. Fresh fruits are used in salads, various culinary preparations, juices, or processed in the form of purees, concentrates, condiments and sauces. Tomato plants are grown worldwide in the field, or in greenhouses. Genetic improvement of this Solanaceous crop has been an on-going process with the objective of gaining high fruit yield, enhanced fruit nutritive value, controlled fruit maturation and ripening, and developing resistance to phytophagous insects, microbial pathogens, and various abiotic stresses. More importantly, with the increase in the world population, the quantum of tomato consumption has considerably increased and farmers, agronomists and horticulturists have had to walk a tight rope to enhance yield without losing sight of the production quality to meet the demands of the fresh market and the processing industry. Of the nearly 3 million hectares under vegetable cultivation the tomato crop occupied one-third of this global area with total tomato production in 1994 reported as 77.5 Mt, averaging 27 t ha\(^{-1}\). Most of the production increases hitherto have been achieved using conventional methods of selection and breeding coupled with improved growth practices: use of fertilizer, improved irrigation, and pest management. Other advancements have been possible through the application of molecular markers to ease selection process and technological innovations such as development of genetically enhanced tomatoes engineered for high quality and resistance to disease and extreme environments. This book presents a critical appraisal of the state-of-the-art findings on this crop in the form of overviews, emphasizing various approaches and strategies used for its improvement through research conducted at various research institutes, organizations and universities world over.

Improvement of a particular crop can best be envisaged when comprehensive information is known of its origin and available genetic resources. The controversy over the taxonomic status of the cultivated tomato \(\text{Lycopersicon esculentum}\) Mill. has been resolved. Application of molecular breeding techniques (RAPDs, RFLPs) and genomics research has now convinced the research community to place tomato under the genus \textit{Solanum}, namely, \textit{Solanum lycopersicum}\ L.\n
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**Preface**

Tomato is one of the most consumed vegetables in the world and is the dietary source of vitamins, minerals and fiber, which are important for human nutrition and health. Fresh fruits are used in salads, various culinary preparations, juices, or processed in the form of purees, concentrates, condiments and sauces. Tomato plants are grown worldwide in the field, or in greenhouses. Genetic improvement of this Solanaceous crop has been an on-going process with the objective of gaining high fruit yield, enhanced fruit nutritive value, controlled fruit maturation and ripening, and developing resistance to phytophagous insects, microbial pathogens, and various abiotic stresses. More importantly, with the increase in the world population, the quantum of tomato consumption has considerably increased and farmers, agronomists and horticulturists have had to walk a tight rope to enhance yield without losing sight of the production quality to meet the demands of the fresh market and the processing industry. Of the nearly 3 million hectares under vegetable cultivation the tomato crop occupied one-third of this global area with total tomato production in 1994 reported as 77.5 Mt, averaging 27 t ha\(^{-1}\). Most of the production increases hitherto have been achieved using conventional methods of selection and breeding coupled with improved growth practices: use of fertilizer, improved irrigation, and pest management. Other advancements have been possible through the application of molecular markers to ease selection process and technological innovations such as development of genetically enhanced tomatoes engineered for high quality and resistance to disease and extreme environments. This book presents a critical appraisal of the state-of-the-art findings on this crop in the form of overviews, emphasizing various approaches and strategies used for its improvement through research conducted at various research institutes, organizations and universities world over.

Improvement of a particular crop can best be envisaged when comprehensive information is known of its origin and available genetic resources. The controversy over the taxonomic status of the cultivated tomato \(\text{Lycopersicon esculentum}\) Mill. has been resolved. Application of molecular breeding techniques (RAPDs, RFLPs) and genomics research has now convinced the research community to place tomato under the genus \textit{Solanum}, namely, \textit{Solanum lycopersicum}\ L.
Genetic Improvement of Solanaceous Crops: Tomato (http://www.sgn.cornell.edu/about/solanum_nomenclature.pl). This implies that one could explore the gene pool among all Solanum species for improvement of this and other Solanaceous crops. Conservation of all tomato genetic resources is, therefore, all the more necessary. Chapter 1 starts with history, origin and early cultivation of tomato. Interestingly, Peru is considered to be a likely place of domestication of tomato and yet another hypothesis sees its first domestication in Mexico. Tomato gene banks have been established in USA and other countries where currently more than 75000 accessions of tomato are preserved. These gene banks maintain data concerning the reproductive biology of conserved accessions, world production scenario of fresh-market as well as processed tomatoes, and descriptive list of characteristics of various collections. Information dealing with these aspects is given in Chapter 2. Role of cytogenetics in evolution and selection of tomato variants with improved traits is elaborated in Chapter 3. This chapter further provides an overview of tomato genomics through genetic maps constructed by applying conventional and molecular breeding techniques in order to assess variability among various tomato genetic resources such as mutants, wild species, intra- and inter-specific populations as well as introgressed lines derived from recombination experiments. By integrating classical gene linkage maps with the high-resolution molecular maps it is now possible to evaluate the degree of similarity in basic genomic structure of tomato sp. Some plants maintain superior traits vigorously only in hybrid form. This phenomenon called heterosis is recognized as one of the primary factors contributing to manifestation of superiority in respect of some quantitative traits of tomato. Chapter 4 describes the strategies for using heterosis for developing tomatoes with certain quantitative traits, whereas Chapter 5 elaborates on improvement of quality traits using traditional and enhanced breeding methods. Tomato fruits are major dietary sources of antioxidant lycopene, and vitamins A and C, besides other micronutrients/antioxidants, which largely contribute to tomato fruit quality. Tomato breeders have been examining a wealth of genetic variability available in the present day heirloom cultivars, land races, and related wild tomato species in respect of various dietary sources. Approaches to genetically enhance tomato fruit’s nutritive value are highlighted in Chapter 6. Molecular markers have proven very useful in selection of elite tomato germplasm, and efforts to best utilize various molecular genetic approaches have led to an understanding of the physiological basis of drought resistance response in tomatoes. An overview of research done on these aspects is provided in Chapters 7 and 8. Recent advances in plant genetic engineering have made it possible to produce transgenic tomato plants with characteristics for a number of improved traits. A general account of genetic engineering
technology applied for production of tomatoes transformed for various traits is given in Chapter 9. Hormonal control of fruit maturation, its molecular basis, and future prospects of applying microarray analysis, as well as proteomics, essentially for producing designer-tomato fruits with enhanced shelf-life are discussed in Chapter 10. Biochemical and molecular mechanisms in fruit ripening have projected insights on existence of molecular links between distinct fruit ripening types in tomato. A number of genes involved in ethylene biosynthesis as well as light signaling are implicated, which reportedly provide targets for manipulation of fruit color, nutrient content, and cell-wall breakdown during the process of ripening. Details of these, based on model systems proposed for fruit ripening of both climacteric and nonclimacteric fruits, are summarized in Chapter 11. An inherent problem encountered in crops, including tomato, is the huge annual yield losses incurred due to diseases caused by pathogens and pests. Molecular breeding coupled with application of transgenic technology has to a greater extent the potential to circumvent this problem for tomato by producing cultivars resistant to bacteria, fungi, viruses, and insects as well as mite pests. Recent findings on these aspects of tomato resistance are reviewed in Chapters 12-15. Finally, the considerable progress made toward understanding the physiological bases of plant tolerance to different abiotic stresses and characterization of tolerant tomato genotypes to stresses, such as salinity, cold and heat, are discussed in Chapter 16.

There has been a long-felt need to have documented, comprehensive information on the improvement of tomato in one place. This book attempts to accomplish this goal. It should be useful not only to breeders, or other specialists, but equally benefit teachers as well as students seeking information on aspects of tomato biology, genetics and biotechnology. It will be apparent to the readers that some authors have used the revised classification of tomato as *Solanum lycopersicum* while others have kept to the older nomenclature, viz., *Lycopersicon esculentum*. We have let both these usages in the book till the transition over the next few years is completed.

This compendium has become a reality only through the expert contributions of the 33 authors from 6 countries and generous support and encouragement from the publishers. We sincerely thank them all.

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Delhi, India

Autar K. Mattoo
Beltsville, USA
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## Abbreviations Used Throughout the Book

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<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>2-ip</td>
<td>6-γ-γ-dimethylamino purine</td>
</tr>
<tr>
<td>ABA</td>
<td>Abscisic acid</td>
</tr>
<tr>
<td>AB-QTL</td>
<td>Advanced backcross QTL analysis</td>
</tr>
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<td>ACC</td>
<td>1-Aminocyclopropane-1-carboxylic acid</td>
</tr>
<tr>
<td>ACO</td>
<td>1-Aminocyclopropane-1-carboxylic acid oxidase</td>
</tr>
<tr>
<td>ACS</td>
<td>1-Aminocyclopropane-1-carboxylic acid synthase</td>
</tr>
<tr>
<td>ADC</td>
<td>Arginine decarboxylase</td>
</tr>
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<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphism</td>
</tr>
<tr>
<td>AIS</td>
<td>Alcohol insoluble acids</td>
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<tr>
<td>AMOVA</td>
<td>Analysis of Molecular Variance</td>
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<tr>
<td>AMV</td>
<td>Alfa mosaic virus</td>
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<tr>
<td>ARDC</td>
<td>Asian Research Development Centre</td>
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<tr>
<td>ARFs</td>
<td>Auxin response factors</td>
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<td>ARS</td>
<td>Agriculture Research Service</td>
</tr>
<tr>
<td>AUX</td>
<td>Auxins</td>
</tr>
<tr>
<td>AVDRDC</td>
<td>Asian Vegetable Research and Development Centre, Taiwan</td>
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<tr>
<td>AVG</td>
<td>Aminoethoxyvinylglycine</td>
</tr>
<tr>
<td>aw</td>
<td>Anthocyanin without marker</td>
</tr>
<tr>
<td>BAC</td>
<td>Bacterial artificial chromosome</td>
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<tr>
<td>BADH</td>
<td>Betaine aldehyde dehydrogenase</td>
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<tr>
<td>BAP</td>
<td>6-benzylaminopurine</td>
</tr>
<tr>
<td>BC</td>
<td>Back cross</td>
</tr>
<tr>
<td>BCTV</td>
<td>Beet curly top virus</td>
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<tr>
<td>BP</td>
<td>Before present</td>
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<tr>
<td>BRs</td>
<td>Brassinosteroids</td>
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<td>CAAS</td>
<td>Institute of Crop Germplasm Resources, China</td>
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<td>CaMV</td>
<td>Cauliflower mosaic virus</td>
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<tr>
<td>CAPS</td>
<td>Cleaved amplified polymorphic sequence</td>
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<tr>
<td>CATIE</td>
<td>Centro Agronomico Tropical de Investigacion y Ensenanza, Costa Rica</td>
</tr>
<tr>
<td>CATIE</td>
<td>Centro Agronomico Tropical de Investigacion y Ensenanza, Costa Rica</td>
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</table>
CBFI  C-repeat/dehydration responsive element biding factor
Gene
CBFI  C-repeat/dehydration responsive element binding gene 1
CDKs  Cyclin dependant kinase
cDNA  Complementary DNA
CGC  Crop Germplasm Committee
CGN  Centre for Genetic Resources, The Netherlands
CHI  Chalcone isomerase
CHS  Chalcone synthase
CIP  International Potato Center, Lima, Peru
CKs  Cytokinins
cM  Centimorgan
CMS  Cytoplasmic male sterility
CMV  Cucumber mosaic virus
CN  Controlled nutrient experiment
CORPOICA  Corporacion Columbiana de Investigacion Agropecuaria, Colombia
COS  Conserved ortholog set
CP  Coat Protein
cpDNA  Chloroplast DNA
CS  Cold stress
CT  Cold tolerance
CTV  Curly Top Virus
DArT  Diversity Array Technology
DMW  Dry matter weight
DNA  Deoxyribonucleic acid
DS  Drought stress
DT  Drought tolerance
DW  Dry weight
EBDC  Ethylene-bis-dithiocarbamate
EC  Electrical conductivity
ELISA  Enzyme-linked immunosorbent-assay
EMBRA  European Molecular Biology and Plant Research Association
EMS  Ethylmethane sulphonate
ER  Extreme resistance
EST  Expressed sequence tag
ETC  Electricity transport chain
ex  Exerted stigma
FAO STAT  FAO statistics base
FAO  Food and Agriculture Organisation
FISH  Fluorescent in situ hybridization
FS  Flavone synthase
FW  Fresh weight
<table>
<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>G:F</td>
<td>Glucose and fructose ratio</td>
</tr>
<tr>
<td>GAs</td>
<td>Gibberellins</td>
</tr>
<tr>
<td>GBSST</td>
<td>Granule bound starch synthase gene 1 waxy</td>
</tr>
<tr>
<td>GCA</td>
<td>General combining ability</td>
</tr>
<tr>
<td>gDW</td>
<td>Gram dry weight</td>
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<tr>
<td>GISH</td>
<td>Genomic in situ hybridization</td>
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<td>GM</td>
<td>Genetically modified</td>
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<td>GMA</td>
<td>Generation Mean Analysis</td>
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<td>GRIN</td>
<td>Germplasm Resources Information Network</td>
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<td>GRSV</td>
<td>Ground nut ring spot virus</td>
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<td>GTP</td>
<td>Guanosine triphosphate</td>
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<tr>
<td>GUS</td>
<td>β-glucuronidase marker</td>
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<tr>
<td>h2</td>
<td>heritability</td>
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<tr>
<td>ha</td>
<td>Hectare</td>
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<tr>
<td>HQT</td>
<td>Hydroxycinnamoyl transferase</td>
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<tr>
<td>HS</td>
<td>Heat stress</td>
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<tr>
<td>HT</td>
<td>Heat tolerance</td>
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<tr>
<td>IAA</td>
<td>Indole acetic acid</td>
</tr>
<tr>
<td>IAC</td>
<td>Institut Agronomico de Campines, Brazil</td>
</tr>
<tr>
<td>IBC</td>
<td>Inbred backcross</td>
</tr>
<tr>
<td>IL</td>
<td>Isogenic line/Introgression line</td>
</tr>
<tr>
<td>INRA</td>
<td>Institut Nationale des Research Agronomiques</td>
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<tr>
<td>IPGRI</td>
<td>International Plant Genetic Resources Institute</td>
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<tr>
<td>ITS</td>
<td>Internal transcribed spacer</td>
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<tr>
<td>JA</td>
<td>Jasmonic acid</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LEA</td>
<td>Late embryogenesis abundance</td>
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<td>LIS</td>
<td>Linalool synthase gene</td>
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<td>LMGV</td>
<td>Lab. De Melhoramento Genetico Vegetale, Brazil</td>
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<td>LTPs</td>
<td>Lipid transfer proteins</td>
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<td>LTs</td>
<td>Low temperatures</td>
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<tr>
<td>M</td>
<td>Mild</td>
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<td>MA</td>
<td>Monosomic addition</td>
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<td>MABC</td>
<td>Marker assisted backcross</td>
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<td>MAS</td>
<td>Marker assisted selection</td>
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<td>Mbp</td>
<td>Mega base pairs</td>
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<td>MCP</td>
<td>1-methylcyclopropene</td>
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<td>MeJA</td>
<td>Methyl jasmonic acid</td>
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<tr>
<td>Meq</td>
<td>Milliequivalent</td>
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<td>MG</td>
<td>Mature green</td>
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<td>MS</td>
<td>Male sterile</td>
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<td>mt DNA</td>
<td>Mitochondrial DNA</td>
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<td>Mt</td>
<td>Million ton</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
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<tr>
<td>N</td>
<td>Necrotic</td>
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<tr>
<td>NAA</td>
<td>1-naphthalene-acetic acid</td>
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<tr>
<td>NAFTA</td>
<td>Northern American Free Trade Agreement</td>
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<td>NBPGR</td>
<td>National Bureau of Plant Genetic Resources India</td>
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<td>NCED</td>
<td>9-cis-epoxycarotenoid dioxygenase</td>
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<td>NCGRP</td>
<td>National Center for Genetic Resources Preservation</td>
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<tr>
<td>Ne</td>
<td>No. of individuals in a theoretically ideal population having the same magnitude of drift as the actual population</td>
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<td>NIL</td>
<td>Near isogenic line</td>
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<td>Neighbor joining analysis</td>
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<td>NOR</td>
<td>Nucleolar Organizing region</td>
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<td>National plant germplasm system</td>
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<td>Neomycin phosphotransferase</td>
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<td>NS</td>
<td>Nonstress conditions</td>
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<td>National Science Foundation</td>
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<td>NSL</td>
<td>National seed laboratory</td>
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<td>nt</td>
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<td>ODC</td>
<td>Ornithine decarbocylase</td>
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<td>p-aminobenzoate</td>
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<td>Polyamines</td>
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<td>Polar auxin transport</td>
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<td>Principal components analysis</td>
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<td>PCR</td>
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<td>Phytoene desaturase</td>
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<td>Pepino mosaic virus</td>
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<td>Polygalacturonase</td>
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<td>Plant growth regulators</td>
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<td>Principal genetic resources unit</td>
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<td>Plant introduction</td>
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<td>Phospholipase D</td>
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<td>Potato leaf roll virus</td>
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<td>Pectinmethyl esterase</td>
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<td>PMI</td>
<td>Phosphomannose isomerase</td>
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<td>PN</td>
<td>Photosynthetic rate</td>
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<td>PPP</td>
<td>Pectinase phosphate pathway</td>
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<td>ps</td>
<td>positional sterile</td>
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<td>PTGS</td>
<td>Post-transcriptional silencing</td>
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<td>PVE</td>
<td>Phenotypic variation explained</td>
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<td>PVX</td>
<td>Potato virus X</td>
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<td>PVY</td>
<td>Potato virus Y</td>
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<td>QTL</td>
<td>Quantitative trait loci</td>
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<td>RAPD</td>
<td>Randomly amplified polymorphic DNA</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>rDNA</td>
<td>Ribosomal DNA</td>
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<td>Repetitive element sequence PCR</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction length fragment polymorphism</td>
</tr>
<tr>
<td>RGA</td>
<td>Resistance gene analog</td>
</tr>
<tr>
<td>RIL</td>
<td>Recombinant inbred line</td>
</tr>
<tr>
<td>RN</td>
<td>Recombination nodules</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribose nucleic acid</td>
</tr>
<tr>
<td>RNAi</td>
<td>RNA interference</td>
</tr>
<tr>
<td>RS</td>
<td>Ringspot</td>
</tr>
<tr>
<td>RSV</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>SAAT</td>
<td>Strawberry alcohol acyltransferase</td>
</tr>
<tr>
<td>SAM</td>
<td>S-adenosyl methionine/S-adenosine-L-methionine</td>
</tr>
<tr>
<td>SARS</td>
<td>Severe acute respiratory syndrome</td>
</tr>
<tr>
<td>SC</td>
<td>Synaptonemal complex/self-compatible</td>
</tr>
<tr>
<td>SCA</td>
<td>Specific combining ability</td>
</tr>
<tr>
<td>SDM</td>
<td>Seedling decapitation method</td>
</tr>
<tr>
<td>SG</td>
<td>Seed germination</td>
</tr>
<tr>
<td>SGN</td>
<td>Solanaceae genomic network</td>
</tr>
<tr>
<td>SI</td>
<td>Self-incompatible</td>
</tr>
<tr>
<td>sl</td>
<td>Stamen less</td>
</tr>
<tr>
<td>SL</td>
<td>Substitution line</td>
</tr>
<tr>
<td>SNP Wave</td>
<td>SNP multiplexed genotyping technology</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SnRK1</td>
<td>Sucrose non-fermenting related kinase 1</td>
</tr>
<tr>
<td>SS</td>
<td>Salt stress</td>
</tr>
<tr>
<td>SSD</td>
<td>Single seed descent</td>
</tr>
<tr>
<td>SSR</td>
<td>Single sequence repeat</td>
</tr>
<tr>
<td>ST</td>
<td>Salt tolerance</td>
</tr>
<tr>
<td>STMS</td>
<td>Selected microsatellite markers</td>
</tr>
<tr>
<td>STS</td>
<td>Silver thiosulphate</td>
</tr>
<tr>
<td>SuSy</td>
<td>Sucrose synthase</td>
</tr>
<tr>
<td>T50</td>
<td>Time to 50% germination</td>
</tr>
<tr>
<td>TB</td>
<td>Tip blight</td>
</tr>
<tr>
<td>TCSV</td>
<td>Tospovirus chlorotic spot virus</td>
</tr>
<tr>
<td>T-DNA</td>
<td>Ti-plasmid DNA segment</td>
</tr>
<tr>
<td>TEV</td>
<td>Tobacco etch virus</td>
</tr>
<tr>
<td>TGRC</td>
<td>Tomato genetic research cooperative</td>
</tr>
<tr>
<td>TI</td>
<td>Tolerance index</td>
</tr>
<tr>
<td>TILLING</td>
<td>Targeting induced local lesions in genomes</td>
</tr>
<tr>
<td>TLCV</td>
<td>Tomato leaf curl virus</td>
</tr>
<tr>
<td>ToMoV</td>
<td>Tomato mottle virus</td>
</tr>
<tr>
<td>ToMV</td>
<td>Tomato mosaic virus</td>
</tr>
<tr>
<td>TSWV</td>
<td>Tomato spotted wilt virus</td>
</tr>
</tbody>
</table>
TYLC  Tomato yellow leaf curl virus
TYTV  Tomato yellow top virus
UPGMA  Unweighted pair group method of arithmetic averages
USDA  United States Department of Agriculture
USDA-ERS  USDA Economic Research Service
USDA-NASS  USDA National Agricultural Statistics Center
UTR  Untranslated region
VIGS  Virus induced gene silencing
VIP  Vegetative insecticidal proteins
VM  Very mild
VS  Vegetative stage
WIS  Water insoluble solids
WUE  Water use efficiency
WVC  World vegetable center
XET  Xyluglucan endotransglycolase
XGH  Xyluglucan hydrolase
XTH  Xyluglucan transglycosylation hydrolysis
YAC  Yeast artificial chromosome
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History, Origin and Early Cultivation of Tomato (Solanaceae)

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INTRODUCTION

Tomatoes rank fourth among the leading world vegetables. In 2001, over 100 million metric tons were produced, with the 15 leading countries being (in descending order) China, US, India, Turkey, Egypt, Italy, Spain, Brazil, Islamic Republic of Iran, Mexico, Greece, Russian Federation, Ukraine, Chile, and Uzbekistan (FAO 2002; Fig. 1.1). There has been a general upward trend in tomato production during the period 1992-2002 (Fig. 1.2). Interestingly, the countries that produce higher yields (Fig. 1.3) do not possess the ideal climate for the tomato crop and have less land area devoted to tomato production (Fig. 1.4). Northern European countries, as well as Canada and New Zealand, produce most of their tomatoes under controlled greenhouse conditions. Tomato consumption has also shown a general increased trend of consumption over a period of time (FAO 2002). Tomatoes supply a mean of 12.1 kg/cap/yr, and tomato consumption is higher in Mediterranean and Arab countries (usually between 40 and 60 kg/cap/yr). Tomatoes are highly popular in Egypt, Italy, Israel, Lebanon, Turkey and United Arab Emirates (60-70 kg/cap/yr), whereas people from Greece and Libya have the highest preference consuming more than 100 kg of tomatoes per capita and year. Tomatoes are also a popular food in Latin and North America.

Tomatoes rank second among the leading vegetables of the US (Ensminger et al. 1995), with a production of 10.25 million metric tons in

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Fig. 1.1 Tomato production worldwide, 2001.

2001. Much of the US production is processed, with major products being canned tomatoes, ketchup, chilli sauce, juice, paste, powder, puree, salad dressings, sauces, soups, and vegetable and juice cocktails.

The US farmgate (point of first sale) value of tomatoes in 2001 was 1.12 billion dollars for fresh tomatoes and 0.54 billion dollars for processed tomatoes ($1.66 billion dollars total) (USDA, National Statistics Service 2002a). California and Florida clearly dominate the US market, with Florida accounting for 40.3% of the fresh US market, and California accounting for 24.1% of the fresh market and 90.7% of the processed market (USDA, National Statistics Service 2002b). Tomato consumption has substantially increased in the US since the beginning of the last century. In 1920, the per capita consumption was only 8.2 kg/yr, which in 1978 increased to
Fig. 1.2 Tomato production worldwide, 1992-2001.
25.5 kg/yr (Rick 1978), and now is 40.5 kg/yr, but mostly of tomatoes in preserved forms (FAO 2002).

Tomato is a rich source of nutrients (Table 1.1). General comments (Ensminger et al. 1995) made in particular from this table are as follows: Fresh tomatoes and tomato juices are high in water and low in calories. Both are good sources of vitamins A and C, but unfortified tomato juice has only about 2-3 the vitamin C content of raw, ripe (red) tomatoes. Similarly, canned tomatoes contain only about 3-4 times the vitamin C content of fresh ripe tomatoes. Ripe tomatoes contain 3-4 times the vitamin A as mature green tomatoes, but otherwise red and green tomatoes are about equal in nutritional value. Tomato puree and plain types of tomato sauce (without added ingredients such as meat or mushrooms) have about twice the solids content and about double the nutritional value of fresh tomatoes and tomato juice. Tomato paste, which has about four times the solids content of fresh tomatoes, is a concentrated source of nutrients, making it a valuable contribution when used in preparation of pastas, pizzas, and other foods. Ketchup and chilli sauce are about equal in
nutritional value, since each item is made with similar ingredients and contains about 32% solids (about 5 times the content of fresh tomatoes and tomato juice). However, the nutrients per calorie of these products are significantly less than those furnished by tomato paste, because the solids content and caloric values are boosted by added salt and sugar.

Tomato popularity and its high level of consumption make this vegetable one of the major sources of vitamins and minerals in human diet, and provides healthy benefits that will be discussed further in the following chapters.

**TAXONOMY**

Since the tomato was introduced to Europe in the sixteenth century, early botanists recognized the close relationships of tomatoes with the genus *Solanum*, and commonly identified them as *S. pomiferum* (Sabine 1820, Luckwill 1943a). Anguillara (1561) identified the newly introduced
tomato as a plant named *Lycopersicon*, which means “wolf peach”, by the Greek naturalist Galen fourteen centuries earlier. However, the actual plant described by Galen is unknown, and it certainly did not refer to any form of tomato because all tomato species are not native of the Old World. Tournefort (1694) was the first to consider cultivated tomatoes within a distinct genus under the early name *Lycopersicon*. He used the multilocular character of the fruit as a criterion to differentiate *Lycopersicon* from *Solanum*. Tournefort listed nine taxa but only seven of them correspond to fasciated-fruited varieties that differed in the color and size of their fruits, and the other two described taxa belong to different Solanaceae (Luckwill 1943a).

Linnaeus (1753) classified tomatoes in the genus *Solanum*, and under the specific name of *Solanum lycopersicum* grouped all the cultivated multilocular forms that Tournefort described as separate species. He also described a second wild species from Peru, *S. peruvianum*. Jussieu (1789), in his natural classification, also included tomatoes in *Solanum*. On the other hand, Miller (1754) reconsidered Tournefort’s classification and formally described the genus *Lycopersicon*. This classification of tomatoes under *Lycopersicon* continued as the prevailing treatment by several classical and

---

**Table 1.1 Chemical composition of tomato fruit (figures for a small tomato of 100 g; after Ensminger et al. 1995).**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>95%</td>
</tr>
<tr>
<td>Food energy</td>
<td>22 kcal</td>
</tr>
<tr>
<td>Protein</td>
<td>1 g</td>
</tr>
<tr>
<td>Fats</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>4.7 g</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Calcium</td>
<td>13.0 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>27.0 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>17.7 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>244.0 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>0.50 mg</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.20 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>0.01 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>900.0 IU</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin E (α-Tocopherol)</td>
<td>0.40 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>23 mg</td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.06 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.04 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.70 mg</td>
</tr>
<tr>
<td>Panthothenic Acid</td>
<td>0.33 mg</td>
</tr>
<tr>
<td>Vit. B-6 (pyridoxine)</td>
<td>0.10 mg</td>
</tr>
<tr>
<td>Folacin (folic acid)</td>
<td>39.00 mcg</td>
</tr>
<tr>
<td>Biotin</td>
<td>4.00 mcg</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td>0</td>
</tr>
</tbody>
</table>

More recently, the phylogenetic relationships within the Solanaceae have been examined with molecular data. Spooner et al. (1993) examined outgroup relationships of tomato to potato and other members of the Solanaceae based on chloroplast DNA restriction site data (Fig. 1.5). Subsequent molecular studies unequivocally supported tomato to be firmly internested in the genus *Solanum* L., then this tomato-potato sister group relationship is now clearly established (Olmstead and Palmer 1997, Bohs and Olmstead 1997, 1999, Peralta and Spooner 2001). Based on these results, a new phylogenetic classification has assigned tomato to the genus *Solanum* (Spooner 2005). This classification of tomatoes in *Solanum* matches the original treatment of Linnaeus (1753), as well as prior taxonomists who insightfully foresaw this generic relationship based on morphological data (Wettstein 1895, MacBride 1962, Seithe 1962, Heine 1976, Fosberg 1987, Child 1990). Börner (1912) also recognized the close affinities among tomatoes and potatoes, and proposed a new genus *Solanopsis* to segregate them. Although most taxonomists today place tomato in *Solanum*, most agronomists and horticulturists do not use this name (see Doco et al. 1997, Shichijo et al. 2001, Van der Heuvel 2001, Weller et al. 2001). Most users of the classification in *Lycopersicon* clearly base their reluctance to use the *Solanum* names on tradition or the practical goal of maintaining familiar names rather than adherence to any particular classification philosophy. In this chapter tomato species are classified in the genus *Solanum* and their comparative *Lycopersicon* synonyms are given in Table 1.2.

Hypotheses of ingroup relationships within tomato also have varied greatly. Müller (1940a), Luckwill (1943a), and Child (1990) classified tomato based on morphological criteria, while Rick (1963, 1979) and Rick et al. (1990b) classified tomato quite differently based on biological (inter-breeding) criteria. Peralta and Spooner (2001) produced a phylogeny of tomato based on DNA sequences of the single-copy GBSSI (*waxy*) gene, and Spooner et al. (2005) based on Amplified Fragment Length Polymorphisms. The results support allogamy, self-incompatibility, and green fruits as primitive in tomatoes, and most closely match the classification of Child (1990). One of the self-incompatible species, the highly polymorphic *Solanum peruvianum* L., was supported to consist of one group of populations from northern Peru and another group of populations from central to southern Peru. A phenetic morphological study by Peralta and Spooner (2003) supported all species, including the “northern” and “southern” group of populations of *S. peruvianum* as distinct taxa. Peralta et al. (2005) used these results, and morphological data, to divide the former *S.
Genetic Improvement of Solanaceous Crops: Tomato

**DISTRIBUTION, HABITATS, MORPHOLOGY, MATING SYSTEMS AND GENETIC RESOURCES OF WILD TOMATOES**

The wild relatives of the cultivated tomato are native of western South America along the coast and high Andes from central Ecuador, through Peru, to northern Chile, and in the Galapagos Islands (Table 1.2). The most likely ancestor of cultivated tomatoes is the wild cherry tomato (usually identified as *S. lycopersicum* var. *cerasiforme*), which is more widespread,
Table 1.2  Comparison of wild tomato species (Solanum L. section Lycopersicon (data compiled from Müller, 1940a; Luckwill 1943a; Esquinas Alcazar 1981; Rick 1982b, 1986b; Taylor 1986; Peralta et al., 2005). The Lycopersicon synonyms follow the Solanum names.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lycopersicon synonyms</th>
<th>Fruit color</th>
<th>Breeding system</th>
<th>Distribution and Habitat</th>
<th>Comments and interesting features for breeding purposes</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. lycopersicum L.</td>
<td>L. esculentum Miller</td>
<td>Red</td>
<td>SC, facultative allogamous</td>
<td>Native from Ecuador and Peru, widespread in America. Wide range of habitats, weed in newly open areas</td>
<td>Moisture-tolerance, resistance to wilt, root-rotting, and leaf-spotting fungi</td>
</tr>
<tr>
<td>S. cheesmaniae (Riley) Fosberg</td>
<td>L. cheesmaniae Riley</td>
<td>Yellow, green, orange, purple</td>
<td>SC, exclusively autogamous</td>
<td>Endemic of the Galápagos Archipelago. From low elevations in the saline seashore up to 500 m in volcanic areas</td>
<td>Closely related to S. galapagense. Salt tolerance, lepidoptera and virus resistances, and genes involved in the retention of fruits and thick pericarp</td>
</tr>
<tr>
<td>S. galapagense</td>
<td>Part of L. cheesmaniae L. Riley (previously known as forma or var. minor)</td>
<td>Pale to deep orange</td>
<td>SC, exclusively autogamous</td>
<td>Endemic of the Galápagos Archipelago. Mostly occurring on coastal lava to within 1 m of high tide mark within range of salt spray, but occasionally inland up to 50 m</td>
<td>Closely related to S. cheesmaniae. Salt tolerance.</td>
</tr>
<tr>
<td>S. pimpinellifolium B. Juss.</td>
<td>L. pimpinellifolium (B. Juss.) Miller</td>
<td>Red</td>
<td>SC, autogamous, facultative allogamous</td>
<td>Central Peru to central chile, dry coastal habitats, 0 – 500 m, but exceptionally up to 1400 m.</td>
<td>Closely related to S. lycopersicum (some natural introgression with it). Contributed to improve color and fruit quality. Insect, nematode, and disease resistances.</td>
</tr>
</tbody>
</table>

(Contd.)
<table>
<thead>
<tr>
<th>Taxon</th>
<th>Species</th>
<th>Color</th>
<th>Habit</th>
<th>Altitude Range</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. arcanum</em></td>
<td><em>S. arcanum</em></td>
<td>Green SI</td>
<td>100-2800 m; N Peru, lomas, dry valleys, and dry rocky slopes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>(L.) Miller</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. chilense</em> (Dunal)</td>
<td><em>L. chilense</em></td>
<td>Small green SI, with purple allogamous stripe</td>
<td>Sea level-3250 m; S Peru to N Chile, grows in dry river beds, survives by deep roots</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reiche</td>
<td><em>(Dunal)</em></td>
<td></td>
<td></td>
<td></td>
<td>Typically erect becoming decumbent; post-syngamic barriers with <em>S. peruvianum</em>. Drought resistance</td>
</tr>
<tr>
<td></td>
<td><em>(L.) chilense</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. chmielewskii</em></td>
<td><em>L. chmielewskii</em></td>
<td>Green SC, facultatively allogamous</td>
<td>1600-3200 m, Pacific side, South-Central Peru to N Bolivia; moist habitats; slightly better-drained sites that <em>S. neorickii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(C. M. Rick, Kesicki, Fobes &amp; M. Holle), D. M. Spooner, G. J. Anderson &amp; R. K. Jansen</em></td>
<td></td>
<td></td>
<td></td>
<td>Sympatric with <em>S. neorickii</em>. Contributed to improve high sugar content in the crop</td>
<td></td>
</tr>
<tr>
<td><em>S. corneliomuelleri</em></td>
<td><em>Part of L. peruvianum (L.) Miller; also known as Lycopersicon glandulosum C. F. Mull.</em></td>
<td>Green SI</td>
<td>Landslides and rocky slopes, (40)200-3300 m, Central to S Peru</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. F. Macbr.</td>
<td><em>(L.) Miller</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. habrochaites</em></td>
<td><em>L. hirsutum</em></td>
<td>Green</td>
<td>Typically SI, 1-2 collections SC, but with later inbreeding depression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Knapp &amp; D. M. Spooner</td>
<td><em>(Dunal)</em></td>
<td></td>
<td></td>
<td></td>
<td>Typically high elevations, (40)200-3000 m, in moist well drained soils; Central Ecuador to Central Peru</td>
</tr>
<tr>
<td><em>S. huaylasense</em></td>
<td><em>Part of L. peruvianum (L.) Miller</em></td>
<td>Green SI</td>
<td>Rocky slopes, (940)1700-3000 m, N Peru, Ancash along Río Santa.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peralta</td>
<td><em>(L.) Miller</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Contd.)
### History, Origin and Early Cultivation of Tomato (Solanaceae)

**S. neorickii**  
*Pale green*  
SC, highly autogamous  
(920)1950-2600 m, Pacific side, South Ecuador to South-central Peru; moist and well-drained rocky environments; more introgression reported with *S. chmielewskii*; yet no natural introgression reported with *S. neorickii*.

**S. pennellii**  
Correll (Correll) D’Arcy  
*Green*  
Usually SI, some SC in Southern range  
sea level to 2300 m; N cent to S cent Peru (8-16 °S); hot dry habitats but subject to dew and fog; (many stomata adaxially, poor root system). Drought resistance; covered with glandular hairs imparts insect resistance; hybridizes unilaterally (as male) with many other species except *S. chilense* or *S. peruvianum*.

**S. peruvianum**  
*L. peruvianum* (L.) Miller  
*Green*  
Typically SI, allogamous,  
Sea level-600 m; Central Peru to N Chile. Coastal lomas formations and occasionally as a weed at fields edges. Virus, bacteria, fungi, aphid, and nematode resistances.
and perhaps more recently distributed into Mexico, Colombia, Bolivia, and other South American countries (Rick and Holle 1990). The prior taxonomies recognized the cherry tomato as *L. esculentum* var. *cerasiforme* or *S. lycopersicon* var. *cerasiforme* but we do not recognize this variety and combine all variants of this species (cultivated and wild) into *S. lycopersicon*. The wild cherry tomato grows spontaneously in tropical and subtropical areas worldwide, where it might have been accidentally introduced or escaped from cultivation.

Wild tomatoes grow in a variety of western South American habitats, from near sea level to over 3,300 m in elevation (Rick 1973, Taylor 1986). These habitats include the arid Pacific coastal lowlands and adjacent lower valleys to mesic uplands in the high Andes. Numerous valleys, formed by rivers draining into the Pacific, characterize the western side of the Andes. Wild tomato populations grow at different altitudes in these narrow and geographically isolated valleys, and are adapted to particular microclimatic and soil conditions. Certainly, the Andean geography, diverse ecological habitats, and different climates contributed to wild tomato diversity (Warnock 1988).

Wild tomatoes are perennial herbaceous plants, although in their natural habitat tomatoes most probably behave as annuals and might die after the first growing season due to frost or drought. They have an erect or prostrate growth habit, and possess taxonomically useful differences in leaf, inflorescence, flower, fruit, and seed characters. Leaves are pinnately dissected with 2-6 opposite or sub-opposite, sessile, subsessile or petiolate pairs of leaflets. There is great interspecific variation in leaf dissection with primary, secondary, tertiary, and interjected leaflets. The basic inflorescence is a cyme with different branching patterns (monochasial, dichotomous, and polychotomous), and with or without axial bracts. Flowers are typically yellow; the anthers are united laterally to form a flask-shaped cone with an elongated sterile tip at the apex (except in *S. pennelli*). Flowers are buzz pollinated. Fruit size, color, and pubescence are variable (Table 1.2), as are seed size, color and development of radial walls of the seed coat cells (Müller 1940a, Luckwill 1943a). Fruits are usually bilocular in the wild species, and bilocular or multilocular in the cultivated varieties.

Mating systems have played an important role in the evolution of wild tomato species, varying from allogamous self-incompatible, to facultative allogamous, and self-compatible, to autogamous and self-compatible (Rick 1963, 1979, 1986a; Table 1.2). The self-incompatibility system in tomatoes is gametophytic and controlled by a single, multiallelic *S* locus (Rick 1982a). Large flowers and greater stigma exsertion from the anther tube have been associated with self-incompatibility resulting in greater outcrossing and genetic variation in wild tomatoes (Rick 1982a). Similarly, in the self-
compatible species *S. pimpinellifolium*, greater outcrossing and genetic variation is related to large flowers and greater stigma exsertion; marginal populations of this species are highly autogamous with little or no genetic variation, bearing small flowers, with little or no stigma exsertion (Rick et al. 1977). Self-incompatibility is most probably regulated by different unlinked genes or gene complexes (Rick 1982a), and changes in mating systems in wild tomatoes occurred from self-incompatibility, as the ancestral condition, to self-compatibility, which probably never reversed to self-incompatibility. Change from self-incompatibility to self-compatibility is expected to have arisen infrequently and independently (Rick 1982a). Hybridization is another possible source of genetic variation. Evidence of natural interspecific hybridization and gene flow among wild self-compatible tomato species have been documented in native sympatric populations of *S. pimpinellifolium* and *S. lycopersicum*, and cultivated tomatoes in Ecuador and Peru (Rick 1958). The reciprocal introgression of traits into both taxa generates complex morphological gradation between them that makes their taxonomic identification difficult (Rick 1958).

The traditional breeding for pure lines in the cultivated tomato has narrowed its genetic base (Stevens and Rick 1986). Fortunately, genetic resources from the primary center of diversity provide a wealth of useful genetic traits to improve the crop (Rick 1982b, 1995). All wild tomato species are diploid \(2n = 2x = 24\) and can be crossed (but sometimes with difficulty) to the cultivated tomato (Rick 1979). They are of great use in breeding programs as sources of disease resistances and agronomic traits (Esquinas Alcazar 1981, Rick 1982b, 1986b, Rick et al. 1987, Stevens and Rick 1986, Laterrot 1989). The International Plant Genetic Resources Institute (IPGRI) recognized the need for maintaining valuable vegetable genetic resources and nominated tomatoes for priority conservation status. Ross (1998) considered that the diversity of tomato is likely to be well conserved, and cited 62,832 accessions maintained in gene banks around the world, although most of these accessions are *S. lycopersicum*. The genetic variation among *S. lycopersicum* accessions at the Asian Research and Development Center (ARDC—one of the largest collections of cultivated tomato germplasm) was evaluated with Random Amplified Polymorphic DNA (RAPDs) by Villand et al. (1998). RAPD diversity was greater in accessions from the primary center (Ecuador, Peru, Chile), and for breeding purposes variation can be obtained at a faster rate by sampling accessions from this area than from other geographic regions. The largest and most important collection of wild species genetic resources exists at the Tomato Genetics Resources Center (TGRC, University of California, Davis).

Tomato also serves as a model organism to understand the basic genetics of diploid plants. Features that enhance the usefulness of tomatoes for genetic studies are: the naturally occurring variability in the species, self-
pollination that lead to the expression of recessive mutations, the possibility of controlled hybridization within and among species, the lack of gene duplication, and the possibility to easily identify the 12 chromosomes (Rick 1978). In recent years there have been great advances in tomato genetics. New methodological approaches like molecular mapping of important agronomical characters have provided powerful tools for the improvement of the tomato crop (Tanksley and McCouch 1997).

DOMESTICATION OF CULTIVATED TOMATOES—PERU OR MEXICO?

Methods for Inferring Location of Crop Origins

Two competing hypotheses have been advanced to ascertain the place of domestication of the cultivated tomato, one from Peru, and another from Mexico. How does one search for origins of crops? The first systematic attempt was outlined by DeCandolle (1886). He used an eclectic approach based on the following four criteria: 1) “Botany”, or observing natural spontaneous geographic distributions of the crop or its putative wild relatives. These data could be gathered from floras or herbaria, but this could be complicated by recent adventive introductions; 2) “Archaeology and paleontology”, gathered from fossil evidence of plant remains in caves, burial sites, or other preserved deposits; 3) “History”, searching for evidence in early accounts of peoples; 4) “Philology”, or linguistic evidence, or comparison of native names of plants to prior languages. DeCandolle, however, placed the least credence on the linguistic evidence. Since DeCandolle’s time, additional techniques have been used in determining the origin of crops which include radiocarbon dating, scanning electron microscopy, palynology, refined archaeological methods as flotation techniques, and genetic and molecular evidence (Smith 1995).

Peruvian Hypothesis

DeCandolle (1886) advanced the Peruvian hypothesis for the site of domestication of tomato. He reviewed botanical (Bauhin 1623, Ruiz and Pavón 1797), linguistic (Roxburgh 1832), and historical (Hernández 1651) evidence and concluded: 1) there were no unambiguous natural records of tomato outside of the Americas before its European discovery there; 2) Bauhin (1623) referred to tomato as “mala peruviana” and “pomi del Peru,” which suggested initial domestication and transport of tomato from Peru to Europe; 3) its origin was from the wild cherry tomato (S. lycopersicum) that by DeCandolle’s time was known to occur from coastal Peru, Mexico, to southwestern US (California); 4) the distribution of cultivated tomato and its progenitor outside of Peru originated by garden escapes; and 5) the
plant was domesticated before the discovery of America but not very long before that. This Peruvian origin was later supported by other authors (Moore 1935, Müller 1940a, b, Luckwill 1943a, b).

**Mexican Hypothesis**

Jenkins (1948) developed the Mexican hypothesis. He pointed out that the first reference to tomato in Europe was made by Matthiolus (1544) who provided a short description of tomato. Matthiolus (1554) amplified the description where he first provided the Italian name “pomi d’oro”, and Latin name “mala aurea.” A later edition of his work (Matthiolus 1586) provided an illustration showing an unambiguously identified tomato plant that made his concept of tomato clear, but there was no reference to its geographical origin. Only later, Anguillara (1561) first used the name “pomi del Peru,” along with the name “pomi d’oro,” but his reference is ambiguous as to whether he was referring to the same plant. Jenkins argued that “pomi del Peru” was used by early botanists to refer to other solanaceous plants such as *Datura stramonium* L. and had nothing to do with tomato, weakening DeCandolle’s linguistic evidence.

Jenkins’s second argument was that there was no evidence for pre-Columbian domestication of tomatoes in South America, yet good evidence for early domestication in Mexico. This comes from a reference from Guilandini (1572) who referred to tomato as “tumatle ex Themistitan,” using an indigenous Mexican name for tomato. Jenkins interpreted the name “Themistitan” as a variant spelling of “Temixtitan” which in turn is a corruption of “Tenochtitlan”, the native name for Mexico City. He therefore concluded that tomatoes came from Mexico. During the seventeenth century the Nahua name “tomatl” was often mentioned by botanists, and variants of this name are used in different languages at present (tomate in Spanish, tomato in English, etc.). Interestingly, the early name “Pomi d’oro” is still used in Italy. According to Jenkins (1948), evidence for early Mexican domestication also came from Hernández (1651) who documented early cultivation of tomato in Mexico at least before 1578 (the year of his death) and possibly from Acosta (1590); although Acosta could be referring to Mexico or Peru. Nevertheless, Yakovleff and Herrera (1935) considered that Acosta documented the uses of tomatoes in ancient Peru.

Jenkins’s third argument was that there was considerably more variation of the landrace cultivars in Mexico than in Peru. Following ideas of Vavilov (1926), Jenkins argued that var. *cerasiform*, the small bilocular fruit form of *S. lycopersicum*, was introduced into Mexico in pre-Columbian times and it was domesticated in the central area that he considered as a secondary center of diversity. Jenkins agreed with DeCandolle (1886) that *S. lycopersicum* was the progenitor of the domesticated cultivars, but disagreed with the place of domestication in Peru.
Our Conclusion

We consider the question of the original site of domestication of cultivated tomato to be unsolved, and likely to forever be so. Like DeCandolle (1886), we consider linguistic evidence to be a weak source of data, and the existing linguistic sources for tomato are scant, ambiguous, and subject to various interpretations. Contrary to Jenkins’s (1948) statements that there are no indigenous Peruvian names for tomato, Horkheimer (1973) documented a Quechua name for tomato (pirca), and Yakovleff and Herrera (1935) cited another Quechua name (pescco-tomate) possibly referring to the small bilocular fruit form of \textit{S. lycopersicum}. The historical evidence also is sparse and ambiguous in their references to tomatoes. From the analysis of the original description by Hernández (1651), it is not clear that the plant cited as “tomatl” referred to the true tomatoes or a native \textit{Physalis} species. Unless some new document is uncovered that clearly identifies introductions of tomato to Europe from a certain area (see McCue 1952, for a comprehensive summary of historical references), the first European site of introduction will forever remain unknown. However, even such a clear reference would not determine a first site of domestication, viz. Mexico vs. Peru.

Jenkins’s (1948) Vavilovian argument of more diversity of cultivars in Mexico is not supported by comparative data (Villand et al. 1998) from South America (Ecuador, Peru and Chile). Tomatoes from Europe and North America share similar isozymes with those from Mexico and Central America, suggesting the tomato was introduced to Europe and North America from Mexico or Central America (Rick and Fobes 1975). Nevertheless, comparisons among genetic variability of primitive tomato cultivars found in Mexico, Central America and Peru, and modern varieties have neither substantiated nor disproved the hypothesis that Mexico was the centre of domestication (Rick et al. 1974, Rick and Fobes 1975, Rick and Holle 1990). Rick and Holle (1990) provided an isozyme study of different accessions of var. \textit{cerasiforme} of the wild cherry tomato (\textit{S. lycopersicum}) from South America, but they did not include cultivars or landraces from Mexico. The only comparative molecular studies (RAPDs and/or nuclear RFLPs) of diversity of landrace cultivars (Williams and St. Clair 1993, Villand et al. 1998) of tomato do not address the Peruvian/Mexican hypothesis.

A molecular study may be useful to elucidate the origin of tomato domestication by comparing a large number of accessions. However, it would be complicated by relative lack of variation within \textit{S. lycopersicum} (including its landraces), and by the difficulty to identify existing landraces from Mexico and Peru as truly native today. The only putative archaeological evidence of tomato is decorated functional ceramics “spindle whorls” produced by the native Quimbaya culture (500-1000 AD) of Colombia (McMeekin 1992). However, our examination of the figures in
this publication do not convince us that these are unequivocally tomato flowers, and could be other Solanum flowers (possibly potato). Like Rick and Holle (1990), we conclude that none of the evidence is conclusive regarding either a Mexican or a Peruvian initial site of domestication, and that tomatoes may have been domesticated independently in both areas.

**EARLY HISTORY OF THE CULTIVATED TOMATO IN EUROPE**

What were the first morphotypes of cultivated tomatoes exported from the Americas and where did they come from? McCue (1952) examined these questions through an extensive search from the literature, herbarium specimens, and early drawings. Despite this extensive search, we still know very little. The first European contact with Mexico was in 1519 (taking of Mexico City), and with Peru in 1531 (completion of the Peruvian conquest). Botanists at that time were mainly interested in the medicinal and culinary properties of plants and had little interest or knowledge of distribution or origin of cultivars. The first tomato references mentioned above were from sixteenth century herbalists, who were mainly interested with the medicinal values or “virtues” of plants, but they knew them only from exchange among botanical gardens.

These early botanists classified new plants by comparison with plants already known in Europe and from classical Greek references. Lycopersicon, the ancient Greek name for the tomato attributed to Galen is a clear example. By this method, Matthiolus (1544) described tomato by comparison to mandrake, a solanaceous plant known to the classical Greek botanist Dioscorides as: “Another species (of mandrake) has been brought to Italy in our time, flattened like the melerose (variety of apple) and segmented, green at first and when ripe of a golden color, which is eaten in the same manner as the egg plant, fried in oil with salt and pepper, like mushrooms.” From this we glean that early introduced tomatoes had yellow fruits. In a later edition of his work, Matthiolus (1554) cited both yellow and red fruits, and mentioned the Italian name for the tomato “pomì d’oro” and its Latin equivalent “mala aurea” or golden apple. Another early common name for tomato is “poma amoris”, or “love apples,” because at that time it was believed that fruits had aphrodisiacal properties. All these ancient names persisted well into the nineteenth century (Moore 1935).

The earliest tomato herbarium specimens also came from this period (McCue 1952). Jerna (1947) reported specimens labeled as “Malus insana, Mandragorae species Poma amoris” attributed to Francesco Petrolini dated between 1550 and 1560. Mattirolo (1899) mentioned another tomato specimen found in the sixteen-volume herbarium of Ulisse Aldrovandi, which was most probably cultivated in Bologna, and is the oldest extant herbarium specimen of tomato, and is now preserved at the Botanical Garden of Bologna.
Georg Oelinger was a Nürnberg apothecarian, an avid plant collector. He cultivated tomatoes in his garden probably as curiosity or as medicinal plants. The complete edition of Oelinger's (1553) work had a picture of red- and yellow-fruited tomatoes, and all the fruits are deeply furrowed (fasciated fruits). It is clear from both illustrations that the flowers had duplications of sepals and petals (6-7-parted).

Dodoens (1553) listed the Latin, German, and French names for the tomatoes along with an illustration of the plant, but he did not mention uses. His later publications (Dodoens 1574, 1583) illustrated round fruits with furrows, flowers with 7-8 petals, and two of the flowers with exserted styles.

The illustrations in L’Obel (1576) and Tabernaemontanus (1591) were similar to Dodens’s (1553). Gesner (1561) mentioned that the tomato fruit was easily grown in Germany, matured early, and had fruits varying in color from gold, red, and white; one illustration showed a plant with round fruits without furrows, and flowers with six sepals.

Teppner (1993) discussed the descriptions and illustrations of early tomatoes cultivated in Europe. He included a copy of Dodens’s (1583) drawing that clearly showed a plant with large, horizontally compressed, furrowed fruits, characteristic of early tomato cultivars. According to Sabine (1820) the cherry tomato must have been introduced at the same time as a large-fruited cultivar. Nevertheless, Aymonin (in Besler 1613) considered that the cherry tomato appeared in Europe around 1625. In Europe, tomatoes initially were cultivated mainly as ornamental plants in gardens, and they were considered inedible or poisonous because they were similar to the poisonous mandrake or belladonna.

Tomatoes were first accepted for culinary purposes in southern Europe (Ray 1673, Miller 1752) during the seventeenth and eighteenth century. Filippo (1811) reported three varieties in Italy and gave instructions for their cultivation. Sabine (1820) reported four varieties of red tomatoes and two of yellow tomatoes that were cultivated in Europe; he also discussed the condition for cultivation in England based on the experience of native gardeners. Alefeld (1866) mentioned seven varieties in Germany. According to McCue (1952), Salmon (1710) mentioned tomatoes for the first time in North America. Although tomato cultivation was not difficult, the crop gained economic importance only by the end of the nineteenth century or beginning of the twentieth century when tomato breeding programs were established (Lehmann 1955, Rick 1978, 1995).

According to Rick (1995), domestication and improvement of tomato fruit production have been accompanied by changes in the position of stigma from the anther tube. The closely related wild species, and older Latin American cultivars (and their wild species progenitor), tend to have
well exserted stigmas. Rick (1995) emphasized that in the absence of appropriate pollinators, flowers with exserted stigmas diminished the percentage of fruit set. Strong artificial selection for less exserted stigmas must have occurred after the tomato was first introduced to Europe, and even more selection under greenhouse culture. As a result, the stigma of most cultivars is shortened and now positioned at the mouth of the anther tube or even completely included in the anther tube. This shortening eliminated outcrossing and increased fruit yield in the modern varieties, but reduced the genetic variation of the crop.

The successful improvement of tomato agronomical traits is based on the understanding of basic genetics, continuous advancement of molecular genetic studies and breeding methods, which will be developed in the following chapters.

SUMMARY

Tomato is a major crop of world economy and supplies essential nutrients in human diets. There have long existed controversies regarding the place of domestication, early history, and taxonomy of tomato. The wild tomato species are native to western South America, from Ecuador south to northern Chile, and the Galapagos Islands. The putative progenitor of the cultivated species (Solanum lycopersicum = Lycopersicon esculentum var. cerasiforme) currently is widespread throughout warm regions of the world, but many of these are recent introductions. There are two competing hypotheses of the place of domestication of tomato, one supporting Peru, another in Mexico. While the Mexican origin is reasonable, we cannot discount a Peruvian origin, or even parallel domestication in both areas. Tomatoes were first recorded outside the Americas in Italy in 1544. They were cultivated first as ornamental or curiosity plants and thought by many to be poisonous. It was first accepted as a vegetable crop in southern Europe during the late sixteenth century. The first European cultivars had yellow to red flattened fruits, with deep furrows, and flowers with stigmas exserted from the anther tube. Derived cultivars had a wider range of fruit colors and shapes, smoother fruits, and stigmas included in the anther tube that led to increased fruit set but reduced the genetic variation of the crop. The taxonomy of tomato always has been controversial. This controversy involves not only generic placement in Lycopersicum or Solanum, but also hypotheses on interspecific relationships. Recent molecular data support treatment of tomato in Solanum (as we treat it here), and support allogamy, self-incompatibility, and green fruits as primitive of tomatoes. These studies support at least two distinct taxa in the formerly recognized S. peruvianum.
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*Not seen in original.*
Genetic Resources of Tomato (Lycopersicon esculentum Mill.) and Wild Relatives

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CLASSIFICATION AND TAXONOMY

The genus and species designation of tomato has been the subject of much debate, consequently, many synonyms exist. The designation most frequently used today for the cultigen is Lycopersicon esculentum Mill., with Solanum lycopersicum L. preferred by some authors. Arguments supporting the transfer of Lycopersicon species into Solanum L. have been made while recognizing the convenience of maintaining the generic designation Lycopersicon for the sake of nomenclatural stability (Peralta and Spooner 2001; see also chapter 1).

The common names used for tomato are numerous. Tomati is the word used by the Indians of Mexico, who have grown the plant for food since prehistoric times. Other names reported by early European explorers were tomatl, tumatle, and tomatas, probably variants of Indian words. Most common names have a root of tomat (used in Danish and Swedish). Tomate is used in French, German, and Portuguese. Tomato is used in English and Spanish. Tomast is used in Dutch and Pomodoro is used in Italian.

Traditional classification (reviewed by Taylor 1986) places tomato within the family Solanaceae (nightshade), sub-family Solanoideae (chromosome number x = 12), and tribe Solaneae. Lycopersicon is one of the smallest genera within this tribe, containing cultivated tomato (Lycopersicon esculentum) and its eight wild relatives (L. pimpinellifolium (L.) Mill., L. cheesmanii L.
Riley, *L. parviflorum* C.M. Rick et al., *L. chmielewskii* C.M. Rick et al., *L. hirsutum* Dunal, *L. chilense* Dunal, *L. pennellii* (Correll) D’Arcy, and *L. peruvianum* (L.) Mill.). Under this classification system *Solanum* is closely related to but distinct from the genus *Lycopersicon*, the two genera being separated on the basis of anther morphology. A comprehensive list of synonyms for *Lycopersicon* species is published on the Germplasm Resources Information Network (GRIN, http://www.ars-grin.gov/). GRIN recognizes *L. glandulosum* C.H. Mull. as a distinct species, whereas many authors treat this taxa as *L. peruvianum var. glandulosum*.

The family Solanaceae contains many plants of economic importance including tomato, potato, eggplant, petunia, tobacco, pepper (*Capsicum*), and *Physalis*. This has promoted detailed studies of many groups but the vast number of species (upwards of 1,000) and their extensive morphological complexity have lent an intractability to comprehensive taxonomic treatments. Consequently, taxonomic studies have focused on subgenera or regional floras (Bohs and Olmstead 1997). *Solanum* contains seven subgenera and 60 to 70 sections (D’Arcy 1991).

During the past decade, molecular genetic techniques have greatly impacted plant taxonomic studies at all levels, including the elucidation of the closest relatives of many crop species (reviewed by Soltis and Soltis 2000). Results of such studies can inform plant breeders, and those interested in comparative genomics, as to which species are potential sources of new alleles or model genetic systems. Recent molecular evidence has placed potato (*S. tuberosum*) and tomato as sister taxa deeply nested within *Solanum*. Under this scheme cultivated tomato is designated as *S. lycopersicum* within section *Lycopersicum*, subgenus *Potatoe*. Molecular evidence supporting this classification is briefly reviewed here.

Restriction site analysis of chloroplast DNA (cpDNA) of *Solanum* subgenus *Potatoe* (including potatoes and pepinos), *Cyphomandra* (tree tomatoes), and *Lycopersicon* (tomatoes), using *Capsicum* and *Datura* as outgroups supported two main clades among the studied taxa: 1) *Solanum* subgenus *Potatoe* and *Lycopersicon*; and 2) other *Solanum* and *Cyphomandra* (Spooner et al. 1993; see also Chapter 1). The authors argued that cpDNA and morphological data supported the transfer of *Lycopersicon* into *Solanum* subgenus *Potatoe*, section *Lycopersicum*, and recognized cultivated tomato as *Solanum lycopersicum* L. The approximately 2 kb chloroplast gene *ndhF* has been found to be useful for phylogenetic studies at inter- and infrageneric levels of plants (Bohs and Olmstead 1997 and references therein). This gene was sequenced in a broad sample of 18 *Solanum* species, species representing five genera from subfamily Solanoideae, and one
outgroup (Nicotiana tabacum L. from subfamily Cestroideae). A strict consensus tree of the 12 most parsimonious trees from unweighted parsimony analysis showed 100% bootstrap support (500 bootstrap replicates) for potato and tomato as sister taxa (Bohs and Olmstead 1997). Presence versus absence of restriction sites for ten restriction enzymes were surveyed for the entire chloroplast genome for 36 broadly sampled Solanum species and 13 outgroup species (Olmstead and Palmer 1997). Main findings of cladistic analysis of 567 variable restriction sites included: 1) monophyly of Solanum, including Lycopersicon and Cyphomandra, supported by 25 restriction site synapomorphies and 100% bootstrap value (100 bootstrap replicates), and 2) three primary clades within Solanum, designated I, II, and III. Clade II consisted of S. muricatum, S. tuberosum, and S. lycopersicum, all within the subgenus Potatoe. This clade was supported by 19 synapomorphies and 100% bootstrap value.

The cpDNA studies of Bohs and Olmstead (1997) and Olmstead and Palmer (1997) discerned three or four primary DNA lineages within broadly sampled Solanum. The latter authors argue that these data are expected to accurately reflect evolutionary relationships within Solanum rather than processes such as lineage sorting or hybridization and introgression. Lineage sorting refers to the differential fixation of shared polymorphisms among descendent species in such a way that obscures phylogenetic relationships. This would require intraspecific polymorphism of cpDNA in a progenitor species, with different haplotypes subsequently becoming fixed in different descendent species. This is purported to be unlikely in these studies because cpDNA evolves slowly, and estimates of cpDNA divergence among closely related species of Solanum are often low (Spooner et al. 1993). Hybridization and introgression are more likely to be problematic in obscuring evolutionary relationships among closely related species than among the distantly related species that were sampled for these studies.

Although tomato and potato are near-relatives and have the same basic chromosome number (x=12), multiple rearrangements prevent them from cross-hybridizing (Bonierbale et al. 1988, Tanksley et al. 1992). Recently, tomato, potato, and capsicum have been extensively studied via molecular mapping in order to understand the evolution of genome structure in the Solanaceae (Livingstone et al. 1999 and references therein).

Phylogenetic Relationships among Lycopersicon Species

Early taxonomic studies subdivided the genus into two groups, Eulycopersicon, which are color-fruiting and Eriolycopersicon, which are
green-fruited species (Muller 1940). Rick (1976) designated species into two groups, the esculentum and the peruvianum complexes, based on their reproductive compatibility with cultivated tomato. Key distinguishing taxonomic characters in the following species descriptions were taken from “A revised key for the Lycopersicon species” (Rick et al. 1990).

**Esculentum Complex**

i) *Lycopersicon esculentum* (Mill.). Cultivated tomato (*L. esculentum* var. *esculentum*) is distributed world-wide. Its precise origin in Mexico and/or Peru is uncertain (see below). Fruit interior is red when ripe with seeds 1.5 mm or longer. Leaf margins are typically serrate. Fruit diameter (3 cm or larger) and number of locules (2-to-many) distinguish it from the cherry tomato (*Lycopersicon esculentum* var. *cerasiforme* (Dunal) A. Gray), another cultivated form of tomato that is derived from *L. esculentum* var. *esculentum* crosses with *L. pimpinellifolium* with a fruit diameter 1.5 to 2.5 cm with 2 locules. Wild and weedy forms of *Lycopersicon esculentum* var. *cerasiforme* are the only wild species found outside of South America (Taylor 1986). It has traditionally been regarded as the most likely direct wild ancestor of the cultigen but molecular genetic evidence has challenged this view (Nesbitt and Tanksley 2002, see below).

ii) *Lycopersicon pimpinellifolium* can reciprocally hybridize with the cultigen and displays natural introgression. It may be a direct ancestor of *L. esculentum* or the two species may have evolved in parallel from a green-fruited ancestor (Rick 1976). Fruit interior is red when ripe with seeds 1.5 mm or longer. Relatively smaller fruit diameter (less than 1.5 cm) and generally undulate or entire leaf margins distinguish this species from *L. esculentum*. In the wild its typical habitat is at relatively low elevations (less than 1000 m) in Peru although there are known exceptions at altitudes of 1200 to 1400 m (Taylor 1986).

iii) *Lycopersicon cheesmanii* can reciprocally hybridize with the cultigen but does not do so in the wild because it is geographically restricted to the Galapagos Islands. Fruit interior is yellow or orange when ripe with seeds 1.0 mm or shorter. Subspecies *L. cheesmanii f. minor* (Hook. f.) C.H. Mull. is found in relatively lower altitude xeric habitats (Taylor 1986) and is characterized by highly ornate and elaborately subdivided leaflets (Muller 1940).

iv) *Lycopersicon parviflorum* is easily reciprocally hybridized with the cultigen although there may be exceptions for some accessions (Taylor 1986). Fruit inside is green or whitish when ripe with seeds 1.0 mm or shorter. Sympodia have two leaves, inflorescences with small or
no bracts, and flowers small (corolla diameter 1.5 cm or less). Its center of diversity is inter-Andean Peru, where it prefers moist habitats along rocky banks of small streams (Taylor 1986). *L. parviflorum* and its sister taxa *Lycopersicon chmielewskii* comprise the *minutum* complex. The highly autogamous inbreeding *L. parviflorum* is thought to have been derived from the primarily outcrossing *L. chmielewskii*.

v) *Lycopersicon chmielewskii* easily hybridizes with the cultigen (Taylor 1986). Fruit interior is green or whitish when ripe. Taxonomic traits distinguishing this species from *L. parviflorum* are larger seeds (1.5 mm or longer) and larger flowers (corolla diameter 2.0 cm or more). It is sympatric with *L. parviflorum* but more limited in its distribution and prefers slightly better-drained habitats (Taylor 1986).

vi) *Lycopersicon hirsutum* can act successfully as the pollen parent when crossed with cultivated tomato but the reciprocal cross does not set fruit. *Lycopersicon hirsutum* f. *glabratum* C.H. Mull. and the cultigen are reciprocally compatible (Taylor 1986). The two forms of *L. hirsutum* are not fully compatible with each other. Fruit interior is green or whitish when ripe and sympodia have three leaves. The *glabratum* biotypes are characterized by less hairy leaves, stems, and fruit, a smaller corolla, less showy flowers, and a tendency to inbreed. *L. hirsutum* typically grows in moist river valleys at the relatively highest elevations for *Lycopersicon* species (500 to 3300 m), with *glabratum* occupying the northern extremes of the distribution. Both are distributed in Ecuador and Peru (Taylor 1986).

vii) *Lycopersicon pennellii* easily hybridizes with cultivated tomato. Fruit interior is green or whitish when ripe and sympodia have two leaves. Inflorescences have large bracts and anthers are free and poricidal. Distribution is relatively restricted along coastal Peru with some populations found in extreme xeric habitats experiencing temperatures of 25° to 30°C (Taylor 1986).

Peruvianum Complex

i) *Lycopersicon chilense* can act as the pollen parent when crossed with the cultigen but viable seeds are rare and embryos must be cultured. In the reciprocal cross, *L. chilense* will not accept *L. esculentum* pollen. Fruit interior is green or whitish when ripe and sympodia have two leaves. Inflorescences have large bracts, anthers are attached in a tube and are dehiscent by lateral apertures. Flowers are congested and anther tubes are straight. Peduncles are longer than 15 cm and plants are erect. *L. chilense* is the most southerly of *Lycopersicon* species
distributed in Chile and southern Peru and prefers extremely arid habitats (Taylor 1986).

ii) *Lycopersicon peruvianum* is genetically and morphologically the most diverse of the *Lycopersicon* species and several varieties have been described (*dentatum, humifusum, peruvianum*). In general, the species exhibits severe barriers to crossing with *L. esculentum*. Fruit interior is green or whitish when ripe and sympodia have two leaves. Inflorescences have large bracts, anthers are attached in a tube and are dehiscent by lateral apertures. Flowers are loosely arrayed with anther tubes generally bending distally. Peduncles are shorter than 15 cm and plants are spreading. Distribution ranges from northern Peru to northern Chile, encompassing a broad range of habitats and including many mountain races that are geographically isolated from each other (Taylor 1986).

iii) *Lycopersicon glandulosum* is synonymous with *Lycopersicon peruvianum* var. *glandulosum*. This is a mountain race common to central Peru. Although it is reproductively compatible with coastal populations of *L. peruvianum* it is distinguished from them by its very thin stems, short dense glandular hairs, and narrow leaflets. It grows at elevations as high as 3000 m where temperatures reach as low as 4° to 8° C (Taylor 1986).

Within the genus *Lycopersicon* closely related interspecies and intraspecies heterogeneity have made the resolution of precise interspecific relationships difficult. Molecular genetic evidence examining relationships has been accumulating during the previous two decades. Speciation within the genus based on these data has been relatively recent. Estimated divergence times for the genus on the basis of pooled silent sites and a rate of $6.03 \times 10^{-9}$ silent substitutions per site year showed that the genus began its initial radiation ~ 7 million years ago. *L. esculentum* and its nearest relatives *L. cheesmanii* and *L. pimpinellifolium* shared a recent common ancestor ~1 million years BP (before present) (Nesbitt and Tanksley 2002).

Molecular markers support three to four major groupings within the genus, reflecting mating system and fruit color, with autogamy and red-orange fruit being true synapomorphs. These results are generally consistent with morphological and crossability data (Rick 1979). Relationships among species within the major groups are not well-resolved. The traditional distinction between the esculentum and peruvianum complexes is somewhat obscured by molecular phylogenetic evidence. This is because of relatively large genetic distances between *L. hirsutum* and *L. pennellii*
and the remaining *Lycopersicon* species, although both species can hybridize with the highly-derived *L. esculentum*, and so are considered to be part of the esculentum complex. The main findings from molecular genetic studies are reviewed here.

The pioneering studies examined organelar genomes. Restriction fragment length polymorphism (RFLP) of chloroplast (cpDNA) and hybridization of mitochondrial (mtDNA) DNA studies supported the transfer of *L. pennellii* from the genus *Solanum* into the genus *Lycopersicon* (Palmer and Zamir 1982, McClean and Hanson 1986). At the subgeneric level parsimony analysis supported red fruit color to be monophyletic in *L. esculentum*, *L. pimpinellifolium*, and *L. cheesmanii* (Palmer and Zamir 1982). cpDNA results also gave evidence for a close relationship of *L. chilense* and *L. chmielewskii* to *L. peruvianum*. This apparent anomaly may be explained by the observation that *L. peruvianum* is a heterogeneous taxon with northern populations being somewhat closely related to the esculentum complex.

Eight *Lycopersicon* species were surveyed for RFLPs using 40 single-copy nuclear probes (Miller and Tanksley 1990). The sample of 156 plants represented two to five plants per accession and with the exception of *L. parviflorum*, multiple accessions per species. Unweighted pair group method using arithmetic averages (UPGMA) analysis using genetic distances based on proportion of shared bands gave two major groupings of species. The first group distinguished the self-incompatible (SI) *L. hirsutum*, *L. pennellii*, and *L. peruvianum* from the self-compatible (SC) *L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, *L. parviflorum*, and *L. chmielewskii* (Table 2.1). The second grouping delineated green versus red-fruited species. An exception was a northern Peruvian representative of *L. peruvianum* var. *humifusum* (LA2150) which grouped with the SC species albeit most distantly. The authors postulated that this accession may be a modern representative of the SI gene pool that gave rise to the SC *L. parviflorum* and *L. chmielewskii*.

Three main groupings resulted from cluster analysis of allozyme data from eight *Lycopersicon* species: i) SC and red-fruited *L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, and *L. peruvianum*, ii) the SC and green-fruited *L. chmielewskii* and *L. parviflorum*, and iii) the SI and green fruited *L. pennellii* and *L. chilense* (Breto et al. 1993).

Random amplified polymorphic DNA (RAPD) markers were used to estimate genetic distances among 22 accessions of *L. peruvianum*; 12 accessions of *L. chilense*; and two accessions from each of *L. esculentum* var. *esculentum*, *L. esculentum* var. *cerasiforme*, *L. pimpinellifolium*, *L. cheesmanii*,
L. chmielewskii, L. parviflorum, L. hirsutum, and L. pennellii (Egashira et al. 2000). Neighbor-joining (NJ) analysis of genetic distances based on 435 RAPD fragments distinguished four groups consisting of three clusters and two outlier taxa; i) L. esculentum, L. pimpinellifolium, L. cheesmanii, and L. cheesmanii var. minor, ii) L. chmielewskii and L. parviflorum, iii) the peruvianum complex L. peruvianum and L. chilense, and iv) L. hirsutum and L. pennellii which were distinct from all other taxa, including each other. Bootstrap support using 1000 bootstrap replicates was generally low (less than 40%) for most of these groups.

Sixteen polymorphic simple sequence repeat (SSR) markers were used to genotype five to ten plants per accession from one to three accessions each of L. esculentum var. esculentum, L. esculentum var. cerasiforme, L. pimpinellifolium, L. cheesmanii, L. parviflorum, L. chmielewskii, L. chilense, L. hirsutum, L. pennellii; and from 11 accessions of L. peruvianum (Alvarez et al. 2001). A NJ tree constructed using eight low-diversity loci (gene diversity less than 0.245) was found to be more reliable than trees constructed using all loci. The authors inferred that relatively more diverse SSR loci were more mutable and exhibited higher degrees of homoplasy among species. SSR results supported genetic relationships based on RFLP (Miller and Tanksley 1990) and morphological (Rick 1979) data. With the low-diversity SSR data the northern accessions of L. peruvianum (LA2334, LA2172, LA1708, LA2157) clustered closer to L. esculentum, L. pimpinellifolium, L. cheesmanii, L. parviflorum, and L. chmielewskii than to the southern L. peruvianum accessions (LA372, LA462, LA1333, LA1373, LA1274, LA1945, LA1955) and L. chilense.

DNA sequence variation of the 5’ portion of granule-bound starch synthase gene (GBSS1 waxy) was examined for three accessions from each of L. esculentum var. cerasiforme, L. cheesmanii, L. chmielewskii, L. parviflorum, L. hirsutum, L. pennellii, L. chilense; five accessions of L. pimpinellifolium; 39 accessions of L. peruvianum one cultigen; plus one to two accessions from each of nine closely related outgroups (Peralta and Spooner 2001). The approximately 1300 nucleotide (nt) region included eight exons and seven introns. A strict consensus tree of 15000 most parsimonious trees did not give good resolution of closely related species. It did support allogamy, self-incompatibility and green fruit as primitive and further supported the splitting of L. peruvianum into two groups with the northern populations close to the self-compatible taxa L. chmielewskii, L. parviflorum, L. cheesmanii, L. pimpinellifolium, and L. esculentum and the southern populations close to L. chilense. L. hirsutum and L. pennellii were distantly related to the remaining
esculentum complex species. *Solanum jungandifolium* and *Solanum ochranthum* were the closest outgroup to *Lycopersicon* with *Solanum lycopersicoides* and *Solanum sitiens* basal to these.

DNA sequences of the approximately 700 nt internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA) from one accession each of *L. esculentum* var. *esculentum*, *L. esculentum* var. *cerasiforme*, *L. cheesmanii*, *L. pimpinellifolium*, *L. parviflorum*; three accessions each from *L. peruvianum*, *L. hirsutum*, and *L. pennellii*; plus one accession from each of seven outgroups were studied using parsimony analysis (Marshall et al. 2001). The single most-parsimonious tree displayed a fully resolved topology but with low bootstrap support on some of the branches. Results showed three clades: i) *esculentum* containing *L. pimpinellifolium*, *L. esculentum* var. *esculentum*, *L. esculentum* var. *cerasiforme*, *L. cheesmanii*, *L. chmielewskii*, and *L. parviflorum*, ii) *peruvianum* containing *L. peruvianum* and *L. chilense*, and iii) *hirsutum* containing *L. hirsutum* and *L. pennellii*. These results reflected mating system (SC versus SI) and fruit color supporting the synapomorphy of both SC and red fruit. The northerly *L. peruvianum* var. *humifusum* (LA2150) was nested within the *peruvianum* clade rather than close to *L. chmielewskii* and *L. parviflorum* (inconsistent with Miller and Tanksley 1990) but with low bootstrap support.

In the first study of *Lycopersicon* relationships using nuclear DNA sequences at multiple loci, four regions were included for a total of approximately 7 kb (Nesbitt and Tanksley 2002). Loci consisted of a fruit weight quantitative trait locus (QTL) *fw2*, the 5’ untranslated region (UTR) of *fw2.2*, *orf44* (an open reading frame of unknown function immediately adjacent to *fw2.2*), three loci on chromosomes other than *fw2.2*: alcohol dehydrogenase 2 (*Adh2*), and two anonymous single-copy loci TG10 and TG11. Species were represented by four accessions of *L. esculentum* var. *esculentum*; three accessions of *L. pimpinellifolium*; and one accession each of *L. cheesmanii*, *L. parviflorum*, *L. hirsutum*, *L. pennellii*, and *L. peruvianum*. The single most-parsimonious tree from data pooled across loci and using *L. pennellii* as the outgroup gave strong support (100% bootstrap values for 100 replicates) for three major groupings: i) *L. esculentum*, *L. cheesmanii*, and *L. pimpinellifolium*, ii) *L. parviflorum* and *L. peruvianum*, and iii) *L. hirsutum* and *L. pennellii*. The *L. peruvianum* accession in this study (LA1708) was a northern type that also clustered close to *L. parviflorum* based on SSR data (Alvarez et al. 2001).

*L. esculentum* var. *cerasiforme* accessions were also sequenced in Nesbitt and Tanksley’s (2002) study but were not used in estimating interspecific
relationships because they introduced many incongruities into the trees. Gene trees of *fw2.2 5’ UTR, Adh2, TG10, and TG11* show ten *L. esculentum var. cerasiforme* alleles to be interspersed among four *L. esculentum var. esculentum* and three *L. pimpinellifolium* alleles. The authors suggested that this apparent admixture of alleles may represent hybridizations between *L. esculentum var. esculentum* and *L. pimpinellifolium* giving rise to *L. esculentum var. cerasiforme*.

To summarize the molecular phylogenetic studies of *Lycopersicon*, there is general support for the following relationships (from derived to ancestral groups): i) red fruit SC *L. esculentum, L. cheesmanii, and L. pimpinellifolium*, ii) green fruit SC species *L. chmielewskii* and *L. parviflorum*, iii) green fruit SI northern *L. peruvianum*, iv) green fruit SI southern *L. peruvianum* and *L. chilense*, and v) green fruit SI *L. hirsutum* and *L. pennellii*.

**ORIGIN AND MOLECULAR DIVERSITY OF CULTIVATED TOMATO**

Cultivated tomato has been documented to have been in existence for only about 400 years (Boswell 1937). There are detailed written accounts of its presumed origin, migration, selection, and introgression (Boswell et al. 1933, Boswell 1937, Jenkins 1948, Stevens and Rick 1986). Peru is the center of origin for the genus and while *L. esculentum var. cerasiforme* was believed to be the direct progenitor of large-fruited cultigens (Bailey 1896), it may more likely be an admixture of cultivated and wild species (Nesbitt and Tanksley 2002). *L. esculentum* is thought to have originally been domesticated and planted in maize fields by ancient Mexicans, although this remains uncertain (Jenkins 1948). Tomato spread to Europe in the early 1500s, initially in Italy and Spain, and thereafter became widely distributed. It was not grown and consumed in large quantities until the late 1700s. Since then it has been selected and bred within a broad range of climates from cool-temperate to tropical. The first cultigens grown in the U.S. came from England and France and the first U.S. improved cultigens were Tilden, released in 1865, and Trophy, released in 1870. Trophy ushered in a new epoch of tomato popularity in the U.S. and is believed to have contributed to the parentage of most of the cultigens developed during the next few decades (Bailey 1896).

Small companies became important sources of seed in late 1800s to 1900s and around 1910 public breeders started introducing disease resistant cultigens, e.g., Tennessee Red and Louisiana Wilt Resistant. These early resistant types dominated the U.S. market in the 1920s and 1930s. In the early 1940s closely related wild species within the genus *Lycopersicon*...
began to be screened for additional disease resistance, and wild sources provided much of the breeding germplasm during subsequent decades (Stevens and Rick 1986). Wild germplasm continues to play a major role in tomato breeding. Recent work has demonstrated that favorable alleles in wild relatives can remain cryptic until expressed in an improved background (Tanksley et al. 1996) and techniques with which to incorporate wild alleles into modern cultigens continue to be refined (Monforte et al. 2001).

Although migration of the cultigen from Latin America to Europe and subsequently throughout the world has been documented, pedigrees for the majority of *L. esculentum* accessions held in germplasm collections are largely unknown. Molecular markers have been used to estimate genetic diversity of sets of cultigens. Two trends have been observed: i) a narrowing of the germplasm base caused by genetic bottlenecks and selection, and ii) an increase in molecular genetic variation in and around regions introgressed from wild relatives.

Genetic variation among accessions originating from primary and secondary centers of diversity was surveyed using 41 RAPD primers (Villard et al. 1998). This study included 21 accessions from the primary center of diversity for the genus (Chile, Peru, and Ecuador), 37 accessions from secondary centers contiguous with the primary center (South America, Central America, and Mexico), and 38 accessions from secondary centers other than South America, Central America, and Mexico (including Asia, Africa, Cuba, Europe, and U.S.). Average genetic distance for all possible 4,560 pairwise comparisons of accessions (estimated as the complement to the simple matching coefficient; Gower 1972) was 0.164 ± 0.084. Genetic diversity based on allele frequencies (Nei 1987) for groups of accessions was estimated. Average genetic diversity was relatively greater in the primary center (0.219) compared to contiguous (0.175) and other (0.137) secondary centers. This supported allozyme data that showed greater diversity in *L. esculentum* from Peru and Ecuador compared to material from secondary centers (Rick and Fobes 1975). There was also more genetic variation in a set of 20 processing tomato cultigens (Villard 1995) compared to random samples of 20 cultigens from this study. The authors suggested that this could be explained by recent introgression of favorable alleles from wild *Lycopersicon* species into processing cultivars. This was consistent with an observed increase in RAPD variation among modern cultigens compared to cultigens released prior to around 1960 (Williams and St. Clair 1993).
Genetic relationships among 19 tomato cultigens from a geographically isolated regions accessions originating from outside the center of origin (1 South Africa, 5 Europe, 1 Russia, 1 China, 1 Australia, 9 from Canada and U.S., and 1 unknown) were studied using 65 polymorphic SSR markers (He et al. 2003). UPGMA clustering based on genetic distances (Nei and Li 1979) did not reveal a clear pattern reflecting geographical origins of accessions. European accessions were widely dispersed throughout the dendrogram, implying that most non-European germplasm is closely related to germplasm of European origin. However, equal numbers of accessions from various geographical regions would need to be compared in order to test this hypothesis.

RAPD markers showed a narrowing of the germplasm base between tomato cultigens bred and released in India during the 1970s compared to the 1990s (Archak et al. 2002). Average gene diversity (Nei 1987) based on 174 RAPD fragments was 0.265 (n = 5 accessions) in pre-1979 material versus 0.068 (n = 7) and 0.118 (n = 8) in two sets of modern material originating from different breeding programs. This was interpreted as hav-

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Self-compatible</th>
<th>Mating System</th>
<th>Cross-compatibility with cultigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. cheesmanii</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. chilense</td>
<td>no</td>
<td>allogamous</td>
<td>can act as male with embryo rescue</td>
</tr>
<tr>
<td>L. chnielewskii</td>
<td>yes</td>
<td>facultative</td>
<td>reciprocal</td>
</tr>
<tr>
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<td>reciprocal</td>
</tr>
<tr>
<td>L. esculentum var. cerasiforme</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. glandulosum</td>
<td>no</td>
<td>allogamous</td>
<td>no</td>
</tr>
<tr>
<td>L. hirsutum</td>
<td>no, except for some biotypes</td>
<td>allogamous or facultative</td>
<td>can act as male</td>
</tr>
<tr>
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<td>reciprocal</td>
</tr>
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<td>L. pennellii</td>
<td>no, except for some biotypes</td>
<td>allogamous or facultative</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. peruvianum</td>
<td>no, except for some biotypes</td>
<td>allogamous or facultative</td>
<td>no, can occasionally be overcome with technical difficulty</td>
</tr>
<tr>
<td>L. pimpinellifolium</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
</tbody>
</table>
ing resulted from a trend towards breeding for a specific type, i.e., determinate plants bearing uniform fruit.

Although the genetic base of cultivated tomato is narrow, researchers have found it possible to distinguish cultivars using small numbers (four to five) of polymorphic loci (Bredemeijer et al. 1998, four SSR loci distinguishing 16 cultivars; Suliman-Pollatschek et al. 2002, four SSR loci distinguishing 10 cultivars; He et al. 2003, five SSR loci distinguishing 19 cultivars).

**ECONOMIC IMPORTANCE**

Tomato is a major vegetable crop in the United States and worldwide. The crop is used both fresh and in processed products. Fresh tomato is eaten by itself, in salads, and is used in many recipes as an ingredient. Processed products include paste, canned tomatoes (diced, crushed, and whole), salsa, ketchup, and as an ingredient in many condiments. Tomatoes are also dried and used for cooking.

Worldwide, tomato is produced for the fresh market and processing on approximately 4 million hectares, with an average yield of 27.2 ton ha\(^{-1}\) and a yearly production of 108.5 million tons (FAOSTAT 2002; Table 2.2). The top five leading tomato producing countries are China, the United States, Turkey, India, and Egypt. Statistics of regions and for leading tomato producers is given in Table 2.2. Tomato area has increased by 38\% and production has increased by 45\% in the past ten years. Most of this worldwide increase in production has come from China, where the area has more than tripled from 0.30 million ha to 0.97 million ha, with an increase in production from 8.5 million tons to 25.5 million tons, propelling China from number two to the number one producer of tomato over the United States. China now accounts for 23.5\% of the world’s tomato production. Leading producers of tomato are listed in Table 2.2.

Production of tomato for fresh market and processing in the United States is given in Table 2.3 (USDA-NASS 1995, 2003). Area of tomato cultivation in the United States for fresh market production has decreased from 53.4 thousand ha to 50.6 thousand ha; however, the area of cultivation for processed products has increased from 110.9 thousand ha to 126.2 thousand ha. Therefore, tomato production for fresh market has decreased in the past ten years from 1.4 million tons to 1.2 million tons; whereas, production of processed tomato has increased in the past ten years from 5.1 million tons to 6.8 million tons.
The value of fresh market tomato produced in the United States is 1.17 billion dollars and the value of processed tomato production is 683 million dollars (Table 2.3). Tomato accounts for approximately 14.5% of the United States, fresh vegetable production value, while it accounts for approximately 50.7% of the processed vegetable production value. Significant production of fresh market tomato is found in 17 states. The two leading producers are Florida and California, which account for 67% of the total value of fresh market tomato production value. There are four primary producers of tomato for the processing market, but California accounts for

### Table 2.2 World production of tomatoes in 1992 and 2002 (FAOSTAT 2002).

<table>
<thead>
<tr>
<th>Location</th>
<th>1992</th>
<th></th>
<th></th>
<th>2002</th>
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<tr>
<td></td>
<td>Area (ha × 10^3)</td>
<td>Yield (ton ha⁻¹)</td>
<td>Production (ton × 10^3)</td>
<td>Area (ha × 10^3)</td>
<td>Yield (ton ha⁻¹)</td>
<td>Production (ton × 10^3)</td>
</tr>
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<td>300</td>
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<td>Leading Countries</td>
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<td>142</td>
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<td>1,820</td>
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<td>1,850</td>
<td>38</td>
<td>45.1</td>
<td>1,700</td>
</tr>
<tr>
<td>Chile</td>
<td>19</td>
<td>40.0</td>
<td>780</td>
<td>20</td>
<td>66.0</td>
<td>1,287</td>
</tr>
<tr>
<td>Ukraine</td>
<td>116</td>
<td>11.2</td>
<td>1,303</td>
<td>105</td>
<td>10.5</td>
<td>1,100</td>
</tr>
<tr>
<td>Uzbekistan</td>
<td>47</td>
<td>29.1</td>
<td>1,370</td>
<td>28</td>
<td>35.7</td>
<td>1,000</td>
</tr>
<tr>
<td>Portugal</td>
<td>20</td>
<td>35.0</td>
<td>700</td>
<td>18</td>
<td>55.2</td>
<td>994</td>
</tr>
<tr>
<td>Morocco</td>
<td>25</td>
<td>35.4</td>
<td>894</td>
<td>19</td>
<td>52.0</td>
<td>991</td>
</tr>
<tr>
<td>Nigeria</td>
<td>40</td>
<td>10.0</td>
<td>400</td>
<td>126</td>
<td>7.0</td>
<td>879</td>
</tr>
</tbody>
</table>
Table 2.3 United States fresh market production of tomatoes 1992 and 2002  
(USDA-NASS, USDA 1995 and 2003)

<table>
<thead>
<tr>
<th>Location</th>
<th>1992</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (ha × 10^3)</td>
<td>Production (ton × 10^3)</td>
</tr>
<tr>
<td></td>
<td>Area (ha × 10^3)</td>
<td>Production (ton × 10^3)</td>
</tr>
<tr>
<td><strong>Total Vegetables</strong></td>
<td>611.8</td>
<td>15,087.1</td>
</tr>
<tr>
<td></td>
<td>679.1</td>
<td>17,659.1</td>
</tr>
<tr>
<td><strong>Tomato</strong></td>
<td>53.4</td>
<td>1,770.5</td>
</tr>
<tr>
<td></td>
<td>50.6</td>
<td>1,692.0</td>
</tr>
<tr>
<td><strong>AL</strong></td>
<td>1.3</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>14.6</td>
</tr>
<tr>
<td><strong>AR</strong></td>
<td>0.5</td>
<td>16.0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>15.2</td>
</tr>
<tr>
<td><strong>CA</strong></td>
<td>14.6</td>
<td>457.2</td>
</tr>
<tr>
<td></td>
<td>15.6</td>
<td>523.9</td>
</tr>
<tr>
<td><strong>FL</strong></td>
<td>20.8</td>
<td>946.1</td>
</tr>
<tr>
<td></td>
<td>18.2</td>
<td>655.2</td>
</tr>
<tr>
<td><strong>GA</strong></td>
<td>1.2</td>
<td>48.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>34.0</td>
</tr>
<tr>
<td><strong>IN</strong></td>
<td>0.5</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>11.2</td>
</tr>
<tr>
<td><strong>MD</strong></td>
<td>0.8</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>12.2</td>
</tr>
<tr>
<td><strong>MI</strong></td>
<td>1.0</td>
<td>15.9</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>16.2</td>
</tr>
<tr>
<td><strong>NJ</strong></td>
<td>1.9</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>34.4</td>
</tr>
<tr>
<td><strong>NY</strong></td>
<td>0.9</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>17.1</td>
</tr>
<tr>
<td><strong>NC</strong></td>
<td>0.6</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>40.4</td>
</tr>
<tr>
<td><strong>OH</strong></td>
<td>1.2</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>112.4</td>
</tr>
<tr>
<td><strong>PA</strong></td>
<td>1.7</td>
<td>32.4</td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>31.8</td>
</tr>
<tr>
<td><strong>SC</strong></td>
<td>1.5</td>
<td>48.3</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>38.0</td>
</tr>
<tr>
<td><strong>TN</strong></td>
<td>1.8</td>
<td>32.7</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>60.1</td>
</tr>
<tr>
<td><strong>TX</strong></td>
<td>1.3</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>8.2</td>
</tr>
<tr>
<td><strong>VA</strong></td>
<td>1.3</td>
<td>59.5</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>68.9</td>
</tr>
</tbody>
</table>

| **Processed** | 585.3 | 12,915.0 | 1,126.4 | 545.9 | 15,528.1 | 1,346.9 |
| **Total Vegetables** | 110.9 | 7,962.8 | 509.4 | 126.2 | 10,574.8 | 683.1 |
| **Tomato** | 97.1 | 7,195.8 | 447.4 | 117.8 | 10,029.8 | 632.4 |
| **CA** | 2.7 | 148.4 | 13.0 | 3.3 | 232.6 | 22.1 |
| **IN** | 2.3 | 165.5 | 12.6 | 1.3 | 101.6 | 9.3 |
| **MI** | 5.9 | 339.9 | 26.0 | 2.5 | 135.7 | 12.1 |
| **OH** | 2.9 | 113.2 | 10.5 | 1.3 | 75.1 | 7.2 |

approximately 93% of the production value. The second producer, Indiana, accounts for only 3% of the production value.

While Florida and California are the number 1 and 2 producers of fresh market tomato, respectively; the value of their production has decreased by approximately 31% in the past ten years with a decrease of the total production value from 83.5% to 50.7%. However, 12 of the other 15 leading production states have increased their production value of fresh market tomato in the past ten years. The largest increases have been in New York, North Carolina, Ohio, and Tennessee. This shows a trend of consumer
preference for locally produced tomatoes because of the perception of higher quality.

The reduction in fresh market tomato can mostly be explained by the increase of imports and the decrease of exports of fresh market tomato by the United States (Table 2.4). In the past ten years fresh market production of tomato in the United States has declined by 78,500 tons. However, the trade deficit of the United States in fresh market tomato production has increased by 634,800 tons. In 2002, most of the United States import of fresh market tomato has come from Mexico, with an increase of imports by 573,400 tons in the past ten years. Imports accounted for approximately 11% of fresh market tomato consumption in the United States in 1992, but by 2002 imports accounted for 32% of the fresh market tomato consumption. Cantliffe (1997) has extensively reviewed the impact of the North American Free Trade Agreement (NAFTA) on Mexican fresh market tomato production and its importation into the United States for the period of 1982 to 1997. The United States has maintained a trade surplus in processed tomato products which has slightly increased in the past ten years (Table 2.4).

Although the majority of fresh market fruit is field grown, there has been an expansion of greenhouse grown tomato. Greenhouse production of fresh market tomato is significant in Europe, especially the Netherlands (Snyder 1996, Table 2.5). Greenhouse production of tomato is predominantly produced with rockwool (Logendra and Janes 1997), moving towards hydroponics (Jensen 1997).

Table 2.4 *United States exports and imports of fresh market and processed tomato (USDA-ERS 2003)*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>166.7</td>
<td>150.6</td>
<td>196.0</td>
<td>860.1</td>
</tr>
<tr>
<td>Paste</td>
<td>73.2</td>
<td>99.1</td>
<td>19.8</td>
<td>17.8</td>
</tr>
<tr>
<td>Canned (whole and pieces)</td>
<td>14.0</td>
<td>34.5</td>
<td>43.3</td>
<td>14.7</td>
</tr>
<tr>
<td>Sauce</td>
<td>59.9</td>
<td>116.1</td>
<td>7.9</td>
<td>124.1</td>
</tr>
<tr>
<td>Catsup and chili sauce</td>
<td>24.5</td>
<td>38.7</td>
<td>7.8</td>
<td>12.9</td>
</tr>
<tr>
<td>Canned pulp (puree)</td>
<td>6.5</td>
<td>16.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
GERMLASM CONSERVATION

Germplasm Collections

Major germplasm collections of tomato are maintained in the United States at the Plant Genetic Resources Unit, United States Department of Agriculture (USDA) at Geneva, NY, and at the Tomato Genetic Resources Center (TGRC) located in the Department of Vegetable Crops at the University of California Davis. The Asian Vegetable Research and Development Center (AVDRC), now referred to as the World Vegetable Center (WVC), located at Tainan, Taiwan is an international center affiliated with the Consultative Group of International Centers, which maintains the major international collection of tomato germplasm.

Worldwide, there are more than 75,000 accessions of Lycopersicon germplasm accessions maintained in more than 120 countries in a number of national institutions (Battencourt and Konopka 1990, updated on web). Table 2.6 lists the largest collections (except the USDA, AVDRC and TGRC which are listed in Table 2.7). Most data reported in the IPGRI listing has been updated since the mid-1990s, with many updates, especially for the larger collections since 2000. This report is available on the Internet at: http://www.ipgri.cgiar.org/germplasm/dbintro.htm. There are URLs available within this report for many of the institutions where large collections of tomato are reported. The countries with the greatest number of germplasm accessions of Lycopersicon, besides the USA, are Brazil, Bulgaria, Canada, China, Colombia, Germany, Hungary, the Philippines, and Spain. These countries have two thousand or more accessions of Lycopersicon species conserved, mostly L. esculentum.

The collections of USDA and AVDRC are mainly cultivated Lycopersicon, though each has significant wild Lycopersicon collections (Table 2.7) with AVDRC conserving 659 accessions of wild Lycopersicon species and the USDA conserving 458 accessions of wild Lycopersicon species. Both of these genebanks have large collections of L. peruvianum and L. pimpinellifolium

<table>
<thead>
<tr>
<th>Country</th>
<th>Hectares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>287</td>
</tr>
<tr>
<td>England/Wales</td>
<td>1214</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>4613</td>
</tr>
<tr>
<td>Spain</td>
<td>12,140</td>
</tr>
<tr>
<td>United States</td>
<td>182</td>
</tr>
</tbody>
</table>

Source: Snyder (1996)

Table 2.5 Greenhouse tomato area in selected countries

Greenhouse tomato area in selected countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Hectares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>287</td>
</tr>
<tr>
<td>England/Wales</td>
<td>1214</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>4613</td>
</tr>
<tr>
<td>Spain</td>
<td>12,140</td>
</tr>
<tr>
<td>United States</td>
<td>182</td>
</tr>
</tbody>
</table>

Source: Snyder (1996)
### Table 2.6 Worldwide *Lycopersicon* germplasm conservation (For USDA, AVDRC, TGRC; see Table 2.7).

<table>
<thead>
<tr>
<th>Country</th>
<th>Institution</th>
<th>No. accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>Australian Tropical Crops &amp; Forages Genetic Resources Centre</td>
<td>1116</td>
</tr>
<tr>
<td>Brazil</td>
<td>Centro Nacional de Pesquisa de Hortali.as, EMBPRA</td>
<td>2070</td>
</tr>
<tr>
<td></td>
<td>Lab. de Melhoramento Genetico Vegetal (LMGV)-CCTA-VENF</td>
<td>508</td>
</tr>
<tr>
<td></td>
<td>Instituto Agronomico de Campinas (IAC)</td>
<td>500</td>
</tr>
<tr>
<td></td>
<td>Departamento de Fitotechnia-Universidad Federal de Vicos</td>
<td>600</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>Institut de resources phytogénétiques ‘K Malkov’</td>
<td>580</td>
</tr>
<tr>
<td>Canada</td>
<td>Saskatoon Research Centre, Agriculture and AgriFood Canada</td>
<td>1897</td>
</tr>
<tr>
<td></td>
<td>Horticultural Experiment Station, Simcoe, Ontario</td>
<td>1070</td>
</tr>
<tr>
<td>China</td>
<td>Institute of Crop Germplasm Resources (CAAS)</td>
<td>1942</td>
</tr>
<tr>
<td>Colombia</td>
<td>Corporacion Columbiana de Investigacion Agropecuaria-CORPOICA</td>
<td>2018</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>Estacion Experimental AGRicola Fabio Baudrit, Univ. de Costa Rica</td>
<td>700</td>
</tr>
<tr>
<td></td>
<td>Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE)</td>
<td>457</td>
</tr>
<tr>
<td>Cuba</td>
<td>Banco de Germoplasma, Inst. de Invest. Fund. en Agricultura</td>
<td>630</td>
</tr>
<tr>
<td>Czech Rep.</td>
<td>Genebank Department-Vegetable Secion Olomouc</td>
<td>1613</td>
</tr>
<tr>
<td>France</td>
<td>Unité Expérientale d’Angers Geves</td>
<td>1254</td>
</tr>
<tr>
<td></td>
<td>Station d’Amélioration des Plantes, INRA Avignon</td>
<td>1246</td>
</tr>
<tr>
<td>Germany</td>
<td>Genebank, Inst. for Plant Genetics and Crop Plant Research (IPK)</td>
<td>3262</td>
</tr>
<tr>
<td>Hungary</td>
<td>Institute for Agrobotany</td>
<td>2043</td>
</tr>
<tr>
<td>India</td>
<td>National Bureau of Plant Genetic Resources (NBPGR)</td>
<td>940</td>
</tr>
<tr>
<td>Italy</td>
<td>Dip. di Agronomia &amp; Genetica Veg. Universita degli Studi de Napoli</td>
<td>804</td>
</tr>
<tr>
<td>Japan</td>
<td>Department of Genetic Resources, Nation. Inst. of Agrobiol. Resour.</td>
<td>452</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>Centre for Genetic Resources, The Netherlands (CGN)</td>
<td>1159</td>
</tr>
<tr>
<td>Nigeria</td>
<td>National Centre for Genetic Resources and Biotechnology, (FMST)</td>
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</tr>
<tr>
<td>Peru</td>
<td>Universidad Nacional Agraria La Molina</td>
<td>936</td>
</tr>
<tr>
<td>Philippines</td>
<td>National Plant Genetic Resources Laboratory, IPB/UPLB</td>
<td>4793</td>
</tr>
<tr>
<td>Poland</td>
<td>Plant Breeding and Acclimatization Institute (IHAR)</td>
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</tr>
<tr>
<td></td>
<td>Plant Genetic Resources Laboratory Research Inst. of Vegt. Crops</td>
<td>427</td>
</tr>
<tr>
<td>Spain</td>
<td>Centro de Recursos Fitogeneticos, INIA</td>
<td>1267</td>
</tr>
<tr>
<td></td>
<td>Experimental Station La Mayora CSIC</td>
<td>801</td>
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<tr>
<td></td>
<td>Univ.Politecéde Valencia, Escuela Té Sup. de Ing.</td>
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</tr>
<tr>
<td></td>
<td>Agron. Banco de Germ plasmo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Banco de Germoplasma de Horticolas-Diputacion General de Aragon</td>
<td>1381</td>
</tr>
<tr>
<td>Turkey</td>
<td>Plant Genetic Resources Dept. Aegean Agricultural Research Inst.</td>
<td>544</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Centre for Introducted Crops Vietnam Inst. Agric. Sci. &amp; Tech.</td>
<td>487</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>Institute of Field and Vegetable Crops</td>
<td>1030</td>
</tr>
</tbody>
</table>
Table 2.7 Lycopersicon holdings of the USDA at Geneva, NY (USDA), the Tomato Genetic Resources Center (TGRC), and the Asian Vegetable Development and Research Center (AVDRC)

<table>
<thead>
<tr>
<th>Species Subtaxa</th>
<th>USDA</th>
<th>TGRC</th>
<th>AVDRC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lycopersicon</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cheesmanii</td>
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<td>39</td>
<td>18</td>
</tr>
<tr>
<td>f. minor</td>
<td>5</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>chilense</td>
<td>1</td>
<td>83</td>
<td>31</td>
</tr>
<tr>
<td>chmielewskii</td>
<td>1</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td><em>esculentum</em></td>
<td>4913</td>
<td>427</td>
<td>5311</td>
</tr>
<tr>
<td>var. <em>cerasiforme</em></td>
<td>267</td>
<td>275</td>
<td>109</td>
</tr>
<tr>
<td>esculentum x esculentum var. *cerasiforme</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>esculentum hybrids</td>
<td>158</td>
<td>*</td>
<td>123</td>
</tr>
<tr>
<td>glandulosum</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>hirsutum</td>
<td>39</td>
<td>76</td>
<td>49</td>
</tr>
<tr>
<td>f. glabratum</td>
<td>21</td>
<td>41</td>
<td>17</td>
</tr>
<tr>
<td>parviflorum</td>
<td>6</td>
<td>53</td>
<td>12</td>
</tr>
<tr>
<td>pennellii</td>
<td>10</td>
<td>40</td>
<td>61</td>
</tr>
<tr>
<td>var. <em>puberulum</em></td>
<td>-</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>peruvianum var. *humifusum</td>
<td>124</td>
<td>155</td>
<td>120</td>
</tr>
<tr>
<td>pimpinellifolium</td>
<td>230</td>
<td>247</td>
<td>315</td>
</tr>
<tr>
<td><em>sp.</em></td>
<td>13</td>
<td>-</td>
<td>1014</td>
</tr>
<tr>
<td><strong>Solanum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>juglandifolium</td>
<td>-</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>lycopersicoides</td>
<td>-</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>ochranthum</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>sitiens</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5809</td>
<td>1557</td>
<td>7231</td>
</tr>
</tbody>
</table>

*See Table 2.8

(Table 2.7). The collection at TGRC has an emphasis on wild species and various genetic stocks though it also has over 700 accessions of *L. esculentum*. Both the USDA and AVDRC have hybrid populations of *L. esculentum* with other *Lycopersicon* species (Table 2.7), while TGRC maintains a significant number of *Lycopersicon* introgression populations (Table 2.8). A small collection of *Solanum* species (Section Petota, sub-section Potatoe, Series Juglandfolium) is also maintained by the TGRC with germplasm conserved for *S. juglandifolium, S. lycopersicoides, S. ochranthum*, and *S. sitiens* (Table 2.7).

Geographical distributions for *Lycopersicon esculentum* accessions maintained by AVDRC and the USDA are given in Table 2.9. The distributions of accessions by country for both collections are similar; with the exception
Table 2.8  Introgression and special purpose populations of TGRC

<table>
<thead>
<tr>
<th>Material</th>
<th>Parental Material</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lycopersicon pennelli</em> introgression lines</td>
<td>LA0716; M-82</td>
<td>76</td>
</tr>
<tr>
<td><em>L. hirsutum</em> introgression lines</td>
<td>LA1777; E-6203 (LA4024)</td>
<td>98</td>
</tr>
<tr>
<td><em>Solanum lycopersicoides</em> introgression lines</td>
<td>LA2951; VF36 (LA0490)</td>
<td>80</td>
</tr>
<tr>
<td><em>L. pennelli</em> alien substitution lines</td>
<td>LA0716</td>
<td>7</td>
</tr>
<tr>
<td><em>L. pimpinellifolium</em> backcross</td>
<td>LA1589, E6203</td>
<td>99</td>
</tr>
<tr>
<td>recombinant inbreds</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. lycopersicoides</em> monsomic addition lines</td>
<td>LA1964; Vendor</td>
<td>10</td>
</tr>
<tr>
<td>High soluble solids derivatives of</td>
<td>LA1208 derivatives</td>
<td>3</td>
</tr>
<tr>
<td><em>L. chmielewskii</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutants derived from <em>L. cheesmannii</em></td>
<td><em>L. esculentum</em> derivates of</td>
<td>5</td>
</tr>
<tr>
<td><em>L. cheesmanii</em> derivative</td>
<td><em>L. cheesmanii</em></td>
<td></td>
</tr>
<tr>
<td><em>L. pimpinellifolium</em> exserted stigma derivative</td>
<td>LA1585</td>
<td>1</td>
</tr>
<tr>
<td><em>S. lycopersicoides</em> hybrid</td>
<td>LA2951; VF36</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>380</td>
</tr>
</tbody>
</table>

that AVDRC has a larger representation from Asian countries, such as India, Korea, Malaysia, the Philippines, Sri Lanka, and Taiwan. Both institutions have large number of accessions from Brazil, Canada, China, El Salvador, the former USSR, Guatemala, Honduras, Hungary, India, Mexico, Peru, Turkey, the USA, and Yugoslavia. There is probably much unintentional duplication between these collections and also for those of Table 2.6. Unwanted redundancy within the USDA collection and among different germplasm collections will be discussed in a later section.

Large genetic resources collections are usually duplicated at a second backup location. Backup germplasm collections are highly desirable since they provide a valuable resource to replace germplasm collections that are lost or destroyed by natural disasters (such as fires, floods etc.), political disturbances or mechanical breakdowns. Both the USDA and the TGRC collections are backed up at the National Center for Genetic Resources Preservation (NCGRP) located at Ft. Collins, Colorado. The backup status of the USDA tomato collection at Ft. Collins is given in Table 2.10. Overall, the USDA collection has an 89% backup, though some of the wild taxa have lower backup rates. The TGRC collection is also almost entirely backed up (95%) at NCGRP (Anonymous 2003).

The majority of *Lycopersicon* germplasm used for improvement of tomato has been wild species germplasm. The TGRC maintains a series of special purpose collections of wild species germplasm (Table 2.11). A number of accessions are available with tolerance to drought, flooding, high
Table 2.9  Number of accessions and Country of origin for Lycopersicon esculentum at the Asian Vegetable Development and Research Center (AVDRC), and the USDA germplasm collection maintained at Geneva, NY (USDA)

<table>
<thead>
<tr>
<th>Country</th>
<th>AVDRC</th>
<th>USDA</th>
<th>Country</th>
<th>AVDRC</th>
<th>USDA</th>
<th>Country</th>
<th>AVDRC</th>
<th>USDA</th>
<th>Country</th>
<th>AVDRC</th>
<th>USDA</th>
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<td>Sweden</td>
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<td>Greece</td>
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<td>6</td>
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<tr>
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<td>-</td>
<td>Guadeloupe</td>
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<td>Nepal</td>
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<td>223</td>
<td>New Caledonia</td>
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<td>1</td>
<td>Uruguay</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Burkina Faso</td>
<td>-</td>
<td>4</td>
<td>Guyana</td>
<td>1</td>
<td>1</td>
<td>New Zealand</td>
<td>3</td>
<td>2</td>
<td>USA</td>
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<td>783</td>
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<td>Nigeria</td>
<td>16</td>
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<td>Hungary</td>
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<td>Pakistan</td>
<td>9</td>
<td>6</td>
<td>Zaire</td>
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<td>1</td>
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<td>Cook Islands</td>
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<td>Indonesia</td>
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<td>44</td>
<td>Iran</td>
<td>61</td>
<td>62</td>
<td>Papua N. Guinea</td>
<td>4</td>
<td>-</td>
<td>Unknown</td>
<td>91</td>
<td>9</td>
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<td>Iraq</td>
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<td>2</td>
<td>Peru</td>
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<td>Total</td>
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<td>Cuba</td>
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<td>7</td>
<td>Israel</td>
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<td>Philippines</td>
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<tr>
<td>Czech Republic</td>
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<td>-</td>
<td>Italy</td>
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<td>94</td>
<td>Poland</td>
<td>46</td>
<td>62</td>
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<td>Czechoslovakia</td>
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<td>10</td>
<td>Jamaica</td>
<td>-</td>
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<td>Puerto Rico</td>
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<td>28</td>
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<td>Denmark</td>
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<td>Romania</td>
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<td>7</td>
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<td>Egypt</td>
<td>4</td>
<td>3</td>
<td>Kazakhstan</td>
<td>-</td>
<td>13</td>
<td>Russian Federation</td>
<td>-</td>
<td>23</td>
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<tr>
<td>El Salvador</td>
<td>410</td>
<td>419</td>
<td>Kenya</td>
<td>1</td>
<td>-</td>
<td>South Yemen</td>
<td>1</td>
<td>-</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
temperatures, aluminum toxicity, chilling injury, salinity-alkalinity, and arthropod damage. Accessions with tolerance are listed in Table 2.10. Lists for these are available on the Internet at: http://tgrc.ucdavis.edu/. These stress tolerant wild species stocks have been extensively utilized in tomato crop improvement.

As mentioned previously, the TGRC maintains a number of introgression populations and special purpose populations, e.g., *Lycopersicon esculentum* X *L. pennellii* and *L. hirsutum* introgression populations are available (Table 2.8). Additionally, TGRC maintains *Solanum lycopersicoides* X *L. esculentum* populations. Various other substitution, backcross recombinant, alien and monosomic addition lines, various mutant populations, and high soluble solid derivatives are also maintained. The majority of the germplasm of *Lycopersicon* maintained at the TGRC is of various genetic stocks (Table 2.12). Monogenic stocks account for approximately 2/3rd of these genetic stocks. There are also large numbers of chromosome markers and miscellaneous marker combinations.
Conservation of genetic resources of tomato in the broad sense encompasses germplasm collection, maintenance, distribution, characterization and evaluation. Collection of tomato germplasm is influenced by the breeding system of the species to be collected and the ease in transfer of traits to the cultigen (Table 2.1). Maintenance of genetic resources of seed crops such as tomatoes involves two separate but inter-related activities; a) long-term maintenance of seed, and b) regeneration of seed as required to maintain sufficient quantities of high-quality seed for storage and distribution. Both of these processes require sufficiently large numbers of plants and or
Genetic Improvement of Solanaceous Crops: Tomato

seeds to avoid the loss of genetic diversity within the collection and to maintain the genetic identity of accessions conserved.

Germplasm characterization and evaluation greatly increases the usefulness of tomato germplasm. Major traits of interest in tomato include quality traits such as soluble solids, yield improvement, and increase of resistance/tolerance to major biotic and abiotic stresses. Conservation of germplasm has the primary goal of providing germplasm for crop improvement and research, and the success of germplasm conservation efforts is measured by the distribution and utilization of the germplasm conserved.

Germplasm Collection

As can be seen from Tables 2.6 and 2.7, the cultivated *Lycopersicon* is well represented in many genebanks around the world. Germplasm acquisition through collections also requires proper sampling procedures to obtain a representative sample for conservation. Outcrossing wild *Lycopersicon* taxa require adequate sampling to obtain a representative sample for conservation while cultivated taxa require less seed per sample.

In the past 20 years there have been a number of collections of the wild *Lycopersicon* species, mostly through the efforts of the TGRC located at Davis, California. Plant collection expeditions have been sponsored by IBPGR in 1980, 1984, 1986, and 1987 (Anonymous 2003) and, additional wild/primitive germplasm collected in 1985, 1995, 1996, and 2001 on trips sponsored by the TGRC and the USDA Plant Exchange Office. The 2001 trip resulted in collection of *L. peruvianum*, *L. chilense*, and two *Solanum* species; *S. lycopersicoides*, and *S. sitiens* from Chile (Chetelat 2001). These expeditions covered areas that were previously poorly represented in existing collections. Collections have also been conducted by researchers from Spain in the Galapagos Islands and in Peru (Nuez and Cuartero 1984).

<table>
<thead>
<tr>
<th>Type</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Translocations</td>
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</tr>
<tr>
<td>Trisomics</td>
<td>31</td>
</tr>
<tr>
<td>Autotetraploids</td>
<td>20</td>
</tr>
<tr>
<td>Chromosome markers</td>
<td>194</td>
</tr>
<tr>
<td>Linkage screening testers</td>
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</tr>
<tr>
<td>Miscellaneous marker combinations</td>
<td>377</td>
</tr>
<tr>
<td>Monogenic stocks</td>
<td>994</td>
</tr>
<tr>
<td>Total</td>
<td>1666</td>
</tr>
</tbody>
</table>

Table 2.12 Various genetic stocks available at TGRC
The Crop Germplasm Committee (CGC) for tomato in the United States National Plant Germplasm System (NPGS) has concluded in its 2003 report (Anonymous 2003) that the status of wild tomato germplasm is in good shape and vastly superior than that of many other crop plants. Recommendations were made for germplasm collection in certain remote areas of Peru, especially in the Rio Maranon watershed because of the presence of *L. peruvianum* and the importance of the genetic diversity found in other populations of *L. peruvianum*. Other acquisition priorities established by this committee include acquisition of germplasm from research projects which have terminated or that are expected to be terminated in the near future. Germplasm from such collections include cultigens, breeding lines, genic and chromosomal variants, and other stocks.

**Regeneration**

To avoid the loss of diversity and genetic identity through genetic drift, mutation, and selection during the regeneration, standards must be established and used for minimal numbers of plants and seeds. The procedures used for regeneration and storage of tomato are dependent on the breeding system of the plant, with cross-pollinated species requiring larger samples during collection, regeneration, and conservation. For self-pollinated species the requirements are determined by needs to maintain a sufficiently large sample for storage to reduce the number of regenerations required and is determined by the crop husbandry of the plant. Cross-pollinated taxa require a sufficient number of plants to adequately represent the accession and to prevent genetic drift during regeneration.

Specifically the USDA collection of *L. esculentum* is maintained by regenerants from approximately 24 plants, with accessions planted in the field without pollination control. Most genebanks also maintain cultivated *Lycopersicon* with similar numbers of plants, usually in the field with no pollination control. With the self-pollinated cultivated tomato, number of plants used for regeneration is mostly determined by numbers of selfings in field operations and by the desired number of seeds obtained for regeneration. Since tomato seed stores well, production of a large amount of seed can significantly reduce the chances of genetic drift, by increasing the time between regenerations. Wild taxa of *Lycopersicon* are both cross and self-pollinated and both self-compatible and self-incompatible (Table 2.1). Some species are facultative with accessions from some areas self-incompatible and others highly self-compatible. Ideally, during regeneration of the cross-pollinated species upwards of 50 plants are used...
for regeneration to obtain a representative sample by reducing the effects of
 genetic drift and selection during the regeneration process.

Regeneration of cultivated tomato is usually conducted by producing
 transplants that are taken to the field once the danger of frost has passed.
 At the USDA in Geneva, these are planted using a transplanter into plastic
 mulch. Seed production requires constant monitoring for diseases with
timely application of pesticides to allow sufficient production of quality,
disease-free seed for maintenance and distribution. Small scale processing
 equipment is used for processing cultivated tomato seed with custom de-
 signed equipment such as in Fig. 2.1, which is used to separate the seed
 and gel from the skin and pulp. This material is then fermented for two to
three days to ease the processing. After this the seed is washed and dried.
Once dried the ‘hair’ is separated from the seed by tumbling in mesh bags
and dried in a clothes-dryer or air only. To reduce incidence of seed borne
viruses (such as TMV) seed is treated with a 20% solution of bleach and
dried. Seed processing for wild taxa is done by hand. Final processing and
storage of seed is discussed in the next section.

Seed Storage Conditions
Methods used for storage are aimed to increase the duration seed remains
viable and useful for distribution. Requirements for long-term storage of
species with orthodox seeds such as tomato have been well established
and are related to moisture content of the seed stored, the type of storage
container and the temperature of the environment used for seed storage

Equations to predict seed longevity in storage have been developed and
refined by Ellis and Roberts (1980) to take into account the variation in
initial seed quality along with seed moisture content and temperature of
storage. The suggested moisture content for storage of orthodox seeds for
genetic conservation is at a moisture content of 5 ± 1%. This moisture
content is achieved at Geneva, NY by drying the seed in a room that is
maintained at 20% relative humidity at a temperature of 4 to 5°C. The
suggested temperature for long-term storage is −20°C. Storage containers
should be airtight but not vacuum-sealed because of damage to seed from
the evacuation process.

Based on the results of seed vigor studies, Zheng et al. (1998) suggested
use of ‘ultra-dry’ (less than 5% moisture content) seed storage for long-
term genetic conservation of plants. They suggested that ultra-dry seed
could be stored at higher temperatures compared to −20°C for long-term
storage in genebanks. Ellis and Roberts (1998), and Walters et al. (1998),
Fig. 2.1 Experimental wet seed processor for separation of tomato seed from small lots of tomato genotypes.
both question the usefulness of ultra-dry seed storage at ambient temperatures as a substitute for long-term cold storage in genebanks. XiangHui et al. (1998) dried tomato seed to 1.5% moisture but found that seed could not be stored for long periods at ambient temperatures. Pandey (1995) found that storage of 2% moisture seeds of tomato in hexylene glycol improved short- or medium term storage at ambient temperatures.

Others (Stanwood and Sova 1995, Iriondo et al. 1992) have made suggestions for use of cryopreservation of seed for long-term genetic conservation (conservation using liquid nitrogen) but there is need for more research before a definitive answer of whether there is any advantage to this for orthodox seeds. Sacks and St. Clair (1996) found that pollen cryopreservation can be used successfully for storage of tomato pollen and for tomato breeding and germplasm storage.

Maintaining Genetic Integrity during Regeneration and Storage
Because seed stocks are depleted through distribution and seeds eventually die during storage, periodic regenerations are necessary. Regeneration and maintenance procedures must minimize genetic changes within accessions. Maintaining genetic integrity involves maintaining genetic identity and genetic diversity. Loss of genetic identity occurs through contamination during regeneration by foreign pollen, seed adulteration during harvesting, threshing, and packaging, and through gene mutations (Steiner et al. 1997). Genetic erosion (or loss of genetic diversity) occurs through genetic drift due to random loss of alleles particularly in small populations and through genetic shifts due to unintentional natural selection.

Most new mutations are rare and random. Some studies indicated that major chromosomal aberrations occurred during seed storage and senescence, especially under desiccating conditions, but these gross mutations did not persist in regenerated populations (Wu et al. 1998). Inadvertent selection for more adapted genotypes or genotypes with relatively more viable seed can occur during regeneration (Wu et al. 1998). Selection acts on specific loci. Without knowing which loci these might be or even if we do know, selection is always difficult to distinguish from genetic drift.

Genetic drift and gene flow are relatively easier to detect than mutation and selection. Several studies have found evidence for one or both of these during regeneration in genebanks (del Rio et al. 1997, Wu et al. 1998, Börner et al. 2000, Parzies et al. 2000). Genetic drift refers to random changes in allele frequencies in an accession caused by random sampling of gametes during sexual reproduction. The rate of drift depends on effective population size ($N_e$), which is defined as the number of individuals in a
Genetic Resources of Tomato (*Lycopersicon esculentum* Mill.) and Wild Relatives

Theoretically ideal population having the same magnitude of drift as the actual population (Hartl and Clark 1989). *Ne* is usually smaller than census size and will be substantially so if an accession undergoes a genetic bottleneck.

Theoretical studies indicate that carefully sampling equal numbers of seeds from as many seed parents as possible can effectively prevent drift during regeneration (Crossa and Vencovsky 1997, Vencovsky and Crossa 1999) but this can become so labor-intensive as to make it impractical (Johnson 1998). An alternative strategy is to sample a single inflorescence per plant, rather than the whole plant, when bulking seed of outcrossing plants, such as outcrossing *Lycopersicon* taxa. This will improve *Ne* providing the relative variation in seeds per spike as less than that of seeds per plant (Johnson 1998). In annual ryegrass (*Lolium multiflorum*) balanced (equal numbers of seeds per plant combined), spike (one inflorescence per plant combined), and bulk (seeds combined proportionally according to seeds per plant) regeneration samples were compared. The first method was clearly superior at maintaining genetic integrity of accessions. The latter two methods maintained diversity of eight isozymes as estimated by heterozygosity and mean numbers of alleles per locus, but allele frequencies shifted using those two methods.

Prevention of genetic drift and contamination in outcrossing species such as most wild *Lycopersicon* spp. requires more resources to be invested in regeneration per accession compared to the self-pollinated *L. esculentum*. Molecular markers showed evidence of genetic drift during regeneration in one of eight wheat (*Triticum aestivum*; Börner et al. 2000) and one of six wild potato (*Solanum jamesii*; del Rio et al. 1997) accessions. In two barley (*Hordeum vulgare*) landraces that had been maintained for over 70 years, *Ne* was estimated to be 4.7 using morphological and isozyme markers, even though census sizes in regeneration plots were routinely 600 plants (Parzies et al. 2000). The authors stated that either intense directional selection or a single bottleneck event could explain the extreme loss of genetic diversity.

The mating system of *L. esculentum* results in homozygosity within accessions. Census sizes of regenerated accessions are typically around 25 plants. This is considered to be large enough to prevent sudden extinction of an accession through rapid fixation of rare, deleterious mutations or an accidentally stressful environmental condition leading to inadvertent selection (Treuren and Hintum 2001). Decline of genetic integrity in a tomato accession would most likely occur from gene flow through contaminating
pollen or mishandling. For seven oat (Avena sativa) lines maintained for 124 years, electrophoresis of storage proteins showed results ranging from no contamination of a line to complete replacement of a line by a foreign phenotype (Steiner et al. 1997). Both mishandling of seed and pollen contamination during maintenance were implicated.

Reducing Redundancy in Collections
Many empirical studies have addressed the question of unintentional duplication of conserved germplasm by examining subsets of collections. Various types of duplications have been defined - identical duplication refers to genetically identical accessions, common duplication denotes accessions derived from a common parental population, partial and compound duplication implies that not all alleles are duplicated, and parental duplication refers to the relationship between a particular cross and the resultant offspring (Hintum and Visser 1995). Although precise language such as this can lead to refined studies of redundancy the use of terms such as these has not become widespread.

A commonly applied experimental approach has been to compare identically or similarly named accessions of a particular species using passport, phenotypic, and/or molecular marker data. The first conclusion that can be reached is that substantial amounts of duplication have been found whenever it was looked for. A study of three European lettuce (Lactuca) collections estimated a mean duplication of 12% within and 37% among the collections based on passport data (Hintum 2000). The author pointed out that this may be an underestimate for two reasons: i) because poorly documented accessions were considered to be distinct, and ii) there may be overrepresentation of certain small fractions of the gene pool due to recent shared ancestry or over-collecting in certain geographical areas. We have evidence for both of these problems in our tomato collection and will likely encounter them in our other vegetable collections.

Studies on duplication often address the pooling of duplicate material and estimating how much genetic variation will be lost by doing so. One strategy for pooling is to maximize the ratio of the similarity within groups to the dissimilarity between groups (Hintum et al. 1996). Many studies have applied this model and examples include all accessions of sorghum (Sorghum bicolor) named “Orange” in the NPGS (Dean et al. 1999), Dutch landraces of B. oleracea accessions at CGN (Hintum et al. 1996), flax (Linum usitatissimum) accessions designated as “breeder’s lines” at CGN (Treuren et al. 2001), all Peruvian sweet potato accessions at CIP (Huamán et al. 1999), and all accessions of cabbage named Golden Acre (B. oleracea var.
capitata L.) in the NPGS (Phippen et al. 1997). In general these studies recommended pooling duplicate accessions based on AMOVA (Analysis of Molecular Variance) in a way that would retain greater than 90% of the total molecular genetic variation. In a pilot study, the USDA at Geneva (unpublished data) applied eight microsatellite markers to six identical San Marzano and six Globe types of tomato and found one of six accessions to be clearly genetically distinct in both cases.

Hintum (2000) has developed methods for quantification of duplications to the level of duplications both within and between germplasm collections along with standard errors of estimates by use of set theory. The definition used for duplicates was accessions with passport data that implies that they are genetically similar or the same. Four lettuce (Lactuca sativa L.) accessions were used to apply the equations developed for estimation of duplications within and between germplasm collections. This study points out that: a) most of the probable duplicates were from the exchange of material between collections, b) most of the duplicate materials consisted of older named varieties, and c) accessions unique to one collection were; i) recently added varieties, ii) recently collected material, and iii) poorly documented duplicates which made their identification difficult. The collections reported in Tables 2.6 and 2.7 probably have a high level of redundancy both within and between genebanks.

The USDA germplasm collection has been surveyed for duplications recently, and 1333 accessions have been found to form 455 sets of putative duplicate accessions. An empirical approach using accession identifiers was used to identify these duplicate accessions. An approach of using passport data to identify the known original source of these accessions to keep as representing the cultigen has been used to reduce duplication for approximately 90% of these accessions. The other 10% of accessions will be grown out at several locations for identification of duplications.

**Development of Core Subsets**

Core subsets are tools for users to efficiently work with a large fraction of the total genetic diversity in a collection. Random sampling methods are applied to develop core subsets. In order to broadly capture diversity, sampling may be from subsets of accessions initially grouped according to phenotype or ecogeographic origin (Li et al. 2002). Phenotypic traits are not always good indicators of genetic variation because they can be influenced by environment or result from independent genetic bases. Molecular markers can help overcome these limitations.
Molecular markers have been used: i) to compare various techniques applied to generate a core (e.g., *Sorghum bicolor*, Grenier et al. 2000), ii) as criteria to decide which accessions to include in a core [e.g., cashew (*Anacardium occidentale*), Dhanaraj et al. 2002, Andean potato (*Solanum phureja*), Ghislain et al. 1999], and iii) to validate that a core is representative of a given base collection [e.g., Mexican common bean (*Phaseolus vulgaris*), Skroch et al. 1998]. Computer simulations, multivariate statistical techniques such as Principal Components Analysis (PCA), and genetic variation measures such as numbers of alleles, genetic diversity, and percentage of rare alleles, are frequently used to establish and validate core subsets.

Molecular markers reflect pedigrees, which may not be significantly correlated with gross morphology. In the Brazilian cassava (*Manihot esculentum*) collection cultigens with similar agronomic traits were very heterogeneous at the molecular level (Carvalho and Schaal 2001). For four major *Sorghum* races, grouping of accessions based on agronomic descriptors did not correlate with groupings produced from RAPD markers (Dahlberg et al. 2002). The authors stated that such correlations should not be expected because in most cases genes underlying an agronomic trait represent a very small fraction of the genome.

Core subsets of *Lycopersicon esculentum* and the wild *Lycopersicon* taxa have been assembled by the USDA-Geneva and TGRC, respectively. The cultivated core subset is a dynamic collection that is being refined. Presently, approximately 200 accessions of cultivated tomato, mostly from the United States, comprise the core collection. The tomato CGC is making efforts to modify this collection to have a balanced representation globally and to have better representation of tomato usage and of plant types. The TGRC at Davis has also established a core collection of wild *Lycopersicon* taxa.

**GENETIC DIVERSITY IN *Lycopersicon* SPECIES**

Mating system, life history traits (e.g., annual versus perennial, longevity, etc.), and ecological factors such as those causing frequent extinction and recolonization, all shape genetic variation within and among populations of a species. For the genus *Lycopersicon*, mating system is the most-extensively studied of these factors. The genus has been viewed as a model system among plants for studying the effect of mating system within-species variation (Stephan and Langley 1998, Baudry et al. 2001), because it consists of closely-related species with a range of mating systems from...
Genetic Resources of Tomato (*Lycopersicon esculentum* Mill.) and Wild Relatives

Selfing (*L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, and *L. parviflorum*) to facultative outcrossing (*L. chmielewskii*) to obligate-outcrossing (*L. pennellii, L. hirsutum, L. chilense*, and *L. peruvianum*). In addition, some biotypes of *L. pennellii, L. hirsutum*, and *L. peruvianum* have been found to be self-compatible (Rick 1982, Taylor 1986).

Neutral theory predicts that polymorphism within a species is a function of mutation rate and effective population size (Kimura 1983). Effective population size is defined as the number of individuals in a theoretically ideal population having the same magnitude of genetic drift as the actual population (Hartl and Clark 1989). Compared to random-mating, selfing is expected to decrease effective population size by one-half and hence the genetic variation by the same amount (Pollak 1987). Additional reasons why selfing (self-pollinated) species are predicted to have reduced variation are related to frequent bottlenecks and reduced recombination. A single self-fertilizing plant can act as a founder for a new population; selfing species may frequently undergo such bottlenecks. A deficiency of double heterozygotes commonly characterizing selfing populations decreases effective recombination rate, thereby increasing linkage disequilibrium. Extensive linkage disequilibrium is associated with reduced variation because selection will effect more of the genome, i.e., the selected locus plus tightly linked, unselected loci (Charlesworth et al. 1993). In the plant genera *Arabidopsis* (Savolainen et al. 1999) and *Leavenworthia* (Liu et al. 1999) selfing species were found to have greater than two-fold reduction in genetic variation relative to outcrossing species.

In the genus *Lycopersicon*, levels of polymorphism for RFLP (Miller and Tanksley 1990), RAPD (Egashira et al. 2000), SSR (Alvarez et al. 2001), and DNA sequences (Baudry et al. 2001) have all been shown to be correlated with mating system.

In an RFLP study, the number of unique restriction fragments and the number of unique restriction patterns were used to estimate genetic diversity of accessions (Miller and Tanksley 1990). SI species *L. hirsutum, L. pennellii*, and *L. peruvianum* were found to be much more diverse than the SC species *L. esculentum, L. pimpinellifolium, L. cheesmanii, L. parviflorum*, and *L. chmielewskii*. Genetic distances among accessions were calculated based on proportion of shared restriction fragments (Nei 1987, equations 5.53 to 5.55). Average genetic distances among accessions were approximately ten-fold greater for *L. peruvianum*, and five-fold greater for *L. hirsutum* and *L. pennellii* than any other SC species. In addition, most of the diversity was distributed among rather than within accessions for the SC species.
Genetic distances based on proportion of shared bands (Nei and Li 1979) between species, between accessions within species, and between plants within an accession were estimated for *L. esculentum* var. *esculentum* and its wild *Lycopersicon* relatives using RAPD markers (Egashira et al. 2000). The facultative outcrosser *L. chmielewskii* contained the highest average within accession variation among the SC species (0.045). This was almost six-fold lower than the highest estimate found among the SI species (0.252 in *L. chilense*). Average genetic distances between accessions within species ranged from 0.036 for *L. esculentum* var. *esculentum* to 0.677 for *L. chilense*. Northern Peruvian accessions of *L. peruvianum* and southern Peruvian accessions of *L. chilense* showed the greatest within-species genetic diversity.

*L. hirsutum*, *L. pennellii*, and *L. peruvianum* were found to be more diverse than *L. esculentum*, *L. pimpinellifolium*, *L. cheesmanii*, *L. parviflorum*, and *L. chmielewskii* in a study of 16 polymorphic SSR loci (Alvarez et al. 2001). Two measures of variation were used: i) numbers of unique alleles (species-specific), and ii) gene diversity based on allele frequencies (Weir 1996). All species except *L. esculentum* harbored at least one unique allele. Sixty-six of 144 alleles (46%) were found to be unique. *L. chilense* contained the relatively highest proportion of unique alleles (0.80) when corrected for differences in numbers of plants sampled per species. Gene diversity within SC species was lowest in *L. esculentum* (0.03) and highest in *L. pimpinellifolium* (0.20). In *L. pimpinellifolium* all gene diversity was partitioned among the three sampled accessions, i.e., alleles were fixed at all loci within accessions of this species. For the SI species, gene diversity ranged from 0.24 in *L. pennellii* to 0.57 in southern representatives of *L. peruvianum*. In contrast to estimates based on RAPDs, SSR markers showed northerly accessions of *L. peruvianum* contain less diversity than southerly accessions (Egashira et al. 2000).

Effects of mating system and recombination on intraspecific DNA sequence polymorphism were studied by comparing *L. chilense*, *L. hirsutum*, *L. peruvianum*, *L. chmielewskii*, and *L. pimpinellifolium* (Baudry et al. 2001). Five plants per species were sampled, except for *L. hirsutum* (three plants), and sequenced at five single-copy genes in chromosomal regions with either high (2.33 x 10^-8 - 2.73 x 10^-8 per site per generation) or low (0.00 - 0.46 x 10^-8 per site per generation) rates of recombination. More than 8 kb of DNA was sequenced per plant, including four anonymous, single-copy cDNA markers ( Tanksley et al. 1992) CT208, CT251, CT268, CT143, and the sucrose accumulator gene *sucr*. Intraspecific polymorphism was estimated as \( \theta \) for non-coding nucleotide sites (Nei 1987). Mating system was
found to have a significant effect on polymorphism. *L. pimpinellifolium* and *L. chmielewskii* had on an average approximately 4 and 40-fold less polymorphism than *L. hirsutum*, the least polymorphic of the SI species. *L. chmielewskii* was the least variable species, with all estimates of $\theta$ equaling zero with the exception of CT143, where $\theta$ was less than 0.01. *L. peruvianum* was the most polymorphic species, with $\theta$ values ranging from approximately 0.01 to greater than 0.03 across loci. In addition, a high proportion (14% - 40% across loci) of fixed differences among the other four species were observed as polymorphisms within *L. peruvianum*. This may represent lineage sorting of alleles among species and imply that *L. peruvianum* is representative of the ancestral species from which other species were derived. Although reduced recombination rate has been found to be significantly correlated with lower polymorphism in other species (e.g., *Drosophila*, Aquadro et al. 1994), in this study recombination and polymorphism were only weakly positively correlated.

Theta estimates for *L. esculentum* across four loci (fw2.2 5’ UTR, Adh2, TG10, and TG11) ranged from 0.0016 - 0.0048 (Nesbitt and Tanksley 2002). These estimates largely reflected polymorphism within *L. esculentum* var. *cerasiforme*. Four modern cultivars of *L. esculentum* var. *esculentum* included in this study contained only one polymorphic site in more than 7 kb of total sequence. The high degree of monomorphism within *L. esculentum* var. *esculentum* has likely resulted from severe bottlenecks. However, polymorphism should be higher within regions of the genome containing introgressed loci from wild relatives (Miller and Tanksley 1990).

To summarize, selfing species of *Lycopersicon* contain significantly less genetic variation relative to outcrossing species. The reduction in variation exceeds the predicted 50% reduction that could be explained by mating system alone. Similar observations have been made in other plant genera (Savolainen et al. 1999, Liu et al. 1999). Additional factors such as founder events, fluctuating population size, population substructure, selection, and linkage must be better understood in order to explain relative levels of genetic variation observed within *Lycopersicon* species (Baudry et al. 2001).

**CHARACTERIZATION AND EVALUATION**

Characterization and evaluation of germplasm of tomato is essential to promote its utilization for crop improvement. The CGC for tomato has identified a number of biotic and abiotic stresses, as well as quality traits as having high priority for evaluation to provide sources for use in improvement programs on tomato (Table 2.13). High priorities have been
established for screening disease resistance for verticillium wilt race 2, bacterial canker, geminiviruses, pepino mosaic virus, spotted wilt, and bacterial spot. High priorities for screening for insect resistances have been established for silverleaf whitefly and nematodes. Priority has also been established for abiotic stresses such as heat and cold. A number of quality traits have been prioritized, especially for soluble solids, flavor, and color.

An international descriptor list has been established for *Lycopersicon* spp. (IPGRI 1996). This list has three major types of descriptors; passport,

<table>
<thead>
<tr>
<th>Type</th>
<th>Priority</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DISEASES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>Verticillium wilt race 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacterial canker</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TYLCV &amp; other geminiviruses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pepino mosaic virus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spotted wilt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacterial spot</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>Late blight</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Corky root</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phytophthora root rot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fruit rots</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CMV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beet curly top virus</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>Bacterial speck race 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PYY</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Target spot</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Powdery mildew</td>
</tr>
<tr>
<td><strong>INSECTS &amp; PESTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>Silverleaf whitefly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nematodes, heat stable</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>Aphids</td>
</tr>
<tr>
<td><strong>ABIOTIC STRESSES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>Cold tolerance</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>Heat tolerance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salinity tolerance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Color disorders</td>
</tr>
<tr>
<td><strong>HORTICULTURAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>Soluble solids</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flavor (need to define components)</td>
</tr>
<tr>
<td>Medium</td>
<td></td>
<td>Antioxidants/nutritional content</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Color</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sugar type</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peelability/dicing</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td>Pectin chemistry</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blossom-end smoothness</td>
</tr>
</tbody>
</table>
characterization and evaluation descriptors. Also included are management descriptors and environment and site descriptors.

The passport descriptors provide the basic identification of the accession which includes the genebank’s accession number along with the other identifiers associated with the accession, such as collection number and/or other institution identifier numbers. If the accession is a variety or cultivar, name and if available, pedigree and breeding method are included in passport descriptors. The taxonomic classification of the accession is also included in the passport descriptors. The other passport descriptors provide information on where an accession was collected, including political and eco-geographic information about the collection site. Eco-geographic information often is very useful in selecting accessions, especially when little or no characterization or evaluation data are available. Management descriptors associated with an accession provide information about seed availability, viability, etc., but are usually not publicly available.

Characterization and evaluation descriptors are often publicly available and provide information to aid users in selecting accessions for use in crop improvement programs. Characterization descriptors have high heritability and are usually only recorded in one environment and include descriptors such as plant type, leaf type, fruit shape, fruit color, among many others. Evaluation descriptors are more detailed and are often replicated over environments. Important evaluation descriptors include biotic and abiotic stress resistance and/or tolerance, quality descriptors such as soluble solids and fruit pH. Other descriptors, especially with evaluation traits, are provided which describe the environment and site where the evaluation was conducted, methodologies used, and person(s) who conducted the research. Examples of these would be the geographical coordinates of the evaluation site, the soil type, weather conditions during the season of the evaluation (temperatures, rainfall), and dates of planting and harvest. This type of data aids in interpretation of many evaluation descriptors.

The United States tomato CGC has established a minimal descriptor list for tomato (Table 2.14). This list was decided in order to provide a guideline for the genebank of which descriptors in the overall list were felt to be of high importance in making selections of germplasm accessions. This was to facilitate provision of a manageable number of descriptors to record during regeneration, since the IPGRI list includes a number of morphological descriptors, many of which are species specific. The minimal descriptor list includes plant descriptors, fruit descriptors, and chemical composition.
**Table 2.14  Minimal descriptor list for tomato.**

I. Plant characteristics

A. Plant growth type
   1. Miniature Dwarf
   2. Dwarf
   3. Determinate
   4. Semi-determinate
   5. Indeterminate

B. Canopy size
   3. Small  [Red Rock]
   5. Intermediate  [Florida MH-1]
   7. Large  [Mountain Pride-determinate; Tropic-indeterminate]

C. Leaf type
   1. Rugose
   2. Potato leaf
   3. Standard
   4. Curled
   5. Others  [Specify in Notes]

D. Flowers per inflorescence
   3. Low
   5. Medium
   7. High

E. Type of inflorescence
   1. Simple
   2. Forked
   3. Compound

F. Number of fruit set
   Recorded on second truss

G. Number of days to maturity
   From sowing until 50% of the plants have at least one fruit ripened

II. Fruit Descriptors

A. Exterior color of immature fruit
   1. Light green
   2. Medium green (Apple)
   3. Dark green (hp, dg)

B. Exterior color of mature fruit
   1. White
   2. Green
   3. Yellow
   4. Gold
   5. Orange
   6. Pink
   7. Red
   8. Other  [Specify in Notes]

C. Exterior mature fruit appearance
   1. Dull
   2. Medium
   3. Glossy

(Contd.)
D. Shoulder Color
   1. Green
   2. Gray Green (light green)
   3. Uniform
E. Mature fruit interior flesh color
   1. White
   2. Green
   3. Yellow
   4. Orange
   5. Pink
   6. Red
   7. Other (Specify)
F. Mature fruit interior flesh color intensity
   1. Pale
   2. Intermediate
   3. Deep
G. Fruit shape
   1. Flattened (oblate)
   2. Slightly flattened (deep oblate)
   3. Globe
   4. Deep Globe
   5. Blocky (square round)
   6. Heart-shaped
   7. Ellipsoid (plum-shaped)
   8. Cylindrical (long oblong)
   9. Pyriform (pear-shaped)
H. Pistil scar
   1. Dot
   2. Stellate
   3. Linear
   4. Irregular
I. Fruit weight (g, average of 10 fruits)
   Checks  [Sweet 100]
   [Red Cherry Large]
   [Roma or New Yorker]
   [Flora-Dade]
   [Tropic]
   [Florida 7060]
J. Uniformity of fruit size
   3. Low
   5. Intermediate
   7. High
K. Fruit firmness
   3. Soft
   5. Medium
   7. Hard
L. Nippled fruit [mature fruits]
   1. Absent

(Contd.)
III. Chemical composition

A. Soluble solids
   Measured with refractometer from 4 fruits

UTILIZATION

The major resource of tomato germplasm for crop improvement in the past 20 years has been the use of the wild species as sources of disease and insect resistance, and for improvement of quality traits (Rick and Chetelat 1995). This is because tomato being a self-pollinated crop has germplasm strongly reduced in variability for domestication and breeding. A thorough summarization of the use of related wild tomato species for crop improvement of tomato through 1995 (with literature citations) has been provided by Rick and Chetelat (1995). Introgression of many disease resistant genes into cultivars has been accomplished through the identification of linked molecular markers (Table 2.15; Causse et al. 2000, Grube et al. 2000). Resistance and/or tolerance has been transferred from wild species of *Lycopersicon* for bacterial, fungal, nematode, viral diseases, and for resistance to parasitic plants (broomrape and dodder). Wild species have also been used as sources of tolerances of abiotic stresses and for improvement of quality traits. Resistance and/or tolerance has also been transferred for insect pests namely, *Coleoptera, Diptera, Homoptera, Lepidoptera*, and *Acarina* arthropods. *L. pennellii* has been found to be a
### Table 2.15  Sources of common disease resistance alleles transferred to cultivated tomato

<table>
<thead>
<tr>
<th>Chr</th>
<th>Locus</th>
<th>Pathogen</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cf-4</td>
<td>Cladosporium fulvum</td>
<td>L. hirsutum 3833 (Univ. of Toronto)</td>
<td>Haanstra et al. (2000), Kerr and Bailey (1964)</td>
</tr>
<tr>
<td>1</td>
<td>Cf-9</td>
<td>Cladosporium fulvum</td>
<td>L. pimpinellifolium PI 126915</td>
<td>Haanstra et al. (1999)</td>
</tr>
<tr>
<td>2</td>
<td>Tm-1</td>
<td>TMV</td>
<td>L. pimpinellifolium, L. hirsutum,</td>
<td>Pelham (1966), Holmes (1954), Frazier and Dennett (1949), Kikuta and Frazier (1947)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L. peruvianum, L. chilense</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>py-1</td>
<td>Pyrenochaeta lycopersici</td>
<td>L. peruvianum var. glandulosum</td>
<td>Doganlar et al. (1998),</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pannevis 02126 K</td>
<td>Laterrot (1993), Laterrot (1983)</td>
</tr>
<tr>
<td>4</td>
<td>Hero</td>
<td>Globodera rostochiensis</td>
<td>L. pimpinellifolium B6173 (LA0121)</td>
<td>Sobczak et al. (2005), Ellis and Maxon-Smith (1971)</td>
</tr>
<tr>
<td>6</td>
<td>Mi</td>
<td>Meloidogyne spp., Macrosiphum</td>
<td>L. peruvianum PI 128657</td>
<td>Yaghoobi et al. (1995)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>euphorbiæ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Ty-1</td>
<td>TYLCV</td>
<td>L. chilense LA 1969</td>
<td>Zamir et al. (1994)</td>
</tr>
<tr>
<td>6</td>
<td>Cf-2</td>
<td>Cladosporium fulvum</td>
<td>L. pimpinellifolium PI 370093</td>
<td>Jones et al. (1992), Kerr et al. (1980), Pitabaldo and Kerr (1980), Langford (1937)</td>
</tr>
<tr>
<td>6</td>
<td>Cf-5</td>
<td>Cladosporium fulvum</td>
<td>L. esculentum x L. pimpinellifolium</td>
<td>Dixon et al. (1998),</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PI 187002</td>
<td>Dickinson et al. (1993), Kerr et al. (1971)</td>
</tr>
<tr>
<td>7</td>
<td>I-1</td>
<td>Fusarium oxysporum f. sp. lycopersici</td>
<td>L. pennellii PI 414773</td>
<td>Scott et al. (2004)</td>
</tr>
</tbody>
</table>

(Contd.)
<table>
<thead>
<tr>
<th>No.</th>
<th>Accession</th>
<th>Disease</th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>I-3</td>
<td>Fusarium oxysporum f. sp. lycopersici</td>
<td>L. pennellii PI 414773</td>
<td>Sarfatti et al. (1989)</td>
</tr>
<tr>
<td>7</td>
<td>Ph-1</td>
<td>Phytophthora infestans</td>
<td>L. esculentum var. cerasiforme PI 108245</td>
<td>Chunwongse et al. (1998), Clayberg et al. (1965), Clayberg et al. (1959), Gallegly and Marvel (1955)</td>
</tr>
<tr>
<td>9</td>
<td>Ve</td>
<td>Verticillium dahliae</td>
<td>Peru Wild (synonymous to Utah 665) PI 303801</td>
<td>Kawchuk et al. (1998), Schable et al. (1951)</td>
</tr>
<tr>
<td>9</td>
<td>Ph-3</td>
<td>Phytophthora infestans</td>
<td>L. pimpinellifolium L3708 (AVDRC)</td>
<td>Chunwongse et al. (1998)</td>
</tr>
<tr>
<td>9</td>
<td>Tm-2’</td>
<td>TMV</td>
<td>L. peruvianum PI 128650</td>
<td>Young et al. (1988)</td>
</tr>
<tr>
<td>9</td>
<td>Sw-5</td>
<td>TSWV</td>
<td>L. peruvianum \textsuperscript{b}</td>
<td>Roselló et al. (1998), Stevens et al. (1992), Stevens (1964)</td>
</tr>
<tr>
<td>10</td>
<td>Ph-2</td>
<td>Phytophthora infestans</td>
<td>L. pimpinellifolium WVa 700</td>
<td>Moreau et al. (1998)</td>
</tr>
<tr>
<td>11</td>
<td>Sm</td>
<td>Stemphylium spp.</td>
<td>L. pimpinellifolium PI 79532</td>
<td>Behare et al. (1991)</td>
</tr>
<tr>
<td>11</td>
<td>I</td>
<td>Fusarium oxysporum f. sp. lycopersici</td>
<td>L. pimpinellifolium PI 79532</td>
<td>Sarfatti et al. (1989)</td>
</tr>
<tr>
<td>11</td>
<td>I-2</td>
<td>Fusarium oxysporum f. sp. lycopersici</td>
<td>L. pimpinellifolium × L. esculentum</td>
<td>Sarfatti et al. (1989)</td>
</tr>
<tr>
<td>12</td>
<td>Mi-3</td>
<td>Meloidogyne incognita, M. javanica</td>
<td>L. peruvianum PI 126443</td>
<td>Yaghoobi et al. (1995)</td>
</tr>
<tr>
<td>12</td>
<td>Lv</td>
<td>Leveillula taurica</td>
<td>L. chilense LA 1969</td>
<td>Chunwongse et al. (1994)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Kerr and Bailey (1964) reported that this accession was lost.

\textsuperscript{b} Stevens et al. (1992) reported that the identity of the original PI number was lost after the death of J.M. Stevens but list PI 126928, PI 126929, PI 126944, PI 128645, PI 128654, PI 129109 and two L. peruvianum var. dentatum accessions with unknown PI numbers as potential sources based on his breeding records.
promising source for drought tolerance and salt tolerance. The work on fruit quality has concentrated on increasing soluble solids content. Higher levels of soluble solids content have been discovered in *L. cheesmanii*, *L. chmielewskii*, and *L. hirsutum*.

A number of studies have identified QTLs in wild species of *Lycopersicon* that provide improvement in the cultivated tomato for horticultural traits. QTLs associated with horticultural yield in *L. pennellii* and *L. hirsutum* were identified by Eshed et al. (1996) and Bernacchi et al. (1998a). *L. hirsutum* alleles were found that gave a 16% increase in total yield and a 20% improvement was achieved by combining introgressions from *L. pennellii*. Introggression lines have been used for mapping of QTL for improved fruit characteristics in *L. chmielewskii* (Frary et al. 2003) and for yield associated QTL using *L. pennellii* (Eshed and Zamir 1995). Materials developed showed promise for improvement of cultivated tomato. Genetic gains from introgressions for desirable wild QTL-alleles from *L. hirsutum* and *L. pimpinellifolium* for quality traits such as fruit firmness, soluble solids content, and brix X red yield have been reported (Bernacchi et al. 1998b).

**SUMMARY**

Cultivated tomato (*Lycopersicon esculentum* Mill.) is an important vegetable, with a worldwide area of 4 million hectares and a production of 108.5 million tons. Tomato cultivation area has increased by 38% and production has increased by 45% in the past ten years, with most of this increase in China, which has increased production from 8.5 to 25.5 million tons, propelling it to the number one tomato producer in the world. Other leading tomato producers are the United States, Turkey, India, and Egypt. In the United States, tomato accounts for 14.5% of the economic value of fresh market vegetable production and 50.7% of the economic value of processed production of vegetables.

Domestication of tomato is relatively recent, within the past 400 years. *L. esculentum* is thought to have originally been domesticated in maize fields by ancient Mexicans, with Peru as the center of diversity for the genus. Tomato spread to Europe in the early 1500s and thereafter became widely dispersed. Tomato has eight (nine by some authorities) related wild species relatives which are extensively utilized for crop improvement.

There are more than 75,000 accessesions of tomato conserved in genebanks around the world, with the largest of these at AVDRC, TGRC, and the USDA genebank at Geneva, NY. These genebanks maintain large collections of the wild relatives in addition to the cultigen. Several collections are also
available of related *Solanum* taxa and *Lycopersicon* introgression populations. While cultivated tomato is self-pollinated, the other taxa provide a mixture from self-pollinated to obligate cross-pollinated, with self compatibility and self-incompatibility. This has led to development of methodologies and standards for maintenance of tomato germplasm to minimize the effects of genetic drift, mutation, and selection.

The cultivated tomato has undergone a narrowing of the germplasm base caused by genetic bottlenecks and selection. The major utilization of tomato germplasm for crop improvement in the past 20 years has been the use of wild species as sources of genetic variation. This has led to a major utilization of wild species introgressions which have resulted in an increase in molecular genetic variation in and around regions that have been introgressed from wild species. Wild species have been used as sources of variation for disease and insect resistances and/or tolerances, abiotic stress tolerances, and for fruit quality.

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INTRODUCTION

As an experimental organism, tomato presents a number of genetic, biological, and economic advantages that have led to its development as a model for cytogenetic and evolutionary studies. The cultivated tomato, *Lycopersicon esculentum* (= *Solanum lycopersicum*), and related wild species traditionally classified as genus *Lycopersicon*, more recently as *Solanum* sect. *Lycopersicon* (Spooner et al. 2005) are diploids, with a chromosome number of 2n=2x=24. Eleven of the 12 chromosomes in the haploid tomato nucleus are metacentric or submetacentric (Lapitan et al. 1989). The exception, chromosome 2, is acrocentric with a heterochromatic short arm consisting primarily of the nucleolus organizing region (NOR). During late prophase of meiosis (diakinesis), only chromosome 2 can be distinguished from the others, by virtue of its association with the nucleolus. However, in early prophase (pachytene), each of the 12 chromosomes can be identified by the position of the centromere, the length of chromatic and achromatic segments, and the pattern of heterochromatic knobs (chromomeres) (Khush 1963). These features are illustrated in corresponding cytological maps for each chromosome in the set (Rick and Butler 1956, Khush and Rick 1968).

The tomato genome is also well defined by genetic maps based on morphological and molecular markers. High density molecular marker maps based on RFLP, SSR, or AFLP markers are available (Tanksley et al. 1992, Pillen et al. 1996, Haanstra et al. 1999, Frary et al. 2005). Estimates for total map length are ~1200-1300 cM. The relatively low haploid DNA content of...
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tomato, ca. 950 Mbp or 0.95 pg per C (Arumuganathan and Earle 1991, Michaelson et al. 1991), makes it well suited for molecular studies. Though larger than Arabidopsis or rice (~125 and 425 Mbp, respectively), the tomato genome is smaller than many other model plant species, such as maize or wheat (~2,500 and 16,000 Mbp, respectively). The average ratio of physical to genetic distance is ~750 kb/cM, a value low enough to make positional cloning of genes practical in most genomic regions. Furthermore, recombination is essentially limited to the euchromatic regions, which constitute less than one fourth of the total DNA content (see below). The tomato genome encodes a total of ~35,000 genes, which are located primarily in euchromatin (Van der Hoeven et al. 2002). Therefore, recombination rates per unit physical distance are substantially higher within the genetically active fraction than in the genome as a whole.

In comparison to other crop plants, many aspects of growth and development in tomato have been beneficial to cytogenetic studies. It is naturally self-pollinated, which simplifies the maintenance of stocks, yet hybridizations are easy to perform and yield large quantities of seed of controlled parentage. Tomato can be grown under a wide range of environmental conditions and propagated through seed or asexually via rooted cuttings. Its photoperiodic insensitivity and relatively short generation time permit the propagation of up to 3 generations per year. The structure of the tomato plant, particularly its compound leaves and sympodial growth habit, allows detection of an enormous array of hereditary variations, such as altered growth habit, leaf shape, texture and color, flower morphology, color and function, and fruit size, shape and color, among others. Tomato also provides a popular model for physiological and biochemical studies of fruit development, quality, and ripening.

As a result of its economic importance as a crop, industry-sponsored research on tomato contributed to early advances in genetics and wide hybridizations. A large number of mutants, aneuploids, and various other spontaneous genetic defects were discovered in growers’ fields (Rick 1945). The relative ease with which it can be transformed by Agrobacterium tumefaciens (Fillatti et al. 1987) has made tomato a popular organism for testing biotechnological approaches to enhancing fruit quality and other traits. As a result of these and other advantages, the first genetically engineered (GMO) food plant marketed in the USA was a tomato (Bruening and Lyons 2000).

Research on tomato has depended to a large extent on genetic resources such as mutants, cytogenetic stocks, and wild species populations (Chetelat 2005). Tomato germplasm is preserved at genebanks such as the C.M. Rick
Tomato Genetics Resource Center (TGRC) and the USDA’s Plant Genetic Resources Unit (PGRU), which provide seed samples to interested researchers worldwide. The TGRC, located at Univ. of California, Davis, maintains over 1,000 monogenic stocks, consisting of spontaneous or induced mutations at 600+ loci affecting most aspects of plant development and morphology (http://tgrc.ucdavis.edu). Over 1,400 other genetic stocks are also available through the TGRC, including several types of trisomics (primary, secondary, tertiary, and compensating), as well as autotetraploids, and derivatives of wild species such as alien additions, substitutions, and introgression lines. Lastly, the TGRC maintains over 1,100 wild species accessions, representing 13-20 species of Lycopersicon and related Solanum taxa collected in their native regions. The PGRU, located in Geneva, New York, maintains a large collection of open-pollinated cultivars, as well as accessions of several wild species (http://www.ars-grin.gov/). The Hebrew University of Jerusalem maintains a large population of mutants useful for the analysis of gene function (http://www.zamir.sgn.cornell.edu/mutants/). Together, these genetic resources provide important tools for analysis of the tomato genome.

The present chapter summarizes and integrates recent advances in the cytogenetics and evolution of tomato, with emphasis on applications in genetics and breeding. The following sections describe novel genetic resources and their uses, genetic maps of the tomato genome, meiotic behavior of wide hybrids, and chromosome structure and evolution. Related topics covered by ‘previous’ reviews include: genome mapping (Pillen et al. 1996), classical and molecular genetics (Rick and Yoder 1988), genetics and breeding (Stevens and Rick 1986, DeVerna and Paterson 1991), and other aspects of tomato cytogenetics (Gill 1983, Quiros 1991). The early literature in this field was summarized in comprehensive reviews by Rick and Khush (1966) and Rick and Butler (1956).

**SOURCES OF VARIATION**

**Hyper- and Hypoploidy**

A rich assortment of hyper- and hypoploid stocks were identified in early work on tomato cytogenetics (Khush 1973). Haploids, monosomics, and segmental deficiencies were induced by irradiation of mature pollen (i.e. post-gametogenesis) and identified by pollination of recessive marker stocks using the pseudo-dominant method (Khush and Rick 1968). However, none could be reliably propagated, sexually or vegetatively, and all are now extinct. Haploids of tomato do not set seed because they rarely produce
viable gametes. Segmental deletions and monosomics cannot be maintained because the gametes with deficiencies are inviable and thus do not transmit to the next generation. Monosomics produce nullisomic gametes (e.g. n-1, n-2, etc), which are eliminated during development of the male or female gametophytes. The only primary monosomic recovered was for chromosome 11, which is one of the shortest chromosomes of the set (Rick and Khush 1961). Although several tertiary monosomics were recovered, none were transmissible to the next generation (Khush and Rick 1966). These results are consistent with the presumed diploid nature of the tomato genome, which, lacking any large scale duplications, does not tolerate deficiencies at the gametophytic stage.

In contrast, hyperploidy is tolerated to a much greater extent, and both triploids and tetraploids are relatively fertile. Spontaneous autotriploids were the most common type of unfruitful ('bull') tomatoes in commercial fields in California (Rick 1945). Pollination of triploids provided useful variants, particularly the trisomics (Khush 1973). However, like the hypoploids, the triploids are genetically unstable since they produce unbalanced gametes, and cannot be maintained through seed. In contrast, autotetraploids are stable, though partially sterile, and 4x stocks of several cultivars and wild species are maintained by the TGRC. Nearly all currently available tomato tetraploids were induced by colchicine treatment or were recovered as spontaneous variants in field cultures. Cultivated tomato and its related wild species are virtually all diploids, in contrast to potato, where a range of ploidy levels (e.g. 2x, 3x, 4x) are found amongst native and domesticated forms. However, there are two known instances of natural tetraploid populations, both in *L. chilense* (= *S. chilense*), one of which also happens to be the northernmost population of this species (Rick 1990).

A complete set of primary trisomics, as well as many secondary, tertiary, and compensating trisomics have been produced in tomato (Khush 1973). The first linkage maps, based on morphological markers, were associated with individual pachytene chromosomes by trisomic segregation analysis. Though no longer the most efficient method for placement of mutant loci on chromosomes—linkage tester stocks are more informative—the trisomics are still useful for assigning molecular markers to their respective chromosomes. For example, chromosomal assignment of RFLP markers was accomplished with the primary trisomics by dosage analysis of hybridization intensity (Young et al. 1987). The primary trisomics were also used to identify individual chromosomes in synaptonemal complex spreads (Sherman and Stack 1992). Secondary and tertiary trisomics have
been useful for determining the positions of centromeres on the genetic map (Pillen et al. 1996).

Allopolyploid hybrids have been created in tomato as vehicles for transferring genes from certain related wild species. This has been a particularly useful strategy for the tomato-like nightshades, *S. lycopersicoides* and *S. sitiens*. In case of *S. lycopersicoides*, the first diploid hybrids were readily obtained by embryo culture, but were highly sterile, due in part to low rates of pairing between homeologous chromosomes (Rick 1951); in contrast, allotetraploid (amphidiploid) hybrids produced by colchicine treatment showed relatively normal meiotic behavior, with preferential pairing among homologues, and much improved fertility. Despite these initial, promising results, little if any progress was made in breeding *S. lycopersicoides* with tomato until the first allotriploid (sesquidiploid) hybrids were reported some 35 years later (Rick et al. 1986). The sesquidiploids eventually yielded a complete series of monosomal alien additions (2n+1), each containing a single extra *S. lycopersicoides* chromosome in the background of *L. esculentum* (Chetelat et al. 1998). Like the primary trisomics, transmission rates and fertility vary widely among the individual monosomal additions. Nonetheless, they are generally fertile enough to be sexually propagated. A limited series of diploid substitution lines, heterozygous for a single *S. lycopersicoides* chromosome, were also derived (Ji and Chetelat 2002).

Allopolyploid hybrids representing the genome of *S. sitiens* (syn. *S. rickii*) have been derived in similar, though more circuitous, fashion. Although *S. sitiens* has not been successfully hybridized with cultivated tomato, it does cross readily with *S. lycopersicoides*, reflecting a close genetic affinity between these species (Rick 1979, 1988b). As a result, *S. sitiens* is also cross compatible with the previously synthesized *L. esculentum—S. lycopersicoides* sesquidiploids (DeVerna et al. 1990). Since the extra *S. lycopersicoides* chromosomes tend to be eliminated in the progeny of the sesquidiploid, it serves as a convenient donor of the *L. esculentum* genome (i.e. acts as a bridging genotype). The resulting diploid *F₁ L. esculentum x S. sitiens* hybrids are highly sterile, but chromosome doubling with colchicine produces more fertile amphidiploids, from which a few monosomal additions, substitutions, and recombinant diploids have been obtained (Pertuze et al. 2003).

**Introgression**

The wild tomato species are potentially rich sources of allelic variation for genetic studies and for cultivar development following introgression.
Crosses between the cultivated tomato and all but two of its wild relatives are feasible, although the ease of hybridizations varies greatly. In contrast to the cultigen, whose early history of domestication and breeding led to severe depletion of its genetic variation, the wild species are far more diverse. Populations of the self-incompatible species, such as *L. peruvianum* (*= S. peruvianum*), are especially heterogeneous, containing more variation within a single accession than all accessions of any one of the self-compatible species, including *L. esculentum* (Miller and Tanksley 1990). In addition, genetic variation within populations of *L. hirsutum* (*= S. habrochaites*) and *L. pimpinellifolium* (*= S. pimpinellifolium*) varies according to geographic location, with populations from the center of each species’ range containing more diversity than those collected at the northern or southern limits (Rick et al. 1977, 1979). Considering that the TGRC alone maintains over 1,100 wild species populations, nearly all of which are cross-compatible with tomato, they represent an amazingly rich and accessible source of genetic variation.

At the phenotypic level, variation is sometimes apparent only in backcross derivatives of interspecific hybrids. In the study of quantitative characters, for example, backcross populations sometimes display ‘transgressive variation’, in which individual genotypic combinations produce phenotypic values exceeding either parental extreme (for example, de Vicente and Tanksley 1993). A similar phenomenon has been observed for qualitative characters, in which mutant phenotypes not expressed by either parent may appear in progeny of wide hybrids (Rick 1967). Such ‘novel variation’ may arise from a number of sources, including genic or plasmatic interactions, latent variation in the wild species, and de novo mutation. For example, the *B* gene for high β-carotene is present in all of the green-fruited species but expressed only in the genetic background of *L. esculentum*, suggesting an interaction between genes determines carotenoid accumulation. Another type of interaction, between the nuclear and cytoplasmic genomes, appears to control expression of cytoplasmic male sterility (CMS) in tomato, as it is only observed when cytoplasm of *L. esculentum* is transferred into the nuclear genome of wild species such as *L. pennellii* (*= S. pennellii*). Latent variation (i.e. residual heterozygosity) in the wild species can also account for novel traits, such as the *old gold* mutant (*B^0^* from *L. chilense*).

The wild species have also been the main sources of marker polymorphisms required for development of high density genetic maps. Using DNA-based markers such as RFLPs and RAPDs, there is generally very little variation detectable within or between *L. esculentum* varieties or landraces.
In contrast, the wild species can be highly variable. For example, a single inbred accession of the wild species *L. pennellii* was polymorphic relative to cultivated tomato for ~71% of AFLP markers (Haanstra et al. 1999). Approximately 81% of RFLPs (probe × RE combinations) were informative in *S. lycopersicoides* vs. tomato (Chetelat et al. 2000).

Mapping populations used for the construction of molecular marker maps in tomato include conventional interspecific F2 or BC populations. Such segregating populations are usually difficult to propagate indefinitely, unless they can be immortalized by tissue culture or other means. Since each unique progeny array is ephemeral, they do not provide an optimal, long term mapping resource. A recombinant inbred line (RIL) population, such as one derived from *L. esculentum* × *L. cheesmanii* *f. minor* (= *S. galapagense*), is a more permanent resource and provides greater map resolution than the corresponding F2 (Paran et al. 1995). However, some of the *L. cheesmanii* RILs had a higher than expected degree of residual heterozygosity and/or reduced fertility. Another type of permanent mapping resource, and one that has been pioneered in tomato, is the introgression line (IL) population. ILs consist of overlapping homozygous chromosome segments introgressed from a wild donor genome into a constant genetic background, in this case, of cultivated tomato. A set of 50 ILs contains an entire *L. pennellii* genome bred into *L. esculentum*, the first such population synthesized for tomato (Eshed and Zamir 1995), with an additional 26 sublines providing increased map resolution (Pan et al. 2000). Similar types of prebred lines have been created for *L. hirsutum* (Monforte and Tanksley 2000a), *L. pimpinellifolium* (Doganlar et al. 2002), and *S. lycopersicoides* (Canady et al. 2005). ILs have a number of advantages for fine mapping of QTLs, gene identification, and related breeding applications (see reviews by Zamir and Eshed 1998; Zamir 2001). Furthermore, ILs tend to have greater viability and fertility than corresponding RILs from the same interspecific hybrids, due to the more limited contribution of wild species genome in each IL. As a result, they provide a more realistic genetic background for evaluation of vegetative and reproductive characters. However, a permanent ‘reference’ mapping population that offers a high level of linkage resolution is not yet available in tomato. Towards this goal, Vision et al. (2001) developed a backcross recombinant inbred population from the cross *L. esculentum* × *L. pennellii*. Using marker assisted selection on a large population, a subset of individual genotypes were chosen to provide maximum map resolution for the population as a whole.
Mutation

Spontaneous and induced mutations affecting development and morphology were essential ingredients of early genetic research on tomato. Mutations provided markers for the first classical maps, for studies of segregation and recombination in wide crosses, and for integration of cytological and genetic maps. The characterization of spontaneous unfruitful (‘bull’) plants occurring in field plantings led to the identification of a large number of useful variants, including male-sterile mutants, trisomics, tetraploids, triploids, haploids, and meiotic defects (Rick 1945). The number of mutants described in tomato increased steadily as a result of these and other studies. The Tomato Genetics Cooperative (TGC) was established by C.M. Rick and associates in 1950 to promote exchange of information and germplasm amongst geneticists, and to coordinate linkage studies (Robinson 1982). First published in 1951, the TGC Report evidenced the accelerating pace of research on tomato genetics: new mutants were described, segregation, allelism and linkage tests reported, and lists of available stocks issued. Large-scale mutagenesis studies, in particular those of Hans Stubbe from Gatersleben, Germany (summarized in Stubbe 1972a, 1972b), greatly expanded the available collections of mutants. At the time of the first comprehensive review (Rick and Butler 1956), only 118 mutant loci were known (of which 56 had been mapped). Today, ~1,200 mutations at 1,000 loci have been described, of which ~400 have been mapped or assigned to a chromosome, and a small but growing number cloned and sequenced. The TGRC currently maintains and distributes over 1,000 monogenic stocks with mutations at over 600 loci, affecting most aspects of development and morphology (Chetelat 2005).

Mutagenesis studies in tomato have employed a variety of artificial means for generating new mutations. The standard methods, widely used in other model organisms, include treatment of seeds or pollen with alkylating agents (mainly EMS) or radiation (primarily X-rays and fast neutrons). Additional mutagenesis strategies that have been used to a limited extent include somaclonal variation (Evans and Sharp 1983, Gavazzi et al. 1987, van den Bulk et al. 1990), and transposon tagging using the maize \textit{Ac}/\textit{Ds} elements (Meissner et al. 1997, 2000). Transposon tagging has several attractive features. First, the gene responsible for a mutant phenotype is readily identified by sequencing DNA on either side of the insertion site (e.g. by inverse PCR). Second, libraries of \textit{Ds} insertions at different positions in the genome have been established (Knapp et al. 1994, Thomas et al. 1994), since transposition of \textit{Ds} occurs preferentially to linked sites, the
probability of tagging a gene in the same region is thereby increased. Thirdly, the chance of identifying \( Ds \) insertions into target genes can be improved by ‘site selected insertional mutagenesis’, a combination of DNA pooling and nested PCR (Cooley et al. 1996).

Despite these improvements, there is still a need for a high throughput mutagenesis system in tomato that will allow gene isolation for functional genomics. Large-scale insertional mutagenesis by T-DNA tagging is not practical in tomato due to limitations in current \textit{Agrobacterium} transformation methods. Recently, EMS mutagenesis has been revived by the development of technology for identifying point mutations. The TILLING (Targeting Induced Local Lesions In Genomes) method screens pooled DNA samples from segregating populations to identify individuals with point mutations in a gene of interest (McCallum et al 2000). EMS mutagenesis, which causes primarily C/G to T/A transitions, has been a highly effective method of generating mutants in tomato. A population of 13,000 M2 families was generated by EMS and fast neutron treatments in cv. M-82, and includes 3,417 catalogued mutant phenotypes (Menda et al. 2004). Examples of allelism with existing mutations suggest the M-82 mutant population is nearly saturated (i.e. likely to contain at least one mutation in each gene). Phenotypes and images of these mutants are available online (http://www. zamir.sgn.cornell.edu/mutants/). The tomato genome – euchromatic regions only – is currently being sequenced by the international ‘SOL’ initiative (http://www.sgn.cornell.edu). Combined with existing EST databases (Van der Hoeven et al. 2002), the increased sequence information will allow more genes to be identified as potential targets based on their sequence alone. Candidate genes can also be identified by comparison of gene or QTL locations in tomato to sequence information from orthologous regions in model species such as tomato and \textit{Arabidopsis} (Ku et al. 2000, 2001).

\textbf{LINKAGE MAPS}

\textbf{Classical Maps}

The ‘classical’ linkage maps of tomato are based on the simultaneous segregation of multiple morphological markers, almost always in intraspecific (\textit{L. esculentum}) crosses. Initially limited to existing mutants of spontaneous origin, establishment of linkage groups was greatly facilitated by a large influx of induced mutations. Cooperation among TGC members in mapping the increasing number of mutant loci was also a key element; for a period of time, each chromosome was ‘assigned’ to a particular
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investigator to work out its linkage relations and develop new marker combinations (Robinson 1982). Efficient detection of linkage was possible with the development of sets of chromosome-specific linkage tester stocks, which combined multiple markers on a single chromosome (see Chetelat and Petersen 2003). Additional linkage tester stocks combined two strategically situated markers on each of two chromosomes, so that in theory a maximum of six segregation tests would be required to detect linkage anywhere in the genome. The number of morphological markers that can be simultaneously and independently genotyped in this fashion is in many cases severely limited by their phenotypic effects. Problems frequently encountered are epistasis among genes controlling related traits, sterility or inviability of multiple marker combinations, and lack of ‘good’ markers (e.g. seedling stage expression) for certain genomic regions. In addition, linkage tests of new morphological markers typically segregate in repulsion phase with respect to the tester combination, which limits precision of recombination fraction estimates for recessive genes. While coupling phase linkage tests, particularly testcrosses, provided greater precision, they require the prior synthesis of new marker combinations (i.e. recombinant genotypes), which becomes limiting with more than just a few markers per chromosome. These factors limited saturation and resolution of the classical linkage map of tomato, as in other organisms. Isozyme markers were integrated with the mutant loci beginning in the 1970’s (Tanksley and Rick 1980). Due to their neutral phenotypes and codominant expression, allozymes overcame many of the limitations of morphological mutants as genetic markers. However, available enzyme staining technology restricted the number of protein markers, and only a few have been added to the map recently (Bernatzky and Tanksley 1986, Chetelat et al. 2000).

At the present time, approximately 400 morphological and isozyme markers have been at least assigned to their respective chromosomes, and the majority have also been positioned within their linkage groups. The last comprehensive linkage summaries are now 19+ years old (Mutschler et al. 1987, Stevens and Rick 1986), and sorely in need of revision. Fortunately, the classical maps for several chromosomes have been updated, usually as a result of integration with molecular markers. These include chromosome 1 (Balint-Kurti et al. 1995, van Tuinen et al. 1997), chromosome 3 (Koornneef et al. 1993, van der Biezen et al. 1994), chromosome 6 (van Wordragen et al. 1996, Weide et al. 1993), chromosome 7 (Burbidge et al. 2001), chromosome 10 (van Tuinen et al. 1997), and chromosome 11 (van Tuinen et al. 1998).
Molecular Marker Maps

The development of molecular linkage maps of the tomato genome based on DNA markers provided many advantages over the existing classical maps. Due to the low level of polymorphism detectable within *L. esculentum* using DNA markers, the molecular linkage maps are based on segregation and recombination in interspecific crosses. F2 progeny from the cross *L. esculentum* × *L. pennellii* have been favored for this purpose because of their relative ease of hybridization, the fertility and normal meiotic behavior of F1 hybrids, and the high polymorphism rate that distinguishes these two species. Because the number of DNA marker loci is not limiting, the molecular linkage maps have high marker density and good saturation of linkage groups. The framework map, based on RFLP markers, contains over 1000 loci, with an average distance between markers of only 1.2 cM (Pillen et al. 1996, Tanksley et al. 1992). This map is also populated with many genes of known function or phenotype, including morphological markers, isozyme loci, and cloned genes. Additionally, the approximate locations of centromeres have been determined for each linkage group (Pillen et al. 1996), providing anchor points to the cytological maps.

In addition to RFLP markers, a number of other DNA marker systems have been used to create linkage maps in tomato, with varying success. AFLPs provide thousands of polymorphic bands, and were used to generate an ultra-dense genetic map of tomato, consisting of over 1200 markers (Haanstra et al. 1999, Spooner et al. 2005). Marker distribution along the chromosomes was decidedly nonrandom, with the majority of AFLPs (particularly the EcoRI-MseI derived markers) tightly clustered in the centromeric regions. AFLPs may thereby provide markers for genomic regions less readily detected by RFLPs (Bonnema et al. 2002). RAPD and SSR markers also map primarily to proximal regions of chromosomes, although SSRs identified in EST sequences are more randomly dispersed (Grandillo and Tanksley 1996, Areshchenkova and Canal 1999, 2002). Despite the greater time and expense of applying RFLP markers, they have the significant advantage of providing multiallelic genetic probes that can be compared across species and populations. This makes RFLPs ideally suited for mapping in tomato, with its rich collection of wild relatives, which provide not only abundant marker variation, but also many traits of interest to breeders. The availability of a set of reference maps and corresponding RFLP markers have provided the genetic tools to expedite a vast array of genetic studies and plant breeding applications in tomato too numerous to summarize here.
An RFLP linkage map has been developed for tomato using conserved ortholog set (COS) markers (Fulton et al. 2002). These represent ESTs that are single or low copy in the tomato genome, and have a high degree of homology to a single ortholog in *Arabidopsis*, as determined by sequence comparisons. Over 1000 COS markers were identified, of which ~550 have been mapped. The COS map is anchored to previous maps with a large number of conventional RFLPs, and includes SSR loci identified within the ESTs sequences. Map resolution has been improved by increasing population size, and total map length is substantially increased over the original molecular map based on RFLPs. Many RFLPs have been converted to CAPS (cleaved amplified polymorphic sequence) markers (Frary et al. 2005). Together with SSRs, these provide a set of framework PCR-based markers. Current versions of these maps, as well as sequence databases, are available through the Solanaceae Genomics Network (SGN) (http://www.sgn.cornell.edu).

Rates of recombination within a given marker interval may vary greatly in tomato due to the influence of several factors. Recombination is generally higher in female than in male gametes (de Vicente and Tanksley 1991, van Oijen et al. 1994, Ganal and Tanksley 1996). Recombination is also elevated in progeny of F1 interspecific hybrids relative to advanced backcross generations (Rick 1969, 1971), and higher in whole chromosomes than in introgressed segments (Paterson et al. 1990, van Wordragen et al. 2000b, Ji and Chetelat 2002). In addition, recombination rates vary according to species divergence. For example, recombination in intraspecific *L. peruvianum* crosses was higher (10% on average) than interspecific *L. esculentum* x *L. pennellii* (van Oijen et al. 1994). Similarly, recombination in *L. esculentum* x *S. lycopersicoides* was ca. 30% lower on average than in *L. esculentum* x *L. pennellii* (Chetelat et al. 2000). Finally, reduced recombination is observed between markers in the centromeric regions, as a result of which marker density (in genetic terms) is higher (Tanksley et al. 1992, see below).

**Comparative Maps of Lycopersicon and Related Solanum Species**

Comparisons of genetic maps from inter- and intraspecific crosses involving *Lycopersicon* spp. indicate nearly complete collinearity between them. Comparative maps of the following species have been developed from interspecific crosses to *L. esculentum*: *L. pimpinellifolium* (Grandillo and Tanksley 1996), *L. cheesmanii f. minor* (Paran et al. 1995), *L. chmielewskii (= S. chmielewskii)* (Paterson et al. 1990), *L. pennellii* (Tanksley et al. 1992), *L. hirsutum* (Bernacchi and Tanksley 1997), *L. parviflorum (= S. neorickii)* (Fulton
et al. 2000), and *L. peruvianum* (van Oijen et al. 1994, Fulton et al. 1997). Although there were significant differences among these maps for total genetic length and/or recombination rates in specific marker intervals, they were all essentially consistent with the framework map in terms of marker order along each chromosome. One noteworthy exception is a region on chromosome 7 which is inverted in *L. pennellii* relative to *L. esculentum* (Van der Knaap et al. 2004). The otherwise strong conservation of gene order indicated by these comparative maps is consistent with observations of normal chromosome pairing and fertility in most F1 interspecific hybrids between *Lycopersicon* species. The genomes of all species in the *Lycopersicon* clade can therefore be considered essentially colinear and homologous.

In contrast, genetic maps of the *S. lycopersicoides* and *S. sitiens* genomes indicate these species have a different genome structure. A genetic map based on BC1 *L. esculentum* x *S. lycopersicoides* showed a genome-wide reduction in recombination of about 30% compared to interspecific *Lycopersicon* maps (Chetelat et al. 2000). No recombination could be detected on the long arm of chromosome 10, suggesting the intergeneric F1 was heterozygous for a structural rearrangement in this region. Following introgression of individual chromosomes into *L. esculentum*, recombination between markers on *S. lycopersicoides* 10L remained undetectable (Ji and Chetelat 2002). To determine the gene order of *S. lycopersicoides* chromosome 10, recombination between homologous chromosomes would be required. A map based on *S. sitiens* x *S. lycopersicoides* is ideal for this purpose, since the two nightshades are closely related and cross-compatible (i.e. their genomes are homologous), yet present a higher rate of marker polymorphism than intraspecific populations of either species. Results of this map showed colinearity with *Lycopersicon* for all regions of the genome, except 10L, where a paracentric inversion distinguishes the two groups (Pertuze et al. 2002). The location of this inversion explains the absence of recombination between *L. esculentum* and *S. lycopersicoides* chromosomes in this region.

Furthermore, the breakpoint of this inversion appears to be identical to the one described for chromosome 10L in cultivated potato (*S. tuberosum*), one of five such rearrangements that distinguish it from tomato (Bonierbale et al. 1988, Tanksley et al. 1992). *S. lycopersicoides* and *S. sitiens* have the same marker order on this chromosome as potato, a configuration that is also observed in pepper (*Capsicum*) and eggplant (*S. melongena*) (Livingstone et al. 1999, Doganlar et al. 2002). Given the close relationship between *Lycopersicon* and the much larger *Solanum* genus (Spooner et al. 2005), the
potato/eggplant/pepper arrangement must be ancestral and the tomato inversion derived. The presence of the potato arrangement in *S. sitiens* and *S. lycopersicoides*, which are among the closest relatives of tomato outside the *Lycopersicon* group, supports this hypothesis. Furthermore, the observed colinearity between tomato, *S. lycopersicoides* and *S. sitiens*, in the regions of the other four potato rearrangements suggests that the 10L inversion evolved most recently, presumably coinciding with divergence of *Lycopersicon* from a *Solanum* ancestor. As such, the 10L inversion is a cytotoxic marker for the *Lycopersicon* genome. These two basic genomes, designated L and S (Fig. 3.1), appear to be the only large scale differences in chromosome structure separating tomato from any of the species with which it is cross-compatible. In comparison, five basic genomes have been postulated for the cultivated potato (A genome) and related *Solanum* species (B, C, D, and E) on the basis of chromosome pairing and fertility in hybrids between them (Matsubayashi 1991).

**MEIOSIS IN WIDE HYBRIDS**

**Hybrids Between Species of *Lycopersicon***

As mentioned above, sexual crosses are possible between cultivated tomato and any of the wild *Lycopersicon* species, although ease of hybridization and fertility of the resulting F₁’s varies greatly (Rick 1979). The red- or orange-fruited species – *L. esculentum*, *L. pimpinellifolium*, and *L. cheesmanii* (= *S. cheesmaniae* or *S. galapagense*) – can be freely intercrossed to form highly fertile hybrids. Crosses between the red/orange and the green-fruited species generally succeed only when the former are used as female parent (unilateral incompatibility), and hybrids are less fertile. Obtaining hybrids with *L. peruvianum* or *L. chilense* can be more problematic: embryo/ovule rescue or other techniques are usually required, and F₁ hybrids between the groups are less fertile.

Despite differences in crossability and hybrid fertility, meiosis is relatively normal in all interspecific combinations examined to date (Rick 1979). In each case, parental chromosomes synapse along their entire length at pachytene, form 12 bivalents with chiasmata at diakinesis, leading to proper alignment at metaphase and regular anaphase of the first division (Afify 1933, Lesley and Lesley 1943, McGuire and Rick 1954, Sawant 1958, Chmielewski 1962, Khush and Rick 1963). Minor differences in chromosome morphology were observed between chromosomes of *L. esculentum* and *L. pennellii*, primarily in the lengths of heterochromatic regions and
Fig. 3.1 Comparative idiograms of the L genome of tomato (Lycopersicon spp.), S genome of the tomato-like nightshades S. lycopersicoides and S. sitiens, and A genome of cultivated potato (Solanum tuberosum). The locations of five paracentric inversions that distinguish these genomes are indicated by arrows. Ancestral chromosome configurations are shown in white, derived inversions in black, and regions of uncertain ancestry in gray, based on results of Livingstone et al. (1999), and Pertuze et al. (2002).
their pattern of chromomeres (Khush and Rick 1963). However, little evidence for structural differentiation has been observed at meiosis in interspecific hybrids. Only occasional abnormalities, such as incomplete pairing, or deficiency or inversion configurations have been detected (Lesley 1950, Rick 1979). In allotetraploid hybrids, heterogenous pairing is only slightly lower than homogenous pairing, and multivalents form at nearly the same rate as in autotetraploids (Rick and Khush 1962, Sybenga et al. 1994). From these observations of meiosis in interspecific hybrids, it appears that speciation within Lycopersicon was accomplished primarily by gene mutation rather than structural rearrangement, a conclusion supported by the strong conservation of marker order on linkage maps described above.

Hybrids With Other Solanum Species

In contrast to the normal meiosis of hybrids within Lycopersicon, meiosis in hybrids involving the more distantly related Solanum species is partially disrupted. Observations in this area are based primarily on F₁ L. esculentum x S. lycopersicoides hybrids, of which the first were synthesized by Rick (1951). Detailed studies were conducted on comparative chromosome morphology and associations in the 2x and 4x intergeneric hybrids (Menzel 1962, 1964, Menzel and Price 1966). Chromosome behavior during early prophase of the 2x hybrid is relatively normal: chromosomes are completely synapsed and form normal synaptonemal complexes at pachytene. However, occasional inversion or deficiency configurations are observed, and the total pachytene complement length of S. lycopersicoides is nearly 1.5 times that of the cultivated tomato. Chromosomes 4, 9, and 10 of S. lycopersicoides were found to be longer than the corresponding chromosomes of L. esculentum, and the resultant bivalents showed unequal pairs. During meiosis, 2x hybrids undergo reduced chiasma formation and produce about four univalents/cell at metaphase I. In 4x allotetraploids, pairing occurs preferentially among homologous chromosomes, resulting in mostly bivalents and a few multivalents. The 4x hybrids also exhibit greater pollen fertility than diploids, and produce a few viable seeds. In allotriploid (sesquidiploid) hybrids, consisting of two genomes of L. esculentum and one of S. lycopersicoides, preferential pairing of the L. esculentum homologues produces 12 bivalents, with the S. lycopersicoides chromosomes forming 12 univalents (Rick et al. 1986). In addition, the condensation of S. lycopersicoides chromosomes during early diakinesis of the sesquidiploids is significantly delayed with respect to their L. esculentum counterparts. These observations of incomplete chromosome pairing and lack of
synchronization during meiosis indicate the chromosomes of \textit{L. esculentum} and \textit{S. lycopersicoides} are homeologous.

A similar relationship exists between the genomes of tomato and \textit{S. sitiens}. Though sexually incompatible with \textit{L. esculentum}, \textit{S. sitiens} crosses easily with its sister taxon \textit{S. lycopersicoides} to form fully fertile hybrids with normal meiotic behavior (Rick 1979, DeVerna et al. 1990, Pertuze et al. 2002). Taking advantage of this chain relationship, sesquidiploid \textit{L. esculentum} - \textit{S. lycopersicoides} hybrids were used as donors of the \textit{L. esculentum} genome in crosses to \textit{S. sitiens}, as described above. Chromosome pairing at diakinesis of the resulting diploid F$_1$ \textit{L. esculentum} x \textit{S. sitiens} hybrids was disrupted, with an average of 5.7 univalents observed per cell. Amphidiploids showed a strong preference for homologous pairing, with a great majority of cells containing 24 bivalents (DeVerna et al. 1990). The results indicate that chromosomes of \textit{S. sitiens} are homeologous with those of \textit{L. esculentum}, and homologous with \textit{S. lycopersicoides}.

While attempts to cross tomato with more distantly related \textit{Solanum} species have failed, somatic hybrids have been produced for some combinations. For example, \textit{L. esculentum} (+) \textit{S. ochranthum} cell fusions resulted in allotetraploid and allohexaploid hybrids (Stommel 2001). Though many were aneuploid, and highly sterile, a few of the 4x hybrids had moderate fertility. Multivalent formation during meiosis in the these hybrids provides evidence of pairing between homeologous chromosomes, a prerequisite for eventual recombination and introgression. Prospects in this area are bolstered by recent success in introducing tomato chromosomes into potato (see next section).

\textbf{Genomic In Situ Hybridization}

The field of molecular cytogenetics has been revolutionized by advances in techniques such as fluorescence in situ hybridization (FISH), which provides powerful tools for investigating chromosome structure and function of complex genomes. A specific application of FISH is genomic \textit{in situ} hybridization (GISH), which utilizes total genomic DNA of one species as a probe to distinguish parental genomes or chromosomes in sexual and somatic hybrids. GISH analysis of hybrids between \textit{Lycopersicon} species has been limited because the taxa involved are closely related, and therefore not easily differentiated by hybridization. GISH experiments using standard stringency conditions usually result in complete hybridization of probe DNA to both genomes if they share a high degree of sequence homology. However, hybridization and/or post-hybridization stringencies can be
increased to effectively differentiate more closely related genomes. For example, GISH of hexaploid *L. esculentum (+) L. peruvianum* somatic hybrids and their diploid derivatives showed extensive pairing and chiasmata formation between chromosomes of the two species (Parokonny et al. 1997). This is not unexpected, based on genetic evidence of recombination in progeny of sexual hybrids (Fulton et al. 1997). Meiosis has been examined by GISH in other hybrids between closely related species, including *L. esculentum x L. pennellii* (Haider Ali 2001) and *S. lycopersicoides x S. sitiens* (see Fig. 3.2B) sexual hybrids.

GISH cytology has been more widely used to analyse meiosis in hybrids between *Lycopersicon* and more distantly related species. For example, *S. lycopersicoides* has been hybridized with *L. esculentum* by somatic cell fusion, in addition to the earlier conventional crosses (Handley et al. 1986, Hossain et al. 1994, Matsumoto et al. 1997). There was little cross hybridization between their genomes, and GISH was a useful tool for determining the genetic constitution of 4x and 6x hybrids and their progeny (Escalante et al. 1998). Pairing between *S. lycopersicoides* and *L. esculentum* chromosomes was studied in monosomic addition and substitution lines, which contain individual alien chromosomes introgressed into cultivated tomato (Ji and Chetelat 2002, Fig. 3.2). In the monosomic additions (2n+1=25), the extra *S. lycopersicoides* chromosome forms a univalent and its tomato counterparts form bivalents in ~90% of meiocytes, indicating a strong preference for homologous pairing. In the corresponding substitution lines (2n=24), pairing between the *S. lycopersicoides* chromosome and its *L. esculentum* homeologue occurs at a higher frequency (up to 90% of cells) due to the absence of homologous partners. However, homeologous pairing was greatly reduced in the substitution for *S. lycopersicoides* chromosome 10, due to a lack of crossing over within the paracentric inversion on 10L that differentiates these genomes.

GISH has also been a useful tool for dissecting the genetic composition of complex hybrids involving more than two parental species (Ji et al. 2004). For example, the amphidiploid *L. esculentum x S. sitiens* hybrid described previously was backcrossed to sesquidiploid *L. esculentum x S. lycopersicoides*, resulting in a complex trigenomic hybrid. A combination of GISH and RFLP analysis revealed the following genetic composition: two genomes of *L. esculentum*, one genome of *S. sitiens*, and two residual chromosomes from *S. lycopersicoides* (Pertuze et al. 2003, Ji et al. 2004). During meiosis, chromosomes of the two *Solanum* parents pair regularly, as do the *L. esculentum* set, while the remaining *S. sitiens* chromosomes are usually unpaired. Though less fertile than a true sesquidiploid, this trigenomic
Fig. 3.2 Examples of the use of genomic in situ hybridization (GISH) for genome analysis of tomato interspecific/intergeneric hybrids, and monosomic addition (MA), substitution (SL) and introgression (IL) lines. (A) Disrupted pairing at metaphase I of F$_1$ L. esculentum x S. lycopersicoides; (B) Partial differentiation at mitotic metaphase of homologous chromosomes in F$_1$ S. lycopersicoides x S. sitiens; (C) S. lycopersicoides SL-8 at diakinesis with pairing between homeologous chromosomes (bivalent; arrow); (D) S. sitiens MA-8 at diakinesis showing pairing between homeologues (bivalent; arrow) and an unpaired L. esculentum chromosome (univalent; arrowhead); (E) S. lycopersicoides SL-7 at pachytene showing the unpaired S. lycopersicoides chromosome (arrow); (F) Heterozygous introgression line containing a segment from S. lycopersicoides chromosome 7 of ~42 cM (TG499 - TG128) in length (arrow). (A, C, E-F) Red = S. lycopersicoides, Blue = L. esculentum; (B) Blue = S. lycopersicoides; Red = S. sitiens; (D) Blue = L. esculentum, Red = S. sitiens. Bars represent 5 µm.
hybrid nonetheless yielded a few monosomic additions, substitutions, and recombinant diploids containing individual *S. sitiens* chromosomes, or segments thereof, in *L. esculentum*. Despite representing only a portion of the *S. sitiens* genome, the introgressions so far obtained demonstrate the feasibility of breeding specific traits from this nightshade into tomato. Virtually ignored in previous searches for disease resistance or other desiderata of interest to breeders, *S. sitiens* is most remarkable for its adaptation to the hyperaridity of its native habitat, the Atacama desert of Chile (Rick 1988b).

The successful regeneration of potato (+) tomato fusion hybrids from protoplasts, first reported by Melchers et al. (1978), raised the possibility of eventual gene transfer between these economically important, yet sexually incompatible, solanaceous crop species. Unfortunately, the initial hybrids were highly sterile, and produced neither fruit nor tubers. Since then, a hexaploid potato (+) tomato fusion hybrid was successfully backcrossed to tetraploid potato, from which a single BC$_1$ plant was generated (Jacobsen et al. 1994). GISH cytology indicated this plant possessed nine tomato chromosomes: at meiosis, they formed three homologous bivalents and three univalents, hence represented only six of the 12 possible tomato chromosomes (Jacobsen et al. 1995). Additional crosses yielded BC$_1$ progeny with different numbers of extra chromosomes, and in which an entire haploid tomato genome was represented (Garriga-Caldere et al. 1997). Following additional backcrosses, a complete set of 12 tomato monosomic additions in a potato background were identified (Garriga-Caldere et al. 1998, Haider Ali et al. 2001). Pairing and recombination between potato and tomato chromosomes was observed, albeit at very low rates, demonstrating the potential for gene transfer between these important solanaceous crops (Garriga-Caldere et al. 1999). A low level of heterogenetic pairing was also observed by Gavrilenko et al. (2001) in 4x *L. esculentum* (+) *S. etuberosum* hybrids; anther culture of the amphidiploid resulted in regeneration of 2x hybrids, suggesting a route for possible transfer of *S. etuberosum* chromosomes into tomato via allohexaploid fusions and androgenic sesquidiploid derivatives.

**CHROMOSOME STRUCTURE AND VARIATION**

**Pachytene Chromosome Structure**

The first studies of tomato pachytene chromosomes revealed the general picture of chromosome morphology (Lesley and Lesley 1935) and identified two chromosomes of the set (Brown 1949). On the basis of centromere positions, and the relative lengths of heterochromatic and euchromatic
regions and heterochromatic knobs, Barton (1950) was able to distinguish each of the 12 tomato chromosomes at pachytene. The chromosomes were numbered from 1 to 12 according to their length, with no. 1 being the longest. The main nucleolar organizing region (NOR) was associated with chromosome 2. A cytological map of the tomato genome was thereby constructed, which incorporated the distinctive features of each chromosome.

The pachytene maps were later integrated with linkage maps by determining the location of genetic markers relative to chromosome landmarks, such as centromeres, telomeres, and breakpoints of various origins. Radiation-induced deletions were used to locate genetic markers on the cytogenetic map of each chromosome (Khush and Rick 1968). Deletions were identified by the pseudo-dominant technique, in which recessive marker stocks were pollinated with irradiated wild type pollen. The appearance of recessive phenotypes in the progeny indicated the loss of the wild type (dominant) alleles. By this method, the positions and lengths of 74 deletions were determined on pachytene chromosomes, and 35 genetically mapped morphological mutations were located on 18 of the 24 chromosome arms. Marker genes were observed to be non-randomly distributed within chromosomes, being located almost exclusively in euchromatin. Genetic data and the distribution of cytological chiasmata pointed to much less recombination in heterochromatin than in euchromatin.

**Ratio of Physical to Genetic Distance**

The ratio of physical to genetic distance is not constant within the tomato genome. Departures from the genome-wide average value (~750 kb/cM) include local recombination hotspots: for example, ~10 kb/cM observed within *Lin5* on chromosome 9 (Fridman et al. 2000), ~24 kb/cM around *ms-14* on chromosome 11 (Gorman et al. 1996), and 55-110 kb/cM near *Cf-4/Cf-9* on chromosome 1 (Bonnema et al. 1997). At the other extreme, lower than average recombination rates are observed near centromeres: for example, ~4-16 Mb/cM around *Tm-2* on chromosome 9 (Ganal et al. 1989) and ~60 Mb/cM around *Mi* on chromosome 6 (Kaloshian et al. 1998). Another manifestation of this recombination suppression is the clustering of random cDNA and genomic clones in proximal regions of most chromosomes on the molecular linkage map ( Tanksley et al. 1992, Pillen et al. 1996). This suppressive effect is most pronounced within alien introgressions, such as those comprising the resistance genes *Mi* and *Tm-2* bred into *L. esculentum* from *L. peruvianum*. Substantially higher recombination rates were observed in the wild species background for the same marker intervals. Prospects for map-based cloning of desirable genes
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or QTLs identified in wild relatives will therefore depend to a large extent on their location within the genome.

**Synaptonemal Complex**

A more accurate estimate of the size and chromatin composition of the tomato genome has been obtained from studies of the synaptonemal complex (SC) from pachytene stage nuclei. Tomato is well suited to cytological visualization of SCs using electron microscopy (Stack 1982). The SC karyotype (like the conventional pachytene version) indicates the pericentromeric regions are composed of large blocks of heterochromatin (Sherman and Stack 1992). Approximately 36% of total SC length is composed of DNA packaged as heterochromatin (Peterson et al. 1996). However, because DNA in heterochromatin is more dense (on a per unit SC length basis), it represents approx. 77% of the total DNA in the tomato genome. These heterochromatic regions of the chromosomes are considered genetically inactive. For example, few mutant loci were mapped to heterochromatin by deletion analysis (Khush and Rick 1968). This means that the effective genome size of tomato (considering only euchromatin) could be as little as 0.22 pg DNA/C, slightly larger than that of *Arabidopsis* (Peterson et al. 1996). However, the tomato genome is composed of mostly single or low copy number sequences: from independent estimates based on hybridization or reassociation studies, this fraction represents ~70% of nuclear DNA (Zamir and Tanksley 1988, Peterson et al. 1998). FISH localization to pachytene chromosomes showed that while euchromatin contains primarily single-copy DNA, a majority of this fraction, as well as most of the repetitive DNA, is located in pericentromeric heterochromatin (Peterson et al. 1999).

The physical basis for centromeric recombination suppression has been examined using recombination nodules (RNs), which are the manifestation of crossing over events on SCs. In a detailed study of the location and frequency of RNs in tomato, Sherman and Stack (1995) elucidated several significant trends. First, RNs are nonrandomly distributed, being located primarily in euchromatin rather than heterochromatin, and absent from the telomere ends of SCs and centromeres. The relatively low frequency of RNs within pericentromeric heterochromatin and near the telomeres explains the clustering of RFLP markers on linkage maps in these regions. Second, RN frequency per unit length of euchromatin is not constant, which might explain the observed gaps and recombination hotspots on genetic maps. Thirdly, a 1:1 relationship between RNs and chiasmata was observed. Assuming each RN is a crossover event, the *L. esculentum* genome would contain a total of ca. 1095 map units, a value similar to those of the classical molecular linkage maps.
Effects of Recombination on Natural Variation

Natural genetic variation at the population level is strongly influenced by mating systems, which can vary among tomato species from obligate outcrossing to complete inbreeding (Rick 1988a). As expected, the strictly allogamous (self-incompatible) species or accessions show vastly more within-population variation than the autogamous or facultative (both self-compatible) groups (Rick et al. 1979, Miller and Tanksley 1990). In addition to the effects of reproductive biology, genetic variation is influenced by gene position along the chromosome. Levels of DNA polymorphism at a locus are positively correlated with the rate of crossing over per unit physical distance (Stephan and Langley 1998). For example, genes close to the centromere (low recombination) tend to have less within species diversity than genes farther away from the centromere. The strongest association between crossing-over and heterozygosity is found in species with intermediate levels of diversity. Relatively speaking, the effect of recombination levels on DNA polymorphism in tomato is weaker than in some species, such as *Drosophila*, and is far less than the influence of mating system (Baudry et al. 2001).

Centromere Mapping

The approximate locations of centromeres on the chromosomes have been determined by several methods (summarized by Pillen et al. 1996). A combination of RFLP mapping and localization of rDNA loci were used for chromosomes 1 and 2, deletion mapping for chromosomes 3 and 6, analysis of the tomato-potato inversion breakpoints for chromosomes 5, 9, 10, 11, and 12, or comparison of cytological, classical and molecular maps for chromosomes 4 and 8. The centromeres of chromosomes 7 and 9 were more precisely localized on the molecular linkage map through dosage analysis in trisomic stocks, including complementary telo-, secondary and tertiary trisomics (Frary et al. 1996). Both centromeres were localized within a cluster of tightly linked markers. To order markers within these clusters, high resolution maps for both centromeric regions were constructed from F2 *L. esculentum* x *L. pennellii* and F2 *L. esculentum* x *L. pimpinellifolium* populations. Similar approaches would be feasible for other chromosomes, for which the pertinent trisomic stocks are available from the TGRC.

Analysis of Genome Structure by FISH

*Repetitive Elements*

*In situ* hybridization (ISH) techniques have been used in tomato to analyse genome organization and to determine the physical localization of DNA
sequences on the chromosomes. ISH involves use of biotin-labeled probes and detection of hybridization signals with colored immuno-chemical precipitates. Fluorescence *in situ* hybridization (FISH) employs a system of coupled fluorochromes to provide higher resolution in detecting target DNA molecules. Both detection methods have been used to map repetitive and single-copy DNA in tomato. Ganal et al. (1989) mapped four classes of repetitive sequences to meiotic metaphase chromosomes using ISH. One of these, TGRI, is a subtelomeric satellite DNA repeat of 162bp. On somatic chromosomes, TGRI is located at 20 of the 24 telomeres, as well as at centromeres and interstitial sites on some chromosomes (Lapitan et al. 1989). TGRI is separated from the telomeric repeat by a few hundred kilobases, and both are found in the heterochromatic terminal knobs observed on pachytene chromosomes (Ganal et al. 1991).

Another class of repeats, the rDNA genes, are located on several tomato chromosomes. The 5S rDNA sequence was localized by FISH on pachytene spreads to the first heterochromatic knob adjacent to the centromere on the short arm of chromosome 1 (Xu and Earle 1996a). The 45S rDNA sequence was estimated to be present in approximately 2300 copies in the tomato genome and was mapped to a distal position on the short arm of chromosome 2 by linkage analysis (Vallejos et al. 1986). Using ISH, hybridization of the 45S sequence was observed at the end of an acrocentric chromosome, presumably chromosome 2 (Ganal et al. 1988). This result agrees with the known location of the main NOR in this region of chromosome 2 (Brown 1949, Barton 1950). Additional rDNA loci were found on chromosomes 6, 9 and 11 (Xu and Earle 1994, 1996b).

### Single and Low Copy Sequences

Unlike repetitive elements, detection of short, single-copy DNA sequences on plant chromosomes using FISH is technically difficult. However, efficient detection of single or low copy sequences is possible using large inserts, such as yeast artificial chromosomes (YACs) or bacterial artificial chromosomes (BACs) (Fuchs et al. 1996). For example, the Colorless non-ripening (*Cnr*) locus was mapped to a small interval on chromosome 2 using FISH of BAC clones to pachytene spreads (Tor et al. 2002).

Peterson et al. (1999) demonstrated the usefulness of synaptonemal complex spreads for detecting very short single copy sequences by FISH. SC spreads have several advantages over mitotic metaphase chromosome preparations for FISH applications: SC spreads are relatively free of debris that can interfere with probe penetration, have relatively decondensed chromatin that is highly accessible to probes, and are about ten times longer than their metaphase counterparts, which permits FISH mapping at higher
resolution. Genomic clones of ~14 kb, containing RFLP probes previously mapped to chromosome 11, were localized by FISH on SC 11. Marker order and the physical distances between them could be ascertained in this fashion.

The resolution of target DNA sequences on chromosomes can be further enhanced by hybridization to extended DNA fibers from interphase nuclei. Initially developed for human DNA, extended fiber FISH has been adapted to several plant species, including tomato (Fransz et al. 1996, Zhong et al. 1996). In this method, genomic DNA fibers from lysed interphase leaf nuclei are uniformly stretched on a microscope slide. DNA molecules thus linearized are stretched to ~3 kb/µm, allowing DNA targets to be mapped by FISH at the level of a few kilobases and a detection sensitivity of only a few hundred base pairs. This can be helpful for ordering markers that cosegregate on genetic maps due to low recombination rates; for example, fiber FISH of BACs spanning the nematode resistance gene Mi showed it is located near the junction of euchromatin and heterochromatin on chromosome 6S (Zhong et al. 1999). Extended fiber FISH was also used to study the molecular and chromosomal organization of individual telomere domains (Zhong et al. 1998).

**CONCLUSIONS AND OUTLOOK**

Continued genetic improvement of cultivated tomato is certain, given its advantageous cytogenetic and biological features, rich germplasm resources, and well-developed genomics infrastructure. The genetic basis of important economic traits, such as disease resistance, yield, and fruit quality, have been thoroughly investigated, resulting in abundant genetic markers—and in many cases the underlying gene sequences themselves—to facilitate their transfer into new varieties. Enlargement of the genetic base of tomato through introgression from related nightshade taxa is expected to provide novel traits. With further development of genomics tools and expansion of sequence databases, tomato will continue to be used as a genetic model for other solanaceous crops.

Tomato and its wild relatives also provide excellent material with which to study the evolution of adaptive traits and reproductive barriers. Speciation within *Lycopersicon* was accomplished primarily by mutation rather than chromosomal rearrangement, and as a result experimental hybridization and introgression between most species are readily accomplished. Another advantage is that genetic variation within and among populations of wild *Lycopersicon* species displays a geographic
pattern of distribution (for example, Rick et al. 1977, 1979; Caicedo and Schaal 2004). This must be due in large part to the geologic history and climate of the native Andean region, which produced habitat differentiation and geographic isolation of populations. Locally adapted populations of wild tomatoes differ in their responses to abiotic stresses, including extremes of moisture, temperature, and salinity, and their susceptibility to diseases and insect pests. In addition, mating systems range from complete autogamy to strict allogamy, and are accompanied by various types of reproductive barriers between species or populations. Tomato is therefore an attractive plant in which to study the molecular and genetic basis of these traits, and their evolutionary significance particularly in native plant populations.

SUMMARY

This chapter summarizes recent advances in the cytogenetics and evolution of tomato, with emphasis on applications in genetics and breeding. As an experimental plant material, the tomato presents a number of genetic, biological, and commercial advantages. Research on tomato has depended to a large extent on genetic resources such as mutants, wild species populations, and other genetic stocks. Recently synthesized introgression line populations representing the genomes of related wild species provide powerful tools for genome analysis and breeding. The genetic base of tomato has been expanded by hybridization and recombination with previously inaccessible tomato-like nightshades. The relatively small genome of tomato is now well delineated with genetic maps of various types. The early classical maps have been superseded by, and in some cases integrated with, high resolution molecular linkage maps based on RFLPs and other types of markers. These maps provide a framework for comparative genetic analysis of the Lycopersicon, and related Solanum species, as well as abundant markers useful in breeding programs. Linkage maps from different tomato species indicate all share the same basic genome structure, consistent with evidence from chromosome pairing in interspecific hybrids, and that speciation must therefore have been accomplished by gene mutation rather than genome rearrangement. Two tomato-like nightshades, Solanum lycopersicoides Dun. and S. sitiens Johnst., differ from the Lycopersicon clade by a paracentric inversion on chromosome 10L, an arrangement which appears to be ancestral to that of tomato. Maps based on conserved ortholog set (COS) markers identify regions of microcolinearity between tomato and Arabidopsis. Advances in cytological methods provide new tools to study genome structure. Fluorescence in situ hybridization (FISH) techniques have
been used for physical mapping of DNA elements, determination of marker order within regions of suppressed recombination, and analysis of chromosome pairing in wide hybrids. Examination of the ultrastructure of synaptonemal complexes (SCs) reveal that recombination nodules, the sites of crossing-over, occur preferentially in euchromatin, and are rare in the pericentromeric heterochromatin. Up to 77% of the tomato genome is heterochromatic and underrepresented on linkage maps. Natural variation within populations of wild species is correlated with rates of crossing over per physical distance along chromosomes; as a result, genes near the centromere tend to be less variable than those in more distal positions. With recent improvements in genomics infrastructure, tomato will continue to serve as a useful model for the genomes of related solanaceous crops.

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INTRODUCTION

The phenomenon of heterosis is defined by Shull (1911) as “the superiority of heterozygous genotypes with respect to one or more characters in comparison with the corresponding homozygotes. Heterosis is the phenotypic result of gene interaction in heterozygotes and thus confined (at least in maximal amount) to that state.” Hence, to observe heterosis, the F₁ must be superior to the two breeding lines that are its parents. Nevertheless, the heterozygote may also be inferior to both homozygotes. This performance is defined as negative heterosis (Jinks 1983).

The expression of hybrid vigor in plant crosses has been recognized for nearly 250 years (Zirkle 1952). The phenomenon received higher degree of attention since the publications of Shull (1908, 1909, 1914), East (1908, 1909) and Jones (1918), which resulted in the development of the first commercial maize hybrids and their introduction in practice at about 1930 (Sprague 1983). Within the years that followed, heterosis was observed in several crops.

Heterosis is now recognized as one of the primary factors that contributed to the success of plant breeding in many crops. Duvick (1997a) reported that maize, sorghum, and sunflower were produced as hybrids in all the industrialized world; hybrid rice was grown extensively in China and was recently introduced in India; many commercial vegetables and flowers were grown almost entirely as hybrids. The application of heterosis was
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determined as one of the greatest achievements in the twentieth century
(Barabas 1992) and as the single greatest applied achievement of the
discipline of genetics (Griffing 1990).

Despite the numerous studies on heterosis, however, and although this
phenomenon is exploited wherever possible, its biological basis
remains unknown and its elucidation is still a major challenge for scientists.
Sprague (1983) mentioned two separate courses that investigations of the
phenomenon of heterosis have followed. The first has been descriptive and
centered primarily on biochemical-physiological studies and during the recent years on molecular studies.

The genetic theories advanced on this subject differ in the relative
importance of overdominance, epistasis and linkage and how they
contribute to hybrid performance. Up to now, none of them is acceptable to
all. Since the rediscovery of Mendelism in 1900 two principal hypotheses
were suggested as the genetic basis of heterosis: dominance and
overdominance. Tsaftaris (1995) summarized these hypotheses as follows:
The dominance hypothesis attributes the increased vigor of heterozygosity
to dominant alleles mainly because of the observed correlation between
recessiveness and detrimental effects. The overdominance hypothesis
assumes that heterozygosity per se is important; i.e. there exist loci, albeit
relatively rare, at which the heterozygote is superior to either homozygote.
Jinks (1954) implicated epistasis in the expression of heterosis and recently
multilocus epistatic interactions are recognized as the third theory of

The last decade has witnessed a period of renewed interest toward
resolving some of the long standing issues related to heterosis. New interest
has been sparked by the application of molecular genetics. The knowledge
and the experience accumulated throughout the years as well as advances
in molecular genetics and technologies provided new tools and stimulated
scientists' endeavors to shed additional light on heterosis. This resulted in
a significant number of studies in which new aspects of the phenomenon
have been evaluated, new approaches to investigate its mechanisms have
been developed and new theories and hypotheses have been advanced
(McDaniel 1986, Griffing 1990, Georgiev 1991, Nienhuis and Sills 1992,
contribution in this respect, however, the opinion of the scientists summarized by Stuber (1999) was that “the causal factors for heterosis are today as obscure as they were 50 years ago”. According to Hallauer (1999) “The exact genetic basis of heterosis may never be known and understood because of allelic interactions: interactions of alleles at a locus, interactions of alleles at different loci, interactions of the nucleus and cytoplasm, and interactions of the genotype and environment. But heterosis will continue to have a major role in the future plant improvement even though our knowledge on its genetic basis is limited.”

Given the present wide use of heterosis in practice on the one hand, and a lack of real understanding of its nature, on the other, recent studies on heterosis center on: a) Increasing knowledge on the genetic mechanisms that includes efforts on physiological, biochemical, and molecular levels. A better understanding of these mechanisms would enhance the ability of the breeders not only to predict the performance of a given hybrid, but also to form new genotypes that might be used directly, without preliminary tests, as F₁ hybrids; b) based on the available knowledge of this phenomenon, increasing the efficiency in the strategies for developing parental lines whose F₁ hybrids would be promising ones; and c) improving the process of hybrid seed production by developing easier and more reliable and efficient technologies.

MANIFESTATION OF HETEROSIS IN TOMATO

Studies on heterosis in tomato were initiated at the beginning of the twentieth century, almost at the same time as those in maize (Hedrick and Booth 1907, East and Hayes 1912). Tomatoes are a self-pollinated inbred crop and there was a view that high level of heterosis could not be manifested in tomato F₁ hybrids. However, heterosis in tomato, as well as in other self-pollinated crops, was observed in several crosses. It provided evidence that this phenomenon was not limited to cross-pollinated crops. One of the theories of this performance was that natural cross-pollination predominated within the wild forms of tomatoes in the centers of their origin. Therefore, crosses between tomato lines might be considered as F₁ between inbred lines of a species which is naturally cross-pollinating (Rick 1950, Rick and Butler 1956).

Heterosis is a widely documented phenomenon in tomato. More than 50-60% of the studies on heterotic performance refer to heterosis for yield and yield components. This percentage was relatively stable even throughout the last 10 years when efforts of tomato breeders strongly
emphasized nutritional value, safety and sensory quality of a food product, tolerance to abiotic stress, etc. Based on these studies, as well as on earlier ones, it might be concluded that the frequency and the level of heterosis for yield and yield components in tomato are relatively high. Kravchenko (1990), for example, reported manifestation of heterosis for yield in 80% and for earliness in 88% of the hybrids studied all over 15 years period. According to Wehner (1997), the level of heterosis for yield in tomato was significant (estimates average 60%). Suresh Kumar et al. (1995) reported 193.55% of heterosis (over superior parent) for one of the yield components - fruit number.

According to Yordanov (1983), besides yield, enhanced plant vigor, earliness, higher adaptability to unfavorable environment and uniformity, were the manifestations of heterosis most often encountered in the tomato. These traits are of significant economic importance. Khanna-Chopra et al. (1993), for example, reported that vigor helped in the efficient utilization of environmental factors. Uniformity is one of the principal benefits of hybrids as this trait in plant structure and maturation permits efficient mechanical harvest. Earliness, generally defined as the number of days from sowing to the appearance of the first ripe fruit (Kemble and Gardner 1992), was characterized by Doganlar et al. (2000) as crucial for regions with short growing season, as desirable for taking advantage of high prices during the early season and as a prerequisite for reducing the heating and lighting expenses of greenhouse-grown tomato. Based on experiments aiming at the development of tomato cultivars adapted to low growing temperature, Nieuwhof (1990) found no important genotype x temperature interactions. This finding suggested that for satisfying the need to reduce energy consumption in greenhouses it was preferable to breed early varieties characterized by rapid growth at normal temperature instead of selecting for adaptation to low growing temperature. In some crosses the magnitude of heterosis for earliness is significant and certainly has, and will be exploited in developing early hybrids. For example, in a study of 92 F1 tomato hybrids between 19 ultra early cultivars used as seed parents and 5 late cultivars. Boe (1988) found that early yield in the F1 hybrids ranged from 52% to 307% when compared to that of the early seed parent.

Genetic studies on tolerance to abiotic stress, nutritive and market quality provide evidence that manifestation of heterosis in tomato is not limited to traits related to plant vigor or yield. Bhatt et al. (1998) reported relatively high levels of heterosis (60.4% and 52.4%) for vitamin C content detected in a number of F1 hybrids. Heterosis for vitamin C was also reported by Dod and Kale (1992), and Mageswari and Natarajan (1999). Heterosis was
observed for total soluble solids and dry matter content (Patil and Patil 1988, Yadav et al. 1988, Shrivastava 1998, Daskaloff et al. 1990, Dod and Kale 1992, Mageswari and Natarajan 1999) and lycopene and β-carotene content in fruits (Chen and Zhao 1990, Amaral Junior et al. 1997). It must be noted that there is also information reporting low level or negative heterosis for the content of compounds related to the nutritive value of tomato such as ascorbic acid, lycopene, total soluble solids, etc. (Chen and Zhao 1990, Wang et al. 1998, Amaral Junior et al. 1999). In a study of a number of tomato lines and hybrids for their suitability to prepare ketchup and paste, manifestation of heterosis for ketchup recovery and paste yield was detected (Jawaharlal et al. 1999). Heterosis for pericarp thickness was described by Patil and Patil (1988), Daskaloff et al. (1990) and Dod and Kale (1992), and for fruit firmness by Wang et al. (1995), Resende et al. (1999) and Atanassova et al. (2005).

Besides traits related to fruit market and nutritive quality, heterosis was reported for characteristics related to plant tolerance to stress, plant physiology etc. Heterotic performance for these traits is very important as it enhances the ability of plants to cope better with the environment, that usually results in higher yield. Experiments demonstrated, for example, that yield gains in hybrid maize were due primarily to improvements in tolerance to abiotic and biotic stress and that the improvement occurred in parental inbreds as well as in their F1 hybrids (Duvick 1997a). In a study of viability of pollen from intra- and inter-specific crosses of tomato, produced at low temperature, Fernández-Muñoz et al. (1995) observed positive heterosis for pollen viability in the cross between *L. esculentum* x *L. pinnatifolium*. Philouze (1997) reported high level of heterosis for yield in the hybrid Monfavet n° 63-5 when grown in non-heated greenhouse. It was found that heterosis was mainly due to higher percentage of fruit set in the hybrid. Under low temperature the fertilizing ability of the hybrid plants pollen was higher than that of the parent lines pollen. Hassan et al. (1999) reported high level of heterosis for tolerance to salinity at seedling stage, that ranged from 24.9% to 100%(based on better parent values). Zhacote and Kharti (1990) observed heterosis for net photosynthetic production in hybrids between cultivated and wild forms of tomato and Titok et al. (1994) reported manifestation of heterosis for chlorophyll content both in leaves and stems, a higher level of heterosis being observed in stems.

Based on these reports, as well as on numerous other studies [some of them reviewed by Yordanov (1983), Kalloo (1988) and Georgiev (1991)], it might be concluded that heterosis in tomato was observed for a large scale of quantitative traits, almost all of them being of breeding interest.
It is commonly assumed that genetic stability (homeostasis) in hybrids refers to reduced genotype - environment interactions. A number of studies provided, however, evidence that the majority of quantitative traits were significantly affected by environmental factors and that heterosis was also dependent on the environment (Yordanov 1983, Russel et al. 1993, Cooper and Podlich 1999, Temperini et al. 2001). Therefore, the evaluation of a given tomato hybrid when grown in different locations is necessary for getting reliable information on its performance. This kind of information is of great importance for tomato growers as it can help them to make intelligent cultivar decisions (Murray et al. 1999).

GENETIC BASIS OF HETEROSIS IN TOMATO

The design and efficiency of breeding programs depends on the relative importance of different types of gene action. Therefore, manipulating heterosis in breeding programs requires knowledge of its quantitative genetic basis. Gene action in tomato, as well as in many other species has been approached by studying the various types of genetic variance in populations and by generation means analysis. Diallel sets of F₁ crosses between collections of tomato lines are usually used for obtaining a preliminary impression of the genetic variation for characters of economic importance. As a result, a relatively large amount of information on the nature of gene action for several quantitative traits in tomato is available, the predominant part of this information concerns total yield, yield components and earliness. Additive and non-additive gene effects have been reported to be important for yield and its main components. It was found that their magnitude varied depending on the genotype and on the environmental conditions (Dod et al. 1992, Natarajan 1992, Sherif and Hussein 1992, Vallejo Cabrera and Estrada 1993, Ramos et al. 1993, Rai et al. 1997, Singh et al. 1998, Surjan et al. 1999). Studies on the genetics of earliness have indicated that dominance plays an important role in this trait (Banerjee and Kalloo 1989, Kemble and Gardner 1992). Information concerning genetics of quantitative characteristics related to seed, cotyledons, leaf, stem, fruit, growth stages, early and total yield etc. was summarized by Georgiev (1991).

As already mentioned, during the last two decades a significant number of genetic studies and breeding programs in tomato emphasized enhancing plant tolerance to abiotic stresses and fruit nutritional value, sensory and market quality. It resulted in determining the genetic basis of characteristics
related to these traits. Data of some of these studies are summarized in Table 4.1 and Table 4.2.

**Table 4.1 Gene expression of some characters related to nutritive and market quality in tomato**

<table>
<thead>
<tr>
<th>Character</th>
<th>Gene action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene content</td>
<td>Dominant</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td>β-carotene content</td>
<td>Additive and non-additive</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td>Vitamin C content</td>
<td>Dominant</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Non-additive</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td>Percentage of reducing sugar</td>
<td>Additive and dominant</td>
<td>Stommel and Haynes (1993)</td>
</tr>
<tr>
<td>Glucose/fructose ratio</td>
<td>Additive</td>
<td>Stommel and Haynes (1993)</td>
</tr>
<tr>
<td>Sugar content</td>
<td>Dominant</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td>Fruit firmness and longevity</td>
<td>Additive</td>
<td>Al-Falluji et al. (1982), Dobhal et al. (1999), Atanassova et al. (2005).</td>
</tr>
<tr>
<td>Pericarp thickness</td>
<td>Additive</td>
<td>Dobhal et al. (1999), Rai et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Additive and non-additive</td>
<td>Singh et al. (1998), Dod et al. (1995)</td>
</tr>
<tr>
<td>TSS</td>
<td>Additive and non-additive</td>
<td>Dobhal et al. (1999), Singh et al. (1998), Dod et al. (1995).</td>
</tr>
<tr>
<td>Reducing sugars content</td>
<td>Additive</td>
<td>Shrivastava (1998 b)</td>
</tr>
<tr>
<td>Dry matter content</td>
<td>Additive</td>
<td>Shrivastava (1998 b)</td>
</tr>
<tr>
<td>Resistance to cuticle cracking</td>
<td>Additive and dominant</td>
<td>Emmons and Scott (1998)</td>
</tr>
</tbody>
</table>

The numerical value recorded for a complex trait (such as total or early yield) is known to be a function of its components. In tomato, for example, earliness is usually divided into four different components and number of fruits per plant and mean fruit weight are considered as the two primary ones determining yield. Hence, the expression of heterosis for a complex trait is also studied and explained on the basis of component interactions. Bos and Sparnaaij (1993) showed that component analysis provided the necessary data for the exploitation of recombinative heterosis in plant breeding. Recombinative heterosis was defined as the phenomenon that the progeny value of complex character exceeded the mid-parent value as a result of the multiplicative relationship between the complex character.
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It was suggested that this form of heterosis may be an important cause of Specific Combining Ability (SCA).

Besides the investigations that focused on acquiring and increasing knowledge on genetic variation for characters of economic importance, a number of studies aiming at getting better understanding of heterosis in tomato have been also carried out during the last decade. These studies might contribute to developing new approaches for more efficient exploitation of heterosis in tomato breeding.

Griffing (1990) tested three heterosis hypotheses in a controlled-nutrient (CN) experiment, with reference to tomato yield and its components for a set of two inbred lines and their hybrid that had previously exhibited heterosis under field conditions. Heterosis was not exhibited by yield or yield components at any of the four nutrient levels. Hence, the total heterosis phenomenon was classified as nutrient-dependent heterosis occurring under field conditions, but not under the nutritional restrictions of the CN experiment. Such a performance fitted the hypothesis suggesting that heterosis was a consequence of a faster hybrid growth rate. Under this hypothesis lack of heterosis at all four levels of CN experiments was due to the CN procedure which forced all three genotypes to have the same growth rate. Under the differential growth rate hypothesis, the F₁ would utilize

Table 4.2  Gene expression of some characters related to tolerance to abiotic stress in tomato

<table>
<thead>
<tr>
<th>Character</th>
<th>Gene action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium use efficiency (based on total plant dry weight) in tomato grown under low-calcium stress</td>
<td>Additive and dominant</td>
<td>Li and Gabelman (1990)</td>
</tr>
<tr>
<td>Low temperature tolerance during germination</td>
<td>Additive</td>
<td>Foolad and Lin (1998)</td>
</tr>
<tr>
<td>Salt tolerance during vegetative growth</td>
<td>Additive and dominant</td>
<td>Foolad (1996)</td>
</tr>
<tr>
<td>Absolute and relative growth under salt stress and Na⁺ and Ca²⁺ accumulations in the leaf</td>
<td>Additive</td>
<td>Foolad (1997)</td>
</tr>
<tr>
<td>Testa effect on germination performance under salt stress</td>
<td>Dominant</td>
<td>Fooland and Jones (1991, 1992)</td>
</tr>
<tr>
<td>Pollen fertility and fruit set under high field temperature</td>
<td>Additive</td>
<td>Dane et al. (1991)</td>
</tr>
</tbody>
</table>

and its components traits. It was suggested that this form of heterosis may be an important cause of Specific Combining Ability (SCA).
Expression of Heterosis by Hybridization

nutrients from a given allocation most quickly while the parent would utilize the nutrients more slowly. It was speculated that the indeterminate pattern of plant development responsible for yield and its components was due to two major gene systems: genes that determined morphogenetic responses and genes that determined growth rate manifestations.

Studies on relationship between assimilatory surface, growth rates and net photosynthetic rate ($P_N$) of tomato hybrid FMHy1 and its parents provided evidence that the hybrid was characterized by the greatest total leaf area and biomass and that heterosis greatly affected the early development of the hybrid (Rao et al. 1992). Greater net assimilation rate of the hybrid during early growth suggested a greater $P_N$ per unit leaf area. It was concluded that early growth of the hybrid and higher growth rates were responsible for higher dry matter production and yield per plant.

In a study of glycolysis, the pentose phosphate pathway (PPP) for oxidation of carbohydrates and the electron transport chain (ETC) in the mitochondria of 5 tomato genotypes and their F1 hybrids differing in yield, Titok et al. (1998) found that glycolysis and PPP, but not ETC, were inhibited in green leaves of the hybrids while in the parental genotypes both these processes were not inhibited. It suggested higher energy potential in the hybrids which produced favorable metabolic conditions for growth and was supposed to be the main reason for improved yield.

Studies on F1 hybrids between line B 317 and a set of isogenic/near isogenic lines (IL/NIL) of tomato cv. Ailsa Craig differing in genes baby lea syndrome (bls), high pigment (hp), sunny (sy), venosa (ven), curly mottled (cm), entire (e), ripening inhibitor (rin), relaxata (rela), lutea (lut) and clausa (cla) showed that heterosis for productivity, early yield and mean fruit weight occurred only in three of the 10 hybrids (Atanassova et al. 2002). The pollen parents of two of these three hybrids (Ailsa Craig ven and Ailsa Craig rela) were characterized as possessing genetically controlled low vitality, i.e., the occurrence of heterosis was more or less limited to F1 hybrids of a given group of mutants. Such results might be consistent with those reported by Strunnikov (1983), who observed high level of heterosis in F1 hybrids of silkworm (Bombyx mori) where one of the populations exhibited genetically controlled low vitality. A hypothesis was drawn out that such populations might possess the so called “compensatory gene complex” (CGC) that might contribute not only to the survival and reproduction of these populations but also to heterosis for some traits in their F1 hybrids.

An attempt of heterosis dissection was made using near-isogenic line TA 523 (L. esculentum) containing a 40-cM introgression at the bottom of
chromosome 1 from *L. hirsutum* (Monforte and Tanksley 2000). A set of recombinant lines (sub NILs) derived from the original NIL TA 523 were developed in order to fine-map the genetic factors included within the original introgression. Analysis of the subNILs revealed that the gene action of the QTL for yield was dominant \((d/a=0.7)\) which eliminated the possibility that yield increase was due to true overdominance at a single gene locus. On the other hand, negative yield effects in other regions of the introgressed segment that would be predicted by the dominance complementation model, were not detected. Epistatic interactions among genetic factors along the introgressed segment were suggested as the cause of yield heterosis.

**STRATEGIES FOR DEVELOPING TOMATO HYBRIDS**

The plant characters chosen as selection criteria depend on the goals of the breeding program. When the trait is characterized by high heritability, a direct selection is possible. Selection for polygenic characters (such as early yield total yield, flavor), influenced also by environment, is complicated. Hence, the development and the selection of parents in hybrid breeding programs can be difficult. One way to facilitate breeding for complex characters is to make them more amenable to improvement by determining and analyzing their components. Most of these components are also of a quantitative nature and may influence each other. According to Bos and Sparnaaij (1993), this not only causes SCA effects, it also causes problems in the identification of markers (phenotypic or molecular) for complex characters. In most cases there is no question of a single marker for complex character: in one genotype a high value for the complex character may be due to high level for component \(x_1\), in a second genotype it may be due to a high level for \(x_2\). Thus, one should look for marker genotypes corresponding with favorable levels of the important component traits, rather than for marker genotypes corresponding with high levels of the complex character.

Components that contribute to the performance of a given complex character in tomato, such as total and early yield, were investigated since the early studies on heterosis in this species (Powers 1945, Burdick 1954, Williams 1959, etc.) and are still largely evaluated till date as well as exploited in breeding programs (Szwadiak and Kordus 1992, Vallejo Cabrera and Estrada 1993, Rai et al. 1997, Wang et al. 1998, Doganlar et al. 2000 etc.). As already mentioned, number of fruits/plant, mean value of fruit weight, plant height etc., were determinant main components for yield.
Based on a number of studies, Doganlar et al. (2000) divided the determinants of earliness into four different components, each one being a heritable trait: 1) days from sowing/transplanting to the first flowering (anthesis); 2) days from anthesis to the first fruit set; 3) days from the first fruit set to the first ripe fruit; 4) days from the first ripe fruit to the end of ripening.

Besides determining the components of a given complex trait, knowledge on the relationships between them is necessary. One way to get such information is to establish correlation between characters. Correlation might be evaluated between components of a complex character: between complex character and one of its components; between components of different complex characters; between a given character and environment; and between physiological character and yield, etc. Based on a significant number of references, Andruchtenko (1987) summarized data on correlation between: a) early and total yield, or yield components and biochemical characteristics of fruit quality; b) fruit weight/firmness and biochemical characteristics of fruit quality; c) environmental factors (e.g., soil and atmosphere) and biochemical characteristics of fruit quality; d) content of fruit compounds and the taste; e) market quality (fruit longevity, firmness, cracking) and characteristics of fruit pericarp, weight, form, etc. Evaluation of relationships between productivity/growth rate and morphological/physiological characteristics of plants grown under unfavorable conditions might be useful in breeding genotypes tolerant to stress (Nieuwhof et al. 1993, Nkansah and Ito 1994, Matsunaga and Monma 2000). Knowledge on the significance of correlation (positive or negative) between two characters might not only help in indirect selection of a trait that is difficult to be controlled all over the breeding cycle, but can also be useful in foreseeing (at least partially) results in some breeding programs. A number of studies, for example, provided evidence that the gain in earliness might cause a reduction in fruit weight (Boe 1988, Banerjee and Kalloo 1989, Lindhout et al. 1994).

A significant number of studies on correlation between different traits in tomato also suggest that the knowledge on the relationship between characters of breeding interest could be useful and exploited in improving parental lines and hybrids. It must be noted, however that the reliability of the genetic correlation established between different traits has been found to vary and is influenced by genotype – environment interactions (Aastveit and Aastveit 1993). This peculiarity needs to be taken into consideration while designing selection strategies.
The real value of the lines designated for developing hybrids, however, is not based on their own performance per se but the performance of their F₁ hybrids. Therefore, the last and the most important step in breeding hybrids is the evaluation of the lines for their combining ability. Diallel sets of F₁ crosses between collections of tomato lines have long been used, and still are used, for selecting the best parents for F₁ and for identifying the best crosses from which to extract improved inbred lines. The biometrical analysis of Griffing (1956) provides information on the combining ability of the parents and the magnitude of additive and non-additive gene action. There is a significant number of studies that refer to general and specific combining ability (GCA and SCA) for a large scale of traits of breeding interest in tomato, such as yield and yield components (Rai et al. 1997, Wang et al. 1998), total soluble solids, dry matter (Dod et al. 1995, Wang et al. 1998, Shrivastava 1998), resistance to cuticle-cracking (Emmons and Scott 1998), etc. GCA and SCA can interact with the environment that would result in changes in parental combining abilities over the environments (Singh 1973). For this reason evaluation of parents in more than one environment is recommended. According to Shattuck et al. (1993), if conducting the diallel in only one environment, plant geneticists should attempt to match the diallel with the environment of interest.

The strategies above mentioned, although widely used in the development of tomato hybrids and their parental lines, are far from being perfect and obligatory for leading to the results expected. Moreover, they are costly because results relate to many years of tests and evaluations not only of the parental lines developed but also of their hybrids. For this reason, enhancing the efficiency of the process of developing parental lines, and hybrids, is and will be of primary importance in the future.

The advent of molecular markers provided tools for mapping genes involved in quantitative trait loci (QTL) and the possibility for plant genetic improvement based on molecular marker assisted selection (MAS). Molecular markers might be used both for trait identification and trait introgression. Therefore, their utility extends throughout all phases of breeding programs. In theory, MAS was shown to produce greater selection gains than phenotypic selection for normally distributed quantitative traits. Even without direct effect of the marker on the quantitative trait locus, detecting linkage between such traits is of interest for increasing the response in selection. Based on a model for estimating the probability of selecting one or more superior genotypes, using MAS, Knapp (1993) found that a breeder for phenotypic selection without applying MAS had to test 1.0 - 16.7 times more progenies than a breeder using MAS. It was concluded
that MAS might substantially decrease the resources needed for a selection goal of a low to moderate heritability trait when both the selection goal and the selection intensity were high.

Tomato has been a model plant for QTL mapping. Several qualitative and quantitative trait loci in tomato have been mapped over the past decade, such as earliness and fruit ripening time (Lindhout et al. 1994, Monforte et al. 1999, Doganlar et al. 2000), total yield (Bernacchi et al. 1998), plant height and fresh mass (Paran et al. 1997), fruit size and shape (Grandillo et al. 1999, van der Knaap and Tanksley 2001), fruit firmness (Bernacchi et al. 1998), lycopene content (Chen et al. 1999), \(\beta\)-carotene accumulation in fruits (Zhang and Stommel 2000), organoleptic quality (Causse et al. 2001, Saliba Colombani et al. 2001), cold and salt tolerance during different developmental stages (Bretó et al. 1994, Foolad et al. 1998a, Foolad et al. 1998b), etc.

Molecular markers (such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and microsatellites (SSRs) are also expected to contribute to easier and more efficient identification of the lines whose crosses would result in promising hybrids. It was commonly assumed that hybrids produced from lines having different origin (i.e. developed from different cultivars) tended to have greater, consistent yield levels than hybrids of inbred lines originating from the same source population. The concept of heterotic groups gradually evolved from empirical evidence of crosses from inbred lines. Theoretically, the more distant the parents, the greater is the number of genes they differ, thereby the greater the potential interaction of the genes in the form of dominance and epistasis, and the greater will be potential for heterosis (Falconer 1989). In this sense genetic diversity might be an important issue in predicting \(F_1\) performance. The assignment of tomato lines to heterotic groups before field testing may allow the breeder to avoid crosses within groups that would result in lower costs.

Genetic diversity can be measured by several means including pedigree data, genotypic origin from contrasting geographic regions, etc. although molecular methodologies are considered as the most reliable ones. The latter might contribute to increasing the accuracy in determining the divergence between the genotypes of interest, that could ultimately lead to improved classification. Comparison of molecular markers with pedigree data of related genotypes, based on theoretical and experimental results is, however, recommended (Melchinger 1993). The idea of exploiting the genetic distance of the parents as an indicator in the pursuit of heterosis in
tomato hybrids is not a new one. Daskaloff (1942) reported high level of heterosis for yield and earliness in F₁ between tomato lines developed on the basis of hybridization between the tomato cultivar Sarya and L. racemigerum (Lange) (accession unknown), lately classified by Muller (Zhutchenko 1973) as L. pimpinellifolium Mill. It was also reported that lines developed on the basis of interspecific hybridizations were characterized by high combining ability of economically important traits. Based on these findings it was concluded that for acquiring heterosis for early and total yield, the hybrids should include lines of different origins (Daskaloff 1955, 1967, Yordanov 1983).

Recently, a number of studies aimed at cultivar identification, determining relatedness, and comparing the magnitude and structure of genetic variation among different tomato accessions have been carried out. RFLP analysis of phylogenetic relationship and genetic variation in the genus Lycopersicon show that the ratio of within vs. between accession diversity was much lower for self-compatible species. It indicated that most of the diversity within these species existed between populations, rather than within populations. Overall the amount of genetic variation in the self-incompatible species far exceeded that found in self-compatible species (Miller and Tanksley 1990). Villand et al. (1998) used RAPD in estimating relationships between accessions collected from Old and New World regions. Differences in RAPD marker frequencies indicated uniqueness of accessions from the Old and New World collections. Accessions from Ecuador, Peru and Chile had a larger magnitude of marker diversity than Old World ones. Comparison of subpopulations of L. esculentum and its subspecies L. esculentum var. cerasiforme indicated that the two were distinct but had similar levels of diversity. Noli et al. (1999) reported results from RAPD analyses of modern and vintage cultivated tomato accessions and eight accessions of wild Lycopersicon species (L. esculentum var. cerasiforme, L. pimpinellifolium and L. peruvianum). Cluster analysis allowed L. esculentum to be clearly distinguished from the wild species. Within L. esculentum two major groups were identified, the first including all the fresh market varieties and vintage processing varieties and the second including most of the modern processing varieties. RAPD analysis did not distinguish cultivars indicated as synonyms or selected from the same standard variety. Bredemeijer et al. (1998) reported that sixteen tomato cultivars were DNA-typed for 20 selected microsatellite markers (STMS) using the fluorescent approach. Length polymorphism among the PCR products was detected with 18 of these markers, yielding gene diversity valued from 0.06 to 0.74. As few as four STMSs were sufficient to differentiate between the 16
cultivars, indicating that these markers were especially suitable for a species like tomato which had low levels of variation as detected by other types of markers. In tomato, a high-density AFLP map has been constructed using an interspecific population (Haanstra et al. 1999). Studies using AFLPs to assess genetic diversity among tomato cultivars demonstrated that AFLP markers were effective for obtaining unique fingerprints of, and assessing genetic diversity among, tomato cultivars (Park et al. 2004).

It has to be noted, however, that studies on the relationship based on marker assisted genetic distance of the parents and heterosis in maize, wheat, soybean, chickpea, oilseed rape and other crops show non-obligatory linearity to heterosis. It might be due to insufficient genome coverage because of the low number of marker loci. It might also suggest that genetic distance at the molecular level as determined by RAPD, RFLPs etc. in some cases could have a limited utility as indicator or predictor of heterotic performance. Several studies on the applicability of molecular markers provide evidence, however, that their application in plant breeding holds promise for increasing the accuracy of prediction of genotypic values (Tsaftaris 1995, Melchinger 1999, Hallauer 1999, Alvarez et al. 2001, Archak et al. 2002, Bredemeijer et al. 2002, He et al. 2003 etc.). According to Melchinger (1999), groupings of germplasm based on molecular marker information can provide the basis for establishing new heterotic groups or broadening the genetic base of existing ones. This must be supplemented by evaluating the performance of crosses among these subgroups to assess their heterotic response, which is essential for identifying heterotic patterns. By using examples from different crops Melchinger (1999) demonstrated that genetic distances based on molecular markers can be used for: a) revealing genetic relationship among different germplasm; b) assessing germplasm to groups and subgroups of similar materials, and c) detecting pedigree relatedness between germplasm.

The recent advent of molecular linkage maps might also make it possible to detect and individually analyze the loci underlying heterosis. The use of molecular markers to identify QTLs responsible for heterosis may help in improving the genetic gain in some selection breeding schemes.

**IMPROVING THE PROCESS OF HYBRID SEED PRODUCTION**

Tomato is a self-pollinated inbred crop, its flower is bisexual and hand emasculation and pollination are used for producing hybrid seed. Biological bases of hybrid seed production including flower morphology
characteristics, anther emasculation, hybridization techniques etc. are reviewed and presented in detail by Yordanov (1983) and Georgiev (1991).

Significant quantities of hybrid seed are produced using a fertile seed parent. Tomato is considered a high value crop grown for either fresh market or processing. According to Duvick (1997b), seeding rates are low compared to the fruit value of this commercial crop. One noteworthy reason is that crossing is performed in countries where labor costs are very low. The quantum of research being carried out to improve the process of hybrid seed production suggests that the present technology is not perfect and does not give itself to easy adaptation to economic needs.

The benefit of incorporating male sterility into hybrid breeding programs was recognized not long after the appreciation of the advantages of heterosis and the detection of male sterile genotypes in tomato. For the first time male sterility was used in tomato hybrid seed production by Rick (1945) and till date this phenomenon is still discussed as the most promising way for facilitating the process of hybrid seed production (Sawhney 1994, 1997, 2004, Gorman and McCormick 1997, Atanassova 2000).

Genetic male sterility occurs widely in *Lycopersicon esculentum*, since cytoplasmic male sterility (CMS) does not occur naturally in the genus *Lycopersicon*. Georgiev (1991) defined the male sterility in tomato as autosterility and classified the sterile mutants into two groups: male sterile [including the male sterile (ms) and stamenless (sl) series] and functional sterile [including positional sterile (ps), positional sterile 2 (ps 2), and exserted stigma (ex)]. Based on anther development and the phenotype, the male sterile mutants in higher plants were classified into structural, sporogenous and functional types (Kaul 1988; Fig. 4.1, Fig. 4.2, Fig. 4.3, Fig. 4.4 and Fig. 5). The majority of the mutants belong to the male sterile (ms) or stamenless (sl) series, while the frequency of the mutations controlling functional sterility is lower (Kaul 1988, Sawhney 1994, Gorman and McCormick 1997, Atanassova et al. 2001).

Over the years it was established that several requirements such as complete male sterility controlled by recessive gene, normal female fertility with no defects in morphology, and stability of sterility expression etc., must be met by designated male sterile plants to be used in breeding programs (Gorman and McCormick 1997, Atanassova et al. 2001). Contrarily, it has been found that each type of male sterility in tomato exhibited not only significant advantages but also significant disadvantages when used in hybrid seed production, (Table 4.3). This table elaborates on several approaches for correcting the disadvantages of using male sterility
Expression of Heterosis by Hybridization

Fig. 4.1 Sporogenous (ms 10) sterility in tomato. Flowers with exserted and non-exserted stigma

Fig. 4.2 Structural (sl) sterility in tomato.

Fig. 4.3 Functional (ps) sterility in tomato.
in tomato hybrid seed production. Despite the numerous attempts of testing almost all these approaches the number of cultivars possessing male sterile seed parent remains rather limited. In our knowledge, (although our
## Table 4.3 Advantages and disadvantages in using different types of male sterility in tomato hybrid seed production

<table>
<thead>
<tr>
<th>Sterility</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Approaches for correcting or escaping disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2. Complete sterility.</td>
<td>2. Occurrence at some periods of high percentage of flowers with non exserted stigma that would require hand emasculation</td>
<td>2. Temporary restoration of fertility by chemical (Shmidt and Shmidt 1981, Ma et al. 1999), or environmental (Masuda et al. 2000) treatment.</td>
</tr>
<tr>
<td></td>
<td>3. Stable expression of sterility independent of the environmental conditions.</td>
<td></td>
<td>3. <em>In vitro</em> micropropagation of male sterile lines</td>
</tr>
<tr>
<td></td>
<td>4. Complete restoration of fertility in F&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Easy transfer of sterility genes to any genotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Structural</td>
<td>The same as in the sporogenous mutants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Functional</td>
<td>1. Easy maintenance by artificial selfing</td>
<td>1. Maintenance of the line as a population of sterile and fertile plants. Sterile plants could be assessed at anthesis</td>
<td>1. Restoration of male fertility to produce pure male sterile seed by hormonal or environmental treatments (Sawhney 1997).</td>
</tr>
<tr>
<td></td>
<td>2. Elimination of the process of emasculation (valid for <em>exserted stigma</em> genotypes)</td>
<td>2. Necessity of stamen emasculation (valid for <em>ps</em> and <em>ps 2</em> sterility in tomato)</td>
<td>1. Combining <em>ps</em> or <em>ps 2</em> with exserted stigma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Undesirable selfing</td>
<td>2. Combining <em>ps</em> or <em>ps 2</em> with short style (Georgiev and Atanassova 1981)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. Use of <em>ps 2 ful</em> recombinants for easier breeding of <em>ps 2</em> lines, (Atanassova 1991)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. Use of RAPD markers linked to <em>ps</em> gene for easier breeding of <em>ps</em> lines (Staniaszek et al. 2000)</td>
</tr>
</tbody>
</table>
information might be incomplete) only male sterile $^{105}$ anthocyanin absent ($ms^{105} aa$), positional sterile ($ps$) and positional sterile 2 ($ps\ 2$) seed parents have found application in release of a number of commercial hybrids during the last two decades (Atanassova, 1999, Staniaszek et al., 2000).

The male sterile ($ms$) and stamenless ($sl$) mutants seem to be the most applicable in breeding programs aimed at the facilitation of hybrid seed production mainly because of their complete male sterility and accessible stigma (Stevens and Rick 1986, Sawhney 1994, Gorman and McCormick 1997). Because of anther deformation, some $ms$ mutants such as $ms\ 10$, $ms\ 15$, $ms\ 32$ exhibit exserted stigma, and are therefore accessible for pollination without emasculation. By developing $ms^{105} aa$ genotypes, the main disadvantage in using $ms$-sterility in hybrid seed production (i.e., assessment of sterile plants at anthesis) was eliminated (Philouze 1974). The anthocyaninless sterile plants are easy to be distinguished since early developmental stages. Moreover, no effect of genes $ms^{105} aa$ on plant and fruit characteristics was established (Gardner 2000). This technology might be applied also if using $ms\ 15$ anthocyanin without ($aw$) genotypes as the two genes are closely linked (Clayberg 1965). According to Jorgensen (1987), tight linkages may be synthesized also through genetic transformation, which would allow introduction of an appropriate marker gene to a random location in each of a large number of plants carrying a suitable $ms$ gene.

Studies on $ms$ mutants and exserted stigma manifestation provided evidence, that the latter, usually a beneficial character in hybrid seed production, might sometimes be harmful. First of all, it is important to note that these mutants were characterized by lower hybrid seed yield (Atanassova 1999), which was suggested probably due to the fast drying of the stigma (Georgiev 1991). Secondly, observations on stigma exsertion variability within a number of $ms$ sterile lines developed at the Station des Plantes Maraîchères, INRA, Monfavet, Avignon, France, showed that the percentage of flowers with accessible stigma strongly varied depending on the environment and on the genotype (Table 4.4, Fig. 4.1). In some genotypes (and this during the period of hybrid seed production), the percentage of flowers without exserted stigma was so high, that for using them in commercial hybrid seed production, anther emasculation would become necessary. These results are consistent with those of Levin et al. (1994). Based on a study of the effects of the $ms\ 10$ gene, polygenes, and their interaction on pistil and anther-cone length, it was concluded that emasculation of the $ms\ 10$ male sterile parent appeared to be unavoidable for the efficient production of hybrid seeds. Removal of the shrunken $ms$ sterile anthers was found, however, to be more difficult and required a
Table 4.4  Manifestation of non-exserted stigma in male sterile tomato lines observed in the experimental fields of the Institute of Genetics, Sofia, during the period of hybrid seed production (May 25 - July 5, 2001)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Percentage of flowers with non-exserted stigma ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May, 25th</td>
</tr>
<tr>
<td>Monalbo  ms 10</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Monalbo  ms 32</td>
<td>10.0 ± 1.3</td>
</tr>
<tr>
<td>Porphyre ms 10</td>
<td>11.4 ± 3.0</td>
</tr>
<tr>
<td>Porphyre ms 32</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td>Monfavet 167 ms 10</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Monfavet 167 ms 32</td>
<td>6.6 ± 1.3</td>
</tr>
</tbody>
</table>

SE* - standard error between means of replicates

longer time than emasculation of fertile flower buds (Atanassova 1999). Recent technologies have made it possible to identify and isolate ms genes and to engineer transgenic male sterile plants (Gorman et al. 1996) as well to chemically induce male sterility (Sakaki and Yamazaki 1990, Cross and Schultz 1997), this particularity should be outlined and taken into consideration. Chen and Tanksley (2001) reported that the fine mapping of stigma exsertion QTLs se2.1 revealed that se2.1 was located in the chromosomal interval between RFLP markers T1301 and T 662 and that the change of style length might be the function of se2.1 genes. This finding might act as tool for more efficacious manifestation of stigma exsertion.

According to Bar and Frankel (1993), some ms mutants (ms 14, ms 17, ms 18, ms 31, ms 33, ms 47) were found to exercise pleiotropic effect on a number of economically important traits such as percentage of early marketable yield, average fruit weight, and total marketable yield. This suggests that detailed studies on ms sterile lines are necessary before including them in breeding programs.

Functional male sterile mutants offer the advantage of reproduction by artificial selfing in order to produce 100% sterile progeny. Despite this advantage they are considered as less promising for use in hybrid seed production because they display two significant disadvantages - occasional lapses in their expressivity resulting in undesirable selfing and except in exserted stigma-sterility, necessity of stamen emasculation. Observations on a large number of ps 2 sterile lines throughout their manipulation in breeding and hybrid seed production have shown, however, that it was possible to deal with the first disadvantage (lapses in ps 2 expressivity) by taking into consideration some specific characteristics related to their performance (Table 4.5; Atanassova 1999, Atanassova et al. 2001).
The need of stamen emasculation is the second disadvantage that usually prevents breeders from using \(ps\ 2\) sterility in breeding programs. Comparative study on the time necessary for the emasculation of floral buds in the fertile plants, and flowers at anthesis in the sterile lines, made it clear that this disadvantage, in terms of limiting factor, was exaggerated. Emasculation during anthesis (as practiced when using a \(ps\ 2\) line as seed parent) was easier and almost twice as rapid as emasculation applied on the fertile floral buds (Atanassova 1999). Anther emasculation could be made even easier if \(ps\ 2\) was combined with short style (Georgiev and Atanassova 1981; Fig. 4.6). Such flowers can be emasculated without using forceps: the anther cone and the petals can be easily separated manually from the flower by plucking out with two fingers the tip of the cone (or the petals) (Fig.4.7). Certainly, the idea of developing genotypes combining \(ps\ 2\) sterility with \textit{exserted stigma} is a tempting one as it would result in efficacious technology: easy maintenance of the seed parent by artificial selfing and no need of anther emasculation. Such a technology, however, could be acceptable only by finding a way to deal with the strong variability in the rate of stigma exsertion. While testing \textit{ex}-lines for their usefulness in

### Table 4.5 Ways of dealing with the lapses in \(ps\ 2\) gene expressivity and its consequences in breeding and hybrid seed production

<table>
<thead>
<tr>
<th>Specific characteristics in the performance of (ps\ 2) lines to be taken into consideration when using (ps\ 2) sterility in breeding and hybrid seed production.</th>
<th>Approaches for dealing with specific characteristics in the performance of (ps\ 2) lines.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The (ps\ 2) gene expressivity varies with the genotypes.</td>
<td>This enables the breeding of (ps\ 2) lines that exhibit very low percentage of selfing.</td>
</tr>
<tr>
<td>2. The percentage of selfing in the (ps\ 2) lines varies within and between the years of growing, being forever lowest during the period of hybrid seed production.</td>
<td>Strict control of this characteristic is necessary throughout the entire breeding process. The percentage of selfing recorded at the end of the growing season, instead at the end of the period of producing hybrid seed, might give a wrong idea on the usefulness of the (ps\ 2) line.</td>
</tr>
<tr>
<td>3. If occurring, anther dehiscence in the (ps\ 2) lines occurs usually after the second day of flowers opening.</td>
<td>Regular emasculation and pollination of the plants at least each two days is necessary. If missing this term, all flowers at anthesis have to be eliminated.</td>
</tr>
<tr>
<td>4. The percentage of selfed seeds when using a (ps\ 2) line as seed parent in producing hybrid seed is significantly lower than the percentage of selfing, observed on the same line.</td>
<td>The usefulness of a (ps\ 2) line has to be evaluated on the basis of both, percentage of selfing and hybridity of the seeds obtained by artificial pollination.</td>
</tr>
</tbody>
</table>
hybrid seed production it was established that it was really difficult to determine and fix the right rate of stigma exsertion. On the one hand, lines possessing 1.0-1.5 mm stigma exsertion were found occasionally to be like normal ones requiring stamen emasculation. On the other hand, F1 hybrids of the lines possessing steadily manifested exserted stigma (2.0 mm or more above the anther cone), performed sometimes as longuistylic, that resulted in lower percentage of fruit setting (Fig. 4.5).
Easier and more rapid anther emasculation is not the only criterion for an efficient hybrid seed production. Hybrid seed yield is also of great importance. Comparative studies on hybrid seed yield obtained from ps 2 sterile lines, depending on the developmental stage of stamen emasculation and pollination, showed that a significantly higher (1.5 to 3 times) hybrid seed yield resulted from pollination at anthesis. This is the stage in which the ps 2-lines are usually manipulated for producing hybrid seed (Atanassova and Georgiev 2002). Therefore, in terms of expenditure and time, use of ps 2 male sterile seed parents proved to be profitable and economically justifiable (Atanassova 1999, Atanassova and Georgiev 2002). These data were also confirmed by the production figures. In Bulgaria, until 1990, about 1 ton of hybrid seed per year (30% of the total quantity of hybrid seed produced) was produced using ms 10 aa and ps 2 seed parents (Georgiev 1991), while presently about 80% of the hybrids released and spread are practically from ps 2 seed parent. Hybrids using ps 2 sterile seed parent were released also in Czech Republic and Moldova (Atanassova 1999, 2000). It suggests that the functional, and more precisely ps 2 male sterility, is not to be underestimated. It should be taken into consideration for elaborating systems or breeding programs aimed at the reduction of the time and costs associated with hybrid seed production.

According to Potaczek and Kubicki (1986) and Staniaszek et al. (2000) the use of positional sterility (ps) in tomato hybrid seed production under Polish climatic conditions was advantageous. The process was found to be cheaper due to labour-saving procedures up to 30% as compared to the traditional method. Two RAPD markers linked to the ps gene were identified. The markers were used for purity determination of maternal lines carrying gene ps gene and F1 hybrids possessing ps sterile seed parent (Staniaszek et al., 2000).

The experience accumulated while applying different types and genes of male sterility in the practice (at least three - ms1035 aa, ps and ps 2) has shown that they should not be categorized as “more or less promising”. Sometimes a given type of sterility might initially look promising but during its manipulation in breeding and hybrid seed production unexpected difficulties may crop up (e.g. the occurrence at times of a high percentage of flowers with non-exserted stigma in some ms tomato lines). Conversely, some mutants or sources of sterility might display undesirable traits, but while using some approaches they could be corrected or eliminated, (e.g. the ps 2 sterility in tomato).

Thus, the application of male sterility in breeding and hybrid seed production in tomato is not merely a theory, but is being practised in a
number of countries. Nevertheless, it is difficult to determine how widely cultivars possessing male sterile seed parent are spread, or what is their percentage based on the total number of hybrid varieties, as it is difficult to track data on the release of varieties possessing a male sterile seed parent. The availability of such hybrids is, however, a fact not to be neglected. It confirms that induction of male sterility is one of the right ways for facilitating hybrid seed production in tomato and a start has been made in this direction.

Economizing hybrid seed production includes not only facilitating its process *per se*, but also increasing its efficiency by improving the quality of the final product, i.e. of the hybrid seed. Besides the high germination ability which is an obligatory characteristic for each kind of commercial seed, the high percentage of hybridity is of primary importance in hybrid seeds. For this reason, breeders are tempted to introduce male sterile seed parents to facilitate the production of hybrid seed. Using male sterile seed parent would be a warranty for production of 100% hybrid seed and would eliminate the necessity of testing the seed for hybridity. In view of the fact that male sterile seed parents could not be widely used in developing tomato hybrids, different morphological markers such as potato leaf (*c*), anthocyaninless of Hoffmann (*ah*), anthocyanin without (*aw*) etc. were introduced in fertile or *ps* 2 seed parents of a number of commercial hybrids (Farkas 1993, Xue 1994, Atanassova et al. 2001). It permitted testing the hybridity of the seed at germination or seedling stage. Recently, some molecular techniques made it possible to rapidly evaluate the purity of the hybrid seed produced (Rom et al. 1995, Paran et al. 1995, Chuang et al. 1999) and probably in the near future these techniques will be widely used for this purpose.

**TOMATO HYBRID ADOPTION IN PRACTICE—HISTORY AND PRESENT SITUATION**

As already mentioned, studies on heterosis in tomato were initiated almost simultaneously with such studies on maize. The introduction of tomato hybrids, however, came into practice 30-35 years later than maize hybrids. The first commercial tomato hybrids were developed much earlier in some countries. In USA, for example, the first hybrid "Burpee Hybrid" was developed by Dr. Oved Shifriss in the early 1940’s, probably 1942, and marketed by the W. Atlee Burpee Co. (Prof. Rick C.M., personal communication). The first tomato hybrid in Bulgaria (Saria x Komet) was developed in 1932 by Prof. Daskaloff, (Daskaloff, 1937) at the Agricultural
Experimental Station (now Institute of Vegetable Crops “Maritza”), Plovdiv, where for the first time large quantities of hybrid seed were produced. Since 1949 and up to the late 1960’s, for example, the number one cultivar for early field production was the hybrid No. 10 x Bizon, developed also by Daskaloff. In Japan one of the first hybrids “Fukuju” was developed by Prof. Fujii (Fujii 1948, 1952) and released in 1938 by Osaka Agricultural Station, (now Osaka Prefectural Agricultural and Forestry Research Station). The first Dutch tomato hybrid Single Cross (Vetomold x Ailsa Craig), combining traits of resistance to *Cladosporium fulvum*, was developed by Bruinsma in 1939 and introduced in 1946. The hybrid exceeded the other varieties by taste and earliness (Anonymous 1992). In France, the first hybrid Fournaise F₁ was developed by Vilmorin and released in 1956 almost simultaneously with the hybrids Monfavet n° 63-5, Monfavet n° 63-4, and Monfavet n°63-18 developed by INRA, (Philouze 1986, 1997). In the early 60’s these hybrids enjoyed a significant success and were rapidly adopted by the producers. In Israel, the first tomato hybrid “Urit” was developed at Volcani Center and released in 1971 (Pilowski et al. 1971). In China, the first tomato hybrid “Beijing Zhaohoug x Aonong No 2” was developed in 1969 at the Zhejiang Agricultural University (Wang et al. 1988).

Tomato hybrids began to take over the market towards the end of sixties or the beginning of seventies. Their use increased dramatically throughout the following decades. In 1997, Duvick (1997 b) reported that 100% of fresh market and 80% of processed tomatoes in USA were F₁ hybrids. At present, there is a similar situation in several countries in Europe, Asia and Australia.

**FUTURE PROSPECTS AND CHALLENGES IN EXPLOITATION OF HETEROSIS IN TOMATO**

The goal of the breeders is to develop hybrids superior in one or a number of traits to the standard hybrid or hybrids previously released and used in practice, and most of them do not develop heterotic hybrids *per se*. In a way each hybrid might be considered as a challenge for developing new, superior hybrids. Meanwhile, consumers and growers demand as well as handling requirements have dramatically changed during the past two decades. Increasing yield, for example, is not any more the major focus of commercial tomato breeding programs. It has shifted to breeding for a complex of traits, such as improved quality, flavor, more efficacious plants coping with abiotic and biotic stresses for at least a substantial part of their life, etc. The longevity of fruit, once considered as a trait of primary importance, is not
now appreciated if not combined with good flavor and/or texture. Hence, the new hybrids developed have to be superior to the previously released ones in terms of traits that the latter already possess plus permanently cumulate new valuable agronomic traits in order to satisfy the demand of consumers for high nutritive quality and food safety, and of growers - for economic profitability and handling requirements. To achieve these objectives plant breeding research should focus on improving fruit flavor, texture, composition and studying the potential of plants to synthesize desirable components by developing new methods that lead to accelerated screening, adjusting the precision of selection stages, minimizing costs etc. The achievement of such ambitious, complex and perhaps even challenging future breeding goals would be difficult (if not impossible) without the extensive use of the phenomenon of heterosis. As already mentioned, heterosis in tomato may be expressed at any developmental stage and observed for a large scale of quantitative traits related to plant productivity, adaptability, physiology and fruit nutritive and market quality. It is commonly known that the phenomenon of heterosis was widely used in developing early and high yield commercial hybrids. It is rather doubtful (as it would be difficult to trace down such information) that heterosis was widely pursued in breeding programs for traits, other than earliness, total yield and yield components.

Hence, given that tomatoes are one of the most important crops in the world because of their volume of consumption, and overall contribution to nutrition, and bearing in mind that the new developed varieties have to satisfy complex consumer and grower demands as well as handling requirements, the manifestation of heterosis for traits related to plant tolerance to biotic and abiotic stresses, photosynthetic efficiency, nutritive and market quality, efficient root system, etc., may be considered as a reserve to be exploited in future breeding programs.

The recent advances in the molecular genetics (tagging and isolation of genes, QTL controlling a given trait, expression of desirable alien genes in transgenic plants, improving the efficiency of breeding via marker assisted selection, etc.) is expected to contribute to the more efficient and extensive exploitation of heterosis in developing tomato hybrids. Combining conventional and molecular breeding techniques might offer help in improving screening efficiency for many traits of agronomic value, estimating genetic diversity, reducing the time for new line development, assessing heterotic groups, and detecting as well as individually analyzing the loci underlying heterosis. The rapidly increasing number of investigations aiming at identification and isolation of male sterile genes
in tomato, engineering transgenic male sterile plants, synthesizing tight linkages between an appropriate marker gene and male sterile gene through genetic transformation might also generate useful approaches contributing to the creation of better systems of hybrid seed production. The fact that the application of male sterility in breeding and hybrid seed production in tomato is no longer a theoretical one, gives ground to believe that such systems would be easily accepted and applied in practice.

The prospects and expectations outlined above need to be taken cautiously. According to Young (2000), although DNA markers hold great promise, realizing this promise remains elusive, as most markers associations are not significantly successful in MAS. At the same time, the effectiveness of MAS in breeding programs would depend also on the genetic determinism of the traits of interest (Hospital et al. 1997) and on its cost (Young 2000). Kearsey and Farquhar (1998) consider that unreliability of QTL location may suggest its false candidacy. Therefore, the new knowledge gained creates new problems to solve, such as developing reliable molecular markers, refining the techniques, decreasing the cost associated with molecular markers assays etc. The capabilities of recently developed molecular techniques suggest their wide application in breeding programs and in the genetic improvement of cultivated plants, including exploitation of heterosis.

In 1908, Shull noted that the efficiency of maize breeding programs would considerably improve by finding a suitable method of predicting hybrid performance before field evaluation. The numerous studies on heterosis and the large experience of exploiting this phenomenon over an entire century, complemented by the rapid advent of molecular techniques during the last decade, make it possible to believe that this endeavor might be on the way to come true.

SUMMARY

Studies on heterosis in tomato were initiated at the beginning of the twentieth century. Although its biological basis remains unknown, this phenomenon is now recognized as one of the primary factors contributing to the success of plant breeding in tomato and many other crops.

This subject is surveyed under the headings: 1. Introduction; 2. Manifestation of heterosis in tomato–Heterosis in tomato is observed for a large scale of economic quantitative traits such as total and early yield and yield components, tolerance to stress, dry matter, vitamin C, lycopene content etc. More than 50-60% of the studies on heterotic performance in tomato
refer, however, to heterosis for yield and yield components; 3. Genetic basis of heterosis in tomato—Manipulating heterosis in breeding programs requires knowledge on its quantitative genetic basis. Data concerning gene action for tolerance to abiotic stresses and fruit nutritional value, sensory and market quality is summarized, and recent studies of heterosis in tomato are reviewed; 4. Strategies for developing tomato hybrids—Making complex characters more amenable to improvement by determining their components as well as relationship between them is analyzed as one way to facilitate breeding for complex characters. Possibilities for tomato genetic improvement based on mapping genes involved in quantitative trait loci (QTL) and more efficient identification of the lines whose crosses would result in promising hybrids, based on molecular markers, are reviewed and discussed; 5. Improving the process of hybrid seed production—Advantages and disadvantages in incorporating different types of genic male sterility into hybrid breeding programs are discussed; 6. Tomato hybrid adoption in practice—History, present situation and information concerning the development of the first tomato hybrids in USA, France, Holland, Israel, Japan, Bulgaria, China is presented; and 7. Future prospects and challenges in exploitation of heterosis in tomato discussed. Achievement of the ambitious and complex breeding goals in tomato improvement will be difficult without the extensive use of heterosis. Combining conventional and molecular breeding techniques might offer help in improving screening efficiency for many traits of agronomic value, estimating genetic diversity, reducing the time for line development, assessing heterotic groups, detecting and individually analyzing the loci underlying heterosis.

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CLASSIFICATION AND TAXONOMY

The genus and species designation of tomato has been the subject of much debate, consequently, many synonyms exist. The designation most frequently used today for the cultigen is Lycopersicon esculentum Mill., with Solanum lycopersicium L. preferred by some authors. Arguments supporting the transfer of Lycopersicon species into Solanum L. have been made while recognizing the convenience of maintaining the generic designation Lycopersicon for the sake of nomenclatural stability (Peralta and Spooner 2001; see also chapter 1).

The common names used for tomato are numerous. Tomati is the word used by the Indians of Mexico, who have grown the plant for food since prehistoric times. Other names reported by early European explorers were tomatl, tumatle, and tomatas, probably variants of Indian words. Most common names have a root of tomat (used in Danish and Swedish). Tomate is used in French, German, and Portuguese. Tomato is used in English and Spanish. Tomast is used in Dutch and Pomodoro is used in
Traditional classification (reviewed by Taylor 1986) places tomato within the family Solanaceae (nightshade), sub-family Solanoideae (chromosome number \(x = 12\)), and tribe Solaneae. Lycopersicon is one of the smallest genera within this tribe, containing cultivated tomato \((L. esculentum)\) and its eight wild relatives \((L. pimpinellifolium, L. parviflorum, L. hirsutum, L. chilense, L. pennellii, L. chmielewskii, L. peruvianum)\). Under this classification system Solanum is closely related to but distinct from the genus Lycopersicon, the two genera being separated on the basis of anther morphology. A comprehensive list of synonyms for Lycopersicon species is published on the Germplasm Resources Information Network (GRIN, http://www.ars-grin.gov/). GRIN recognizes \(L. glandulosum\) C.H. Mull. as a distinct species, whereas many authors treat this taxa as \(L. peruvianum\) var. glandulosum.

The family Solanaceae contains many plants of economic importance including tomato, potato, eggplant, petunia, tobacco, pepper (Capsicum),...
and Physalis. This has promoted detailed studies of many
groups but the
vast number of species (upwards of 1,000) and their
extensive morphological
complexity have lent an intractability to comprehensive
taxonomic
treatments. Consequently, taxonomic studies have focused on
subgenera
or regional floras (Bohs and Olmstead 1997). Solanum
contains seven
subgenera and 60 to 70 sections (D'Arcy 1991).
During the past decade, molecular genetic techniques have
greatly
impacted plant taxonomic studies at all levels, including
the elucidation of
the closest relatives of many crop species (reviewed by
Soltis and Soltis
2000). Results of such studies can inform plant breeders,
and those interested
in comparative genomics, as to which species are potential
sources of new
alleles or model genetic systems. Recent molecular evidence
has placed
potato (S. tuberosum) and tomato as sister taxa deeply
nested within Solanum.
Under this scheme cultivated tomato is designated as S.
lycopersicum within
section Lycopersicum, subgenus Potatoe. Molecular evidence
supporting this
classification is briefly reviewed here.
Restriction site analysis of chloroplast DNA (cpDNA) of
Solanum
subgenus Potatoe (including potatoes and pepinos),
Cyphomandra (tree
tomatoes), and Lycopersicon (tomatoes), using Capsicum and
Datura as
outgroups supported two main clades among the studied taxa:
1) Solanum
subgenus Potatoe and Lycopersicon; and 2) other Solanum and
Cyphomandra
(Spooner et al. 1993; see also Chapter 1). The authors
argued that cpDNA
and morphological data supported the transfer of
Lycopersicon into Solanum
subgenus Potatoe, section Lycopersicum, and recognized
cultivated tomato
as Solanum lycopersicum L. The approximately 2 kb
chloroplast gene ndhF
has been found to be useful for phylogenetic studies at
inter- and
therein). This gene was sequenced in a broad sample of 18
Solanum species,
species representing five genera from subfamily
Solanoideae, and one
outgroup (Nicotiana tabacum L. from subfamily Cestroideae).
A strict
consensus tree of the 12 most parsimonious trees from
unweighted
parsimony analysis showed 100% bootstrap support (500
bootstrap
replicates) for potato and tomato as sister taxa (Bohs and
Olmstead 1997).
Presence versus absence of restriction sites for ten
restriction enzymes were
surveyed for the entire chloroplast genome for 36 broadly
sampled Solanum
species and 13 outgroup species (Olmstead and Palmer 1997). Main findings of cladistic analysis of 567 variable restriction sites included: 1) monophyly of Solanum, including Lycopersicon and Cyphomandra, supported by 25 restriction site synapomorphies and 100% bootstrap value (100 bootstrap replicates), and 2) three primary clades within Solanum, designated I, II, and III. Clade II consisted of S. muricatum, S. tuberosum, and S. lycopersicum, all within the subgenus Potatoe. This clade was supported by 19 synapomorphies and 100% bootstrap value.

The cpDNA studies of Bohs and Olmstead (1997) and Olmstead and Palmer (1997) discerned three or four primary DNA lineages within broadly sampled Solanum. The latter authors argue that these data are expected to accurately reflect evolutionary relationships within Solanum rather than processes such as lineage sorting or hybridization and introgression. Lineage sorting refers to the differential fixation of shared polymorphisms among descendant species in such a way that obscures phylogenetic relationships. This would require intraspecific polymorphism of cpDNA in a progenitor species, with different haplotypes subsequently becoming
fixed in different descendant species. This is purported to be unlikely in these studies because cpDNA evolves slowly, and estimates of cpDNA divergence among closely related species of Solanum are often low (Spooner et al. 1993). Hybridization and introgression are more likely to be problematic in obscuring evolutionary relationships among closely related species than among the distantly related species that were sampled for these studies.

Although tomato and potato are near-relatives and have the same basic chromosome number (x=12), multiple rearrangements prevent them from cross-hybridizing (Bonierbale et al. 1988, Tanksley et al. 1992). Recently, tomato, potato, and capsicum have been extensively studied via molecular mapping in order to understand the evolution of genome structure in the Phylogenetic Relationships among Lycopersicon Species Early taxonomic studies subdivided the genus into two groups, Eulycopersicon, which are color-fruited and Eriolycopersicon, which are green-fruited species (Muller 1940). Rick (1976) designated species into two groups, the esculentum and the peruvianum complexes, based on their
reproductive compatibility with cultivated tomato. Key distinguishing taxonomic characters in the following species descriptions were taken from “A revised key for the Lycopersicon species” (Rick et al. 1990).

Esculentum Complex

i) Lycopersicon esculentum (Mill.). Cultivated tomato (L. esculentum var. esculentum) is distributed world-wide. Its precise origin in Mexico and/or Peru is uncertain (see below). Fruit interior is red when ripe with seeds 1.5 mm or longer. Leaf margins are typically serrate. Fruit diameter (3 cm or larger) and number of locules (2-to-many) distinguish it from the cherry tomato (Lycopersicon esculentum var. cerasiforme (Dunal) A. Gray), another cultivated form of tomato that is derived from L. esculentum var. esculentum crosses with L. pimpinellifolium with a fruit diameter 1.5 to 2.5 cm with 2 locules. Wild and weedy forms of Lycopersicon esculentum var. cerasiforme are the only wild species found outside of South America (Taylor 1986). It has traditionally been regarded as the most likely direct wild ancestor of the cultigen but molecular genetic evidence has challenged this view (Nesbitt and Tanksley 2002, see below).

ii) Lycopersicon pimpinellifolium can reciprocally hybridize with the cultigen and displays natural introgression. It may be a direct ancestor of L. esculentum or the two species may have evolved in parallel from a green-fruited ancestor (Rick 1976). Fruit interior is red when ripe with seeds 1.5 mm or longer. Relatively smaller fruit diameter (less than 1.5 cm) and generally undulate or entire leaf margins distinguish this species from L. esculentum. In the wild its typical habitat is at relatively low elevations (less than 1000 m) in Peru although there are known exceptions at altitudes of 1200 to 1400 m (Taylor 1986).

iii) Lycopersicon cheesmanii can reciprocally hybridize with the cultigen but does not do so in the wild because it is geographically restricted to the Galapagos Islands. Fruit interior is yellow or orange when ripe with seeds 1.0 mm or shorter. Subspecies L. cheesmanii f. minor (Hook. f.) C.H. Mull. is found in relatively lower altitude xeric habitats (Taylor 1986) and is characterized by highly ornate and elaborately subdivided leaflets (Muller 1940).
iv) *Lycopersicon parviflorum* is easily reciprocally hybridized with the cultigen although there may be exceptions for some accessions (Taylor 1986). Fruit inside is green or whitish when ripe with seeds 1.0 mm or shorter. Sympodia have two leaves, inflorescences with small or no bracts, and flowers small (corolla diameter 1.5 cm or less). Its center of diversity is inter-Andean Peru, where it prefers moist habitats along rocky banks of small streams (Taylor 1986). *L. parviflorum* and its sister taxa *Lycopersicon chmielewskii* comprise the minutumcomplex. The highly autogamous inbreeding *L. parviflorum* is thought to have been derived from the primarily outcrossing *L. chmielewskii*.

v) *Lycopersicon chmielewskii* easily hybridizes with the cultigen (Taylor 1986). Fruit interior is green or whitish when ripe. Taxonomic traits distinguishing this species from *L. parviflorum* are larger seeds (1.5 mm or longer) and larger flowers (corolla diameter 2.0 cm or more). It is sympatric with *L. parviflorum* but more limited in its distribution and prefers slightly better-drained habitats (Taylor 1986).

vi) *Lycopersicon hirsutum* can act successfully as the pollen parent when crossed with cultivated tomato but the reciprocal cross does not set fruit. *Lycopersicon hirsutum* f. *glabratum* C.H. Mull. and the cultigen are reciprocally compatible (Taylor 1986). The two forms of *L. hirsutum* are not fully compatible with each other. Fruit interior is green or whitish when ripe and sympodia have three leaves. The *glabratum* biotypes are characterized by less hairy leaves, stems, and fruit, a smaller corolla, less showy flowers, and a tendency to inbreed. *L. hirsutum* typically grows in moist river valleys at the relatively highest elevations for *Lycopersicon* species (500 to 3300 m), with *glabratum* occupying the northern extremes of the distribution. Both are distributed in Ecuador and Peru (Taylor 1986).

vii) *Lycopersicon pennellii* easily hybridizes with cultivated tomato. Fruit interior is green or whitish when ripe and sympodia have two leaves. Inflorescences have large bracts and anthers are free and poricidal. Distribution is relatively restricted along coastal Peru with some populations found in extreme xeric habitats experiencing temperatures of 25º to 30º C (Taylor 1986).

Peruvianum Complex
i) Lycopersicon chilense can act as the pollen parent when crossed with the cultigen but viable seeds are rare and embryos must be cultured. In the reciprocal cross, L. chilense will not accept L. esculentum pollen. Fruit interior is green or whitish when ripe and sympodia have two leaves. Inflorescences have large bracts, anthers are attached in a tube and are dehiscent by lateral apertures. Flowers are congested and anther tubes are straight. Peduncles are longer than 15 cm and plants are erect. L. chilense is the most southerly of Lycopersicon species distributed in Chile and southern Peru and prefers extremely arid habitats (Taylor 1986).

ii) Lycopersicon peruvianum is genetically and morphologically the most diverse of the Lycopersicon species and several varieties have been described (dentatum, humifusum, peruvianum). In general, the species exhibits severe barriers to crossing with L. esculentum. Fruit interior is green or whitish when ripe and sympodia have two leaves. Inflorescences have large bracts, anthers are attached in a tube and are dehiscent by lateral apertures. Flowers are loosely arrayed with anther tubes generally bending distally. Peduncles are shorter than 15 cm and plants are spreading. Distribution ranges from northern Peru to northern Chile, encompassing a broad range of habitats and including many mountain races that are geographically isolated from each other (Taylor 1986).

iii) Lycopersicon glandulosum is synonymous with Lycopersicon peruvianum var. glandulosum. This is a mountain race common to central Peru. Although it is reproductively compatible with coastal populations of L. peruvianum it is distinguished from them by its very thin stems, short dense glandular hairs, and narrow leaflets. It grows at elevations as high as 3000 m where temperatures reach as low as 4º to 8º C (Taylor 1986).

Within the genus Lycopersicon closely related interspecies and intraspecies heterogeneity have made the resolution of precise interspecific relationships difficult. Molecular genetic evidence examining relationships has been accumulating during the previous two decades. Speciation within the genus based on these data has been relatively recent.
Estimated divergence times for the genus on the basis of pooled silent sites and a rate of $6.03 \times 10^{-9}$ silent substitutions per site year showed that the genus began its initial radiation ~ 7 million years ago. *L. esculentum* and its nearest relatives *L. cheesmanii* and *L. pimpinellifolium* shared a recent common ancestor ~1 million years BP (before present) (Nesbitt and Tanksley 2002).

Molecular markers support three to four major groupings within the genus, reflecting mating system and fruit color, with autogamy and red orange fruit being true synapomorphs. These results are generally consistent with morphological and crossability data (Rick 1979). Relationships among species within the major groups are not well-resolved. The traditional distinction between the esculentum and peruvianum complexes is somewhat obscured by molecular phylogenetic evidence. This is because of relatively large genetic distances between *L. hirsutum* and *L. pennellii* and the remaining *Lycopersicon* species, although both species can hybridize with the highly-derived *L. esculentum*, and so are considered to be part of the esculentum complex. The main findings from molecular genetic studies
are reviewed here.

The pioneering studies examined organellar genomes. Restriction fragment length polymorphism (RFLP) of chloroplast (cpDNA) and hybridization of mitochondrial (mtDNA) DNA studies supported the transfer of *L. pennellii* from the genus *Solanum* into the genus *Lycopersicon* (Palmer and Zamir 1982, McClean and Hanson 1986). At the subgeneric level parsimony analysis supported red fruit color to be monophyletic in *L. esculentum*, *L. pimpinellifolium*, and *L. cheesmanii* (Palmer and Zamir 1982). cpDNA results also gave evidence for a close relationship of *L. chilense* and *L. chmielewskii* to *L. peruvianum*. This apparent anomaly may be explained by the observation that *L. peruvianum* is a heterogeneous taxon with northern populations being somewhat closely related to the *esculentum* complex.

Eight *Lycopersicon* species were surveyed for RFLPs using 40 single copy nuclear probes (Miller and Tanksley 1990). The sample of 156 plants represented two to five plants per accession and with the exception of *L. parviflorum*, multiple accessions per species. Unweighted pair group method using arithmetic averages (UPGMA) analysis using genetic distances based
on proportion of shared bands gave two major groupings of species. The
first group distinguished the self-incompatible (SI) *L. hirsutum*, *L. pennellii*,
and *L. peruvianum* from the self-compatible (SC) *L. esculentum*, *L.
pimpinellifolium*, *L. cheesmanii*, *L. parviflorum*, and *L.
chmielewskii* (Table 2.1).
The second grouping delineated green versus red-fruited species. An
exception was a northern Peruvian representative of *L. peruvianum* var.
humifusum (LA2150) which grouped with the SC species albeit
distantly. The authors postulated that this accession may be a modern
representative of the SI gene pool that gave rise to the SC *L. parviflorum* and
*L. chmielewskii*.

Three main groupings resulted from cluster analysis of allozyme data
from eight *Lycopersicon* species: i) SC and red-fruited *L. esculentum*, *L.
pimpinellifolium*, *L. cheesmanii*, and *L. peruvianum*, ii) the SC and green-fruiting
*L. chmielewskii* and *L. parviflorum*, and iii) the SI and green-fruiting *L. pennellii*
and *L. chilense* (Breto et al. 1993).
Random amplified polymorphic DNA (RAPD) markers were used to
estimate genetic distances among 22 accessions of *L. peruvianum*; 12 acces-
sions of *L. chilense*; and two accessions from each of *L. esculentum* var.
esculentum, L. esculentum var. cerasiforme, L. pimpinellifolium, L. cheesmanii, L. chmielewskii, L. parviflorum, L. hirsutum, and L. pennellii (Egashira et al. 2000). Neighbor-joining (NJ) analysis of genetic distances based on 435 RAPD fragments distinguished four groups consisting of three clusters and two outlier taxa; i) L. esculentum, L. pimpinellifolium, L. cheesmanii, and L. cheesmanii var. minor, ii) L. chmielewskii and L. parviflorum, iii) the peruvianum complex L. peruvianum and L. chilense, and iv) L. hirsutum and L. pennellii which were distinct from all other taxa, including each other.

Bootstrap support using 1000 bootstrap replicates was generally low (less than 40%) for most of these groups.

Sixteen polymorphic simple sequence repeat (SSR) markers were used to genotype five to ten plants per accession from one to three accessions each of L. esculentum var esculentum, L. esculentum var cerasiforme, L. pimpinellifolium, L. cheesmanii, L. parviflorum, L. chmielewskii, L. chilense, L. hirsutum, L. pennellii; and from 11 accessions of L. peruvianum (Alvarez et al. 2001). A NJ tree constructed using eight low-diversity loci (gene diversity less than 0.245) was found to be more reliable than trees constructed using
all loci. The authors inferred that relatively more diverse SSR loci were more mutable and exhibited higher degrees of homoplasy among species.

SSR results supported genetic relationships based on RFLP (Miller and Tanksley 1990) and morphological (Rick 1979) data. With the low-diversity SSR data the northern accessions of L. peruvianum (LA2334, LA2172, LA1708, LA2157) clustered closer to L. esculentum, L. pimpinellifolium, L. cheesmanii, L. parviflorum, and L. chmielewskii than to the southern L. peruvianum accessions (LA372, LA462, LA1333, LA1373, LA1274, LA1945, LA1955) and L. chilense.

DNA sequence variation of the 5’ portion of granule-bound starch synthase gene (GBSS1 waxy) was examined for three accessions from each of L. esculentum var. cerasiforme, L. cheesmanii, L. chmielewskii, L. parviflorum, L. hirsutum, L. pennellii, L. chilense; five accessions of L. pimpinellifolium; 39 accessions of L. peruvianum one cultigen; plus one to two accessions from each of nine closely related outgroups (Peralta and Spooner 2001). The approximately 1300 nucleotide (nt) region included eight exons and seven introns. A strict consensus tree of 15000 most parsimonious trees did not
give good resolution of closely related species. It did support allogamy,

self-incompatibility and green fruit as primitive and further supported the

splitting of L. peruvianum into two groups with the northern populations

close to the self-compatible taxa L. chmielewskii, L. parviflorum, L. cheesmanii,

L. pimpinellifolium, and L. esculentum and the southern populations close to

L. chilense. L. hirsutum and L. pennellii were distantly related to the remaining

esculentum complex species. Solanum jungandifolium and Solanum

ochranthum were the closest outgroup to Lycopersicon with Solanum

lycopersicoides and Solanum sitiens basal to these.

DNA sequences of the approximately 700 nt internal transcribed spacer

(ITIS) region of nuclear ribosomal DNA (rDNA) from one accession each of

L. esculentum var. esculentum, L. esculentum var. cerasiforme, L. cheesmanii, L.
pimpinellifolium, L. parviflorum; three accessions each from L. peruvianum, L.
hirsutum, and L. pennellii; plus one accession from each of seven outgroups

were studied using parsimony analysis (Marshall et al. 2001). The single

most-parsimonious tree displayed a fully resolved topology but with low

bootstrap support on some of the branches. Results showed three clades: i)
esculentum containing L. pimpinellifolium, L. esculentum var. esculentum, L. esculentum var. cerasiforme, L. cheesmanii, L. chmielewskii, and L. parviflorum,

ii) peruvianum containing L. peruvianum and L. chilense, and iii) hirsutum containing L. hirsutum and L. pennellii. These results reflected mating system (SC versus SI) and fruit color supporting the synapomorphy of both SC and red fruit. The northerly L. peruvianum var. humifusum (LA2150) was nested within the peruvianum clade rather than close to L. chmielewskii and L. parviflorum (inconsistent with Miller and Tanksley 1990) but with low bootstrap support.

In the first study of Lycopersicon relationships using nuclear DNA sequences at multiple loci, four regions were included for a total of approximately 7 kb (Nesbitt and Tanksley 2002). Loci consisted of a fruit weight quantitative trait locus (QTL) fw2, the 5' untranslated region (UTR) of fw2.2, orf44 (an open reading frame of unknown function immediately adjacent to fw2.2), three loci on chromosomes other than fw2.2: alcohol dehydrogenase 2 (Adh2), and two anonymous single-copy loci TG10 and TG11. Species were represented by four accessions of L. esculentum var.
esculentum; three accessions of L. pimpinellifolium; and one accession each of L. cheesmanii, L. parviflorum, L. hirsutum, L. pennellii, and L. peruvianum.

The single most-parsimonious tree from data pooled across loci and using L. pennellii as the outgroup gave strong support (100% bootstrap values for 100 replicates) for three major groupings: i) L. esculentum, L. cheesmanii, and L. pimpinellifolium, ii) L. parviflorum and L. peruvianum, and iii) L. hirsutum and L. pennellii. The L. peruvianum accession in this study (LA1708) was a northern type that also clustered close to L. parviflorum based on SSR data (Alvarez et al. 2001).

L. esculentum var. cerasiforme accessions were also sequenced in Nesbitt and Tanksley’s (2002) study but were not used in estimating interspecific relationships because they introduced many incongruities into the trees.

Gene trees of fw2.2 5’ UTR, Adh2, TG10, and TG11 show ten L. esculentum var. cerasiforme alleles to be interspersed among four L. esculentum and three L. pimpinellifolium alleles. The authors suggested that this apparent admixture of alleles may represent hybridizations between L. esculentum var. esculentum and L. pimpinellifolium giving rise to L. esculentum.
var. cerasiforme.

To summarize the molecular phylogenetic studies of Lycopersicon, there is general support for the following relationships (from derived to ancestral groups): i) red fruit SC L. esculentum, L. cheesmanii, and L. pimpinellifolium, ii) green fruit SC species L. chmielewskii and L. parviflorum, iii) green fruit SI northern L. peruvianum, iv) green fruit SI southern L. peruvianum and L. chilense, and v) green fruit SI L. hirsutum and L. pennellii.

ORIGIN AND MOLECULAR DIVERSITY OF CULTIVATED TOMATO

Cultivated tomato has been documented to have been in existence for only about 400 years (Boswell 1937). There are detailed written accounts of its presumed origin, migration, selection, and introgression (Boswell et al. 1933, Boswell 1937, Jenkins 1948, Stevens and Rick 1986). Peru is the center of origin for the genus and while L. esculentum var. cerasiforme was believed to be the direct progenitor of large-fruited cultigens (Bailey 1896), it may more likely be an admixture of cultivated and wild species (Nesbitt and Tanksley 2002). L. esculentum is thought to have originally been domesticated and planted in maize fields by ancient Mexicans, although this remains
uncertain (Jenkins 1948). Tomato spread to Europe in the early 1500s, initially in Italy and Spain, and thereafter became widely distributed. It was not grown and consumed in large quantities until the late 1700s. Since then it has been selected and bred within a broad range of climates from cool-temperate to tropical. The first cultigens grown in the U.S. came from England and France and the first U.S. improved cultigens were Tilden, released in 1865, and Trophy, released in 1870. Trophy ushered in a new epoch of tomato popularity in the U.S. and is believed to have contributed to the parentage of most of the cultigens developed during the next few decades (Bailey 1896).

Small companies became important sources of seed in late 1800s to 1900s and around 1910 public breeders started introducing disease resistant cultigens, e.g., Tennessee Red and Louisiana Wilt Resistant. These early resistant types dominated the U.S. market in the 1920s and 1930s. In the early 1940s closely related wild species within the genus Lycopersicon began to be screened for additional disease resistance, and wild sources provided much of the breeding germplasm during subsequent decades.
Wild germplasm continues to play a major role in tomato breeding. Recent work has demonstrated that favorable alleles in wild relatives can remain cryptic until expressed in an improved background (Tanksley et al. 1996) and techniques with which to incorporate wild alleles into modern cultivars continue to be refined (Monforte et al. 2001).

Although migration of the cultigen from Latin America to Europe and subsequently throughout the world has been documented, pedigrees for the majority of L. esculentum accessions held in germplasm collections are largely unknown. Molecular markers have been used to estimate genetic diversity of sets of cultivars. Two trends have been observed: i) a narrowing of the germplasm base caused by genetic bottlenecks and selection, and ii) an increase in molecular genetic variation in and around regions introgressed from wild relatives.

Genetic variation among accessions originating from primary and secondary centers of diversity was surveyed using 41 RAPD primers (Villand et al. 1998). This study included 21 accessions from the primary center of diversity for the genus (Chile, Peru, and
from secondary centers contiguous with the primary center (South America, Central America, and Mexico), and 38 accessions from secondary centers other than South America, Central America, and Mexico (including Asia, Africa, Cuba, Europe, and U.S.). Average genetic distance for all possible 4,560 pairwise comparisons of accessions (estimated as the complement to the simple matching coefficient; Gower 1972) was 0.164 ± 0.084. Genetic diversity based on allele frequencies (Nei 1987) for groups of accessions was estimated. Average genetic diversity was relatively greater in the primary center (0.219) compared to contiguous (0.175) and other (0.137) secondary centers. This supported allozyme data that showed greater diversity in L. esculentum from Peru and Ecuador compared to material from secondary centers (Rick and Fobes 1975). There was also more genetic variation in a set of 20 processing tomato cultigens (Villand 1995) compared to random samples of 20 cultigens from this study. The authors suggested that this could be explained by recent introgression of favorable alleles from wild Lycopersicon species into processing cultivars. This was consistent
with an observed increase in RAPD variation among modern cultigens compared to cultigens released prior to around 1960 (Williams and St. Clair 1993).

Genetic relationships among 19 tomato cultigens from a geographically isolated regions accessions originating from outside the center of origin (1 South Africa, 5 Europe, 1 Russia, 1 China, 1 Australia, 9 from Canada and U.S., and 1 unknown) were studied using 65 polymorphic SSR markers (He et al. 2003). UPGMA clustering based on genetic distances (Nei and Li 1979) did not reveal a clear pattern reflecting geographical origins of accessions. European accessions were widely dispersed throughout the dendrogram, implying that most non-European germplasm is closely related to germplasm of European origin. However, equal numbers of accessions from various geographical regions would need to be compared in order to test this hypothesis.

RAPD markers showed a narrowing of the germplasm base between tomato cultigens bred and released in India during the 1970s compared to the 1990s (Archak et al. 2002). Average gene diversity (Nei 1987) based on 174 RAPD fragments was 0.265 (n = 5 accessions) in pre-1979
versus 0.068 (n = 7) and 0.118 (n = 8) in two sets of modern material originating from different breeding programs. This was interpreted as havTable 2.1 Reproductive biology of Lycopersicon species.

Table 2.1 Reproductive biology of Lycopersicon species.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Self-compatible</th>
<th>Mating System</th>
<th>Cross-compatibility with cultigen</th>
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<tr>
<td>L. cheesmanii</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. chilense</td>
<td>no</td>
<td>allogamous</td>
<td>can act as male with embryo rescue</td>
</tr>
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<td>L. chmielewskii</td>
<td>yes</td>
<td>facultative</td>
<td>reciprocal</td>
</tr>
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<td>L. esculentum var.</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. esculentum</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
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<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. cerasiforme</td>
<td>no</td>
<td>allogamous</td>
<td>no</td>
</tr>
<tr>
<td>L. glandulosum</td>
<td>no</td>
<td>allogamous</td>
<td>no</td>
</tr>
<tr>
<td>L. hirsutum</td>
<td>no, except allogamous</td>
<td>can act as male</td>
<td></td>
</tr>
<tr>
<td>f. typicum</td>
<td>for some or biotypes facultative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. hirsutum</td>
<td>yes</td>
<td>facultative</td>
<td>reciprocal</td>
</tr>
<tr>
<td>f. glabratum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. parviflorum</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
<tr>
<td>L. pennellii</td>
<td>no, except allogamous</td>
<td>reciprocal for some or biotypes facultative</td>
<td></td>
</tr>
<tr>
<td>f. typicum</td>
<td>for some or biotypes facultative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. peruvianum</td>
<td>no, except allogamous</td>
<td>no, can occasionally be for some or overcome with technical biotypes facultative difficulty</td>
<td></td>
</tr>
<tr>
<td>L. pimpinellifolium</td>
<td>yes</td>
<td>autogamous</td>
<td>reciprocal</td>
</tr>
</tbody>
</table>

ing resulted from a trend towards breeding for a specific type, i.e., determi
nate plants bearing uniform fruit.

Although the genetic base of cultivated tomato is narrow, researchers have found it possible to distinguish cultivars using small numbers (four to five) of polymorphic loci (Bredemeijer et al. 1998, four SSR loci distinguishing 16 cultivars; Suliman-Pollatschek et al. 2002, four SSR loci distinguishing 10 cultivars; He et al. 2003, five SSR loci distinguishing 19 cultivars).

ECONOMIC IMPORTANCE

Tomato is a major vegetable crop in the United States and worldwide. The crop is used both fresh and in processed products. Fresh tomato is eaten by itself, in salads, and is used in many recipes as an ingredient. Processed products include paste, canned tomatoes (diced, crushed, and whole), salsa, ketchup, and as an ingredient in many condiments. Tomatoes are also dried and used for cooking.

Worldwide, tomato is produced for the fresh market and processing on approximately 4 million hectares, with an average yield of 27.2 ton ha⁻¹ and a yearly production of 108.5 million tons (FAOSTAT 2002; Table 2.2).

The top five leading tomato producing countries are China, the United
States, Turkey, India, and Egypt. Statistics of regions and for leading to
tomato producers is given in Table 2.2. Tomato area has
increased by 38%
and production has increased by 45% in the past ten years.
Most of this
worldwide increase in production has come from China, where
the area
has more than tripled from 0.30 million ha to 0.97 million
ha, with an
increase in production from 8.5 million tons to 25.5
million tons, propel
ling China from number two to the number one producer of
tomato over
the United States. China now accounts for 23.5% of the
world's tomato
production. Leading producers of tomato are listed in Table
2.2.
Production of tomato for fresh market and processing in the United
States is given in Table 2.3 (USDA-NASS 1995, 2003). Area of
cultivation in the United States for fresh market
production has decreased
from 53.4 thousand ha to 50.6 thousand ha; however, the
area of cultivation
for processed products has increased from 110.9 thousand ha
to 126.2
thousand ha. Therefore, tomato production for fresh market
has decreased
in the past ten years from 1.4 million tons to 1.2 million
tons; whereas,
production of processed tomato has increased in the past
ten years from
The value of fresh market tomato produced in the United States is 1.17 billion dollars and the value of processed tomato production is 683 million dollars (Table 2.3). Tomato accounts for approximately 14.5% of the United States, fresh vegetable production value, while it accounts for approximately 50.7% of the processed vegetable production value. Significant production of fresh market tomato is found in 17 states. The two leading producers are Florida and California, which account for 67% of the total value of fresh market tomato production value. There are four primary producers of tomato for the processing market, but California accounts for

<table>
<thead>
<tr>
<th>Location</th>
<th>Area (ha*10^3)</th>
<th>Yield (ton ha^-1)</th>
<th>Production (ton*10^3)</th>
</tr>
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<tr>
<td>World</td>
<td>2,883</td>
<td>25.9</td>
<td>74,757</td>
</tr>
<tr>
<td>Africa</td>
<td>442</td>
<td>20.2</td>
<td>8,918</td>
</tr>
<tr>
<td>North and Central America</td>
<td>329</td>
<td>37.9</td>
<td>12,476</td>
</tr>
<tr>
<td>South America</td>
<td>150</td>
<td>30.0</td>
<td>4,496</td>
</tr>
<tr>
<td>Asia</td>
<td>1,254</td>
<td>23.7</td>
<td>29,768</td>
</tr>
<tr>
<td>Europe</td>
<td>698</td>
<td>26.8</td>
<td>18,688</td>
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</table>
Leading Countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Value</th>
<th>Percentage</th>
<th>Production Value</th>
<th>Percentage</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>27.9</td>
<td>8,501</td>
<td>974</td>
<td>26.1</td>
<td>25,466</td>
</tr>
<tr>
<td>United States of America</td>
<td>59.2</td>
<td>9,730</td>
<td>177</td>
<td>69.4</td>
<td>12,267</td>
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<tr>
<td>Turkey</td>
<td>37.5</td>
<td>6,450</td>
<td>225</td>
<td>40.0</td>
<td>9,000</td>
</tr>
<tr>
<td>India</td>
<td>15.7</td>
<td>4,850</td>
<td>520</td>
<td>14.3</td>
<td>7,420</td>
</tr>
<tr>
<td>Egypt</td>
<td>30.8</td>
<td>4,694</td>
<td>181</td>
<td>35.0</td>
<td>6,329</td>
</tr>
<tr>
<td>Italy</td>
<td>46.6</td>
<td>5,483</td>
<td>123</td>
<td>49.3</td>
<td>6,055</td>
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<tr>
<td>Spain</td>
<td>47.4</td>
<td>2,647</td>
<td>60</td>
<td>65.2</td>
<td>3,878</td>
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<td>Brazil</td>
<td>41.0</td>
<td>2,141</td>
<td>62</td>
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<td>3,518</td>
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<td>Iran, Islamic Rep. of</td>
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<td>Mexico</td>
<td>16.5</td>
<td>1,677</td>
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<td>Russian Federation</td>
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<td>Chile</td>
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<td>700</td>
<td>20</td>
<td>66.0</td>
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<td>Ukraine</td>
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<td>1,303</td>
<td>105</td>
<td>10.5</td>
<td>1,100</td>
</tr>
<tr>
<td>Uzbekistan</td>
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<td>28</td>
<td>35.7</td>
<td>1,000</td>
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<tr>
<td>Portugal</td>
<td>35.0</td>
<td>700</td>
<td>10</td>
<td>55.2</td>
<td>994</td>
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<tr>
<td>Morocco</td>
<td>35.4</td>
<td>894</td>
<td>19</td>
<td>52.0</td>
<td>991</td>
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<tr>
<td>Nigeria</td>
<td>10.0</td>
<td>400</td>
<td>126</td>
<td>7.0</td>
<td>879</td>
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</table>

approximately 93% of the production value. The second producer, India, accounts for only 3% of the production value.

While Florida and California are the number 1 and 2 producers of fresh
market tomato, respectively; the value of their production has decreased by
approximately 31% in the past ten years with a decrease of the total pro
duction value from 83.5% to 50.7%. However, 12 of the other 15 leading
production states have increased their production value of fresh market
tomato in the past ten years. The largest increases have been in New York,
North Carolina, Ohio, and Tennessee. This shows a trend of consumer

Location (ha 10^3 ) (ton 10^3 ) ($ 10^6 ) (ha 10^3 )

<table>
<thead>
<tr>
<th>Location</th>
<th>Fresh Market</th>
<th>Vegetable</th>
<th>Tomato</th>
<th>AL</th>
<th>AR</th>
<th>CA</th>
<th>FL</th>
<th>GA</th>
<th>IN</th>
<th>MD</th>
<th>MI</th>
<th>NJ</th>
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<tbody>
<tr>
<td></td>
<td>Area Production Value</td>
<td>Area Production Value</td>
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<tr>
<td>Location</td>
<td>(ha 10^3 )</td>
<td>(ton 10^3 )</td>
<td>($ 10^6 )</td>
<td>(ha 10^3 )</td>
<td>(ton 10^3 )</td>
<td>($ 10^6 )</td>
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<tr>
<td>Fresh Market</td>
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<td>15,087.1</td>
<td>5,889.3</td>
<td>679.1</td>
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<td>Tomato</td>
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<td>50.6</td>
<td>1,692.0</td>
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<tr>
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<td>6.5</td>
<td>0.5</td>
<td>14.6</td>
<td>7.4</td>
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<tr>
<td>AR</td>
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<td>9.1</td>
<td>0.5</td>
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<td>CA</td>
<td>14.6</td>
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<td>343.7</td>
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<td>FL</td>
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<tr>
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<tr>
<td>State</td>
<td>Total Vegetables</td>
<td>Tomato</td>
<td>CA</td>
<td>IN</td>
<td>MI</td>
<td>OH</td>
<td>Other states</td>
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<tr>
<td>-------</td>
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<td>2.3</td>
<td>5.9</td>
<td>2.9</td>
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<td>509.4</td>
<td>447.4</td>
<td>3.3</td>
<td>1.3</td>
<td>10.5</td>
<td>26.0</td>
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<td></td>
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</tr>
<tr>
<td>SC</td>
<td>545.9</td>
<td>126.2</td>
<td>117.8</td>
<td>3.3</td>
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<td>26.0</td>
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<td>TN</td>
<td>15,528.1</td>
<td>10,547.8</td>
<td>10,029.8</td>
<td>2.7</td>
<td>2.3</td>
<td>5.9</td>
<td>113.2</td>
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</tr>
<tr>
<td>TX</td>
<td>1,346.9</td>
<td>768.1</td>
<td>632.4</td>
<td>22.1</td>
<td>22.1</td>
<td>22.1</td>
<td>22.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>VA</td>
<td>1,346.9</td>
<td>683.1</td>
<td>632.4</td>
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<td>22.1</td>
<td>22.1</td>
<td>22.1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Processed

The reduction in fresh market tomato can mostly be explained by the increase of imports and the decrease of exports of fresh market tomato by the United States (Table 2.4). In the past ten years fresh market production of tomato in the United States has declined by 78,500 tons.
However, the trade deficit of the United States in fresh market tomato production has increased by 634,800 tons. In 2002, most of the United States import of fresh market tomato has come from Mexico, with an increase of imports by 573,400 tons in the past ten years. Imports accounted for approximately 11% of fresh market tomato consumption in the United States in 1992, but by 2002 imports accounted for 32% of the fresh market tomato consumption. Cantliiffe (1997) has extensively reviewed the impact of the North American Free Trade Agreement (NAFTA) on Mexican fresh market tomato production and its importation into the United States for the period of 1982 to 1997. The United States has maintained a trade surplus in processed tomato products which has slightly increased in the past ten years (Table 2.4).

Although the majority of fresh market fruit is field grown, there has been an expansion of greenhouse grown tomato. Greenhouse production of fresh market tomato is significant in Europe, especially the Netherlands (Snyder 1996, Table 2.5). Greenhouse production of tomato is predominantly produced with rockwool (Logendra and Janes 1997), moving to
wards hydroponics (Jensen 1997).

Table 2.4 United States exports and imports of fresh market and processed tomato (USDA-ERS 2003) Exports (ton $\times 10^3$)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>166.7</td>
<td>150.6</td>
<td>Fresh</td>
<td>196.0</td>
<td>860.1</td>
</tr>
<tr>
<td>Paste</td>
<td>73.2</td>
<td>99.1</td>
<td>Paste</td>
<td>19.8</td>
<td>17.8</td>
</tr>
<tr>
<td>Canned</td>
<td>14.0</td>
<td>34.5</td>
<td>Prepared</td>
<td>43.3</td>
<td>14.7</td>
</tr>
<tr>
<td>(whole and (excluding pieces sauce) Sauce)</td>
<td>59.9</td>
<td>116.1</td>
<td>Sauce (including 7.9 124.1 pulp and puree)</td>
<td>7.9</td>
<td>124.1</td>
</tr>
<tr>
<td>Catsup and chili sauce</td>
<td>24.5</td>
<td>38.7</td>
<td>Dried</td>
<td>7.8</td>
<td>12.9</td>
</tr>
<tr>
<td>Canned pulp</td>
<td>6.5</td>
<td>16.1</td>
<td>(puree)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GERMPLASM CONSERVATION

Germplasm Collections

Major germplasm collections of tomato are maintained in the United States at the Plant Genetic Resources Unit, United States Department of Agriculture (USDA) at Geneva, NY, and at the Tomato Genetic Resources Center (TGRC) located in the Department of Vegetable Crops at the University of California Davis. The Asian Vegetable Research and Development Center (AVRDC), now referred to as the World Vegetable Center (WVC), located at
Tainan, Taiwan is an international center affiliated with the Consultative Group of International Centers, which maintains the major international collection of tomato germplasm.

Worldwide, there are more than 75,000 accessions of Lycopersicon germplasm accessions maintained in more than 120 countries in a number of national institutions (Battencourt and Konopka 1990, updated on web).

Table 2.6 lists the largest collections (except the USDA, AVDRC and TGRC which are listed in Table 2.7). Most data reported in the IPGRI listing has been updated since the mid-1990s, with many updates, especially for the larger collections since 2000. This report is available on the Internet at:

http://www.ipgri.cgiar.org/germplasm/dbintro.htm. There are URLs available within this report for many of the institutions where large collections of tomato are reported. The countries with the greatest number of germplasm accessions of Lycopersicon, besides the USA, are Brazil, Bulgaria, Canada, China, Colombia, Germany, Hungary, the Philippines, and Spain. These countries have two thousand or more accessions of Lycopersicon species conserved, mostly L. esculentum.
The collections of USDA and AVDRC are mainly cultivated Lycopersicon, though each has significant wild Lycopersicon collections (Table 2.7) with AVDRC conserving 659 accessions of wild Lycopersicon species and the USDA conserving 458 accessions of wild Lycopersicon species. Both of these genebanks have large collections of L. peruvianum and L. pimpinellifolium Table 2.5 Greenhouse tomato area in selected countries

Country Hectares

Canada 287

England/Wales 1214

The Netherlands 4613

Spain 12,140

United States 102

Source: Snyder (1996)

Table 2.6 Worldwide Lycopersicon germplasm conservation (For USDA, AVDRC, TGRC; see Table 2.7). No.

Country Institution accessions

Australia Australian Tropical Crops & Forages Genetic Resources Centre 1116

Brazil Centro Nacional de Pesquisa de Hortali̇as, EMBRAPA 2070 Lab. de Melhoramento Genetico Vegetal (LMGV)-CCTA-VENF 500 Instituto Agronomico de Campinas (IAC) 500 Departamento de Fitotechnia-Universidad Federal de Vicosa 600

Bulgaria Institut de resources phytogénétiques 'K Malkov' 500

Canada Saskatoon Research Centre, Agriculture and AgriFood Canada 1897 Horticultural Experiment Station, Simcoe, Ontario 1070

Table 2.7 Wild Lycopersicon accessions.
China Institute of Crop Germplasm Resources (CAAS) 1942

Colombia Corporacion Columbiana de Investigacion Agropecuaria 2018 CORPOICA

Costa Rica Estacion Experimental Agricola Fabio Baudrit, Univ. de 700 Costa Rica Centro Agronomico Tropical de Investigacion y Ensenanza 457 (CATIE)

Cuba Banco de Germoplasma, Inst. de Invest. Fund. en Agricultura 630

Czech Rep. Genebank Department-Vegetable Secion Olomouc 1613

France Unité Expérimentale d’Angers Geves 1254 Station d’Amélioratoin des Plantes, INRA Avignon 1246

Germany Genebank, Inst. for Plant Genetics and Crop Plant Research 3262 (IPK)

Hungary Institute for Agrobotany 2043

India National Bureau of Plant Genetic Resources (NBPGR) 940

Italy Dip. di Agronomia & Genetica Veg. Universita degli Studi 804 de Napoli

Japan Department of Genetic Resources, Nation. Inst. of AgrobioI. 452 Resour.

The Netherlands Centre for Genetic Resources, The Netherlands (CGN) 1159

Nigeria National Centre for Genetic Resources and Biotechnology, 451 (FMST)

Peru Universidad Nacional Agraria La Molina 936

Philippines National Plant Genetic Resources Laboratory, IPB/UPLB 4793

Poland Plant Breeding and Acclimatization Institute (IHAR) 419 Plant Genetic Resources Laboratory Research Inst. of Veegt. Crops 427

Spain Centro deRecursos Fitogeneticos, INIA 1267

Experimental Station La Mayora CSIC 801 Univ.Politecde Valencia, Escuela Té Sup. de Ing. 1405 Agron. Banco de Germplasm Banco de Germoplasma de Horticolas-Diputacion General 1381 de Aragon
Table 2.7 Lycopersicon holdings of the USDA at Geneva, NY (USDA), the Tomato Genetic Resources Center (TGRC), and the Asian Vegetable Development and Research Center (AVDRC)

<table>
<thead>
<tr>
<th>Species Subtaxa</th>
<th>USDA</th>
<th>TGRC</th>
<th>AVDRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopersicon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cheesmanii</td>
<td>7</td>
<td>39</td>
<td>18</td>
</tr>
<tr>
<td>cheesmanii f. minor</td>
<td>5</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>chilense</td>
<td>1</td>
<td>83</td>
<td>31</td>
</tr>
<tr>
<td>chmielewskii</td>
<td>1</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>esculentum</td>
<td>4913</td>
<td>427</td>
<td>5311</td>
</tr>
<tr>
<td>esculentum var. cerasiforme</td>
<td>267</td>
<td>275</td>
<td>109</td>
</tr>
<tr>
<td>esculentum x esculentum</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>var. cerasiforme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>esculentum hybrids</td>
<td>158</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>glandulosum</td>
<td>12</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>hirsutum</td>
<td>39</td>
<td>76</td>
<td>49</td>
</tr>
<tr>
<td>hirsutum f. glabratum</td>
<td>21</td>
<td>41</td>
<td>17</td>
</tr>
<tr>
<td>parviflorum</td>
<td>6</td>
<td>53</td>
<td>12</td>
</tr>
<tr>
<td>pennellii</td>
<td>10</td>
<td>40</td>
<td>61</td>
</tr>
<tr>
<td>pennellii var. puberulum</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>peruvianum</td>
<td>124</td>
<td>155</td>
<td>120</td>
</tr>
<tr>
<td>peruvianum var. humifusum</td>
<td>2</td>
<td>11</td>
<td>4</td>
</tr>
</tbody>
</table>
pimpinellifolium 230 247 315
sp. 13 1014
Solanum
juglandifolium 7
lycopersicoides 16
ochranthum 4
sitiens 5
Total 5809 1557 7231

*See Table 2.8

(Table 2.7). The collection at TGRC has an emphasis on wild species and various genetic stocks though it also has over 700 accessions of L. esculentum.

Both the USDA and AVDRC have hybrid populations of L. esculentum with other Lycopersicon species (Table 2.7), while TGRC maintains a significant number of Lycopersicon introgression populations (Table 2.8). A small collection of Solanum species (Section Petota, sub-section Potatoe, Series Juglandfolium) is also maintained by the TGRC with germplasm conserved for S. juglandifolium, S. lycopersicoides, S. ochranthum, and S. sitiens (Table 2.7).

Geographical distributions for Lycopersicon esculentum accessions maintained by AVDRC and the USDA are given in Table 2.9. The distributions of accessions by country for both collections are similar;
with the exception Table 2.8 Introgression and special purpose populations of TGRC

Material Parental Material Number

Lycopersicon pennelli introgression lines LA0716; M-82 76

L. hirsutum introgression lines LA1777; E-6203 (LA4024) 98

Solanum lycopersicoides introgression lines LA2951; VF36 (LA0490) 80

L. pennellii alien substitution lines LA0716 7

L. pimpinellifolium backcross LA1589, E6203 99

recombinant inbreds

S. lycopersicoides monsomic addition lines LA1964; Vendor 10

High soluble solids derivatives of LA1200 derivatives 3

L. chmielewskii

Mutants derived from L. cheesmanii L. esculentum derivatives of S L. cheesmanii

L. pimpinellifolium exserted stigma LA1505 1

derivative

S. lycopersicoides hybrid LA2951; VF36 1

Total 380

that AVDRC has a larger representation from Asian countries, such as India, Korea, Malaysia, the Philippines, Sri Lanka, and Taiwan. Both insti

tutions have large number of accessions from Brazil, Canada, China, El Salvador, the former USSR, Guatemala, Honduras, Hungary, India, Mexico, Peru, Turkey, the USA, and Yugoslavia. There is probably much uninten
tional duplication between these collections and also for those of Table 2.6.

Unwanted redundancy within the USDA collection and among different germplasm collections will be discussed in a later section.

Large genetic resources collections are usually duplicated at a second backup location. Backup germplasm collections are highly desirable since they provide a valuable resource to replace germplasm collections that are lost or destroyed by natural disasters (such as fires, floods etc.), political disturbances or mechanical breakdowns. Both the USDA and the TGRC collections are backed up at the National Center for Genetic Resources Preservation (NCGRP) located at Ft. Collins, Colorado. The backup status of the USDA tomato collection at Ft. Collins is given in Table 2.10. Overall, the USDA collection has an 89% backup, though some of the wild taxa have lower backup rates. The TGRC collection is also almost entirely backed up (95%) at NCGRP (Anonymous 2003).

The majority of Lycopersicon germplasm used for improvement of tomato has been wild species germplasm. The TGRC maintains a series of special purpose collections of wild species germplasm (Table 2.11). A number of accessions are available with tolerance to drought,
flooding, high Genetic Resources of Tomato (Lycopersicon esculentum Mill.) and Wild Relatives Research Center (AVDRC), and the USDA germplasm collection maintained at Geneva, NY (USDA)

<table>
<thead>
<tr>
<th>Country</th>
<th>AVDRC</th>
<th>USDA Country</th>
<th>AVDRC</th>
<th>USDA Country</th>
<th>AVDRC</th>
<th>USDA Country</th>
<th>AVDRC</th>
<th>USDA Country</th>
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<tr>
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<td>Ethiopia</td>
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<td>Sri Lanka</td>
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<td>Former USSR</td>
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<td>Georgia</td>
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<td>Mauritius</td>
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<td>Taiwan</td>
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<td>Mexico</td>
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<td>137</td>
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<td>10</td>
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<td>New Caledonia</td>
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<td>Panama</td>
<td>50</td>
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</tr>
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<td>Costa Rica</td>
<td>46</td>
<td>44</td>
<td>Iran</td>
<td>61</td>
<td>62</td>
<td>Papua N. Guinea</td>
<td>4 Unknown</td>
<td>91</td>
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</table>
Table 2.10 Backup of the USDA Lycopersicon collection at Geneva, NY

<table>
<thead>
<tr>
<th>Species</th>
<th>Subtaxa</th>
<th>No. Acc.</th>
<th>Backup</th>
<th>%Backup</th>
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<td>5</td>
<td>3</td>
<td>42.86</td>
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<td>L. chilense</td>
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<td>1</td>
<td>1</td>
<td>100.0</td>
</tr>
<tr>
<td>L. chmielewskii</td>
<td></td>
<td>1</td>
<td>1</td>
<td>100.0</td>
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<td>4362</td>
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</tr>
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<td>267</td>
<td>264</td>
<td>68.9</td>
</tr>
<tr>
<td>L. esculentum x esculentum</td>
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<td>158</td>
<td>158</td>
<td>100.0</td>
</tr>
<tr>
<td>var. L. cerasiforme</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. esculentum hybrids</td>
<td></td>
<td>150</td>
<td>150</td>
<td>100.0</td>
</tr>
<tr>
<td>L. glandulosum</td>
<td></td>
<td>12</td>
<td>10</td>
<td>83.3</td>
</tr>
<tr>
<td>L. hirsutum</td>
<td></td>
<td>39</td>
<td>23</td>
<td>76.2</td>
</tr>
<tr>
<td>L. hirsutum f. glabratum</td>
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<td>21</td>
<td>16</td>
<td>59.0</td>
</tr>
<tr>
<td>L. parviflorum</td>
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<td>6</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>L. pennellii</td>
<td></td>
<td>10</td>
<td>3</td>
<td>30.0</td>
</tr>
</tbody>
</table>
L. pennellii var. puberulum
L. peruvianum 124 95 76.6
L. peruvianum var. humifusum 2 2 100.0
L. pimpinellifolium 230 219 95.2
L. sp. 13 1 7.7
Total 5809 5169 89.0

temperatures, aluminum toxicity, chilling injury, salinity-alkalinity, and arthropod damage. Accessions with tolerance are listed in Table 2.10. Lists for these are available on the Internet at:
http://tgrc.ucdavis.edu/. These stress tolerant wild species stocks have been extensively utilized in tomato crop improvement.

As mentioned previously, the TGRC maintains a number of introgression populations and special purpose populations, e.g., Lycopersicon esculentum X L. pennellii and L. hirsutum introgression populations are available (Table 2.8). Additionally, TGRC maintains Solanum lycopersicoides X L. esculentum populations. Various other substitution, backcross recombinant, alien and monosomic addition lines, various mutant populations, and high soluble solid derivatives are also maintained. The majority of the germplasm of Lycopersicon maintained at the TGRC is of various genetic stocks.
Monogenic stocks account for approximately 2/3 of these genetic stocks. There are also large numbers of chromosome markers and miscellaneous marker combinations.

Conservation

Conservation of genetic resources of tomato in the broad sense encompasses germplasm collection, maintenance, distribution, characterization and evaluation. Collection of tomato germplasm is influenced by the breeding system of the species to be collected and the ease in transfer of traits to the cultigen (Table 2.1). Maintenance of genetic resources of seed crops such as tomatoes involves two separate but inter-related activities; a) long-term maintenance of seed, and b) regeneration of seed as required to maintain sufficient quantities of high-quality seed for storage and distribution.

Both of these processes require sufficiently large numbers of plants and or Table 2.11 TGRC stress tolerant wild species stocks

Stress Taxon Accessions


Flooding L. esculentum var. LA1421 a cerasiforme S. juglandifolium b LA2120 S. ochranthum b LA2682

High temperature L. esculentum LA2661, LA2662, LA3120, LA3320

Aluminum L. esculentum var. LA2710 c cerasiforme


Arthropod L. hirsutum LA0407 a L. pennellii LA0716 a

a others available

b Listed as general characteristic of taxon

Suspected

seeds to avoid the loss of genetic diversity within the collection and to maintain the genetic identity of accessions conserved.

Germplasm characterization and evaluation greatly increases the usefulness of tomato germplasm. Major traits of interest in tomato include quality traits such as soluble solids, yield improvement, and increase of resistance/tolerance to major biotic and abiotic stresses. Conservation of germplasm has the primary goal of providing germplasm for crop improve ment and research, and the success of germplasm conservation efforts is measured by the distribution and utilization of the germplasm conserved.

Germplasm Collection

As can be seen from Tables 2.6 and 2.7, the cultivated Lycopersicon is well
represented in many genebanks around the world. Germplasm acquisition through collections also requires proper sampling procedures to obtain a representative sample for conservation. Outcrossing wild Lycopersicon taxa require adequate sampling to obtain a representative sample for conservation while cultivated taxa require less seed per sample.

In the past 20 years there have been a number of collections of the wild Lycopersicon species, mostly through the efforts of the TGRC located at Davis, California. Plant collection expeditions have been sponsored by IBPGR in 1980, 1984, 1986, and 1987 (Anonymous 2003) and, additional wild/primitive germplasm collected in 1985, 1995, 1996, and 2001 on trips sponsored by the TGRC and the USDA Plant Exchange Office. The 2001 trip resulted in collection of L. peruvianum, L. chilense, and two Solanum species: S. lycopersicoides, and S. sitiens from Chile (Chetelat 2001). These expeditions covered areas that were previously poorly represented in existing collections. Collections have also been conducted by researchers from Spain in the Galapagos Islands and in Peru (Nuez and Cuartero 1984). Table 2.12 Various genetic stocks available at TGRC
Type Number

Translocations 37
Trisomics 31
Autotetraploids 20
Chromosome markers 194
Linkage screening testers 13
 Miscellaneous marker combinations 377
Monogenic stocks 994
Total 1666

The Crop Germplasm Committee (CGC) for tomato in the United States

National Plant Germplasm System (NPGS) has concluded in its 2003 report 

(Anonymous 2003) that the status of wild tomato germplasm is in good
shape and vastly superior than that of many other crop plants.

Recommendations were made for germplasm collection in certain remote
areas of Peru, especially in the Rio Maranon watershed because of the
presence of L. peruvianum and the importance of the genetic diversity found
in other populations of L. peruvianum. Other acquisition priorities
established by this committee include acquisition of germplasm from
research projects which have terminated or that are expected to be
terminated in the near future. Germplasm from such collections include
cultigens, breeding lines, genic and chromosomal variants, and other stocks.

Regeneration

To avoid the loss of diversity and genetic identity through genetic drift, mutation, and selection during the regeneration, standards must be established and used for minimal numbers of plants and seeds. The procedures used for regeneration and storage of tomato are dependent on the breeding system of the plant, with cross-pollinated species requiring larger samples during collection, regeneration, and conservation. For self-pollinated species, the requirements are determined by needs to maintain a sufficiently large sample for storage to reduce the number of regenerations required and is determined by the crop husbandry of the plant. Cross-pollinated taxa require a sufficient number of plants to adequately represent the accession and to prevent genetic drift during regeneration. Specifically, the USDA collection of L. esculentum is maintained by regenerants from approximately 24 plants, with accessions planted in the field without pollination control. Most genebanks also maintain cultivated Lycopersicon with similar numbers of plants, usually in the field with no
pollination control. With the self-pollinated cultivated tomato, number of

plants used for regeneration is mostly determined by

numbers of selfings

in field operations and by the desired number of seeds

obtained for

regeneration. Since tomato seed stores well, production of a large amount

of seed can significantly reduce the chances of genetic drift, by increasing

the time between regenerations. Wild taxa of Lycopersicon are both cross

and self-pollinated and both self-compatible and self-incompatible

(Table 2.1). Some species are facultative with accessions from some areas

self-incompatible and others highly self-compatible. Ideally, during

regeneration of the cross-pollinated species upwards of 50 plants are used

for regeneration to obtain a representative sample by reducing the effects of

genetic drift and selection during the regeneration process.

Regeneration of cultivated tomato is usually conducted by producing

transplants that are taken to the field once the danger of frost has passed.

At the USDA in Geneva, these are planted using a transplanter into plastic

mulch. Seed production requires constant monitoring for diseases with

timely application of pesticides to allow sufficient production of quality,
disease-free seed for maintenance and distribution. Small scale processing

equipment is used for processing cultivated tomato seed with custom de

signed equipment such as in Fig. 2.1, which is used to separate the seed

and gel from the skin and pulp. This material is then fermented for two to

three days to ease the processing. After this the seed is washed and dried.

Once dried the ‘hair’ is separated from the seed by tumbling in mesh bags

and dried in a clothes-dryer or air only. To reduce incidence of seed borne

viruses (such as TMV) seed is treated with a 20% solution of bleach and

dried. Seed processing for wild taxa is done by hand. Final processing and

storage of seed is discussed in the next section.

Seed Storage Conditions

Methods used for storage are aimed to increase the duration seed remains

viable and useful for distribution. Requirements for long-term storage of

species with orthodox seeds such as tomato have been well established

and are related to moisture content of the seed stored, the type of storage

container and the temperature of the environment used for seed storage


Equations to predict seed longevity in storage have been
developed and refined by Ellis and Roberts (1980) to take into account the variation in initial seed quality along with seed moisture content and temperature of storage. The suggested moisture content for storage of orthodox seeds for genetic conservation is at a moisture content of 5 ± 1%. This moisture content is achieved at Geneva, NY by drying the seed in a room that is maintained at 20% relative humidity at a temperature of 4 to 5°C. The suggested temperature for long-term storage is -20°C. Storage containers should be airtight but not vacuum-sealed because of damage to seed from the evacuation process.

Based on the results of seed vigor studies, Zheng et al. (1998) suggested use of ‘ultra-dry’ (less than 5% moisture content) seed storage for long-term genetic conservation of plants. They suggested that ultra-dry seed could be stored at higher temperatures compared to -20°C for long-term storage in genebanks. Ellis and Roberts (1998), and Walters et al. (1998), Genetic Resources of Tomato (Lycopersicon esculentum Mill.) and Wild Relatives 51 Fig. 2.1 Experimental wet seed processor for separation of tomato seed from small lots of tomato genotypes both question the usefulness of ultra-dry seed storage at ambient tempera
tures as a substitute for long-term cold storage in genebanks. XiangHui et al. (1998) dried tomato seed to 1.5% moisture but found that seed could not be stored for long periods at ambient temperatures. Pandey (1995) found that storage of 2% moisture seeds of tomato in hexylene glycol improved short- or medium term storage at ambient temperatures.

Others (Stanwood and Sova 1995, Iriondo et al. 1992) have made suggestions for use of cryopreservation of seed for long-term genetic conservation (conservation using liquid nitrogen) but there is need for more research before a definitive answer of whether there is any advantage to this for orthodox seeds. Sacks and St. Clair (1996) found that pollen cryopreservation can be used successfully for storage of tomato pollen and for tomato breeding and germplasm storage.

Maintaining Genetic Integrity during Regeneration and Storage

Because seed stocks are depleted through distribution and seeds eventually die during storage, periodic regenerations are necessary. Regeneration and maintenance procedures must minimize genetic changes within accessions. Maintaining genetic integrity involves maintaining genetic identity and genetic diversity. Loss of genetic identity occurs...
through contamination during regeneration by foreign pollen, seed adulteration during harvesting, threshing, and packaging, and through gene mutations (Steiner et al. 1997). Genetic erosion (or loss of genetic diversity) occurs through genetic drift due to random loss of alleles particularly in small populations and through genetic shifts due to unintentional natural selection.

Most new mutations are rare and random. Some studies indicated that major chromosomal aberrations occurred during seed storage and senescence, especially under desiccating conditions, but these gross mutations did not persist in regenerated populations (Wu et al. 1998). Inadvertent selection for more adapted genotypes or genotypes with relatively more viable seed can occur during regeneration (Wu et al. 1998). Selection acts on specific loci. Without knowing which loci these might be or even if we do know, selection is always difficult to distinguish from genetic drift.

Genetic drift and gene flow are relatively easier to detect than mutation and selection. Several studies have found evidence for one or both of these during regeneration in genebanks (del Rio et al. 1997, Wu et al. 1998,
Genetic drift refers to random changes in allele frequencies in an accession caused by random sampling of gametes during sexual reproduction. The rate of drift depends on effective population size (Ne), which is defined as the number of individuals in a theoretically ideal population having the same magnitude of drift as the actual population (Hartl and Clark 1989). Ne is usually smaller than census size and will be substantially so if an accession undergoes a genetic bottleneck.

Theoretical studies indicate that carefully sampling equal numbers of seeds from as many seed parents as possible can effectively prevent drift during regeneration (Crossa and Vencovsky 1997, Vencovsky and Crossa 1999) but this can become so labor-intensive as to make it impractical (Johnson 1998). An alternative strategy is to sample a single inflorescence per plant, rather than the whole plant, when bulking seed of outcrossing plants, such as outcrossing Lycopersicon taxa. This will improve Ne providing the relative variation in seeds per spike as less than that of seeds per plant (Johnson 1998).
(equal numbers of seeds per plant combined), spike (one inflorescence per plant combined), and bulk (seeds combined proportionally according to seeds per plant) regeneration samples were compared. The first method was clearly superior at maintaining genetic integrity of accessions. The latter two methods maintained diversity of eight isozymes as estimated by heterozygosity and mean numbers of alleles per locus, but allele frequencies shifted using those two methods.

Prevention of genetic drift and contamination in outcrossing species such as most wild Lycopersicon spp. requires more resources to be invested in regeneration per accession compared to the self-pollinated L. esculentum.

Molecular markers showed evidence of genetic drift during regeneration in one of eight wheat (Triticum aestivum; Börner et al. 2000) and one of six wild potato (Solanum jamesii; del Rio et al. 1997) accessions. In two barley (Hordeum vulgare) landraces that had been maintained for over 70 years, Ne was estimated to be 4.7 using morphological and isozyme markers, even though census sizes in regeneration plots were routinely 600 plants (Parzies et al. 2000). The authors stated that either intense directional
selection or a single bottleneck event could explain the extreme loss of genetic diversity.

The mating system of L. esculentum results in homozygosity within accessions. Census sizes of regenerated accessions are typically around 25 plants. This is considered to be large enough to prevent sudden extinction of an accession through rapid fixation of rare, deleterious mutations or an accidentally stressful environmental condition leading to inadvertent selection (Treuren and Hintum 2001). Decline of genetic integrity in a tomato accession would most likely occur from gene flow through contaminating pollen or mishandling. For seven oat (Avena sativa) lines maintained for 124 years, electrophoresis of storage proteins showed results ranging from no contamination of a line to complete replacement of a line by a foreign phenotype (Steiner et al. 1997). Both mishandling of seed and pollen contamination during maintenance were implicated.

Reducing Redundancy in Collections

Many empirical studies have addressed the question of unintentional duplication of conserved germplasm by examining subsets of collections.

Various types of duplications have been defined - identical duplication
refers to genetically identical accessions, common duplication denotes accessions derived from a common parental population, partial and compound duplication implies that not all alleles are duplicated, and parental duplication refers to the relationship between a particular cross and the resultant offspring (Hintum and Visser 1995). Although precise language such as this can lead to refined studies of redundancy the use of terms such as these has not become widespread.

A commonly applied experimental approach has been to compare identically or similarly named accessions of a particular species using passport, phenotypic, and/or molecular marker data. The first conclusion that can be reached is that substantial amounts of duplication have been found whenever it was looked for. A study of three European lettuce (Lactuca) collections estimated a mean duplication of 12% within and 37% among the collections based on passport data (Hintum 2000). The author pointed out that this may be an underestimate for two reasons: i) because poorly documented accessions were considered to be distinct, and ii) there may be overrepresentation of certain small fractions of the gene pool due to recent
shared ancestry or over-collecting in certain geographical areas. We have
evidence for both of these problems in our tomato
collection and will likely
encounter them in our other vegetable collections.

Studies on duplication often address the pooling of
duplicate material

and estimating how much genetic variation will be lost by
doing so. One

strategy for pooling is to maximize the ratio of the
similarity within groups
to the dissimilarity between groups (Hintum et al. 1996).
Many studies

have applied this model and examples include all accessions
of sorghum
(Sorghum bicolor) named “Orange” in the NPGS (Dean et al.
1999), Dutch

landraces of B. oleracea accessions at CGN (Hintum et al.
1996), flax (Linum
usitatissimum) accessions designated as “breeder’s lines”
at CGN (Treuren
et al. 2001), all Peruvian sweet potato accessions at CIP
(Huamán et al.
1999), and all accessions of cabbage named Golden Acre (B.
oleracea var.
capitata L.) in the NPGS (Phippen et al. 1997). In general
these studies

recommended pooling duplicate accessions based on AMOVA
(Analysis
of Molecular Variance) in a way that would retain greater
than 90% of the
total molecular genetic variation. In a pilot study, the
USDA at Geneva
(unpublished data) applied eight microsatellite markers to six identical San Marzano and six Globe types of tomato and found one of six accessions to be clearly genetically distinct in both cases.

Hintum (2000) has developed methods for quantification of duplications to the level of duplications both within and between germplasm collections along with standard errors of estimates by use of set theory. The definition used for duplicates was accessions with passport data that implies that they are genetically similar or the same. Four lettuce (Lactuca sativa L.) accessions were used to apply the equations developed for estimation of duplications within and between germplasm collections. This study points out that: a) most of the probable duplicates were from the exchange of material between collections, b) most of the duplicate materials consisted of older named varieties, and c) accessions unique to one collection were; i) recently added varieties, ii) recently collected material, and iii) poorly documented duplicates which made their identification difficult. The collections reported in Tables 2.6 and 2.7 probably have a high level of redundancy both within and between genebanks.
The USDA germplasm collection has been surveyed for duplications recently, and 1333 accessions have been found to form 455 sets of putative duplicate accessions. An empirical approach using accession identifiers was used to identify these duplicate accessions. An approach of using passport data to identify the known original source of these accessions to keep as representing the cultigen has been used to reduce duplication for approximately 90% of these accessions. The other 10% of accessions will be grown out at several locations for identification of duplications.

Development of Core Subsets

Core subsets are tools for users to efficiently work with a large fraction of the total genetic diversity in a collection. Random sampling methods are applied to develop core subsets. In order to broadly capture diversity, sampling may be from subsets of accessions initially grouped according to phenotype or ecogeographic origin (Li et al. 2002). Phenotypic traits are not always good indicators of genetic variation because they can be influenced by environment or result from independent genetic bases.

Molecular markers can help overcome these limitations. Molecular markers have been used: i) to compare various techniques
applied to generate a core (e.g., Sorghum bicolor, Grenier et al. 2000), ii) as criteria to decide which accessions to include in a core (e.g., cashew (Anacardium occidentale), Dhanaraj et al. 2002, Andean potato (Solanum phureja), Ghislain et al. 1999), and iii) to validate that a core is representative of a given base collection (e.g., Mexican common bean (Phaseolus vulgaris), Skroch et al. 1998]. Computer simulations, multivariate statistical techniques such as Principal Components Analysis (PCA), and genetic variation measures such as numbers of alleles, genetic diversity, and percentage of rare alleles, are frequently used to establish and validate core subsets.

Molecular markers reflect pedigrees, which may not be significantly correlated with gross morphology. In the Brazilian cassava (Manihot esculentum) collection cultigens with similar agronomic traits were very heterogeneous at the molecular level (Carvalho and Schaal 2001). For four major Sorghum races, grouping of accessions based on agronomic descriptors did not correlate with groupings produced from RAPD markers (Dahlberg et al. 2002).

The authors stated that such correlations should not be expected because
in most cases genes underlying an agronomic trait represent a very small fraction of the genome.

Core subsets of Lycopersicon esculentum and the wild Lycopersicon taxa have been assembled by the USDA-Geneva and TGRC, respectively. The cultivated core subset is a dynamic collection that is being refined. Presently, approximately 200 accessions of cultivated tomato, mostly from the United States, comprise the core collection. The tomato CGC is making efforts to modify this collection to have a balanced representation globally and to have better representation of tomato usage and of plant types. The TGRC at Davis has also established a core collection of wild Lycopersicon taxa.

GENETIC DIVERSITY IN Lycopersicon SPECIES

Mating system, life history traits (e.g., annual versus perennial, longevity, etc.), and ecological factors such as those causing frequent extinction and recolonization, all shape genetic variation within and among populations of a species. For the genus Lycopersicon, mating system is the most-extensively studied of these factors. The genus has been viewed as a model system among plants for studying the effect of mating
system within-spe
cies variation (Stephan and Langley 1998, Baudry et al. 2001), because it
consists of closely-related species with a range of mating systems from
selfing (L. esculentum, L. pimpinellifolium, L. cheesmanii, and L. parviflorum)
to facultative outcrossing (L. chmielewskii) to obligate-outcrossing (L. pennelli,
L. hirsutum, L. chilense, and L. peruvianum). In addition, some biotypes of L.
pennelli, L. hirsutum, and L. peruvianum have been found to be self-compat
ible (Rick 1982, Taylor 1986).
Neutral theory predicts that polymorphism within a species is a function
of mutation rate and effective population size (Kimura 1983). Effective
population size is defined as the number of individuals in a theoretically
ideal population having the same magnitude of genetic drift as the actual
population (Hartl and Clark 1989). Compared to random-mating, selfing is
expected to decrease effective population size by one-half and hence the
genetic variation by the same amount (Pollak 1987). Additional reasons
why selfing (self-pollinated) species are predicted to have reduced variation
are related to frequent bottlenecks and reduced recombination. A single
self-fertilizing plant can act as a founder for a new
population: selfing

species may frequently undergo such bottlenecks. A deficiency of double heterozygotes commonly characterizing selfing populations decreases effective recombination rate, thereby increasing linkage disequilibrium.

Extensive linkage disequilibrium is associated with reduced variation because selection will effect more of the genome, i.e., the selected locus plus tightly linked, unselected loci (Charlesworth et al. 1993).

In the plant genera Arabidopsis (Savolainen et al. 1999) and Leavenworthia (Liu et al. 1999) selfing species were found to have greater than two-fold reduction in genetic variation relative to outcrossing species.

In the genus Lycopersicon, levels of polymorphism for RFLP (Miller and Tanksley 1990), RAPD (Egashira et al. 2000), SSR (Alvarez et al. 2001), and DNA sequences (Baudry et al. 2001) have all been shown to be correlated with mating system.

In an RFLP study, the number of unique restriction fragments and the number of unique restriction patterns were used to estimate genetic diversity of accessions (Miller and Tanksley 1990). SI species L. hirsutum, L. pennellii, and L. peruvianum were found to be much more diverse than the SC species.
L. esculentum, L. pimpinellifolium, L. cheesmanii, L. parviflorum, and L. chmielewskii. Genetic distances among accessions were calculated based on proportion of shared restriction fragments (Nei 1987, equations 5.53 to 5.55). Average genetic distances among accessions were approximately ten-fold greater for L. peruvianum, and five-fold greater for L. hirsutum and L. pennelli than any other SC species. In addition, most of the diversity was distributed among rather than within accessions for the SC species.

Genetic distances based on proportion of shared bands (Nei and Li 1979) between species, between accessions within species, and between plants within an accession were estimated for L. esculentum var. esculentum and its wild Lycopersicon relatives using RAPD markers (Egashira et al. 2000). The facultative outcrosser L. chmielewskii contained the highest average within accession variation among the SC species (0.045). This was almost six-fold lower than the highest estimate found among the SI species (0.252 in L. chilense). Average genetic distances between accessions within species ranged from 0.006 for L. esculentum var. esculentum to 0.677 for L. chilense. Northern Peruvian accessions of L. peruvianum and
southern Peruvian accessions of L. chilense showed the greatest within-species genetic diversity.

L. hirsutum, L. pennellii, and L. peruvianum were found to be more diverse than L. esculentum, L. pimpinellifolium, L. cheesmanii, L. parviflorum, and L. chmielewskii in a study of 16 polymorphic SSR loci (Alvarez et al. 2001).

Two measures of variation were used: i) numbers of unique alleles (species-specific), and ii) gene diversity based on allele frequencies (Weir 1996).

All species except L. esculentum harbored at least one unique allele. Sixty-six of 144 alleles (46%) were found to be unique. L. chilense contained the relatively highest proportion of unique alleles (0.80) when corrected for differences in numbers of plants sampled per species. Gene diversity within SC species was lowest in L. esculentum (0.03) and highest in L. pimpinellifolium (0.20). In L. pimpinellifolium all gene diversity was partitioned among the three sampled accessions, i.e., alleles were fixed at all loci within accessions of this species. For the SI species, gene diversity ranged from 0.24 in L. pennellii to 0.57 in southern representatives of L. peruvianum. In contrast to estimates based on RAPDs, SSR
markers showed northerly accessions of L. peruvianum contain less diversity than southerly accessions (Egashira et al. 2000).

Effects of mating system and recombination on intraspecific DNA sequence polymorphism were studied by comparing L. chilense, L. hirsutum, L. peruvianum, L. chmielewskii, and L. pimpinellifolium (Baudry et al. 2001).

Five plants per species were sampled, except for L. hirsutum (three plants), and sequenced at five single-copy genes in chromosomal regions with either high (2.33 x 10^{-8} - 2.73 x 10^{-8} per site per generation) or low (0.00 - 0.46 x 10^{-8} per site per generation) rates of recombination. More than 8 kb of DNA was sequenced per plant, including four anonymous, single-copy cDNA markers (Tanksley et al. 1992) CT208, CT251, CT268, CT143, and the sucrose accumulator gene sucr. Intraspecific polymorphism was estimated as $\pi$ for non-coding nucleotide sites (Nei 1987). Mating system was found to have a significant effect on polymorphism. L. pimpinellifolium and L. chmielewskii had on an average approximately 4 and 40-fold less polymorphism than L. hirsutum, the least polymorphic of the SI species. L. chmielewskii was the least variable species, with all
estimates of \( \theta \) equalling zero with the exception of CT143, where \( \theta \) was less than 0.01. L. peruvianum was the most polymorphic species, with \( \theta \) values ranging from approximately 0.01 to greater than 0.03 across loci. In addition, a high proportion (14% - 40% across loci) of fixed differences among the other four species were observed as polymorphisms within L. peruvianum. This may represent lineage sorting of alleles among species and imply that L. peruvianum is representative of the ancestral species from which other species were derived. Although reduced recombination rate has been found to be significantly correlated with lower polymorphism in other species (e.g., Drosophila, Aquadro et al. 1994), in this study recombination and polymorphism were only weakly positively correlated.

Theta estimates for L. esculentum across four loci (fw2.2 5’ UTR, Adh2, TG10, and TG11) ranged from 0.0016 - 0.0048 (Nesbitt and Tanksley 2002).

These estimates largely reflected polymorphism within L. esculentum var. cerasiforme. Four modern cultigens of L. esculentum var. esculentum included in this study contained only one polymorphic site in more than 7 kb of total sequence. The high degree of monomorphism within
L. esculentum var. esculentum has likely resulted from severe bottlenecks.

However, polymorphism should be higher within regions of the genome containing introgressed loci from wild relatives (Miller and Tanksley 1990).

To summarize, selfing species of Lycopersicon contain significantly less genetic variation relative to outcrossing species. The reduction in variation exceeds the predicted 50% reduction that could be explained by mating system alone. Similar observations have been made in other plant genera (Savolainen et al. 1999, Liu et al. 1999). Additional factors such as founder events, fluctuating population size, population substructure, selection, and linkage must be better understood in order to explain relative levels of genetic variation observed within Lycopersicon species (Baudry et al. 2001).

CHARACTERIZATION AND EVALUATION

Characterization and evaluation of germplasm of tomato is essential to promote its utilization for crop improvement. The CGC for tomato has identified a number of biotic and abiotic stresses, as well as quality traits as having high priority for evaluation to provide sources for use in improvement programs on tomato (Table 2.13). High priorities have been
established for screening disease resistance for verticillium wilt race 2,
bacterial canker, geminiviruses, pepino mosaic virus, spotted wilt, and
bacterial spot. High priorities for screening for insect resistances have been
established for silverleaf whitefly and nematodes. Priority has also been
established for abiotic stresses such as heat and cold. A number of quality
traits have been prioritized, especially for soluble solids, flavor, and color.
An international descriptor list has been established for Lycopersicon
spp. (IPGRI 1996). This list has three major types of descriptors: passport,
Table 2.13 Tomato problems where genetic improvements would benefit U.S. production.

Type Priority Description

DISEASES High Verticillium wilt race 2 Bacterial canker TLYCV & other geminiviruses Pepino mosaic virus Spotted wilt Bacterial spot Medium Late blight Corky root Phytophthora root rot Fruit rots CMV Beet curly top virus Low Bacterial speck race 2 PVY Target spot Powdery mildew

INSECTS & PESTS (new screening protocols important) High Silverleaf whitefly Nematodes, heat stable Medium Aphids

ABIOTIC STRESSES High Cold tolerance Medium Heat tolerance Salinity tolerance Color disorders

HORTICULTURAL High Soluble solids Flavor (need to define components) Medium Antioxidants/nutritional content Color Sugar type Peelability/dicing Low Pectin chemistry Blossom-end smoothness

coloration and evaluation descriptors. Also included are manage
ment descriptors and environment and site descriptors.

The passport descriptors provide the basic identification of the accession which includes the genebank’s accession number along with the other identifiers associated with the accession, such as collection number and/or or other institution identifier numbers. If the accession is a variety or cultivar, name and if available, pedigree and breeding method are included in passport descriptors. The taxonomic classification of the accession is also included in the passport descriptors. The other passport descriptors provide information on where an accession was collected, including political and eco-geographic information about the collection site. Eco-geographic information often is very useful in selecting accessions, especially when little or no characterization or evaluation data are available. Management descriptors associated with an accession provide information about seed availability, viability, etc., but are usually not publicly available.

Characterization and evaluation descriptors are often publicly available and provide information to aid users in selecting accessions for use in crop improvement programs. Characterization descriptors have high
heritability and are usually only recorded in one environment and include descriptors such as plant type, leaf type, fruit shape, fruit color, among many others. Evaluation descriptors are more detailed and are often replicated over environments. Important evaluation descriptors include biotic and abiotic stress resistance and/or tolerance, quality descriptors such as soluble solids and fruit pH. Other descriptors, especially with evaluation traits, are provided which describe the environment and site where the evaluation was conducted, methodologies used, and person(s) who conducted the research. Examples of these would be the geographical coordinates of the evaluation site, the soil type, weather conditions during the season of the evaluation (temperatures, rainfall), and dates of planting and harvest. This type of data aids in interpretation of many evaluation descriptors.

The United States tomato CGC has established a minimal descriptor list for tomato (Table 2.14). This list was decided in order to provide a guideline for the genebank of which descriptors in the overall list were felt to be of high importance in making selections of germplasm accessions. This was
to facilitate provision of a manageable number of descriptors to record during regeneration, since the IPGRI list includes a number of morphological descriptors, many of which are species specific. The minimal descriptor list includes plant descriptors, fruit descriptors, and chemical composition. Table 2.14 Minimal descriptor list for tomato.

I. Plant characteristics


D. Flowers per inflorescences 3. Low 5. Medium 7. High

E. Type of inflorescence 1. Simple 2. Forked 3. Compound

F. Number of fruit set Recorded on second truss

G. Number of days to maturity From sowing until 50% of the plants have at least one fruit ripened

II. Fruit Descriptors

A. Exterior color of immature fruit 1. Light green 2. Medium green (Apple) 3. Dark green (hp, dg)


I. Fruit weight (g, average of 10 fruits) Checks [Sweet 100] [Red Cherry Large] [Roma or New Yorker] [Flora-Dade] [Tropic] [Florida 7060]


L. Nippled fruit [mature fruits] 1. Absent
   (Contd.) (Contd.) 2. Present 3. Inverted

M. Presence/absence of jointless pedicel 1. Absent 2. Present 3. Arthritic (not complete)


P. Concentric cracking 0. Absent 3. Slight 5. Intermediate 7. Severe


III. Chemical composition

A. Soluble solids Measured with refractometer from 4 fruits
   (Contd.)

UTILIZATION

The major resource of tomato germplasm for crop improvement in the past 20 years has been the use of the wild species as sources of disease and
insect resistance, and for improvement of quality traits (Rick and Chetelat 1995). This is because tomato being a self-pollinated crop has germplasm strongly reduced in variability for domestication and breeding. A thorough summarization of the use of related wild tomato species for crop improvement of tomato through 1995 (with literature citations) has been provided by Rick and Chetelat (1995). Introgression of many disease resistant genes into cultivars has been accomplished through the identification of linked molecular markers (Table 2.15; Causse et al. 2000, Grube et al. 2000). Resistance and/or tolerance has been transferred from wild species of Lycopersicon for bacterial, fungal, nematode, viral diseases, and for resistance to parasitic plants (broomrape and dodder). Wild species have also been used as sources of tolerances of abiotic stresses and for improvement of quality traits. Resistance and/or tolerance has also been transferred for insect pests namely, Coleoptera, Diptera, Homoptera, Lepidoptera, and Acarina arthropods. L. pennellii has been found to be a Genetic Resources of Tomato (Lycopersicon esculentum Mill.) and Wild Relatives 65 Chr Locus Pathogen Source Reference
1 Cf-4 Cladosporium fulvum L. hirsutum 3833 (Univ. of Toronto) a Haanstra et al. (2000), Kerr and Bailey (1964)

1 Cf-9 Cladosporium fulvum L. pimpinellifolium PI 126915 Haanstra et al. (1999)

2 Tm-1 TMV L. pimpinellifolium, L. hirsutum, Levesque et al. (1990), L. peruvianum, L. chilense Pelham (1966), Holmes (1954), Frazier and Dennett (1949), Kikuta and Frazier (1947)


4 Hero Globodera rostochiensis L. pimpinellifolium B6173 (LA0121) Sobczak et al. (2005), Ellis and MaxonSmith (1971)


6 Mi Meloidogyne spp., Macrosiphum L. peruvianum PI 128657 Yaghoobi et al. et al. (1995) (Meu-1) euphorbiae

6 Ty-1 TYLCV L. chilense LA 1969 Zamir et al. (1994)

6 Cf-2 Cladosporium fulvum L. pimpinellifolium PI 370093 Jones et al. (1992), Kerr et al. (1980), Pitbaldo and Kerr (1980), Langford (1937)

6 Cf-5 Cladosporium fulvum L. esculentum x L. pimpinellifolium Dixon et al. (1998), PI 187002 Dickinson et al. (1993), Kerr et al. (1971)

7 I-1 Fusarium oxysporum f. sp. lycopersici L. pennellii PI 414773 Scott et al. (2004) (Contd.) 6 Genetic Improvement of Solanaceous Crops: Tomato

7 I-3 Fusarium oxysporum f. sp. lycopersici L. pennellii PI 414773 Sarfatti et al. (1989)

7 Ph-1 Phytophera infestans L. esculentum var. cerasiforme Chunwongse et al. (1998), PI 108245 Clayberg et al. (1965), Clayberg et al. (1959), Gallegly and Marvel (1955)

9 Ve Verticillium dahliae Peru Wild (synonymous to Utah 665) Kauchuk et al. (1998), PI 303801 Schaible et al. (1951)
9 Ph-3 Phytopthera infestans L. pimpinellifolium L3708 (AVDRC) Chunwongse et al. (1998)

9 Tm-2 a TMV L. peruvianum PI 128650 Young et al. (1988)


9 Sw-5 TSWV L. peruvianum b Roselló et al. (1998), Stevens et al. (1992), Stevens (1964)

10 Ph-2 Phytopthera infestans L. pimpinellifolium WVa 700 Moreau et al. (1998)

11 Sm Stemphylium spp. L. pimpinellifolium PI 79532 Behare et al. (1991)

11 I Fusarium oxysporum f. sp. lycopersici L. pimpinellifolium PI 79532 Sarfatti et al. (1989)

11 I-2 Fusarium oxysporum f. sp. lycopersici L. pimpinellifolium L. esculentum Sarfatti et al. (1989) PI 126915

12 Mi-3 Meloidogyne incognita, M. javanica L. peruvianum PI 126443 Yaghoobi et al. (1995)

12 Lv Leveillula taurica L. chilense LA 1969 Chunwongse et al. (1994)

a

Kerr and Bailey (1964) reported that this accession was lost.

b

Stevens et al. (1992) reported that the identity of the original PI number was lost after the death of J.M. Stevens but list PI 126928, PI 126929, PI 126944, PI 128645, PI 128654, PI 129109 and two L. peruvianum var. dentatum accessions with unknown PI numbers as potential sources based on his breeding records.

promising source for drought tolerance and salt tolerance.
The work on fruit quality has concentrated on increasing soluble solids content. Higher levels of soluble solids content have been discovered in L. cheesmanii, L. chmielewskii, and L. hirsutum.

A number of studies have identified QTLs in wild species of Lycopersicon that provide improvement in the cultivated tomato for horticultural traits.

QTLs associated with horticultural yield in L. pennellii and L. hirsutum were identified by Eshed et al. (1996) and Bernacchi et al. (1998a). L. hirsutum alleles were found that gave a 16% increase in total yield and a 20% improvement was achieved by combining introgressions from L. pennellii.

Introgression lines have been used for mapping of QTL for improved fruit characteristics in L. chmielewskii (Frary et al. 2003) and for yield associated QTL using L. pennellii (Eshed and Zamir 1995). Materials developed showed promise for improvement of cultivated tomato. Genetic gains from introgressions for desirable wild QTL-alleles from L. hirsutum and L. pimpinellifolium for quality traits such as fruit firmness, soluble solids content, and brix X red yield have been reported (Bernacchi et al. 1998b).

SUMMARY
Cultivated tomato (Lycopersicon esculentum Mill.) is an important vegetable, with a worldwide area of 4 million hectares and a production of 108.5 million tons. Tomato cultivation area has increased by 38% and production has increased by 45% in the past ten years, with most of this increase in China, which has increased production from 8.5 to 25.5 million tons, propelling it to the number one tomato producer in the world. Other leading tomato producers are the United States, Turkey, India, and Egypt. In the United States, tomato accounts for 14.5% of the economic value of fresh market vegetable production and 50.7% of the economic value of processed production of vegetables.

Domestication of tomato is relatively recent, within the past 400 years. L. esculentum is thought to have originally been domesticated in maize fields by ancient Mexicans, with Peru as the center of diversity for the genus. Tomato spread to Europe in the early 1500s and thereafter became widely dispersed. Tomato has eight (nine by some authorities) related wild species relatives which are extensively utilized for crop improvement.

There are more than 75,000 accessions of tomato conserved in genebanks.
around the world, with the largest of these at AVDRC, TGRC, and the
USDA genebank at Geneva, NY. These genebanks maintain large
collections
of the wild relatives in addition to the cultigen. Several
collections are also
available of related Solanum taxa and Lycopersicon
introgression
populations. While cultivated tomato is self-pollinated, the other taxa
provide a mixture from self-pollinated to obligate
cross-pollinated, with
self compatibility and self-incompatibility. This has led
to development of
methodologies and standards for maintenance of tomato
germplasm to
minimize the effects of genetic drift, mutation, and
selection.

The cultivated tomato has undergone a narrowing of the
germplasm
base caused by genetic bottlenecks and selection. The major
utilization of
tomato germplasm for crop improvement in the past 20 years
has been the
use of wild species as sources of genetic variation. This
has led to a major
utilization of wild species introgressions which have
resulted in an in
crease in molecular genetic variation in and around regions
that have been
introgressed from wild species. Wild species have been used
as sources of
variation for disease and insect resistances and/or
tolerances, abiotic stress
tolerances, and for fruit quality.


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INTRODUCTION

As an experimental organism, tomato presents a number of genetic, bio
tological, and economic advantages that have led to its development as a
model for cytogenetic and evolutionary studies. The cultivated tomato,
Lycopersicon esculentum (= Solanum lycopersicum), and related wild species
traditionally classified as genus Lycopersicon, more recently as Solanum sect.

Lycopersicon (Spooner et al. 2005) are diploids, with a chromosome number
of 2n=2x=24. Eleven of the 12 chromosomes in the haploid tomato nucleus
are metacentric or submetacentric (Lapitan et al. 1989). The exception, chro
mosome 2, is acrocentric with a heterochromatic short arm consisting
primarily of the nucleolus organizing region (NOR). During late prophase
of meiosis (diakinesis), only chromosome 2 can be distinguished from the
others, by virtue of its association with the nucleolus. However, in early
prophase (pachytene), each of the 12 chromosomes can be identified by the
position of the centromere, the length of chromatic and achromatic seg
ments, and the pattern of heterochromatic knobs (chromonemes) (Khush
1963). These features are illustrated in corresponding
cytological maps for each chromosome in the set (Rick and Butler 1956, Khush and Rick 1968).

The tomato genome is also well defined by genetic maps based on morphological and molecular markers. High density molecular marker maps based on RFLP, SSR, or AFLP markers are available ( Tanksley et al. 1992, Pillen et al. 1996, Haanstra et al. 1999, Frary et al. 2005). Estimates for total map length are ~1200-1300 cM. The relatively low haploid DNA content of tomato, ca. 950 Mbp or 0.95 pg per C (Arumuganathan and Earle 1991, Michaelson et al. 1991), makes it well suited for molecular studies. Though larger than Arabidopsis or rice (~125 and 425 Mbp, respectively), the tomato genome is smaller than many other model plant species, such as maize or wheat (~2,500 and 16,000 Mbp, respectively). The average ratio of physical to genetic distance is ~750 kb/cM, a value low enough to make positional cloning of genes practical in most genomic regions. Furthermore, recombination is essentially limited to the euchromatic regions, which constitute less than one fourth of the total DNA content (see below). The tomato genome encodes a total of ~35,000 genes, which are located
primarily in euchromatin (Van der Hoeven et al. 2002). Therefore, recombination rates per unit physical distance are substantially higher within the genetically active fraction than in the genome as a whole.

In comparison to other crop plants, many aspects of growth and development in tomato have been beneficial to cytogenetic studies. It is naturally self-pollinated, which simplifies the maintenance of stocks, yet hybridizations are easy to perform and yield large quantities of seed of controlled parentage. Tomato can be grown under a wide range of environmental conditions and propagated through seed or asexually via rooted cuttings. Its photoperiodic insensitivity and relatively short generation time permit the propagation of up to 3 generations per year. The structure of the tomato plant, particularly its compound leaves and sympodial growth habit, allows detection of an enormous array of hereditary variations, such as altered growth habit, leaf shape, texture and color, flower morphology, color and function, and fruit size, shape and color, among others. Tomato also provides a popular model for physiological and biochemical studies of fruit development, quality, and ripening.
As a result of its economic importance as a crop, industry-sponsored research on tomato contributed to early advances in genetics and wide hybridizations. A large number of mutants, aneuploids, and various other spontaneous genetic defects were discovered in growers' fields (Rick 1945).

The relative ease with which it can be transformed by Agrobacterium tumefaciens (Fillatti et al. 1987) has made tomato a popular organism for testing biotechnological approaches to enhancing fruit quality and other traits. As a result of these and other advantages, the first genetically engineered (GMO) food plant marketed in the USA was a tomato (Bruening and Lyons 2000).

Research on tomato has depended to a large extent on genetic resources such as mutants, cytogenetic stocks, and wild species populations (Chetelat 2005). Tomato germplasm is preserved at genebanks such as the C.M. Rick Tomato Genetics Resource Center (TGRC) and the USDA’s Plant Genetic Resources Unit (PGRU), which provide seed samples to interested researchers worldwide. The TGRC, located at Univ. of California, Davis, maintains over 1,000 monogenic stocks, consisting of spontaneous or
induced mutations at 600+ loci affecting most aspects of plant development

and morphology (http://tgrc.ucdavis.edu). Over 1,400 other genetic stocks

are also available through the TGRC, including several types of trisomics

(primary, secondary, tertiary, and compensating), as well as autotetraploids,

and derivatives of wild species such as alien additions, substitutions, and

introgression lines. Lastly, the TGRC maintains over 1,100 wild species

accessions, representing 13-20 species of Lycopersicon and related Solanum
taxa collected in their native regions. The PGRU, located in Geneva, New York, maintains a large collection of open-pollinated cultivars, as well as

accessions of several wild species (http://www.ars-grin.gov/). The Hebrew University of Jerusalem maintains a large population of mutants useful for

the analysis of gene function (http://www.zamir.sgn.cornell.edu/mutants/). Together, these genetic resources provide important tools for

analysis of the tomato genome.

The present chapter summarizes and integrates recent advances in the
cytogenetics and evolution of tomato, with emphasis on applications in

genetics and breeding. The following sections describe novel genetic
resources and their uses, genetic maps of the tomato genome, meiotic behavior of wide hybrids, and chromosome structure and evolution. Related topics covered by 'previous' reviews include: genome mapping (Pillen et al. 1996), classical and molecular genetics (Rick and Yoder 1988), genetics and breeding (Stevens and Rick 1986, DeVerne and Paterson 1991), and other aspects of tomato cytogenetics (Gill 1983, Quiros 1991). The early literature in this field was summarized in comprehensive reviews by Rick and Khush (1966) and Rick and Butler (1956).

SOURCES OF VARIATION

Hyper- and Hypoploidy

A rich assortment of hyper- and hypoploid stocks were identified in early work on tomato cytogenetics (Khush 1973). Haploids, monosomics, and segmental deficiencies were induced by irradiation of mature pollen (i.e. post-gametogenesis) and identified by pollination of recessive marker stocks using the pseudo-dominant method (Khush and Rick 1968). However, none could be reliably propagated, sexually or vegetatively, and all are now extinct. Haploids of tomato do not set seed because they rarely produce viable gametes. Segmental deletions and monosomics cannot
be maintained

because the gametes with deficiencies are inviable and thus do not trans

mit to the next generation. Monosomics produce nullisomic gametes (e.g. n-1, n-2, etc), which are eliminated during development of the male or female gametophytes. The only primary monosomic recovered was for chro

mosome 11, which is one of the shortest chromosomes of the set (Rick and Khush 1961). Although several tertiary monosomics were recovered, none were transmissible to the next generation (Khush and Rick 1966). These results are consistent with the presumed diploid nature of the tomato genome, which, lacking any large scale duplications, does not tolerate deficiencies at the gametophytic stage.

In contrast, hyperploidy is tolerated to a much greater extent, and both triploids and tetraploids are relatively fertile. Spontaneous autotriploids were the most common type of unfruitful ('bull') tomatoes in commercial fields in California (Rick 1945). Pollination of triploids provided useful variants, particularly the trisomics (Khush 1973). However, like the hy

ploids, the triploids are genetically unstable since they produce unbalanced gametes, and cannot be maintained through seed.
In contrast, autotetraploids are stable, though partially sterile, and 4x stocks of several cultivars and wild species are maintained by the TGRC. Nearly all currently available tomato tetraploids were induced by colchicine treatment or were recovered as spontaneous variants in field cultures. Cultivated tomato and its related wild species are virtually all diploids, in contrast to potato, where a range of ploidy levels (e.g. 2x, 3x, 4x) are found amongst native and domesticated forms. However, there are two known instances of natural tetraploid populations, both in L. chilense (= S. chilense), one of which also happens to be the northernmost population of this species (Rick 1990).

A complete set of primary trisomics, as well as many secondary, tertiary, and compensating trisomics have been produced in tomato (Khush 1973). The first linkage maps, based on morphological markers, were associated with individual pachytene chromosomes by trisomic segregation analysis. Though no longer the most efficient method for placement of mutant loci on chromosomes—linkage tester stocks are more informative—the trisomics are still useful for assigning molecular
markers to their respective chromosomes. For example, chromosomal assignment of RFLP markers was accomplished with the primary trisomics by dosage analysis of hybridization intensity (Young et al. 1987). The primary trisomics were also used to identify individual chromosomes in synaptonemal complex spreads (Sherman and Stack 1992). Secondary and tertiary trisomics have been useful for determining the positions of centromeres on the genetic map (Pillen et al. 1996).

Allopolyploid hybrids have been created in tomato as vehicles for transferring genes from certain related wild species. This has been a particularly useful strategy for the tomato-like nightshades, S. lycopersicoides and S. sitiens. In case of S. lycopersicoides, the first diploid hybrids were readily obtained by embryo culture, but were highly sterile, due in part to low rates of pairing between homeologous chromosomes (Rick 1951); in contrast, allotetraploid (amphidiploid) hybrids produced by colchicine treatment showed relatively normal meiotic behavior, with preferential pairing among homologues, and much improved fertility. Despite these initial, promising results, little if any progress was made
in breeding S.

lycopersicoides with tomato until the first allotriploid (sesquidiploid) hy

brids were reported some 35 years later (Rick et al. 1986). The sesquidiploids

eventually yielded a complete series of monosomic alien

additions (2n+1),

each containing a single extra S. lycopersicoides chromosome in the back

ground of L. esculentum (Chetelat et al. 1998). Like the

primary trisomics,

transmission rates and fertility vary widely among the

individual mono

sonic additions. Nonetheless, they are generally fertile enough to be

sexually propagated. A limited series of diploid

substitution lines, het

erozygous for a single S. lycopersicoides chromosome, were

also derived (Ji

and Chetelat 2002).

Allopolyploid hybrids representing the genome of S. sitiens

(syn. S. rickii)

have been derived in similar, though more circuitous,

fashion. Although S.

sitiens has not been successfully hybridized with

cultivated tomato, it does

cross readily with S. lycopersicoides, reflecting a close

genetic affinity between

these species (Rick 1979, 1988b). As a result, S. sitiens is also cross

compatible with the previously synthesized L. esculentum-S.

lycopersicoides

sesquidiploids (DeVerna et al. 1990). Since the extra S.
lycopersicoides

chromosomes tend to be eliminated in the progeny of the sesquidiploid, it

serves as a convenient donor of the L. esculentum genome (i.e. acts as a bridging genotype). The resulting diploid F₁ L. esculentum × S. sitiens hybrids

are highly sterile, but chromosome doubling with colchicine produces more fertile amphidiploids, from which a few monosomic additions, substitutions, and recombinant diploids have been obtained (Pertuze et al. 2003).

Introgression

The wild tomato species are potentially rich sources of allelic variation for genetic studies and for cultivar development following introgression.

Crosses between the cultivated tomato and all but two of its wild relatives are feasible, although the ease of hybridizations varies greatly. In contrast to the cultigen, whose early history of domestication and breeding led to severe depletion of its genetic variation, the wild species are far more diverse. Populations of the self-incompatible species, such as L. peruvianum (= S. peruvianum), are especially heterogeneous, containing more variation within a single accession than all accessions of any one of the self-compat
ible species, including L. esculentum (Miller and Tanksley 1990). In addi-
tion, genetic variation within populations of L. hirsutum
(= S. habrochaites)
and L. pimpinellifolium (= S. pimpinellifolium) varies
according to geographic
location, with populations from the center of each species’
range containing more diversity than those collected at the northern or
southern limits
(Rick et al. 1977, 1979). Considering that the TGRC alone
maintains over
1,100 wild species populations, nearly all of which are
cross-compatible
with tomato, they represent an amazingly rich and
accessible source of
genetic variation.
At the phenotypic level, variation is sometimes apparent
only in backcross
derivatives of interspecific hybrids. In the study of
quantitative characters,
for example, backcross populations sometimes display
‘transgressive
variation’, in which individual genotypic combinations
produce phenotypic
values exceeding either parental extreme (for example, de
Vicente and
Tanksley 1993). A similar phenomenon has been observed for
qualitative
characters, in which mutant phenotypes not expressed by
either parent
may appear in progeny of wide hybrids (Rick 1967). Such
‘novel variation’
may arise from a number of sources, including genic or plasmatic interactions, latent variation in the wild species, and de novo mutation.

For example, the B gene for high β-carotene is present in all of the green fruited species but expressed only in the genetic background of L. esculentum, suggesting an interaction between genes determines carotenoid accumulation. Another type of interaction, between the nuclear and cytoplasmic genomes, appears to control expression of cytoplasmic male sterility (CMS) in tomato, as it is only observed when cytoplasm of L. esculentum is transferred into the nuclear genome of wild species such as L. pennellii (= S. pennellii). Latent variation (i.e. residual heterozygosity) in the wild species can also account for novel traits, such as the old gold mutant (B og) from L. chilense.

The wild species have also been the main sources of marker polymorphisms required for development of high density genetic maps. Using DNA based markers such as RFLPs and RAPDs, there is generally very little variation detectable within or between L. esculentum varieties or landraces (Miller and Tanksley 1990, Villand et al. 1998, Williams and St. Clair 1993).
In contrast, the wild species can be highly variable. For example, a single
inbred accession of the wild species L. pennellii was polymorphic relative
to cultivated tomato for ~71% of AFLP markers (Haanstra et al. 1999).

Approximately 81% of RFLPs (probe x RE combinations) were informative
in S. lycopersicoides vs. tomato (Chetelat et al. 2000).

Mapping populations used for the construction of molecular
marker maps in tomato include conventional interspecific F 2 or
BC populations.

Such segregating populations are usually difficult to propagate indefinitely,
unless they can be immortalized by tissue culture or other means. Since
each unique progeny array is ephemeral, they do not provide an optimal,
long term mapping resource. A recombinant inbred line (RIL) population,
such as one derived from L. esculentum x L. cheesmanii f. minor (= S.
galapagense), is a more permanent resource and provides greater map
resolution than the corresponding F 2 (Paran et al. 1995).

However, some of the L. cheesmanii RILs had a higher than expected degree of
residual heterozygosity and/or reduced fertility. Another type of permanent
mapping resource, and one that has been pioneered in tomato, is the
introgression line (IL) population. ILs consist of overlapping homozygous chromosome segments introgressed from a wild donor genome into a constant genetic background, in this case, of cultivated tomato. A set of 50 ILs contains an entire L. pennellii genome bred into L. esculentum, the first such population synthesized for tomato (Eshed and Zamir 1995), with an additional 26 sublines providing increased map resolution (Pan et al. 2000).

Similar types of prebred lines have been created for L. hirsutum (Monforte and Tanksley 2000a), L. pimpinellifolium (Doganlar et al. 2002), and S. lycopersicoides (Canady et al. 2005). ILs have a number of advantages for fine mapping of QTLs, gene identification, and related breeding applications (see reviews by Zamir and Eshed 1998; Zamir 2001). Furthermore, ILs tend to have greater viability and fertility than corresponding RILs from the same interspecific hybrids, due to the more limited contribution of wild species genome in each IL. As a result, they provide a more realistic genetic background for evaluation of vegetative and reproductive characters.

However, a permanent ‘reference’ mapping population that offers a high level of linkage resolution is not yet available in tomato. Towards this goal,
Vision et al. (2001) developed a backcross recombinant inbred population from the cross L. esculentum x L. pennellii. Using marker assisted selection on a large population, a subset of individual genotypes were chosen to provide maximum map resolution for the population as a whole.

Mutation

Spontaneous and induced mutations affecting development and morphology were essential ingredients of early genetic research on tomato. Mutations provided markers for the first classical maps, for studies of segregation and recombination in wide crosses, and for integration of cytological and genetic maps. The characterization of spontaneous unfruitful (‘bull’) plants occurring in field plantings led to the identification of a large number of useful variants, including male-sterile mutants, trisomics, tetraploids, triploids, haploids, and meiotic defects (Rick 1945).

The number of mutants described in tomato increased steadily as a result of these and other studies. The Tomato Genetics Cooperative (TGC) was established by C.M. Rick and associates in 1950 to promote exchange of information and germplasm amongst geneticists, and to coordinate linkage
studies (Robinson 1982). First published in 1951, the TGC Report evidenced
the accelerating pace of research on tomato genetics: new mutants were
described, segregation, allelism and linkage tests reported, and lists of
available stocks issued. Large-scale mutagenesis studies, in particular those
of Hans Stubbe from Gatersleben, Germany (summarized in Stubbe 1972a,
1972b), greatly expanded the available collections of mutants. At the time
of the first comprehensive review (Rick and Butler 1956), only 118 mutant
loci were known (of which 56 had been mapped). Today, ~1,200 mutations
at 1,000 loci have been described, of which ~400 have been mapped or
assigned to a chromosome, and a small but growing number cloned and
sequenced. The TGRC currently maintains and distributes over 1,000
monogenic stocks with mutations at over 600 loci, affecting most aspects of
development and morphology (Chetelat 2005).
Mutagenesis studies in tomato have employed a variety of artificial
means for generating new mutations. The standard methods, widely used
in other model organisms, include treatment of seeds or pollen with
alkylating agents (mainly EMS) or radiation (primarily X-rays and fast
neutrons). Additional mutagenesis strategies that have been used to a limited extent include somaclonal variation (Evans and Sharp 1983, Gavazzi et al. 1987, van den Bulk et al. 1990), and transposon tagging using the maize Ac/Ds elements (Meissner et al. 1997, 2000). Transposon tagging has several attractive features. First, the gene responsible for a mutant phenotype is readily identified by sequencing DNA on either side of the insertion site (e.g. by inverse PCR). Second, libraries of Ds insertions at different positions in the genome have been established (Knapp et al. 1994, Thomas et al. 1994), since transposition of Ds occurs preferentially to linked sites, the probability of tagging a gene in the same region is thereby increased.

Thirdly, the chance of identifying Ds insertions into target genes can be improved by 'site selected insertional mutagenesis', a combination of DNA pooling and nested PCR (Cooley et al. 1996).

Despite these improvements, there is still a need for a high throughput mutagenesis system in tomato that will allow gene isolation for functional genomics. Large-scale insertional mutagenesis by T-DNA tagging is not practical in tomato due to limitations in current Agrobacterium
transformation methods. Recently, EMS mutagenesis has been
revived by
the development of technology for identifying point
mutations. The
TILLING (Targeting Induced Local Lesions In Genomes) method
screens
pooled DNA samples from segregating populations to identify
individuals
with point mutations in a gene of interest (McCallum et al
2000). EMS
mutagenesis, which causes primarily C/G to T/A transitions,
has been a
highly effective method of generating mutants in tomato. A
population of
13,000 M2 families was generated by EMS and fast neutron
treatments in
cv. M-82, and includes 3,417 catalogued mutant phenotypes
(Menda et al.
2004). Examples of allelism with existing mutations suggest
the
M-82 mutant population is nearly saturated (i.e. likely to
contain at least
one mutation in each gene). Phenotypes and images of these
mutants are
available online (http://www.
zamir.sgn.cornell.edu/mutants/). The
tomato genome – euchromatic regions only – is currently
being sequenced
by the international ‘SOL’ initiative (http://www.sgn.cornell.edu).
Combined with existing EST databases (Van der Hoeven et al.
2002), the
increased sequence information will allow more genes to be
identified as
potential targets based on their sequence alone. Candidate
genes can also
be identified by comparison of gene or QTL locations in
tomato to sequence
information from orthologous regions in model species such
as tomato
and Arabidopsis (Ku et al. 2000, 2001).

LINKAGE MAPS

Classical Maps

The ‘classical’ linkage maps of tomato are based on the
simultaneous
segregation of multiple morphological markers, almost
always in
intraspecific (L. esculentum) crosses. Initially limited to
existing mutants of
spontaneous origin, establishment of linkage groups was
greatly facilitated
by a large influx of induced mutations. Cooperation among
TGC members
in mapping the increasing number of mutant loci was also a
key element;
for a period of time, each chromosome was ‘assigned’ to a
particular
investigator to work out its linkage relations and develop
new marker
combinations (Robinson 1982). Efficient detection of
linkage was possible
with the development of sets of chromosome-specific linkage
tester stocks,
which combined multiple markers on a single chromosome (see
Chetelat
and Petersen 2003). Additional linkage tester stocks
combined two strategically situated markers on each of two chromosomes, so that in theory a maximum of six segregation tests would be required to detect linkage anywhere in the genome. The number of morphological markers that can be simultaneously and independently genotyped in this fashion is in many cases severely limited by their phenotypic effects. Problems frequently encountered are epistasis among genes controlling related traits, sterility or inviability of multiple marker combinations, and lack of ‘good’ markers (e.g. seedling stage expression) for certain genomic regions. In addition, linkage tests of new morphological markers typically segregate in repulsion phase with respect to the tester combination, which limits precision of recombination fraction estimates for recessive genes. While coupling phase linkage tests, particularly testcrosses, provided greater precision, they require the prior synthesis of new marker combinations (i.e. recombinant genotypes), which becomes limiting with more than just a few markers per chromosome. These factors limited saturation and resolution of the classical linkage map of tomato, as in other organisms. Isozyme markers were
integrated with the mutant loci beginning in the 1970’s (Tanksley and Rick 1980). Due to their neutral phenotypes and codominant expression, allozymes overcame many of the limitations of morphological mutants as genetic markers. However, available enzyme staining technology restricted the number of protein markers, and only a few have been added to the map recently (Bernatzky and Tanksley 1986, Chetelat et al. 2000).

At the present time, approximately 400 morphological and isozyme markers have been at least assigned to their respective chromosomes, and the majority have also been positioned within their linkage groups. The last comprehensive linkage summaries are now 19+ years old (Mutschler et al. 1987, Stevens and Rick 1986), and sorely in need of revision. Fortunately, the classical maps for several chromosomes have been updated, usually as a result of integration with molecular markers. These include chromosome 1 (Balint-Kurti et al. 1995, van Tuinen et al. 1997), chromosome 3 (Koorneef et al. 1993, van der Biezen et al. 1994), chromosome 6 (van Wordragen et al. 1996, Weide et al. 1993), chromosome 7 (Burbidge et al. 2001), chromosome 10 (van Tuinen et al. 1997), and chromosome 11 (van Tuinen et al. 1998).
Molecular Marker Maps

The development of molecular linkage maps of the tomato genome based on DNA markers provided many advantages over the existing classical maps. Due to the low level of polymorphism detectable within L. esculentum using DNA markers, the molecular linkage maps are based on segregation and recombination in interspecific crosses. F2 progeny from the cross L. esculentum x L. pennellii have been favored for this purpose because of their relative ease of hybridization, the fertility and normal meiotic behavior of F1 hybrids, and the high polymorphism rate that the distinguishes these two species. Because the number of DNA marker loci is not limiting, the molecular linkage maps have high marker density and good saturation of linkage groups. The framework map, based on RFLP markers, contains over 1000 loci, with an average distance between markers of only 1.2 cM (Pillen et al. 1996, Tanksley et al. 1992). This map is also populated with many genes of known function or phenotype, including morphological markers, isozyme loci, and cloned genes. Additionally, the approximate locations of centromeres have been determined for each linkage group.
(Pillen et al. 1996), providing anchor points to the
cytological maps.

In addition to RFLP markers, a number of other DNA marker
systems

have been used to create linkage maps in tomato, with
varying success.

AFLPs provide thousands of polymorphic bands, and were used
to generate

an ultra-dense genetic map of tomato, consisting of over
1200 markers

(Haanstra et al. 1999, Spooner et al. 2005). Marker
distribution along the

chromosomes was decidedly nonrandom, with the majority of
AFLPs

(particularly the EcoRI-MseI derived markers) tightly
clustered in the

centromeric regions. AFLPs may thereby provide markers for
genomic

regions less readily detected by RFLPs (Bonnema et al.
2002). RAPD and

SSR markers also map primarily to proximal regions of
chromosomes,

although SSRs identified in EST sequences are more randomly
dispersed

(Grandillo and Tanksley 1996, Areshchenkova and Ganal 1999,
2002).

Despite the greater time and expense of applying RFLP
markers, they have

the significant advantage of providing multiallelic genetic
probes that can

be compared across species and populations. This makes
RFLPs ideally

suited for mapping in tomato, with its rich collection of
wild relatives,

which provide not only abundant marker variation, but also

many traits of

interest to breeders. The availability of a set of

reference maps and

corresponding RFLP markers have provided the genetic tools
to expedite a

vast array of genetic studies and plant breeding

applications in tomato too

numerous to summarize here.

An RFLP linkage map has been developed for tomato using

conserved

ortholog set (COS) markers (Fulton et al. 2002). These

represent ESTs that

are single or low copy in the tomato genome, and have a

high degree of

homology to a single ortholog in Arabidopsis, as determined

by sequence

comparisons. Over 1000 COS markers were identified, of

which ~550 have

been mapped. The COS map is anchored to previous maps with

a large

number of conventional RFLPs, and includes SSR loci

identified within

the ESTs sequences. Map resolution has been improved by

increasing popu

lation size, and total map length is substantially

increased over the origi

nal molecular map based on RFLPs. Many RFLPS have been

converted to

CAPS (cleaved amplified polymorphic sequence) markers

(Frary et al. 2005).

Together with SSRs, these provide a set of framework
PCR-based markers.

Current versions of these maps, as well as sequence databases, are available through the Solanaceae Genomics Network (SGN) (http://www.sgn.cornell.edu).

Rates of recombination within a given marker interval may vary greatly in tomato due to the influence of several factors. Recombination is generally higher in female than in male gametes (de Vicente and Tanksley 1991, van Oijen et al. 1994, Ganal and Tanksley 1996). Recombination is also elevated in progeny of F 1 interspecific hybrids relative to advanced backcross generations (Rick 1969, 1971), and higher in whole chromosomes than in introgressed segments (Paterson et al. 1990, van Wordragen et al. 1996, Chetelat and Meglic 2000, Monforte and Tanksley 2000b, Ji and Chetelat 2002). In addition, recombination rates vary according to species divergence. For example, recombination in intraspecific L. peruvianum crosses was higher (10% on average) than interspecific L. esculentum x L. pennellii (van Oijen et al. 1994). Similarly, recombination in L. esculentum x S. lycopersicoides was ca. 30% lower on average than in L. esculentum x L. pennellii (Chetelat et al. 2000). Finally, reduced recombination is observed
between markers in the centromeric regions, as a result of which marker density (in genetic terms) is higher (Tanksley et al. 1992, see below).

Comparative Maps of Lycopersicon and Related Solanum Species

Comparisons of genetic maps from inter- and intraspecific crosses involving Lycopersicon spp. indicate nearly complete colinearity between them.

Comparative maps of the following species have been developed from interspecific crosses to L. esculentum: L. pimpinellifolium (Grandillo and Tanksley 1996), L. cheesmanii f. minor (Paran et al. 1995), L. chmielewskii (= S. chmielewskii) (Paterson et al. 1990), L. pennellii (Tanksley et al. 1992), L. hirsutum (Bernacchi and Tanksley 1997), L. parviflorum (= S. neorickii) (Fulton et al. 2000), and L. peruvianum (van Oijen et al. 1994, Fulton et al. 1997).

Although there were significant differences among these maps for total genetic length and/or recombination rates in specific marker intervals, they were all essentially consistent with the framework map in terms of marker order along each chromosome. One noteworthy exception is a region on chromosome 7 which is inverted in L. pennellii relative to L. esculentum (Van der Knaap et al. 2004). The otherwise
strong conservation of gene order indicated by these comparative maps is consistent with observations of normal chromosome pairing and fertility in most F1 interspecific hybrids between Lycopersicon species. The genomes of all species in the Lycopersicon clade can therefore be considered essentially colinear and homologous.

In contrast, genetic maps of the S. lycopersicoides and S. sitiens genomes indicate these species have a different genome structure. A genetic map based on BC1 L. esculentum x S. lycopersicoides showed a genome-wide reduction in recombination of about 30% compared to interspecific Lycopersicon maps (Chetelat et al. 2000). No recombination could be detected on the long arm of chromosome 10, suggesting the intergeneric F1 was heterozygous for a structural rearrangement in this region. Following introgression of individual chromosomes into L. esculentum, recombination between markers on S. lycopersicoides 10L remained undetectable (Ji and Chetelat 2002). To determine the gene order of S. lycopersicoides chromosome 10, recombination between homologous chromosomes would be required. A map based on S. sitiens x S. lycopersicoides is
ideal for this purpose, since the two nightshades are closely related and cross-compatible (i.e. their genomes are homologous), yet present a higher rate of marker polymorphism than intraspecific populations of either species. Results of this map showed colinearity with Lycopersicon for all regions of the genome, except 10L, where a paracentric inversion distinguishes the two groups (Pertuze et al. 2002). The location of this inversion explains the absence of recombination between L. esculentum and S. lycopersicoides chromosomes in this region.

Furthermore, the breakpoint of this inversion appears to be identical to the one described for chromosome 10L in cultivated potato (S. tuberosum), one of five such rearrangements that distinguish it from tomato (Bonierbale et al. 1988, Tanksley et al. 1992). S. lycopersicoides and S. sitiens have the same marker order on this chromosome as potato, a configuration that is also observed in pepper (Capsicum) and eggplant (S. melongena) (Livingstone et al. 1999, Doganlar et al. 2002). Given the close relationship between Lycopersicon and the much larger Solanum genus (Spooner et al. 2005), the potato/eggplant/pepper arrangement must be ancestral and
the tomato

inversion derived. The presence of the potato arrangement in S. sitiens and
S. lycopersicoides, which are among the closest relatives of tomato outside

the Lycopersicon group, supports this hypothesis. Furthermore, the observed
colinearity between tomato, S. lycopersicoides and S. sitiens, in the regions of

the other four potato rearrangements suggests that the 10L inversion
evolved most recently, presumably coinciding with divergence of

Lycopersicon from a Solanum ancestor. As such, the 10L inversion is a cyto
taxonomic marker for the Lycopersicon genome. These two

basic genomes,
designated L and S (Fig. 3.1), appear to be the only large scale differences

in chromosome structure separating tomato from any of the species with

which it is cross-compatible. In comparison, five basic genomes have been

postulated for the cultivated potato (A genome) and related Solanum spe
cies (B, C, D, and E) on the basis of chromosome pairing and fertility in

hybrids between them (Matsubayashi 1991).

MEIOSIS IN WIDE HYBRIDS

Hybrids Between Species of Lycopersicon

As mentioned above, sexual crosses are possible between cultivated tomato
and any of the wild Lycopersicon species, although ease of hybridization
and fertility of the resulting F1’s varies greatly (Rick 1979). The
red- or orange-fruited species – L. esculentum, L. pimpinellifolium, and L.
cheesmanii (= S. cheesmaniae or S. galapagense) – can be freely intercrossed to
form highly fertile hybrids. Crosses between the red/orange and the green
fruited species generally succeed only when the former are used as female
parent (unilateral incompatibility), and hybrids are less fertile. Obtaining
hybrids with L. peruvianum or L. chilense can be more problematic: embryo/
 ovule rescue or other techniques are usually required, and F1 hybrids
between the groups are less fertile.

Despite differences in crossability and hybrid fertility, meiosis is rela
tively normal in all interspecific combinations examined to date (Rick 1979).

In each case, parental chromosomes synapse along their entire length at
pachytene, form 12 bivalents with chiasmata at diakinesis, leading to proper
alignment at metaphase and regular anaphase of the first division (Afify
1933, Lesley and Lesley 1943, McGuire and Rick 1954, Sawant 1958,
some morphology were observed between chromosomes of L. esculentum
and L. pennellii, primarily in the lengths of heterochromatic regions and


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INTRODUCTION

The phenomenon of heterosis is defined by Shull (1911) as “the superiority of heterozygous genotypes with respect to one or more characters in comparison with the corresponding homozygotes. Heterosis is the phenotypic result of gene interaction in heterozygotes and thus confined (at least in maximal amount) to that state.” Hence, to observe heterosis, the F1 must be
superior to the two breeding lines that are its parents. Nevertheless, the heterozygote may also be inferior to both homozygotes. This performance is defined as negative heterosis (Jinks 1983).

The expression of hybrid vigor in plant crosses has been recognized for nearly 250 years (Zirkle 1952). The phenomenon received higher degree of attention since the publications of Shull (1908, 1909, 1914), East (1908, 1909) and Jones (1918), which resulted in the development of the first commercial maize hybrids and their introduction in practice at about 1930 (Sprague 1983). Within the years that followed, heterosis was observed in several crops.

Heterosis is now recognized as one of the primary factors that contributed to the success of plant breeding in many crops. Duvick (1997a) reported that maize, sorghum, and sunflower were produced as hybrids in all the industrialized world; hybrid rice was grown extensively in China and was recently introduced in India; many commercial vegetables and flowers were grown almost entirely as hybrids. The application of heterosis was determined as one of the greatest achievements in the
twentieth century

(Barabas 1992) and as the single greatest applied achievement of the discipline of genetics (Griffing 1990).

Despite the numerous studies on heterosis, however, and although this phenomenon is exploited wherever possible, its biological basis remains unknown and its elucidation is still a major challenge for scientists.

Sprague (1983) mentioned two separate courses that investigations of the phenomenon of heterosis have followed. The first has been descriptive and concerned with properties, frequency and the magnitude of the observed effects. The second course centered primarily on biochemical-physiological studies and during the recent years on molecular studies.

The genetic theories advanced on this subject differ in the relative importance of overdominance, epistasis and linkage and how they contribute to hybrid performance. Up to now, none of them is acceptable to all. Since the rediscovery of Mendelism in 1900 two principal hypotheses were suggested as the genetic basis of heterosis: dominance and overdominance. Tsaftaris (1995) summarized these hypotheses as follows:

The dominance hypothesis attributes the increased vigor of heterozygosity
to dominant alleles mainly because of the observed correlation between recessiveness and detrimental effects. The overdominance hypothesis assumes that heterozygosity per se is important; i.e. there exist loci, albeit relatively rare, at which the heterozygote is superior to either homozygote.

Jinks (1954) implicated epistasis in the expression of heterosis and recently multilocus epistatic interactions are recognized as the third theory of heterosis (Allard 1996, Stuber 1999, Monforte and Tanksley 2000).

The last decade has witnessed a period of renewed interest toward resolving some of the long standing issues related to heterosis. New interest has been sparked by the application of molecular genetics. The knowledge and the experience accumulated throughout the years as well as advances in molecular genetics and technologies provided new tools and stimulated scientists' endeavors to shed additional light on heterosis. This resulted in a significant number of studies in which new aspects of the phenomenon have been evaluated, new approaches to investigate its mechanisms have been developed and new theories and hypotheses have been advanced (McDaniel 1986, Griffing 1990, Georgiev 1991, Nienhuis and
Sills 1992,

Khanna-Chopra et al. 1993, Verma and Chahal 1993, Houle
1994, Stuber
1995, David
Kafka 1998,
Goodnight
significant
contribution in this respect, however, the opinion of the
scientists
summarized by Stuber (1999) was that “the causal factors
for heterosis are
today as obscure as they were 50 years ago”. According to
Hallauer (1999)
“The exact genetic basis of heterosis may never be known
and understood
because of allelic interactions: interactions of alleles at
a locus, interactions
of alleles at different loci, interactions of the nucleus
and cytoplasm, and
interactions of the genotype and environment. But heterosis
will continue
to have a major role in the future plant improvement even
though our
knowledge on its genetic basis is limited.”

Given the present wide use of heterosis in practice on the
one hand, and
a lack of real understanding of its nature, on the other, recent studies on
heterosis center on: a) Increasing knowledge on the genetic
mechanisms that includes efforts on physiological, biochemical, and molecular levels.

A better understanding of these mechanisms would enhance the ability of the breeders not only to predict the performance of a given hybrid, but also to form new genotypes that might be used directly, without preliminary tests, as F 1 hybrids; b) based on the available knowledge of this phenomenon, increasing the efficiency in the strategies for developing parental lines whose F 1 hybrids would be promising ones; and c) improving the process of hybrid seed production by developing easier and more reliable and efficient technologies.

MANIFESTATION OF HETEROSIS IN TOMATO

Studies on heterosis in tomato were initiated at the beginning of the twentieth century, almost at the same time as those in maize (Hedrick and Booth 1907, East and Hayes 1912). Tomatoes are a self-pollinated inbred crop and there was a view that high level of heterosis could not be manifest in tomato F 1 hybrids. However, heterosis in tomato, as well as in other self-pollinated crops, was observed in several crosses. It provided evidence that this phenomenon was not limited to cross-pollinated crops.
One of the theories of this performance was that natural cross-pollination predominated within the wild forms of tomatoes in the centers of their origin. Therefore, crosses between tomato lines might be considered as F1 between inbred lines of a species which is naturally cross-pollinating (Rick 1950, Rick and Butler 1956).

Heterosis is a widely documented phenomenon in tomato. More than 50-60% of the studies on heterotic performance refer to heterosis for yield and yield components. This percentage was relatively stable even throughout the last 10 years when efforts of tomato breeders strongly emphasized nutritional value, safety and sensory quality of a food product, tolerance to abiotic stress, etc. Based on these studies, as well as on earlier ones, it might be concluded that the frequency and the level of heterosis for yield and yield components in tomato are relatively high. Kravchenko (1990), for example, reported manifestation of heterosis for yield in 80% and for earliness in 88% of the hybrids studied all over 15 years period.

According to Wehner (1997), the level of heterosis for yield in tomato was significant (estimates average 60%). Suresh Kumar et al. (1995) reported
193.55% of heterosis (over superior parent) for one of the yield components

- fruit number.

According to Yordanov (1983), besides yield, enhanced plant vigor,

earliness, higher adaptability to unfavorable environment and uniformity,

were the manifestations of heterosis most often encountered in the tomato.

These traits are of significant economic importance. Khanna-Chopra et al. (1993), for example, reported that vigor helped in the efficient utilization of environmental factors. Uniformity is one of the principal benefits of hybrids as this trait in plant structure and maturation permits efficient mechanical harvest. Earliness, generally defined as the number of days from sowing to the appearance of the first ripe fruit (Kemble and Gardner 1992), was characterized by Doganlar et al. (2000) as crucial for regions with short growing season, as desirable for taking advantage of high prices during the early season and as a prerequisite for reducing the heating and lighting expenses of greenhouse-grown tomato. Based on experiments aiming at the development of tomato cultivars adapted to low growing temperature, Nieuwhof (1990) found no important genotype x temperature interactions.
This finding suggested that for satisfying the need to reduce energy consumption in greenhouses it was preferable to breed early varieties characterized by rapid growth at normal temperature instead of selecting for adaptation to low growing temperature. In some crosses the magnitude of heterosis for earliness is significant and certainly has, and will be exploited in developing early hybrids. For example, in a study of 92 F1 tomato hybrids between 19 ultra early cultivars used as seed parents and 5 late cultivars, Boe (1988) found that early yield in the F1 hybrids ranged from 52% to 307% when compared to that of the early seed parent.

Genetic studies on tolerance to abiotic stress, nutritive and market quality provide evidence that manifestation of heterosis in tomato is not limited to traits related to plant vigor or yield. Bhatt et al. (1998) reported relatively high levels of heterosis (60.4% and 52.4%) for vitamin C content detected in a number of F1 hybrids. Heterosis for vitamin C was also reported by Dod and Kale (1992), and Mageswari and Natarajan (1999). Heterosis was observed for total soluble solids and dry matter content (Patil and Patil 1988, Yadav et al. 1988, Shrivastava 1998 a, Dasksloff et
al. 1990, Dod and Kale 1992, Mageswari and Natarajan 1999) and lycopene and \( \beta \)-carotene content in fruits (Chen and Zhao 1990, Amaral Junior et al. 1997). It must be noted that there is also information reporting low level or negative heterosis for the content of compounds related to the nutritive value of tomato such as ascorbic acid, lycopene, total soluble solids, etc. (Chen and Zhao 1990, Wang et al. 1998, Amaral Junior et al. 1999). In a study of a number of tomato lines and hybrids for their suitability to prepare ketchup and paste, manifestation of heterosis for ketchup recovery and paste yield was detected (Jawaharlal et al. 1999). Heterosis for pericarp thickness was described by Patil and Patil (1988), Daskaloff et al. (1990) and Dod and Kale (1992), and for fruit firmness by Wang et al. (1995), Resende et al. (1999) and Atanassova et al. (2005).

Besides traits related to fruit market and nutritive quality, heterosis was reported for characteristics related to plant tolerance to stress, plant physiology etc. Heterotic performance for these traits is very important as it enhances the ability of plants to cope better with the environment, that usually results in higher yield. Experiments demonstrated,
for example, that yield gains in hybrid maize were due primarily to

improvements in tolerance to abiotic and biotic stress and that the

improvement occurred in parental inbreds as well as in their F1 hybrids

(Duvick 1997a). In a study of viability of pollen from intra- and inter

specific crosses of tomato, produced at low temperature, Fernàndez-Muñoz

et al. (1995) observed positive heterosis for pollen viability in the cross

between L. esculentum x L. pimpinellifolium. Philouze (1997) reported high

level of heterosis for yield in the hybrid Monfavet n o 63-5 when grown in

non-heated greenhouse. It was found that heterosis was mainly due to

higher percentage of fruit set in the hybrid. Under low temperature the

fertilizing ability of the hybrid plants pollen was higher than that of the

parent lines pollen. Hassan et al. (1999) reported high level of heterosis for

tolerance to salinity at seedling stage, that ranged from 24.9% to 100%(based

on better parent values). Zhacote and Kharti (1990) observed heterosis for

net photosynthetic production in hybrids between cultivated and wild

forms of tomato and Titok et al. (1994) reported manifestation of heterosis
for chlorophyll content both in leaves and stems, a higher level of heterosis being observed in stems.

Based on these reports, as well as on numerous other studies [some of them reviewed by Yordanov (1983), Kalloo (1988) and Georgiev (1991)], it might be concluded that heterosis in tomato was observed for a large scale of quantitative traits, almost all of them being of breeding interest.

It is commonly assumed that genetic stability (homeostasis) in hybrids refers to reduced genotype - environment interactions. A number of studies provided, however, evidence that the majority of quantitative traits were significantly affected by environmental factors and that heterosis was also dependent on the environment (Yordanov 1983, Russel et al. 1993, Cooper and Podlich 1999, Temperini et al. 2001). Therefore, the evaluation of a given tomato hybrid when grown in different locations is necessary for getting reliable information on its performance. This kind of information is of great importance for tomato growers as it can help them to make intelligent cultivar decisions (Murray et al. 1999).

GENETIC BASIS OF HETEROSIS IN TOMATO

The design and efficiency of breeding programs depends on the relative
importance of different types of gene action. Therefore, manipulating
heterosis in breeding programs requires knowledge of its quantitative
genetic basis. Gene action in tomato, as well as in many other species has
been approached by studying the various types of genetic variance in
populations and by generation means analysis. Diallel sets of F1 crosses
between collections of tomato lines are usually used for obtaining a
preliminary impression of the genetic variation for characters of economic
importance. As a result, a relatively large amount of information on the
nature of gene action for several quantitative traits in tomato is available,
the predominant part of this information concerns total yield, yield
components and earliness. Additive and non-additive gene effects have
been reported to be important for yield and its main components. It was
found that their magnitude varied depending on the genotype and on the
environmental conditions (Dod et al. 1992, Natarajan 1992, Sherif and
al. 1997, Singh et al. 1998, Surjan et al. 1999). Studies on the genetics of
earliness have indicated that dominance plays an important
role in this

trait (Banerjee and Kalloo 1989, Kemble and Gardner 1992). Information concerning genetics of quantitative characteristics related to seed, cotyledons, leaf, stem, fruit, growth stages, early and total yield etc. was summarized by Georgiev (1991).

As already mentioned, during the last two decades a significant number of genetic studies and breeding programs in tomato emphasized enhancing plant tolerance to abiotic stresses and fruit nutritional value, sensory and market quality. It resulted in determining the genetic basis of characteristics related to these traits. Data of some of these studies are summarized in Table 4.1 and Table 4.2.

Table 4.1 Gene expression of some characters related to nutritive and market quality in tomato

<table>
<thead>
<tr>
<th>Character</th>
<th>Gene action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycopene content</td>
<td>Dominant</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td>-carotene content</td>
<td>Additive and non-additive</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td>Vitamin C content</td>
<td>Dominant</td>
<td>Daskaloff et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>Non-additive</td>
<td></td>
</tr>
<tr>
<td>Percentage of reducing sugar</td>
<td>Additive and dominant</td>
<td>Stommel and Haynes (1993)</td>
</tr>
<tr>
<td>Glucose/fructose ratio</td>
<td>Additive</td>
<td>Stommel and Haynes (1993)</td>
</tr>
</tbody>
</table>
Sugar content Dominant Daskaloff et al. (1990)

Fruit firmness and longevity Additive Al-Falluji et al. (1982), Dobhal et al. (1999), Atanassova et al. (2005).


Reducing sugars content Additive Shrivastava (1998 b)

Dry matter content Additive Shrivastava (1998 b)

Resistance to cuticle Additive and dominant Emmons and Scott (1998)

cracking

The numerical value recorded for a complex trait (such as total or early yield) is known to be a function of its components. In tomato, for example,

earliness is usually divided into four different components and number of fruits per plant and mean fruit weight are considered as the two primary ones determining yield. Hence, the expression of heterosis for a complex trait is also studied and explained on the basis of component interactions.

Bos and Sparnaaij (1993) showed that component analysis provided the necessary data for the exploitation of recombinative heterosis in plant breeding. Recombinative heterosis was defined as the
phenomenon that

the progeny value of complex character exceeded the mid-parent value as

a result of the multiplicative relationship between the complex character

and its components traits. It was suggested that this form of heterosis may

be an important cause of Specific Combining Ability (SCA).

Besides the investigations that focused on acquiring and increasing

knowledge on genetic variation for characters of economic importance, a

number of studies aiming at getting better understanding of heterosis in

tomato have been also carried out during the last decade. These studies

might contribute to developing new approaches for more efficient exploita

tion of heterosis in tomato breeding.

Griffing (1990) tested three heterosis hypotheses in a controlled-nutrient

(CN) experiment, with reference to tomato yield and its components for a

set of two inbred lines and their hybrid that had previously exhibited

heterosis under field conditions. Heterosis was not exhibited by yield or

ty yield components at any of the four nutrient levels. Hence, the total heterosis

phenomenon was classified as nutrient-dependent heterosis occurring

under field conditions, but not under the nutritional restrictions of the CN
experiment. Such a performance fitted the hypothesis suggesting that heterosis was a consequence of a faster hybrid growth rate. Under this hypothesis lack of heterosis at all four levels of CN experiments was due to the CN procedure which forced all three genotypes to have the same growth rate. Under the differential growth rate hypothesis, the F1 would utilize Table 4.2 Gene expression of some characters related to tolerance to abiotic stress in tomato

<table>
<thead>
<tr>
<th>Character</th>
<th>Gene action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium use efficiency</td>
<td>Additive and Li and Gabelman (1990)</td>
<td>(based on total plant dominant weight) in tomato grown under low-calcium stress</td>
</tr>
<tr>
<td>Low temperature tolerance</td>
<td>Additive Foolad and Lin (1998)</td>
<td>during germination</td>
</tr>
<tr>
<td>Salt tolerance during</td>
<td>Additive and Foolad (1996)</td>
<td>vegetative growth dominant</td>
</tr>
<tr>
<td>Absolute and relative</td>
<td>Additive Foolad (1997)</td>
<td>under salt stress and Na + and Ca 2 accumulations in the leaf</td>
</tr>
<tr>
<td>Testa effect on</td>
<td>Dominant Fooland and Jones</td>
<td></td>
</tr>
</tbody>
</table>
Pollen fertility and fruit set Additive Dane et al. (1991) under high field temperature nutrients from a given allocation most quickly while the parent would utilize the nutrients more slowly. It was speculated that the indeterminate pattern of plant development responsible for yield and its components was due to two major gene systems: genes that determined morphogenetic responses and genes that determined growth rate manifestations.

Studies on relationship between assimilatory surface, growth rates and net photosynthetic rate ($P_N$) of tomato hybrid FMHy1 and its parents provided evidence that the hybrid was characterized by the greatest total leaf area and biomass and that heterosis greatly affected the early development of the hybrid (Rao et al. 1992). Greater net assimilation rate of the hybrid during early growth suggested a greater $P_N$ per unit leaf area. It was concluded that early growth of the hybrid and higher growth rates were responsible for higher dry matter production and yield per plant.

In a study of glycolysis, the pentose phosphate pathway (PPP) for
oxidation of carbohydrates and the electron transport chain (ETC) in the
mitochondria of 5 tomato genotypes and their F1 hybrids
differing in yield.

Titok et al. (1998) found that glycolysis and PPP, but not
ETC, were inhibited

in green leaves of the hybrids while in the parental
genotypes both these

processes were not inhibited. It suggested higher energy
potential in the

hybrids which produced favorable metabolic conditions for
growth and

was supposed to be the main reason for improved yield.

Studies on F1 hybrids between line B 317 and a set of
isogenic/near

isogenic lines (IL/NIL) of tomato cv. Ailsa Craig differing
in genes baby lea

syndrome (bls), high pigment (hp), sunny (sy), venosa
(ven), curly mottled (cm),

entire (e), ripening inhibitor (rin), relaxata (rela),
lutea (lut) and clausa (clau)

showed that heterosis for productivity, early yield and
mean fruit weight

occurred only in three of the 10 hybrids (Atanassova et al.
2002). The

pollen parents of two of these three hybrids (Ailsa Craig
ven and Ailsa

Craig rela) were characterized as possessing genetically
controlled low

vitality, i.e., the occurrence of heterosis was more or
less limited to F1 hybrids

of a given group of mutants. Such results might be
consistent with those
reported by Strunnikov (1983), who observed high level of heterosis in F1 hybrids of silkworm (Bombyx mori) where one of the populations exhibited genetically controlled low vitality. A hypothesis was drawn out that such populations might possess the so called “compensatory gene complex” (CGC) that might contribute not only to the survival and reproduction of these populations but also to heterosis for some traits in their F1 hybrids.

An attempt of heterosis dissection was made using near-isogenic line TA 523 (L. esculentum) containing a 40-cM introgression at the bottom of chromosome 1 from L. hirsutum (Monforte and Tanksley 2000). A set of recombinant lines (sub NILs) derived from the original NIL TA 523 were developed in order to fine-map the genetic factors included within the original introgression. Analysis of the subNILs revealed that the gene ac tion of the QTL for yield was dominant (d/a=0.7) which eliminated the possibility that yield increase was due to true overdominance at a single gene locus. On the other hand, negative yield effects in other regions of the introgressed segment that would be predicted by the dominance comple mentation model, were not detected. Epistatic interactions among genetic
factors along the introgressed segment were suggested as the cause of yield heterosis.

STRATEGIES FOR DEVELOPING TOMATO HYBRIDS

The plant characters chosen as selection criteria depend on the goals of the breeding program. When the trait is characterized by high heritability, a direct selection is possible. Selection for polygenic characters (such as early yield total yield, flavor), influenced also by environment, is complicated.

Hence, the development and the selection of parents in hybrid breeding programs can be difficult. One way to facilitate breeding for complex characters is to make them more amenable to improvement by determining and analyzing their components. Most of these components are also of a quantitative nature and may influence each other. According to Bos and Sparnaaïj (1993), this not only causes SCA effects, it also causes problems in the identification of markers (phenotypic or molecular) for complex characters. In most cases there is no question of a single marker for complex character: in one genotype a high value for the complex character may be due to high level for component x 1 , in a second genotype it may be due to
a high level for x 2. Thus, one should look for marker genotypes corresponding with favorable levels of the important component traits, rather than for marker genotypes corresponding with high levels of the complex character.

Components that contribute to the performance of a given complex character in tomato, such as total and early yield, were investigated since the early studies on heterosis in this species (Powers 1945, Burdick 1954, Williams 1959, etc.) and are still largely evaluated till date as well as exploited in breeding programs (Szwadiak and Kordus 1992, Vallejo Cabrera and Estrada 1993, Rai et al. 1997, Wang et al. 1998, Doganlar et al. 2000, etc.). As already mentioned, number of fruits/plant, mean value of fruit weight, plant height etc., were determinant main components for yield.

Based on a number of studies, Doganlar et al. (2000) divided the determinants of earliness into four different components, each one being a heritable trait: 1) days from sowing/transplanting to the first flowering (anthesis); 2) days from anthesis to the first fruit set; 3) days from the first fruit set to the first ripe fruit; 4) days from the first ripe fruit to the end of
Besides determining the components of a given complex trait, knowledge on the relationships between them is necessary. One way to get such information is to establish correlation between characters. Correlation might be evaluated between components of a complex character: between complex character and one of its components; between components of different complex characters; between a given character and environment; and between physiological character and yield, etc. Based on a significant correlation between: a) early and total yield, or yield components and biochemical characteristics of fruit quality; b) fruit weight/ firmness and biochemical characteristics of fruit quality; c) environmental factors (e.g. soil and atmosphere) and biochemical characteristics of fruit quality; d) content of fruit compounds and the taste; e) market quality (fruit longevity, firmness, cracking) and characteristics of fruit pericarp, weight, form, etc.

Evaluation of relationships between productivity/growth rate and morphological/physiological characteristics of plants grown under unfavorable conditions might be useful in breeding genotypes tolerant to ripening.
stress (Nieuwhof et al. 1993, Nkansah and Ito 1994, Matsunaga and Monma 
2000). Knowledge on the significance of correlation (positive or negative) 
between two characters might not only help in indirect selection of a trait 
that is difficult to be controlled all over the breeding cycle, but can also be 
useful in foreseeing (at least partially) results in some breeding programs. 

A number of studies, for example, provided evidence that the gain in 
earliness might cause a reduction in fruit weight (Boe 
1988, Banerjee and 
Kalloo 1989, Lindhout et al. 1994). A significant number of studies on correlation between different traits 
in tomato also suggest that the knowledge on the relationship between 
characters of breeding interest could be useful and exploited in improving 
parental lines and hybrids. It must be noted, however that the reliability of 
the genetic correlation established between different traits has been found 
to vary and is influenced by genotype - environment interactions (Aastveit 
and Aastveit 1993). This peculiarity needs to be taken into consideration 
while designing selection strategies. The real value of the lines designated for developing hybrids, however, 
is not based on their own performance per se but the
performance of their
F1 hybrids. Therefore, the last and the most important
step in breeding
hybrids is the evaluation of the lines for their combining
ability. Diallel
sets of F1 crosses between collections of tomato lines
have long been used,
and still are used, for selecting the best parents for F1,
and for identifying
the best crosses from which to extract improved inbred
lines. The biometrical
analysis of Griffing (1956) provides information on the
combining ability
of the parents and the magnitude of additive and
non-additive gene action.
There is a significant number of studies that refer to
general and specific
combining ability (GCA and SCA) for a large scale of traits
of breeding
interest in tomato, such as yield and yield components (Rai
et al. 1997,
Wang et al. 1998), total soluble solids, dry matter (Dod et
al. 1995, Wang et
al. 1998, Shrivastava 1998), resistance to cuticle-cracking
(Emmons and
Scott 1998), etc. GCA and SCA can interact with the
environment that
would result in changes in parental combining abilities
over the
environments (Singh 1973). For this reason evaluation of
parents in more
than one environment is recommended. According to Shattuck
et al. (1993),
if conducting the diallel in only one environment, plant geneticists should

try to match the diallel with the environment of interest.

The strategies above mentioned, although widely used in the development of tomato hybrids and their parental lines, are far from being perfect and obligatory for leading to the results expected. Moreover, they are costly because results relate to many years of tests and evaluations not only of the parental lines developed but also of their hybrids. For this reason, enhancing the efficiency of the process of developing parental lines, and hybrids, is and will be of primary importance in the future.

The advent of molecular markers provided tools for mapping genes involved in quantitative trait loci (QTL) and the possibility for plant genetic improvement based on molecular marker assisted selection (MAS).

Molecular markers might be used both for trait identification and trait introgression. Therefore, their utility extends throughout all phases of breeding programs. In theory, MAS was shown to produce greater selection gains than phenotypic selection for normally distributed quantitative traits.

Even without direct effect of the marker on the quantitative trait locus,
detecting linkage between such traits is of interest for increasing the
response in selection. Based on a model for estimating the probability of
selecting one or more superior genotypes, using MAS, Knapp (1993) found
that a breeder for phenotypic selection without applying MAS had to test
1.0 - 16.7 times more progenies than a breeder using MAS. It was concluded
that MAS might substantially decrease the resources needed for a selection
goal of a low to moderate heritability trait when both the selection goal
and the selection intensity were high.
Tomato has been a model plant for QTL mapping. Several qualitative
and quantitative trait loci in tomato have been mapped over the past de
cade, such as earliness and fruit ripening time (Lindhout et al. 1994,
Monforte et al. 1999, Doganlar et al. 2000), total yield (Bernacchi et al.
1998), plant height and fresh mass (Paran et al. 1997), fruit size and shape
(Grandillo et al. 1999, van der Knaap and Tanksley 2001), fruit firmness
(Bernacchi et al. 1998), lycopene content (Chen et al. 1999),
-carotene accumulation in fruits (Zhang and Stommel 2000), organoleptic
quality (Causse et al. 2001, Saliba Colombani et al. 2001), cold and salt
tolerance during different developmental stages (Bretó et al. 1994, Foolad et al. 1990a, Foolad et al. 1990b), etc.

Molecular markers (such as restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and microsatellites (SSRs) are also expected to contribute to easier and more efficient identification of the lines whose crosses would result in promising hybrids. It was commonly assumed that hybrids produced from lines having different origin (i.e. developed from different cultivars) tended to have greater, consistent yield levels than hybrids of inbred lines originating from the same source population. The concept of heterotic groups gradually evolved from empirical evidence of crosses from inbred lines. Theoretically, the more distant the parents, the greater is the number of genes they differ, thereby the greater the potential interaction of the genes in the form of dominance and epistasis, and the greater will be potential for heterosis (Falconer 1989). In this sense genetic diversity might be an important issue in predicting F1 performance. The assignment of tomato lines to heterotic groups before field testing may
allow the breeder to avoid crosses within groups that would result in lower costs.

Genetic diversity can be measured by several means including pedigree data, genotypic origin from contrasting geographic regions, etc. although molecular methodologies are considered as the most reliable ones.

The latter might contribute to increasing the accuracy in determining the divergence between the genotypes of interest, that could ultimately lead to improved classification. Comparison of molecular markers with pedigree data of related genotypes, based on theoretical and experimental results is, however, recommended (Melchinger 1993). The idea of exploiting the genetic distance of the parents as an indicator in the pursuit of heterosis in tomato hybrids is not a new one. Daskaloff (1942) reported high level of heterosis for yield and earliness in F1 between tomato lines developed on the basis of hybridization between the tomato cultivar Sarya and L. racemigerum (Lange) (accession unknown), lately classified by Muller (Zhutchenko 1973) as L. pimpinellifolium Mill. It was also reported that lines developed on the basis of interspecific hybridizations were character
ized by high combining ability of economically important traits. Based on these findings it was concluded that for acquiring heterosis for early and total yield, the hybrids should include lines of different origins (Daskaloff 1955, 1967, Yordanov 1983).

Recently, a number of studies aimed at cultivar identification, determining relatedness, and comparing the magnitude and structure of genetic variation among different tomato accessions have been carried out. RFLP analysis of phylogenetic relationship and genetic variation in the genus Lycopersicon show that the ratio of within vs. between accession diversity was much lower for self-compatible species. It indicated that most of the diversity within these species existed between populations, rather than within populations. Overall the amount of genetic variation in the self-incompatible species far exceeded that found in self-compatible species (Miller and Tanksley 1990). Villand et al. (1998) used RAPD in estimating relationships between accessions collected from Old and New World regions. Differences in RAPD marker frequencies indicated uniqueness of accessions from the Old and New World collections. Accessions from
Ecuador, Peru and Chile had a larger magnitude of marker diversity than Old World ones. Comparison of subpopulations of L. esculentum and its subspecies L. esculentum var. cerasiforme indicated that the two were distinct but had similar levels of diversity. Noli et al. (1999) reported results from RAPD analyses of modern and vintage cultivated tomato accessions and eight accessions of wild Lycopersicon species (L. esculentum var. cerasiforme, L. pimpinellifolium and L. peruvianum). Cluster analysis allowed L. esculentum to be clearly distinguished from the wild species. Within L. esculentum two major groups were identified, the first including all the fresh market varieties and vintage processing varieties and the second including most of the modern processing varieties. RAPD analysis did not distinguish cultivars indicated as synonyms or selected from the same standard variety.

Bredemeijer et al. (1998) reported that sixteen tomato cultivars were DNA typed for 20 selected microsatellite markers (STMS) using the fluorescent approach. Length polymorphism among the PCR products was detected with 18 of these markers, yielding gene diversity valued from 0.06 to 0.74.

As few as four STMSs were sufficient to differentiate between the 16
cultivars, indicating that these markers were especially suitable for a species like tomato which had low levels of variation as detected by other types of markers. In tomato, a high-density AFLP map has been constructed using an interspecific population (Haanstra et al. 1999). Studies using AFLPs to assess genetic diversity among tomato cultivars demonstrated that AFLP markers were effective for obtaining unique fingerprints of, and assessing genetic diversity among, tomato cultivars (Park et al. 2004).

It has to be noted, however, that studies on the relationship based on marker assisted genetic distance of the parents and heterosis in maize, wheat, soybean, chickpea, oilseed rape and other crops show non-obligatory linearity to heterosis. It might be due to insufficient genome coverage because of the low number of marker loci. It might also suggest that genetic distance at the molecular level as determined by RAPD, RFLPs etc. in some cases could have a limited utility as indicator or predictor of heterotic performance. Several studies on the applicability of molecular markers provide evidence, however, that their application in plant breeding holds promise for increasing the accuracy of prediction of
genotypic values
According to Melchinger (1999), groupings of germplasm based on molecular marker information can provide the basis for establishing new heterotic groups or broadening the genetic base of existing ones. This must be supplemented by evaluating the performance of crosses among these subgroups to assess their heterotic response, which is essential for identifying heterotic patterns.

By using examples from different crops Melchinger (1999) demonstrated that genetic distances based on molecular markers can be used for: a) revealing genetic relationship among different germplasm; b) assessing germplasm to groups and subgroups of similar materials, and c) detecting pedigree relatedness between germplasm.

The recent advent of molecular linkage maps might also make it possible to detect and individually analyze the loci underlying heterosis. The use of molecular markers to identify QTLs responsible for heterosis may help in improving the genetic gain in some selection breeding schemes.

IMPROVING THE PROCESS OF HYBRID SEED PRODUCTION
Tomato is a self-pollinated inbred crop, its flower is bisexual and hand emasculation and pollination are used for producing hybrid seed. Biological bases of hybrid seed production including flower morphology characteristics, anther emasculation, hybridization techniques etc. are reviewed and presented in detail by Yordanov (1983) and Georgiev (1991).

Significant quantities of hybrid seed are produced using a fertile seed parent. Tomato is considered a high value crop grown for either fresh market or processing. According to Duvick (1997b), seeding rates are low compared to the fruit value of this commercial crop. One noteworthy reason is that crossing is performed in countries where labor costs are very low. The quantum of research being carried out to improve the process of hybrid seed production suggests that the present technology is not perfect and does not give itself to easy adaptation to economic needs.

The benefit of incorporating male sterility into hybrid breeding programs was recognized not long after the appreciation of the advantages of heterosis and the detection of male sterile genotypes in tomato. For the first time male sterility was used in tomato hybrid seed production by Rick
(1945) and till date this phenomenon is still discussed as the most promising way for facilitating the process of hybrid seed production (Sawhney 1994, 1997, 2004, Gorman and McCormick 1997, Atanassova 2000).

Genetic male sterility occurs widely in Lycopersicon esculentum, since cytoplasmic male sterility (CMS) does not occur naturally in the genus Lycopersicon. Georgiev (1991) defined the male sterility in tomato as autosterility and classified the sterile mutants into two groups: male sterile [including the male sterile (ms) and stamenless (sl) series] and functional sterile [including positional sterile (ps), positional sterile 2 (ps 2), and exserted stigma (ex)]. Based on anther development and the phenotype, the male sterile mutants in higher plants were classified into structural, sporogenous and functional types (Kaul 1988; Fig. 4.1, Fig. 4.2, Fig. 4.3, Fig. 4.4 and Fig. 5). The majority of the mutants belong to the male sterile (ms) or stamenless (sl) series, while the frequency of the mutations controlling functional sterility is lower (Kaul 1988, Sawhney 1994, Gorman and McCormick 1997, Atanassova et al. 2001).

Over the years it was established that several requirements such as
complete male sterility controlled by recessive gene, normal female fertility

with no defects in morphology, and stability of sterility expression etc.,

must be met by designated male sterile plants to be used in breeding programs (Gorman and McCormick 1997, Atanassova et al. 2001).

Contrarily, it has been found that each type of male sterility in tomato exhibited not only significant advantages but also significant disadvantages when used in hybrid seed production, (Table 4.3). This table elaborates on several approaches for correcting the disadvantages of using male sterility.

Fig. 4.1 Sporogenous (ms 10) sterility in tomato. Flowers with exserted and non-exserted stigma Fig. 4.2 Structural (sl) sterility in tomato. Fig. 4.3 Functional (ps) sterility in tomato.

Fig. 4.4 Functional (ps 2) sterility in tomato. Longitudinal section of tomato anther cones; on the left: ps 2-indehiscent anthers; on the right: fertile anthers

Fig. 4.5 Functional (ex) sterility in tomato. On the left: fertile flower (P 2 ); on the right: exserted stigma sterile flower (P 1 ); in the middle: flower with exserted stigma (F 1 )

in tomato hybrid seed production. Despite the numerous attempts of testing almost all these approaches the number of cultivars possessing male sterile seed parent remains rather limited. In our knowledge, (although our Expression of Heterosis by Hybridization 131

Sterility Advantages Disadvantages Approaches for correcting or escaping disadvantages
Sporogenous

Structural

Functional 1. Elimination of the process of emasculating (stigma accessible because of anther deformations) 2. Complete sterility. 3. Stable expression of sterility independent of the environmental conditions. 4. Complete restoration of fertility in F 1. 5. Easy transfer of sterility genes to any genotype. The same as in the sporogenous mutants 1. Easy maintenance by artificial selfing. 2. Elimination of the process of emasculation (valid for exserted stigma genotypes). 1. Maintenance of the line as a population of sterile and fertile plants. Sterile plants could be assessed at anthesis. 2. Occurrence at some periods of high percentage of flowers with non exserted stigma that would require hand emasculation. 1. Maintenance of the line as a population of sterile and fertile plants. Sterile plants could be assessed at anthesis. 1. Necessity of stamen emasculation (valid for ps and ps 2 sterility in tomato). 2. Undesirable selfing. 1. Maintenance of the line as a population of sterile and fertile plants. Sterile plants could be assessed at anthesis. 2. Occurrence at some periods of high percentage of flowers with non exserted stigma that would require hand emasculation.
applicable in breeding programs aimed at the facilitation of hybrid seed production mainly because of their complete male sterility and accessible stigma (Stevens and Rick 1986, Sauhney 1994, Gorman and McCormick 1997). Because of anther deformation, some ms mutants such as ms 10, ms 15, ms 32 exhibit exserted stigma, and are therefore accessible for pollination without emasculation. By developing ms10 35 aa genotypes, the main disadvantage in using ms-sterility in hybrid seed production (i.e., assessment of sterile plants at anthesis) was eliminated (Philouze 1974).

The anthocyaninless sterile plants are easy to be distinguished since early developmental stages. Moreover, no effect of genes ms10 35 aa on plant and fruit characteristics was established (Gardner 2000). This technology might be applied also if using ms 15 anthocyanin without (aw) genotypes as the two genes are closely linked (Clayberg 1965). According to Jorgensen (1987), tight linkages may be synthesized also through genetic transformation, which would allow introduction of an appropriate marker gene to a random location in each of a large number of plants carrying a suitable ms gene.

Studies on ms mutants and exserted stigma manifestation
provided evidence, that the latter, usually a beneficial character in hybrid seed production, might sometimes be harmful. First of all, it is important to note that these mutants were characterized by lower hybrid seed yield (Atanassova 1999), which was suggested probably due to the fast drying of the stigma (Georgiev 1991). Secondly, observations on stigma exsertion variability within a number of ms sterile lines developed at the Station des Plantes Maraîchères, INRA, Monfavet, Avignon, France, showed that the percentage of flowers with accessible stigma strongly varied depending on the environment and on the genotype (Table 4.4, Fig. 4.1). In some genotypes (and this during the period of hybrid seed production), the percentage of flowers without exserted stigma was so high, that for using them in commercial hybrid seed production, anther emasculation would become necessary. These results are consistent with those of Levin et al. (1994). Based on a study of the effects of the ms 10 gene, polygenes, and their interaction on pistil and anther-cone length, it was concluded that emasculation of the ms 10 male sterile parent appeared to be unavoidable
for the efficient production of hybrid seeds. Removal of the shrunken ms sterile anthers was found, however, to be more difficult and required a

Table 4.4 Manifestation of non-exserted stigma in male sterile tomato lines observed in the experimental fields of the Institute of Genetics, Sofia, during the period of hybrid seed production (May 25 - July 5, 2001)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Percentage of flowers with non-exserted stigma ± SE*</th>
<th>May 25th</th>
<th>June 5th</th>
<th>June 15th</th>
<th>July 25th</th>
<th>July 5th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monalbo ms 10</td>
<td>3.2 ± 0.2</td>
<td>27.4 ± 5.7</td>
<td>64.7 ± 8.2</td>
<td>67.5 ± 11.0</td>
<td>16.6 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Monalbo ms 32</td>
<td>10.0 ± 1.3</td>
<td>33.2 ± 4.9</td>
<td>63.0 ± 9.4</td>
<td>69.6 ± 7.4</td>
<td>21.5 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>Porphyre ms 10</td>
<td>11.4 ± 3.0</td>
<td>44.0 ± 4.1</td>
<td>59.6 ± 7.6</td>
<td>92.4 ± 6.0</td>
<td>90.2 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Porphyre ms 32</td>
<td>9.5 ± 1.7</td>
<td>41.4 ± 7.6</td>
<td>87.1 ± 8.3</td>
<td>74.2 ± 5.9</td>
<td>56.4 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>Monfavet 167 ms 10</td>
<td>1.5 ± 0.2</td>
<td>13.7 ± 2.8</td>
<td>1.8 ± 0.1</td>
<td>14.0 ± 0.0</td>
<td>9.2 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Monfavet 167 ms 32</td>
<td>6.6 ± 1.3</td>
<td>3.9 ± 0.8</td>
<td>19.6 ± 4.1</td>
<td>3.4 ± 0.5</td>
<td>5.0 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

SE* - standard error between means of replicates

longer time than emasculation of fertile flower buds (Atanassova 1999).

Recent technologies have made it possible to identify and isolate ms genes and to engineer transgenic male sterile plants (Gorman et al. 1996) as well to chemically induce male sterility (Sakaki and Yamazaki 1990, Cross and Schultz 1997), this particularity should be outlined and taken into consideration. Chen and Tanksley (2001) reported that the
fine mapping of stigma exsertion QTLs se2.1 revealed that se2.1 was located in the chromosomal interval between RFLP markers T1301 and T662 and that the change of style length might be the function of se2.1 genes. This finding might act as tool for more efficacious manifestation of stigma exsertion.

According to Bar and Frankel (1993), some ms mutants (ms 14, ms 17, ms 18, ms 31, ms 33, ms 47) were found to exercise pleiotropic effect on a number of economically important traits such as percentage of early marketable yield, average fruit weight, and total marketable yield. This suggests that detailed studies on ms sterile lines are necessary before including them in breeding programs.

Functional male sterile mutants offer the advantage of reproduction by artificial selfing in order to produce 100% sterile progeny. Despite this advantage they are considered as less promising for use in hybrid seed production because they display two significant disadvantages - occasional lapses in their expressivity resulting in undesirable selfing and except in exserted stigma-sterility, necessity of stamen emasculation. Observations on a large number of ps 2 sterile lines throughout their
manipulation in breed

ing and hybrid seed production have shown, however, that it was possible
to deal with the first disadvantage (lapses in ps 2 expressivity) by taking
into consideration some specific characteristics related to their performance
(Table 4.5; Atanassova 1999, Atanassova et al. 2001).
The need of stamen emasculation is the second disadvantage that
usually prevents breeders from using ps 2 sterility in breeding programs.
Comparative study on the time necessary for the emasculation of floral
buds in the fertile plants, and flowers at anthesis in the sterile lines, made
it clear that this disadvantage, in terms of limiting factor, was exaggerated.
Emasculation during anthesis (as practiced when using a ps 2 line as seed
parent) was easier and almost twice as rapid as emasculation applied on
the fertile floral buds (Atanassova 1999). Anther emasculation could be
made even easier if ps 2 was combined with short style
(Georgiev and Atanassova 1981; Fig. 4.6). Such flowers can be emasculated
without using forceps: the anther cone and the petals can be easily separated manually
from the flower by plucking out with two fingers the tip of the cone (or the
petals) (Fig. 4.7). Certainly, the idea of developing
genotypes combining ps 2 sterility with exserted stigma is a tempting one as it would result in efficacious technology: easy maintenance of the seed parent by artificial selfing and no need of anther emasculation. Such a technology, however, could be acceptable only by finding a way to deal with the strong variability in the rate of stigma exsertion. While testing ex-lines for their usefulness in Table 4.5 Ways of dealing with the lapses in ps 2 gene expressivity and its consequences in breeding and hybrid seed production

Specific characteristics in the performance of Approaches for dealing with specific ps 2 lines to be taken into consideration when characteristics in the performance of ps 2 using ps 2 sterility in breeding and hybrid seed production.

1. The ps 2 gene expressivity varies This enables the breeding of ps 2 lines that with the genotypes exhibit very low percentage of selfing.

2. The percentage of selfing in the Strict control of this characteristic is ps 2 lines varies within and between necessary throughout the entire breeding the years of growing, being forever process. The percentage of selfing recorded lowest during the period of hybrid at the end of the growing season, instead seed production. at the end of the period of producing hybrid seed, might give a wrong idea on the use fulness of the ps 2 line.

3. If occurring, anther dehiscence in Regular emasculation and pollination of the ps 2 lines occurs usually after the plants at least each two days is the second day of flowers opening, necessary. If missing this term, all flowers at anthesis have to be eliminated.
4. The percentage of selfed seeds when The usefulness of a ps 2 line has to be using a ps 2 line as seed parent in evaluated on the basis of both, percentage producing hybrid seed is significantly of selfing and hybridity of the seeds lower than the percentage of selfing, obtained by artificial pollination. observed on the same line.

hybrid seed production it was established that it was really difficult to
determine and fix the right rate of stigma exsertion. On the one hand, lines
possessing 1.0-1.5 mm stigma exsertion were found occasionally to be like
normal ones requiring stamen emasculation. On the other hand, F 1 hybrids
of the lines possessing steadily manifested exserted stigma (2.0 mm or
more above the anther cone), performed sometimes as longuistylic, that
resulted in lower percentage of fruit setting (Fig. 4.5).

Fig. 4.6 Longitudinal section of tomato flowers with normal and short style.

Fig. 4.7 Emasculation in tomato ps 2- line possessing relatively low level stigma without using forceps

Easier and more rapid anther emasculation is not the only criterion for
an efficient hybrid seed production. Hybrid seed yield is also of great im
portance. Comparative studies on hybrid seed yield obtained from ps 2
sterile lines, depending on the developmental stage of stamen emascula
tion and pollination, showed that a significantly higher (1.5 to 3 times)
hybrid seed yield resulted from pollination at anthesis. This is the stage in
which the ps 2-lines are usually manipulated for producing hybrid seed

(Atanassova and Georgiev 2002). Therefore, in terms of expenditure and
time, use of ps 2 male sterile seed parents proved to be profitable and

These data were also confirmed by the production figures. In Bulgaria,
until 1990, about 1 ton of hybrid seed per year (30% of the total quantity of
hybrid seed produced) was produced using ms 10 aa and ps 2 seed parents
(Georgiev 1991), while presently about 80% of the hybrids released and
spread are practically from ps 2 seed parent. Hybrids using ps 2 sterile
seed parent were released also in Czech Republic and Moldova (Atanassova
1999, 2000). It suggests that the functional, and more precisely ps 2 male
sterility, is not to be underestimated. It should be taken into consideration
for elaborating systems or breeding programs aimed at the reduction of the
time and costs associated with hybrid seed production.

According to Potaczek and Kubicki (1986) and Staniaszek et al. (2000)
the use of positional sterility (ps) in tomato hybrid seed production under
Polish climatic conditions was advantageous. The process was found to
be cheaper due to labour-saving procedures up to 30% as compared to the traditional method. Two RAPD markers linked to the ps gene were identified. The markers were used for purity determination of maternal lines carrying gene ps gene and F 1 hybrids possessing ps sterile seed parent (Staniaszek et al., 2000).

The experience accumulated while applying different types and genes of male sterility in the practice (at least three - ms10 35 aa, ps and ps 2) has shown that they should not be categorized as “more or less promising”.

Sometimes a given type of sterility might initially look promising but during its manipulation in breeding and hybrid seed production unexpected difficulties may crop up (e.g. the occurrence at times of a high percentage of flowers with non-exserted stigma in some ms tomato lines).

Conversely, some mutants or sources of sterility might display undesirable traits, but while using some approaches they could be corrected or eliminated, (e.g. the ps 2 sterility in tomato).

Thus, the application of male sterility in breeding and hybrid seed production in tomato is not merely a theory, but is being practised in a number of countries. Nevertheless, it is difficult to
determine how widely

cultivars possessing male sterile seed parent are spread, or what is their

percentage based on the total number of hybrid varieties, as it is difficult to

track data on the release of varieties possessing a male sterile seed parent.

The availability of such hybrids is, however, a fact not to be neglected. It

confirms that induction of male sterility is one of the right ways for

facilitating hybrid seed production in tomato and a start has been made in

this direction.

Economizing hybrid seed production includes not only facilitating its

process per se, but also increasing its efficiency by improving the quality of

the final product, i.e. of the hybrid seed. Besides the high germination

ability which is an obligatory characteristic for each kind of commercial

seed, the high percentage of hybridity is of primary importance in hybrid

seeds. For this reason, breeders are tempted to introduce male sterile seed

parents to facilitate the production of hybrid seed. Using male sterile seed

parent would be a warranty for production of 100% hybrid seed and would

eliminate the necessity of testing the seed for hybridity. In view of the fact

that male sterile seed parents could not be widely used in
developing tomato hybrids, different morphological markers such as potato leaf (c),
anthocyaninless of Hoffmann (ah), anthocyanin without (aw) etc. were introduced
in fertile or ps 2 seed parents of a number of commercial hybrids (Farkas 1993, Xue 1994, Atanassova et al. 2001). It permitted testing the hybridity
of the seed at germination or seedling stage. Recently, some molecular techniques made it possible to rapidly evaluate the purity of the hybrid
seed produced (Rom et al. 1995, Paran et al. 1995, Chuang et al. 1999) and
probably in the near future these techniques will be widely used for this purpose.

TOMATO HYBRID ADOPTION IN PRACTICE—HISTORY AND PRESENT SITUATION

As already mentioned, studies on heterosis in tomato were initiated almost simultaneously with such studies on maize. The introduction of tomato
hybrids, however, came into practice 30-35 years later than maize hybrids.
The first commercial tomato hybrids were developed much earlier in some
countries. In USA, for example, the first hybrid "Burpee Hybrid" was
developed by Dr. Oved Shifriss in the early 1940's, probably 1942, and
marketed by the W. Atlee Burpee Co. (Prof. Rick C.M.,
personal
communication). The first tomato hybrid in Bulgaria (Saria
× Komet) was
developed in 1932 by Prof. Daskaloff, (Daskaloff, 1937) at
the Agricultural
Experimental Station (now Institute of Vegetable Crops
“Maritza”), Plovdiv,
where for the first time large quantities of hybrid seed
were produced.
Since 1949 and up to the late 1960’s, for example, the
number one cultivar
for early field production was the hybrid No. 10 × Bizon,
developed also
by Daskaloff. In Japan one of the first hybrids "Fukuju" was
developed by
Prof. Fujii (Fujii 1948, 1952) and released in 1938 by
Osaka Agricultural
Station, (now Osaka Prefectural Agricultural and Forestry
Research Station).
The first Dutch tomato hybrid Single Cross (Vetoemold x
Ailsa Craig),
combining traits of resistance to Cladosporium fulvum, was
developed by
Bruinsma in 1939 and introduced in 1946. The hybrid
exceeded the other
varieties by taste and earliness (Anonymous 1992). In
France, the first hybrid
Fournaise F 1 was developed by Vilmorin and released in
1956 almost
simultaneously with the hybrids Monfavit n o 63-5,
Monfavit n o 63-4, and
Monfavit n o 63-18 developed by INRA, (Philouze 1986,
1997). In the early
60’s these hybrids enjoyed a significant success and were rapidly adopted by the producers. In Israel, the first tomato hybrid “Urith” was developed at Volcani Center and released in 1971 (Pilowski et al. 1971). In China, the first tomato hybrid “Beijing Zhaohougu x Aonong No 2” was developed in 1969 at the Zhejiang Agricultural University (Wang et al. 1988).

Tomato hybrids began to take over the market towards the end of sixties or the beginning of seventies. Their use increased dramatically throughout the following decades. In 1997, Duvick (1997 b) reported that 100% of fresh market and 80% of processed tomatoes in USA were F 1 hybrids. At present, there is a similar situation in several countries in Europe, Asia and Australia.

FUTURE PROSPECTS AND CHALLENGES IN EXPLOITATION OF HETEROSIS IN TOMATO

The goal of the breeders is to develop hybrids superior in one or a number of traits to the standard hybrid or hybrids previously released and used in practice, and most of them do not develop heterotic hybrids per se. In a way each hybrid might be considered as a challenge for developing new, superior hybrids. Meanwhile, consumers and growers demand as well as
handling

requirements have dramatically changed during the past two decades.

Increasing yield, for example, is not any more the major focus of commercial tomato breeding programs. It has shifted to breeding for a complex of traits, such as improved quality, flavor, more efficacious plants coping with abiotic and biotic stresses for at least a substantial part of their life, etc. The longevity of fruit, once considered as a trait of primary importance, is not now appreciated if not combined with good flavor and/or texture. Hence, the new hybrids developed have to be superior to the previously released ones in terms of traits that the latter already possess plus permanently cumulate new valuable agronomic traits in order to satisfy the demand of consumers for high nutritive quality and food safety, and of growers - for economic profitability and handling requirements. To achieve these objectives plant breeding research should focus on improving fruit flavor, texture, composition and studying the potential of plants to synthesize desirable components by developing new methods that lead to accelerated screening, adjusting the precision of selection stages, minimizing costs etc.
The achievement of such ambitious, complex and perhaps even challenging future breeding goals would be difficult (if not impossible) without the extensive use of the phenomenon of heterosis. As already mentioned, heterosis in tomato may be expressed at any developmental stage and observed for a large scale of quantitative traits related to plant productivity, adaptability, physiology and fruit nutritive and market quality. It is commonly known that the phenomenon of heterosis was widely used in developing early and high yield commercial hybrids. It is rather doubtful (as it would be difficult to trace down such information) that heterosis was widely pursued in breeding programs for traits, other than earliness, total yield and yield components.

Hence, given that tomatoes are one of the most important crops in the world because of their volume of consumption, and overall contribution to nutrition, and bearing in mind that the new developed varieties have to satisfy complex consumer and grower demands as well as handling requirements, the manifestation of heterosis for traits related to plant tolerance to biotic and abiotic stresses, photosynthetic efficiency, nutritive
and market quality, efficient root system, etc., may be considered as a reserve to be exploited in future breeding programs.

The recent advances in the molecular genetics (tagging and isolation of genes, QTL controlling a given trait, expression of desirable alien genes in transgenic plants, improving the efficiency of breeding via marker assisted selection, etc.) is expected to contribute to the more efficient and extensive exploitation of heterosis in developing tomato hybrids. Combining conventional and molecular breeding techniques might offer help in improving screening efficiency for many traits of agronomic value, estimating genetic diversity, reducing the time for new line development, assessing heterotic groups, and detecting as well as individually analyzing the loci underlying heterosis. The rapidly increasing number of investigations aiming at identification and isolation of male sterile genes in tomato, engineering transgenic male sterile plants, synthesizing tight linkages between an appropriate marker gene and male sterile gene through genetic transformation might also generate useful approaches contributing to the creation of better systems of hybrid seed production. The fact that the
application of male sterility in breeding and hybrid seed production in
tomato is no longer a theoretical one, gives ground to believe that such
systems would be easily accepted and applied in practice.
The prospects and expectations outlined above need to be
taken cautiously. According to Young (2000), although DNA markers hold great
promise, realizing this promise remains elusive, as most markers
associations are not significantly successful in MAS. At the same time, the
effectiveness of MAS in breeding programs would depend also on the
genetic determinism of the traits of interest (Hospital et al. 1997) and on its
cost (Young 2000). Kearsey and Farquhar (1998) consider that unreliability
of QTL location may suggest its false candidacy. Therefore, the new
knowledge gained creates new problems to solve, such as developing
reliable molecular markers, refining the techniques, decreasing the cost
associated with molecular markers assays etc. The capabilities of recently
developed molecular techniques suggest their wide application in breeding
programs and in the genetic improvement of cultivated plants, including
exploitation of heterosis.
In 1908, Shull noted that the efficiency of maize breeding
programs

would considerably improve by finding a suitable method of predicting

hybrid performance before field evaluation. The numerous studies on

heterosis and the large experience of exploiting this phenomenon over an

total century, complemented by the rapid advent of molecular techniques

during the last decade, make it possible to believe that this endeavor might

be on the way to come true.

SUMMARY

Studies on heterosis in tomato were initiated at the beginning of the

twentieth century. Although its biological basis remains unknown, this

phenomenon is now recognized as one of the primary factors contributing

to the success of plant breeding in tomato and many other crops.

This subject is surveyed under the headings: 1. Introduction; 2.

Manifestation of heterosis in tomato—Heterosis in tomato is observed for a

large scale of economic quantitative traits such as total and early yield and

yield components, tolerance to stress, dry matter, vitamin C, lycopene content

etc. More than 50-60% of the studies on heterotic performance in tomato

refer, however, to heterosis for yield and yield components; 3. Genetic basis
of heterosis in tomato—Manipulating heterosis in breeding programs

requires knowledge on its quantitative genetic basis. Data concerning gene

action for tolerance to abiotic stresses and fruit nutritional value, sensory

and market quality is summarized, and recent studies of heterosis in tomato

are reviewed; 4. Strategies for developing tomato hybrids—Making complex

characters more amenable to improvement by determining their components

as well as relationship between them is analyzed as one way to facilitate

breeding for complex characters. Possibilities for tomato genetic

improvement based on mapping genes involved in quantitative trait loci (QTL) and more efficient identification of the lines whose crosses would

result in promising hybrids, based on molecular markers, are reviewed

and discussed; 5. Improving the process of hybrid seed production—

Advantages and disadvantages in incorporating different types of genic

male sterility into hybrid breeding programs are discussed; 6. Tomato hybrid

adoption in practice—history, present situation and information concerning

the development of the first tomato hybrids in USA, France, Holland, Israel,

Japan, Bulgaria, China is presented; and 7. Future
prospects and challenges
in exploitation of heterosis in tomato discussed.
Achievement of the
ambitious and complex breeding goals in tomato improvement will be
difficult without the extensive use of heterosis. Combining conventional
and molecular breeding techniques might offer help in improving screening
efficiency for many traits of agronomic value, estimating genetic diversity,
reducing the time for line development, assessing heterotic groups, detecting
and individually analyzing the loci underlying heterosis.

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INTRODUCTION

During the past century, plant breeding and genetics have contributed significantly to improve nutritive value of horticultural and agronomic crops. Crop nutritive value is influenced by available cultivars, as well as production and postharvest environments. Significant strides have been made, especially in altering content of macronutrients such as proteins, carbohydrates, and oils in crop plants. Current research on improving phytonutrient content includes greater focus on study and assignment of
health-promoting properties to micronutrients as well. A vast number of phytonutrients have been identified which are believed to impart health benefits. Relatively few of these phytonutrients, however, are well proven to impart this function. Of this large group of compounds, the genetics of biosynthesis and accumulation is well characterized in relatively few examples. The knowledge required by plant breeders and geneticists to improve plant nutritive value requires input from many disciplines to contribute to the improvement of health benefits. These disciplines include nutrition, food science, medicine, postharvest physiology, molecular biology, and biochemistry.

Tigchelaar (1987) identified four alternatives to enhance the contribution of horticultural crops to human nutrition. These include: 1) genetic enhancement of significant nutrients, 2) improvement of fruit quality to encourage greater per capita consumption, 3) facilitate culture to encourage greater production and availability, and 4) reduce the seasonality of supply to encourage consumption. This review focuses on genetic enhancement of tomato fruit nutritive quality. Varietal development programs have long
focused on selection for yield and disease resistance. Enhancement of
constituents that are not major yield components take on new importance
today in crop improvement programs as market demands support these
efforts. A survey conducted in 1990 identified 11 crucial issues affecting
horticulture and ranked food quality and safety as the number one issue in
terms of present importance (Mitchell 1990). Survey respondents encouraged
the development and production of crops having higher nutritional value.
Current high levels of consumer health awareness suggest that a survey
created today would yield similar priorities.
The cultivated tomato, Solanum lycopersicum*, is an extremely popular
and versatile vegetable crop. The tomato fruit is utilized fresh and as a
processed product. It boasts a United States commercial crop value of nearly
2 billion dollars (United States Department of Agriculture 2005). World
wide, over 115 million metric tons of tomatoes were produced in 2004 for
fresh and processed consumption on approximately 4.4 million hectares
(Food and Agriculture Organization of the United Nations 2004). The
United States is one of the leading world tomato producers, second only to
China. In 2004, China and United States produced 30.1 million and 12.4 million metric tons of tomatoes, respectively (Food and Agriculture Organization of the United Nations 2004). Tomato ranks second in importance among commercial vegetable crops in the United States in terms of yield and consumption, and is the favorite homegrown crop of four out of five backyard gardeners. Yearly per capita consumption of fresh tomatoes increased 15% between the early 1990s and the early 2000s to nearly 18 pounds per person. Conversely, use in processed products declined 9% to 68 pounds per person (United States Department of Agriculture 2005).

By virtue of the volume of tomato products that are consumed, the tomato crop makes a significant dietary contribution to human health (Table 6.1).

A typical tomato fruit, for example, contains only intermediate levels of provitamin A, carotenoids and vitamin C, yet makes important contributions to the dietary intake of vitamin A (9.5%) and vitamin C (11.5%) (Senti and Rizek 1975, United States Department of Agriculture 2002). An average carrot, by comparison, contains provitamin A levels 75 to 85 times higher than that of a typical red pigmented tomato, and supplies 14% of the total
U.S. vitamin A consumption. Vitamin A deficiency is a major cause of childhood mortality and is also a likely contributing factor to maternal health.

* Author has adopted new taxonomic nomenclature Solanum lycopersicum of cultivated tomato (in lieu of Lycopersicon esculentum) in this chapter and also treated other Lycopersicon species under the genus Solanum based on recent publication of Spooner (2005)

Table 6.1 Nutrient content of fresh tomatoes: red, ripe, raw, year-round average.

Nutrient Value per 100g of Nutrient Value per 100g of edible portion edible portion

**Proximates**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>94.5 g</td>
</tr>
<tr>
<td>Protein</td>
<td>0.88 g</td>
</tr>
<tr>
<td>Total fat (lipid)</td>
<td>0.20 g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>3.92 g</td>
</tr>
<tr>
<td>Fiber, total dietary</td>
<td>1.2 g</td>
</tr>
<tr>
<td>Sugars, total</td>
<td>2.63 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.00 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.25 g</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.37 g</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>833 IU</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>0.08 mg</td>
</tr>
<tr>
<td>Folate, total</td>
<td>15 mcg</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
</tr>
<tr>
<td>Zn-Tocopherol</td>
<td>0.54 mg</td>
</tr>
</tbody>
</table>

*Calcium* 10 mg, *Vitamin K* 7.9 mcg

*Iron* 0.27 mg
Magnesium 11 mg Lipids
Phosphorous 24 mg Fatty acids,
Potassium 237 mg total saturated 0.05 g
Sodium 5 mg total monounsaturated 0.05 g
Zinc 0.17 mg total polyunsaturated 0.14 g
Copper 0.06 mg Phytosterols 7 mg
Manganese 0.11 mg
Selenium 0 mcg Carotenoids β-carotene 449 mcg β-carotene 101 mcg Lycopene 2573 mcg Lutein + zeaxanthin 123 mcg
Adapted from: United States Department of Agriculture, National Nutrient Database

Deaths in developing countries (World Health Organization 2005). Tomatoes
are a major dietary source of lycopene, the red pigment in tomato fruit that
is associated with dietary health benefits (Gerster 1997). In addition to
these well-known phytonutrients, other compounds in tomato fruit with
antioxidant properties include chlorogenic acid, rutin, plastoquinones,
also contribute carbohydrates, fiber, flavor compounds, minerals, proteins,
and glycoalkaloids to the diet (Davies and Hobson 1981, Gundersen et al.
2001).

Various aspects of the inheritance of tomato fruit
nutritive constituents have been condensed in larger reviews of tomato genetics and fruit quality (e.g., Stevens 1986, Stevens and Rick.1986, Tigchelaar 1987, Berry and Uddin 1991). The present chapter reviews new developments relevant to prior treatments of the subject and highlights new applications of molecular genetics to enhancement of tomato nutritive value.

MICRONUTRIENTS

Considerable genetic variation exists in tomato for micronutrients with antioxidant activity (Hanson et al. 2004, Schauer et al. 2004). A number of these micronutrients, particularly carotenoids, have been the subject of considerable research due to their contribution to the quality of fresh and processed tomato products. Increased recognition of their health promoting properties has stimulated new research and provided added justification for ongoing investigations in this area.

Carotenoids

Horticultural crops are the main source of dietary carotenoids and much of the world dietary vitamin A is derived from vegetable carotenes. Tomato fruit color is determined by carotenoid content. Enhanced fruit pigmentation has long been a goal in tomato cultivar development
programs due to the positive association between the intensity of pigmentation and perceived product quality. Recognition of the health benefits associated with carotenoid consumption provides added justification for improving tomato fruit pigmentation (Paiva and Russell 1999). In addition to \( \beta \)-carotene, which is a provitamin A carotenoid, lycopene has received increasing attention for its potential health benefits due to recent research that demonstrated positive associations between tomato products and improved human health (Giovannucci et al. 1995). Consumption of tomato products is associated with decreased risk of cardiovascular disease and certain cancers, including prostate and cervical cancer (Clinton 1998, Gerster 1997, Giovannucci 1999).

Recent discussions note the positive association between lycopene and health, but also emphasize that there are a family of compounds in tomato that are of benefit (Laquatra et al. 2005). Relative to carotenoids, studies on the colorless carotenoid phytofluene demonstrated that it is more bioavailable than expected, based on its concentrations in tomatoes (Paetau et al. 1998). These studies suggest that phytofluene may play a synergistic
role with other carotenoids in protecting human health.

Total carotenoid content of a typical tomato fruit varies between 70 and 190 µg/g fresh weight (Gross 1991). Carotenes constitute 70 to 95% of the total carotenoids. Lycopene, the red pigment in tomato fruit, is the predominant carotene, comprising up to 90% of the total carotenoids. Its colorless polyene precursors, phytoene and phytofluene account for 15 to 30% of total carotenoids. Lesser amounts of the provitamin A carotenoid, -carotene (2 to 15%), plus -carotene, -carotene, -carotene, lutein, and neurosporene can be measured. Within the tomato fruit, the outer pericarp tissue contains the highest concentration of carotenoids. Lycopene levels in the outer pericarp may be six-fold higher than that in the locular tissue (Gross 1991). Levels of carotenoids in tomato products are dependent on the variety and on growing conditions. Summer- and winter-greenhouse grown fruits are lower in carotene content than fruits produced outdoors during summer months. Fruit picked green and ripened in storage may be much lower in carotene than vine-ripened fruit (Gould 1992).

The identification and cloning of genes (e.g. cDNA) that code for nearly all of the enzymes required for carotenoid biosynthesis in
plants has stimulated considerable interest in engineering plants with altered carotenoid content (Cunningham and Gantt 1998). Numerous successful and unsuccessful efforts to alter fruit carotenoid composition by manipulating expression of carotenogenesis transgenes have been reported.

Early studies employing constitutively expressed phytoene synthase in transgenic tomatoes caused ectopic production of carotenoids and dwarfism (Fray et al. 1995). Dwarfism in these plants was inversely related to expression of phytoene synthase, a likely diversion of geranyl geranyl pyrophosphate away from the gibberellin biosynthetic pathway. Subsequent studies have focused on expression of the bacterial carotenoid genes, crtl encoding phytoene desaturase and crtB encoding phytoene synthase, in transgenic plants (Romer et al. 2000, Fraser et al. 2002). This strategy resulted in successful modifications of fruit carotenoid content without the adverse effects associated with constitutive expression of the tomato phytoene synthase Psy1 cDNA. Expression of crtl resulted in a three-fold increase in -carotene content (Romer et al. 2000). Total carotenoid content was not
increased and transgene expression did not affect plant growth and
development. Expression of crtB in transgenic plants resulted in 2 to 4-fold
increases in total carotenoid content. Levels of phytoene, lycopene,
carotene, and lutein were increased 2.4-, 1.8-, and 2.2-fold, respectively
(Fraser et al. 2002). Rosati et al. (2000) successfully altered carotenoid levels
in transgenic plants with constructs intended to up-regulate or down
regulate the expression of the lycopene \( \beta \)-cyclase gene. Expression of an
Arabidopsis lycopene \( \beta \)-cyclase cDNA resulted in increased \( \beta \)-carotene
content. Conversely, transformation with a tomato antisense lycopene
cyclase cDNA construct reduced lycopene \( \beta \)-cyclase activity and was
accompanied by a slight increase in lycopene content. In a related study,
overexpression of the genes encoding the Arabidopsis lycopene \( \beta \)-cyclase
and pepper \( \beta \)-carotene hydroxylase, under the control of a phytoene
desaturase (Pds) tomato fruit specific promoter, produced transformants
with significantly increased levels of \( \beta \)-carotene and the xanthophylls
cryptoxanthin, and zeaxanthin (Dharmapuri et al. 2002). The xanthophyll
overproduction trait was inherited as a single dominant character. Overall,
these studies demonstrate that expression of foreign transgenes that influence carotenoid biosynthesis can successfully overcome the co suppression reported to occur when using tomato coding sequences.

Utilizing a novel approach, Mehta et al. (2002) demonstrated that fruit specific expression of an yeast S-adenosylmethionine decarboxylase gene in transgenic plants resulted in increased ripening-specific accumulation of spermidine and spermine, levels of which normally decline in ripening fruit, and ethylene levels that were higher than those observed in non transformants. Increased polyamine accumulation was associated with a 2- to 3-fold increase in lycopene content. Elucidation of the mechanism by which higher polyamines influence carotenoid accumulation in an ethylene independent manner should reveal new strategies to improve fruit quality and nutritive value.

Mutants

A large genetic database exists for simply inherited genes that influence carotenoid content in tomato. The elucidation of carotenoid biochemistry was aided in large part through the study of single gene mutants of tomato.
Based upon the results of inheritance studies in tomato and on the information available at that time on the structures of proposed intermediates, Porter and Lincoln (1950) detailed the first biochemical pathway of carotenoid biosynthesis in 1950. Subsequent modifications and extensions of that pathway have occurred (Porter and Anderson 1962, 1967, Goodwin 1971, Jones and Porter 1986, Hirschberg 2001). Carotene biosynthesis in higher plants has been reviewed extensively in recent years (Bramley 1992, Bartley and Scolnik 1994, Hirschberg 2001). The molecular genetics of carotenoid biosynthesis in plants has advanced rapidly due to the wealth of information available from bacterial and fungal models and extension of this knowledge to plant genomes (Piechulla et al. 1985).

More than 20 genes have been characterized in tomato that influence the type, amount, or distribution of fruit carotenoids depicted in Table 6.2 (Khudairi 1972, Darby 1978, Stommel 1992a). Carotenoid profiles in these variant genotypes have allowed speculation on points of gene action in the carotenoid biosynthetic pathway. Many of the available color variants were first identified as mutants that arose spontaneously in cultivars of
S. lycopersicum. Color mutants also occur in the wild tomato species, and in the case of the green-fruited species, are often manifested transgressively upon introduction into S. lycopersicum.

Table 6.2 Selected loci that influence tomato fruit carotenoid, anthocyanin, and carbohydrate content.

<table>
<thead>
<tr>
<th>Mutant (symbol)</th>
<th>Fruit Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotenoid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apricot (at)</td>
<td>Fruit yellow with pinkish blush Jenkins and McKinney (1955)</td>
<td></td>
</tr>
<tr>
<td>Beta (B)</td>
<td>Fruit flesh orange; increased Lincoln and Porter (1950), α-carotene, reduced lycopene Tomes et al. (1954), Ronen et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Beta modifier (Mo B)</td>
<td>&gt;90% of total carotenes as Tomes et al. (1954), α-carotene (Mo B), ca. 50% of Zhang and Stommel (2000) total carotenes as α-carotene (Mo B +); action specific to B.</td>
<td></td>
</tr>
<tr>
<td>Colorless skin (y)</td>
<td>Unpigmented fruit epidermis; Rick and Butler (1956) Colorless skin over red flesh results in pink fruit</td>
<td></td>
</tr>
<tr>
<td>Dark green (dg)</td>
<td>Immature fruit dark green; Konsler (1973), increased levels of carotenoids Levin et al. (2003) in mature fruit</td>
<td></td>
</tr>
<tr>
<td>Delta (Del)</td>
<td>Orange-red flesh; enhanced Tomes (1963), α-carotene and β-carotene Ronen et al. (1999) fractions</td>
<td></td>
</tr>
<tr>
<td>Diospyros (dps)</td>
<td>Fruit tissue is dusky orange Rick (1967)</td>
<td></td>
</tr>
<tr>
<td>Ghost (gh)</td>
<td>Phytoene synthesis normal, Rick et al. (1959), no colored carotenoids Scolnik et al. (1987)</td>
<td></td>
</tr>
<tr>
<td>Green flesh (gf)</td>
<td>Chlorophyll retained in ripe Kerr (1958a) fruit, normal lycopene synthesis; fruit reddish-brown</td>
<td></td>
</tr>
<tr>
<td>Green ripe (Gr)</td>
<td>Green-pigmented flesh in Kerr (1958b), Barry ripe fruit et al. (2005)</td>
<td></td>
</tr>
</tbody>
</table>
High pigment 1 (hp1) Immature fruit dark green; Clayberg et al. (1960), Van increased levels of carotenoids Tuinen et al. (1997), Liu in mature fruit et al. (2004)

High pigment 2 (hp2) Similar to hp1 Yen et al. (1997), Mustilli et al. (1999)

Intensified Enhanced lycopene synthesis Rick (1974)

pigmentation (Ip)

Old gold crimson (og c) Enhanced red color; increased Thompson et al. (1967), lycopene, reduced β-carotene Ronen et al. (2000)

Red yellow (ry) Modifier for red color in yellow Young (1956) fruit

Sherry (sh) Fruit flesh yellow with reddish Zscheile and Lesley (1967) tinge

Tangerine (t) Orange fruit; colored carotenoids MacArthur (1934), principally prolycopene Isaacson et al. (2002)

Yellow flesh (r) Reduced polyenes, very low Rick & Butler (1956), levels of carotenes; fruit flesh Fray and Grierson (1993) yellow (Contd.)

An example of such a transgressive segregant is the Beta (B) allele, first characterized in progeny descended from a cross between S. lycopersicum and the green-fruitied species S. habrochaites* (Lincoln et al. 1943, Kohler et al. 1947). Introgression of the B allele into S. lycopersicum increases β-carotene content at the expense of lycopene. Inheritance studies suggested that high concentrations of β-carotene found in orange-pigmented fruit were controlled by a single gene exhibiting incomplete dominance (Lincoln and Porter 1950). Subsequent studies by Tomes et al. (1954)
determined that B was dominant but subject to influence by a modifier gene, Mo B, that segregated independently of B. Utilizing molecular markers linked to B and Mo B, genotypic evaluations discounted incomplete dominance to explain inheritance of fruit carotene content, but revealed that B and Mo B were linked on chromosome 6 and did not segregate as independent genes as originally proposed (Zhang and Stommel 2000). In the presence of the homozygous recessive form of the allele, Mo B Mo B, α-carotene represents more than 90% of the total carotene content and fruit are orange pigmented (Tomes et al. 1954). Expression of the dominant Mo B + form of the allele, however, reduces α-carotene content to more than 50% of the total carotenoids and increases lycopene to less than 50% of total carotenoids resulting in red-orange pigmented fruit. Orange-fruited accessions of S. cheesmaniae, S. galapagense, S. pimpinelli folium, S. chilense, and S. chmielewskii containing high concentrations of α-carotene have also been described (Rick 1956, Manuelyan et al. 1975, Chalukova 1988, Stommel and Haynes 1994).

Anthocyanin

Anthocyanin fruit (Aft) Variable purple pigmentation;
Giorgiev (1972), anthocyanin in skin and outer Jones et al. (2003) pericarp

Atroviolacium (atv) Excess anthocyanin on fruit Rick (1964)

Aubergine (Abg) Fruit epidermis purple, Rick et al. (1994) particularly on shoulder

Carbohydrate

Apoplastic invertase (LIN5) Increased hexose sugars Fridman et al. (2000)

Fructose glucose ratio (Fgr) Increased fructose to Schaffer et al. (1999) glucose ratio

Fructokinase 2 (FK2) Modifier of Fgr Levin et al. (2000)


Transient starch Increased transient starch Schaffer et al. (2000)

accumulation accumulation

(AGPaseL1)

(Contd.)

Name changed from L. hirsutum

The inheritance of β-carotene content in these wild tomato species is consistent with that described for the dominant B gene from S. habrochaites.

Molecular analysis revealed that B encodes a novel lycopene β-cyclase that converts lycopene to β-carotene (Ronen et al. 2000). Increased transcription of B in the Beta mutant results in conversion of lycopene to carotene and orange pigmented fruit. Based upon variable expression of
lycopene-\(\alpha\)-cyclase transgenes in fruit tissue of transformed plants, Ronen et al. (2000) suggested that variation observed in tissue-specific \(\alpha\)-carotene accumulation could be attributed to differences in the rate of gene expression as a result of additional sequence elements upstream to the promoter.

Use of \(B\) resulted in the development of the fresh market tomato varieties Caro-Red (Tomes and Quackenbush 1958) and Caro-Rich (Tigchelaar and Tomes 1974) that contain nearly ten times the \(\alpha\)-carotene of normal red fruited tomato varieties. Consumer preference for red fruit pigmentation has unfortunately, limited widespread use of these cultivars. Increased consumer education may be required to ensure consumption of high carotene containing varieties. Breakage of the close genetic linkage between \(B\) and sp, the gene for indeterminate growth habit, has eliminated this limitation of \(B\) for use in cultivars intended for commercial production (Stommel 2001, Stommel et al. 2005).

In contrast with \(B\), the recessive old gold crimson mutant (og \(c\)) enhances lycopene content at the expense of \(\alpha\)-carotene (Thompson et al. 1967, Lee and Robinson 1980). Ronen et al. (2000) demonstrated that og \(c\) is an allele
of B and that null mutations in the B gene are responsible for the old gold crimson phenotype. Despite up to 40% reductions in \&carotene levels in crimson plants, crimson cultivars have been developed for the market for their desirable dark red pigmentation. The relatively recent recognition of health benefits attributable to lycopene in the diet has negated any negative consequences of the loss in nutrients from reduced levels of \&carotene.

Under low temperature conditions, plants expressing og c exhibit orange flower pigmentation, thus facilitating screening in segregating populations.

Introgression of the monogenic recessive high pigment (non-allelic hp1 and hp2, mapping to chromosome 2 and 1, respectively; Van Tuinen et al. 1997, Yen et al. 1997) mutant alleles enhance total fruit carotenoid content without significantly altering the relative percentage of different carotenoid constituents (Cookson et al. 2003). As a result, levels of a number of carotenoids can be increased without altering desirable red fruit pigmentation.

These mutants are light hypersensitive and characterized by seedlings with higher anthocyanin levels and short hypocotyls as well as higher fruit and foliage pigmentation (Mochizuki and Kamimura 1984). Within
hp1 and hp2 loci, two mutant alleles have been identified that exhibit varying photoresponsiveness: hp1 and hp1 w and hp2 and hp2 j (Kerckhoffs and Kendrick 1997). A third mutant exhibiting exaggerated photoresponsiveness is the dark green (dg) mutant (Konsler 1973). Recent evidence indicates that dg is allelic to hp2 (Levin et al. 2003). Incorporation of these alleles boosts total carotene content 30 to 50%. α-carotene content of dg lines is approximately 50% greater than that found in hp lines and 250% greater than that typical of normal red-fruited tomatoes. All three mutants produce dark green immature fruit due to increased chlorophyll content, but mature green fruit of dg mutants is characteristically much darker green in comparison to hp1 and hp2 fruit (Baker and Tomes 1964, Palmieri et al. 1978). Both dg and hp mutations also increase fruit firmness and ascorbic acid levels (Jarret et al. 1984). Undesirable pleiotropic effects that include slow seed germination, increased seedling mortality, brittle stems and premature defoliation is associated with these mutants and has thus far limited their practical use. Plants expressing both crimson and high pigment alleles pro...
duce fruit with lycopene levels three to four times that of conventional red-fruited tomatoes. If utilized as a source of natural lycopene, the negative effect of hp on yield can be offset by the industries high dollar value of the extracted pigments.

The hp2 mutant allele encodes the tomato homolog of the nuclear protein DEETIOLATED1 (DET1) from Arabidopsis that is involved in light signal transduction (Mustilli et al. 1999). Similarly, dg encodes a novel allele of the DET1 gene (Levin et al. 2003). Recent studies demonstrated that hp1 is a mutation in a tomato UV-DAMAGED DNA-BINDING PROTEIN 1 (DDB1) homolog whose Arabidopsis counterpart interacts with DET1 (Liu et al. 2004). Studies on these high pigment mutants reveal that light signal transduction regulates the carotenoid pathway in a manner that affects total fruit carotenoid content and that genes encoding components of light signal transduction may provide new genetic tools for manipulating fruit nutritional value (Liu et al. 2004, Yen et al. 1997). Liu et al. (2004) demonstrated that two tomato light signal transduction genes, LeHY5 and LeCOP1LIKE, are positive and negative regulators of fruit pigmentation,
respectively. Further studies (Levin et al. 2004) report additional putative
light responsive genes that modulate carotenoid profiles in fruit of these
light hypersensitive tomato mutants.

The gene Intensified Pigmentation, which has effects similar to that of
dg and hp, was described in progeny descended from an S. lycopersicum x
S. chmielewskii cross (Rick 1974). Fruit expressing Ip also exhibit dark green
immature fruit and intensified carotenoid pigmentation in ripe fruit.

Unlike dg and hp, Ip behaves as a dominant gene and appears to have
reduced detrimental effects on seed germination and plant vigor.

A variety of mutants affecting tomato fruit carotenoid content distinct
from the high pigment and high \( \beta \)-carotene types have been characterized
and may have beneficial effects in enhancing or evaluating fruit nutritional
composition. The recessive gene r (Yellow Flesh) is responsible for yellow
fruit flesh and results in greatly reduced levels of polyenes and very low
levels of colored carotenoids (Rick and Butler 1956). Many yellow-fleshed
home garden type tomatoes have been developed. The r locus corresponds
to a null mutation for a chromoplast-specific phytoene synthase, Psy1 (Fray
and Grierson 1993), thus accounting for the lack of carotenoid accumulation in yellow-fruited tomatoes and green-fruited species. A second phytoene synthase gene, Psy2, has been identified which is also expressed in ripening tomato fruit (Bartley and Scolnik 1993). Its transcripts are relatively more abundant, however, in mature leaves. Phytoene synthase has been described as the “pacemaker” in carotenoid synthesis in ripening fruit and is regulated at the level of transcription (Fraser et al., 1994). Lois et al. (2000) have proposed that a second gene, DXS, encoding the first enzyme of isoprenoid synthesis in the plastids, works in concert with Psy1 to control fruit carotenoid synthesis. The dominant delta (Del) allele conditions increased \( \beta \)-carotene and reduced lycopene content, resulting in reddish-orange colored fruit (Tomes 1963). Ronen et al. (1999) demonstrated cosegregation of the Crtl-e locus encoding \( \beta \)-cyclase with the Del mutation located on chromosome 12. Cyclase converts lycopene to \( \beta \)-carotene. Transcript for Crtl-e was shown to increase 30-fold in ripening fruit of the Del mutant. Del does not alter carotenoid composition or Crtl-e mRNA in leaves or flowers, thus indicat
ing that Del is likely an allele of the gene for \( \beta \)-cyclase (Ronen et al. 1999).

The recessive tangerine \((t)\) mutant conditions orange fruit color due to

the accumulation of poly-cis-lycopene, also referred to as prolycopene

(MacArthur 1934, Tomes 1963). Fruit of the tangerine mutant also exhibit

elevated phytoene and phytofluene. Trans-lycopene is the principal form

of lycopene in red tomato fruit. Located on chromosome 10, a clone of the

tangerine gene, designated CRTISO, was shown to encode an authentic

carotenoid isomerase that is required during carotenoid desaturation

(Isaacson et al. 2002). CRTISO is a redox-type enzyme structurally related
to the bacterial-type phytoene desaturase CRTI. Analysis of two alleles of

tangerine demonstrated that in one case, loss of function in CRTISO was

attributable to a deletion mutation in CRTISO, and in the second, expression

of this gene was impaired. CRTISO from tomato is normally expressed in

all green tissues but is upregulated during fruit ripening and in flowers.

Unlike orange-pigmented fruit that accumulate \( \beta \)-carotene due to the Beta

allele, tangerine mutants do not have increased pro-vitamin A carotenoids.

Evidence that cis-lycopene is more bioavailable, than trans-lycopene (Unlu
et al. 2003, Boileau et al. 1999), has focused considerable interest on this
mutant in human nutrition-related studies. Pigment compositions of nearly
all orange-fleshed cultivars available for commercial and home use attribute
their color to the tangerine allele.

Incorporation of the recessive y allele results in a colorless epidermis
lacking the normal yellow pigmentation (Rick and Butler 1956). Presence
of y in red-fleshed genotypes results in a pink fruit phenotype. Pink culti
vars are popular in home garden and specialty markets. The combination
of y with r (yellow flesh) results in pale yellow or “white” fruit. Several
novel cultivars, such as White Queen, owe their pigmentation to this gene
combination. Plants with the recessive apricot (at) allele bear fruit that are
coloristically yellow with a pinkish blush at maturity (Jenkins and
Mackinney 1955). Variations on this include a modifier gene for red color
in yellow fruit (ry; Young 1956) and sherry (sh; Zscheile and Lesley 1967).

Additional color variants include the diospyros (dps) mutant with dusky
orange fruit.

Presence of the recessive green flesh (gf) allele prevents breakdown of
chlorophyll that normally occurs in maturing fruit (Kerr 1958a). Retention of green chlorophylls and the red pigment, lycopene, results in reddish brown colored fruit. Anthers of gf plants exhibit a characteristic lemon green pigmentation. Green ripe (Gr) results in green-pigmented flesh in ripe fruit (Kerr 1958b). Barry et al. (2005) mapped the dominant Gr locus to the long arm of chromosome 1 and determined that Gr is an ethylene insensitive mutant that may encode a novel ethylene signaling component. Novel heirloom cultivars have been described with unusual pigmentation patterns. ‘Green Grape’, for example, exhibits yellow/green exterior and green interior attributed to a lesion in up-regulation of the carotenoid biosynthetic pathway. Expression of the ghost (gh) allele on chromosome 11 results in fruit that contain only phytoene and no colored carotenoids due to a block in the desaturation of phytoene (Rick et al. 1959, Scolnik et al. 1987). Expression of gh may be quite variable. Similar to fruit color, leaf tissue is generally white or light yellow, although mosaics of white and green are not uncommon.

Quantitative Trait Loci

The described monogenic mutants have a dramatic effect on
fruit pigmentation. Nonetheless, they have not contributed widely to carotenoid pigmentation in commercial cultivars. Extensive genetic and molecular characterization of simply inherited tomato pigment mutants has not established a molecular genetic basis for quantitatively inherited variation in fruit pigmentation.

Analogous to fruit firmness, soluble solids, and other fruit quality traits, quantitative trait loci (QTL) associated with variation in fruit pigmentation have been described that begin to explain dissimilarity in intensity of red pigmentation in modern tomato cultivars. Numerous QTL introgressed from S. pimpinellifolium (Tanksley and Nelson 1996, Chen et al. 1999), S. habrochaites (Bernacchi et al. 1998, Kabelka et al. 2004, Yates et al. 2004), S. peruvianum (Fulton et al. 1997, Yates et al. 2004), and S. neorickii (Fulton et al. 2000) have been described that influence fruit color. Analysis of QTL identified in a S. lycopersicum cross also revealed loci associated with enhanced fruit color (Saliba-Colombani et al. 2001, Causse et al. 2002). Not surprisingly, these QTL may have negative or positive effects on ripe fruit color and epistasis as well as pleiotropy may occur.
Whereas QTL studies often focus upon the positive effect of loci introgressed from wild relatives of tomato, Kabelka et al. (2004) identified loci from *S. lycopersicum* that contributed to improved tomato color and noted that the trend for introgressions from *S. habrochaites* was to shift the population toward undesirable color. Encouragingly, all of these studies identify some QTL associated with fruit qualities that have also been identified for quality attributes by others in different interspecific tomato crosses. These conserved major loci and minor loci with positive epistatic effects will be of great interest in marker-assisted breeding strategies to improve tomato quality and nutritive value.

**Vitamins**

Vitamin A and vitamin C are the principal vitamins in tomato fruit. The tomato makes important contributions to the dietary intake of these vitamins. Tomatoes also provide moderate levels of folate and potassium in the diet and lesser amounts of vitamin E and several water-soluble vitamins.

Vitamin A -carotene is the principal provitamin A carotenoid and is an essential
nutrient in the human diet because of its retinoid activity (Tee 1992, Omenn et al. 1994). Epidemiological evidence suggests that increased intake of high β-carotene containing fruits and vegetables may be associated with a reduced risk of heart disease and certain cancers (Ziegler 1989, Doll 1990, Block et al. 1992, Omenn et al. 1994). Vitamin A deficiency has been described as one of the most serious nutritional disorders of children in the world, especially in developing countries (Sommer 1997, World Health Organization 2005). Traditional breeding and transgene strategies to improve β-carotene content have yielded germplasm with increased levels of this provitamin A carotenoid (see Carotenoids).

Vitamin C

Fruits and vegetables supply approximately 91% of the vitamin C in the U.S. food supply. In tomato, considerable variation exists among cultivars and wild species accessions for ascorbic acid content, thereby providing good opportunities for genetic enhancement of fruit vitamin C potential.

Within the Solanum section Lycopersicon, ascorbic acid levels are reported to range from 10-120 mg/100 g fresh weight (Lambeth et al. 1966, Hobson and Davies 1971). Depending upon cultivar, environment,
fruit maturity

and postharvest treatment, ascorbic acid comprises 40-90% of the organic
acids (Bradley 1946, Carangal et al. 1954, Davies 1965, McClendon et al. 1959). Exposure of fruit to sunlight is an important factor affecting the
level of ascorbic acid (Hassan and McCollum 1954, Dumas et al. 2003).

Malic acid is the other principal organic acid and ranges from 10-60% of
that of ascorbate (Davies 1965). Ascorbic acid content typically increases
as fruits mature, reaching a maximum just before full red color, and
decreasing with full ripeness and overmaturity (Malewski and Markakis 1971). A comparison of fresh versus processed purees found that processing
caused a 3% to 17% loss in ascorbic acid content (Warnock 1983). Warnock (1983) suggested that genotypic differences accounted for the degree of
ascorbate loss upon processing.

Stevens (1972) evaluated the inheritance of citrate and malate in two
tomato accessions. The inheritance of citrate and malate concentration was
controlled by a single gene for each compound. The genes are linked and
exhibit 18% recombination. The dominant form of the alleles condition a
high concentration of citrate and a low concentration of
malate. Since high malate concentrations are often associated with sour fruit taste, high citrate to malate ratios are preferred.

Tomato cultivars such as ‘DoubleRich’ with double the normal vitamin C content (ca. 50 mg/100g) were developed via an interspecific cross between the cultivated tomato and the ascorbic acid-rich wild species *S. peruvianum*. An association between high vitamin C and poor yield, due primarily to small fruit size, has stymied commercial use of these cultivars (Stevens and Rick 1986). Fruit of high pigment (hp) genotypes also contain increased levels of vitamin C. Unfortunately, undesirable effects of hp on yield have likewise limited its usefulness in increasing vitamin C. Andrews et al. (2004) proposed that increased levels of ascorbic acid in developing fruit may increase the tolerance of hp-1 fruit to photooxidative injury.

Modern tomato hybrids have come under scrutiny for alleged lower vitamin C content and inferior nutritional quality. Contrary to this notion, a controlled study demonstrated that newer cultivars contained approximately 25% more vitamin C than those developed twenty years earlier (Burge et al. 1975, Matthews et al. 1973). This suggests that further
incremental gains in genetic improvement of tomato vitamin C content may yet be realized without compromising yield.

Despite the wealth of information documenting the importance of vitamin C to human health, relatively little information has been available about the pathway(s) leading to its biosynthesis in plants. This contrasts with a well-understood pathway in animals (Burns 1967). Wheeler et al. (1998) provided evidence that mannose and galactose are efficient precursors for ascorbic acid in plants and demonstrated the existence of a galactose dehydrogenase which is active in converting galactose to ascorbate precursors, thus filling a major gap in plant carbohydrate metabolism. Agius et al. (2003) recently isolated the gene GaIUR which encodes an NADPH-dependent D-galacturonate reductase and demonstrated that biosynthesis of ascorbic acid in strawberry fruit occurs through galacturonic acid, a component of cell wall pectins. Overexpression of GaIUR in Arabidopsis thaliana enhanced vitamin C levels two- to three fold, thus demonstrating the potential to engineer increased vitamin C levels in tomato. In light of the negative effects on yield often associated
with high vitamin C lines produced through conventional hybridization,
characterization of transgene effects on fruit yield and other quality attributes must be evaluated.

Folate

Folate in the diet comes mainly from plant sources. Whereas green leafy vegetables and legume seeds are folate-rich, folate concentrations in other vegetables are considerably lower (Scott et al. 2000, Konings et al. 2001).

Dietary fortification and supplementation with folic acid is practiced to offset birth defects, anemia, and increased risk of vascular disease and certain cancers (Lucock 2000, Krishnaswamy and Nair 2001, Molloy and Scott 2001). Nonetheless, folate deficiency is a serious concern in poor countries and is common in wealthy countries as well (de Bree et al. 1997, Konings et al. 2001, Krishnaswamy and Nair 2001). The biosynthetic pathway of folate is well characterized (Hanson and Gregory 2002, Goyer et al. 2004), and thus provides opportunities to engineer enhanced folate content in dietary foods (Zhang et al. 2003, Hossain et al. 2004).

Folates are tripartite molecules composed of pteridine and p-aminobenzoate (PABA) plus one or more glutamate moieties.
Overexpression of GTP cyclohydrolase I in fruit of tomato transformants resulted in a 3- to 140-fold increase in levels of the folate precursor, pteridine, and an average 2-fold increase in folate content (de la Garza et al. 2004). Transformants with intermediate levels of pteridine exhibited the highest levels of folate. Transformants that accumulated increased folate also exhibited depleted levels of PABA. Exogenous supply of PABA resulted in additional 10-fold increases in folate content, suggesting that additional modifications in the folate biosynthetic pathway to increase levels of this limiting compound may further boost fruit folate content.

Glycoalkaloids

Tomato belongs to the poisonous nightshade family and was long regarded as toxic. Although widely consumed in Europe by the mid-eighteenth century, tomatoes did not gain acceptance in the United States until the late 1700's due to the presumption that the fruits were poisonous. Glycoalkaloids and their toxic effects are commonly associated with Solanaceous species. Tomato accumulates the glycoalkaloids β-tomatine and dehydrotomatine in a 10 : 1 ratio (Madhavi and Salunkhe 1998). These compounds act as toxins or feeding deterrents to insect
pests of tomato

and are toxic to several pathogenic microorganisms that affect the crop. In

striking contrast with potato glycoalkaloids, the tomatine compounds in
tomato appear to be less toxic for human consumption, presumably because

they are eliminated from the body as an insoluble tomatine-cholesterol

complex formed in the digestive tract (Kozukue and Friedman 2003).

Friedman and Levin (1995) note that pickled and fried green
tomato fruit

have relatively high levels of tomatine in comparison to
red ripe fruit and

are widely consumed in many countries. Tomatine has a high

affinity for
cholesterol in vitro, and when administered orally to hamsters, significantly

reduced plasma cholesterol (Friedman et al. 2000a, b). High
tomatine

containing green tomatoes had a significantly greater
effect on reducing

LDL cholesterol and triglyceride levels than feeding low
tomatine

containing red tomatoes. Friedman (2002) notes the need for additional

studies to further substantiate studies suggesting a beneficial role for dietary
tomatine in enhancing the immune system, and in cancer chemotherapy.

Levels of tomatine compounds are influenced by the stage of tomato
fruit maturity. Immature fruit contain up to 500 mg of \( \alpha \)-tomatine per kg fresh weight of tomato fruit. Coincident with reduction in chlorophyll content of ripening tomato fruit, tomatine concentration declines 100-fold in breaker stage or ripe fruit to approximately 5 mg per kg fresh fruit weight (Kozukue and Friedman 2003). Courtney and Lambeth (1977) identified elevated levels of tomatine in fruit of the green-fruited species noting that the highest levels were found in an S. chmielewskii, S. habrochaites, S. cheesmaniae, S. pimpinellifolium, and S. peruvianum, noting that the highest levels were found in an S. chmielewskii accession. According to Rick et al. (1994), an unusual S. lycopersicum mutant that produces bitter fruit retains high tomatine levels in ripe fruit (500-5000 mg/kg dry weight). Retention of tomatine in these fruits is controlled by a single recessive gene and presumably encodes a defective tomatine-degrading enzyme that is normally active in ripening fruit. Consumption of these fruits by natives of Peru, apparently without acute toxic effects, further supports the notion that tomatine may be a lesser dietary toxin than once believed.

Considerable attention has been devoted to study of tomatine in vegeta
tive tomato tissue. Juvik et al. (1982) identified considerable variation for

foliar tomatine concentration, noting that levels were highest in an acces-
sion of S. peruvianum. Tomatine concentrations of S. lycopersicum and S.
pimpinellifolium accessions were moderately higher than levels in S.
lycopersicum. Inheritance studies demonstrated that foliar tomatine con-
centration was controlled by two codominant alleles at a single locus (Juvik
and Stevens 1982). Tomatine levels in S. lycopersicoides were up to 3.5% of
tissue dry weight (Oleszek et al. 1986).

Minerals

The roles of minerals in plant foods that have a positive effect on human

health or nutrition are well established. There is a wealth of literature that

provides evidence of their importance from research in animal model sys-
tems as well as from clinical and epidemiological studies (Lachance 1998).

Tomato mineral composition is greatly influenced by plant nutrition,

and as a result, has been well characterized in the context of mineral
deficiency and the effect of these conditions on plant health. There is

significant genotypic variation for mineral content in tomato fruit.

Potassium, together with nitrate and phosphorous,
constitutes approximately 93% of the total inorganic fruit constituents (Hobson and Davies 1971). Phosphorous and potassium are the two most well studied constituents. The concentration of other minerals in the fruit is low. Hobson and Davies (1971) summarize prior reviews of tomato mineral composition.

Although characterizations of mineral content, and in some cases, inheritance studies have been conducted, little effort has been made to improve fruit mineral composition.

Phosphorous

Phosphorous is the major inorganic anion in tomato fruit. Phosphorous levels in 25 divergent tomato accessions ranged from 3.1 to 6.7 mM (Paulson and Stevens 1974). Uptake of phosphorous is highly dependent on root growth. An inheritance study of tomato fruit phosphorous concentration found a strong genotype-environment interaction that was not attributed to variation in available soil phosphorous (Stevens and Paulson 1973). The study suggested that few genes are involved in genetic control of fruit phosphorous levels and that additive and dominance effects, in addition to epistatic interactions, contribute to observed phenotypes.
Potassium

The major inorganic cation in tomato fruit is potassium. Stevens (1972) reported potassium concentrations that ranged from 45.2 to 86.7 meq/liter among 55 divergent tomato lines. Potassium deficiency may contribute to poor fruit color and reduced acid content (Bradley 1946, Carangal et al. 1954). Significant positive correlations between potassium content and the total and titratable acidities have been reported (Hobson and Davies 1971).

Factors which increase potassium content of the fruit result in a corresponding increase in organic acids in an effort to maintain fruit pH.

Calcium

Over 30 physiological plant disorders have been associated with calcium deficiency (Maynard 1979). In tomato, calcium deficiency contributes to fruit blossom end rot. Good cultural practices have been the primary means for producers to control blossom end rot. Heritable differences for calcium utilization efficiency in tomato have been reported (Giordano et al. 1982).

The potential to manipulate mineral composition in tomato using transgene approaches is well-demonstrated by a recent report wherein calcium con
tent was increased up to 50% via expression of an Arabidopsis H^+/Ca^{2+}
transporter in carrot (Park et al. 2004).

Amino Acids

Reports on the amino acid composition of tomato fruit vary considerably.

Variation in amino acid composition is likely due to both genotypic
differences and varying plant nutrition (Freeman and Woodbridge 1960,
Davies 1966a). Glutamic acid is the predominant amino acid of ripe fruit.

Glutamic acid, α-aminobutyric acid, glutamine, and aspartic acid account
for approximately 80% of the total free amino acids (Freeman and
Woodbridge 1960). Glutamic acid content rises sharply, and aspartic acid
increases to a lesser extent, during fruit ripening. Interest in the amino acid
content of tomato centers on the influence of these nutrients on fruit flavor.

Attempts to modify fruit amino acid composition have been limited to
studies evaluating the effect of different fertilizer regimes. Applications of
nitrogen generally increased glutamic and aspartic acid content (Carangal
et al. 1954, Davies 1964). Apart from serine and threonine, which reach
peak levels before fruit fully ripen, other amino acids decrease during rip
ening, presumably for protein synthesis. Free amino acids
comprise a significant portion of the total amino acids of immature and mature tomato (Friedman 2002). Free lysine and methionine constitute 20-25% and 12-18% of total lysine and methionine, respectively. Lysine:arginine ratios are more than double that reported for cereal proteins and are comparable to that reported for legume proteins. Methionine:glycine ratios are greater than those reported for legume proteins and are similar to that for animal proteins. The essential amino acid content of tomato is considered to be of good quality, being similar to soy protein (Friedman and Brandon 2001).

Considerable diversity in amino acid content was identified among tomato and its wild relatives (Schauer et al. 2004). Levels of amino acids in fruit of wild species were generally lower in wild species in comparison to the cultivated form and amino acid metabolite ratios varied across species.

Phenolic Compounds

More than 4,000 phenolic phytochemicals have been identified (King and Young 1999). Flavonoids, phenolic acids, and polyphenols are the main classes of dietary phenolics. Flavonoids, which include anthocyanins, are the largest group of plant phenols and have been the
subject of consider
able research since they impart color to many horticultural commodities.

Various health-promoting effects have been ascribed to plant phenolic constituents. Vinson et al. (1998) determined that vegetables high in phenolic compounds have antioxidant quality comparable to that of pure phenols and superior to that of the antioxidant vitamins A, C and E. In a survey of S. lycopersicum and S. pimpinellifolium accessions, total phenolics content was most closely associated with measures of antioxidant activity (Hanson et al. 2004). Consumption of foods rich in dietary phenolics are believed to contribute to reduced radical-mediated pathogeneses such as carcinogenesis and atherosclerosis (Ames et al. 1993, Sawa et al. 1999). Phenolic compounds extracted from plant tissue have also been demonstrated to have significant hypolipidemic action (Sudheesh et al. 1997). The average per capita consumption of vegetable phenols in the U.S. is estimated at approximately 218 mg per day of catechin equivalents, which equates to three times the recommended intake of vitamins C, E and ß-carotene antioxidants (Vinson et al. 1998).

Flavonoids
Flavonoids comprise a large group of secondary plant metabolites and include anthocyanins, flavonols, flavones, catechins, and flavonones (Harborne 1994, Harborne and Williams 2000). Many are present in plants as sugar conjugates. As food constituents, flavonoids are believed to have health promoting properties because of their antioxidant and free radical scavenging activity (Shahidi and Wanasundara 1992, Pietta 2000). A multitude of epidemiological and in vitro studies provide increasing evidence that flavonoids impart dietary health benefits. Epidemiological studies suggest that their consumption is associated with a reduced risk of cardiovascular disease (Hertog et al. 1997), cancer (Knekt et al. 1997, Wattenburg 1990), and dementia (Commenges et al. 2000). In vitro studies demonstrate that flavonols can induce human protective enzyme systems (Nijveldt et al. 2001), have effects on antiplatelet aggregation (Rice-Evans et al. 1997), and reduce blood viscosity. Flavonols are also associated with reduced inflammatory responses and allergic reactions (Cook and Samman 1996), and antiviral, antituberculosis, and antimalarial activities (Harborne and Williams 2000). Daily flavone and flavonol intake in
western countries, expressed as aglycones, is estimated at approximately 25 mg per day (Hertog et al. 1993).

Nearly 98% of flavonols detected in tomato fruit were found to occur in the peel (Stewart et al. 2000). Tomatoes contain low levels of quercetin and kaempferol conjugates. The principal quercetin conjugate is rutin. Recent studies demonstrate that naringenin chalcone, a precursor to quercetin and kaempferol conjugates, is the main tomato fruit flavonoid and that a metabolic block at this step in the biosynthetic pathway results in low quercetin and kaempferol content (Muir et al. 2001). Total flavonol content in fruit of fresh market cultivars ranged from 1.3 to 203 microgram per gram of fresh weight (Stewart et al. 2000, Crozier et al. 1997). Flavonol concentrations in fruit of large-fruited genotypes were low, whereas the highest concentration of flavonols occurred in small cherry tomato fruit produced in warm sunny climates. Tomato juice and tomato puree contained 14 to 16 microgram per ml and 70 microgram per gram fresh weight, respectively. Whereas fresh tomatoes contained primarily flavonol conjugates, processed tomato products contained significant
amounts of free flavonols. Cooking reduced levels of conjugated quercetin 35% to 82%, depending on the cooking method employed (Crozier et al. 1997). This may be a critical factor for bioavailability of different flavonols. Quercetin -glucoside, for example, is more easily absorbed than its aglycone, quercetin (Aziz et al. 1998).

In addition to colorless flavonols, novel heirloom cultivars such as 'Cherokee Purple' may exhibit red/purple flesh attributable to fruit anthocyanin accumulation (Table 6.2). Whereas anthocyanins normally occur in vegetative tissues of tomato, anthocyanin pigmentation in fruit is atypical. Fruit of tomato genotypes with the dominant anthocyanin fruit (Aft; formerly Af) gene accumulate elevated levels of anthocyanin in the fruit skin and outer pericarp tissues as well, resulting in purple fruit pigmentation (Giorgiev 1972, Jones et al. 2003). Anthocyanins in the Aft mutant are predominantly petunidin, and lesser amounts of malvidin and delphinidin (Jones et al. 2003). The recessive atroviolacium (atv; Rick 1964) and dominant Aubergine (Abg; Rick et al. 1994) loci also result in varying degrees of anthocyanin accumulation and purple pigmentation.
Numerous studies have been directed toward manipulation of the flavonoid biosynthetic pathway. The majority of these have focused on alteration of anthocyanin pigmentation for flower and foliage color in ornamental crops (Mol et al. 1998, Dixon and Steele 1999, Forkmann and Martens 2001). Naturally occurring genetic diversity for these compounds in tomato, and a well-characterized biosynthetic pathway, make tomato an attractive crop in which to develop cultivars with enhanced levels of flavonoids and expanded range of health benefiting properties.

Heterologous overexpression in tomato of a Petunia chi-a gene encoding chalcone isomerase resulted in up to 78-fold increased levels of flavonols in fruit peel (Muir et al. 2001). Increased flavonol content was due to significant increases in peel concentration of quercetin glycosides and smaller increases in kaempferol glycosides. The altered phenotype exhibited stable inheritance and produced no demonstrable negative effects on plant growth and development. Flavonol levels in fruit of transgenic tomato lines overexpressing chalcone isomerase contained flavonol levels
similar to those found in onions, a crop with naturally high flavonol content. Further studies revealed that concomitant expression of the sequences encoding chalcone synthase and flavonol synthase from Petunia were sufficient to achieve kaempferol accumulation in tomato flesh (Colliver et al. 2002).

Introduction of the maize transcription factor genes LC and C1 similarly resulted in upregulation of the flavonoid pathway in tomato fruit flesh (Bovy et al. 2002). These fruits accumulated high levels of kaempferol and lesser amounts of naringenin in their flesh. Absence of anthocyanins in LC/C1 fruit was believed to be attributable to insufficient expression of the gene encoding flavonone-3’5’-hydroxylase, although other explanations including altered enzyme substrate specificity are plausible (Bovy et al. 2002).

Conventional approaches to develop flavonoid-rich tomato genotypes have been reliant on phenotypic evaluation of fruit color and/or biochemical analysis of flavonoid constituents. Utilizing information from molecular genetic studies of anthocyanin biosynthesis in tomato, Willits et al. (2005) devised an alternative strategy based upon gene expression assays of
candidate loci to identify exotic tomato genotypes that may be useful in a traditional breeding program for improving fruit flavonoid content.

Previously discussed transgene studies demonstrated that chalcone isomerase (CHI) is the primary block to synthesis of quercetin in tomato peel and that additional blocks may occur at chalcone synthase (CHS), flavanone hydroxylases, and flavonol synthase (FS) in fruit flesh (Muir et al. 2001, Colliver et al. 2002). Screening of a diverse set of Solanum section Lycopersicon accessions for flavonol gene expression identified two S. chilense accessions and a single S. pennellii accession that expressed CHI in the fruit peel, and also expressed CHS, CHI, and FLS in the fruit flesh (Willits et al. 2005). Introgression of the S. pennellii accession into tomato produced progeny that accumulated high levels of quercetin in both the fruit flesh and the fruit peel. The study is novel in its approach to identifying valuable accessions for enhancing fruit nutritive value and further highlights the untapped genetic diversity available in wild tomato germplasm.

The rich genetic diversity present in tomato and its wild relatives, together with the potential to manipulate biosynthetic pathways via introduction
of foreign genes, provides potentially limitless opportunities to alter or introduce tomato fruit constituents that may contribute to fruit nutritive value. Resveratrol, an antioxidant with non-vitamin activity, is abundant in grape and several other species. It has recently been induced in tomato via expression of a grape stilbene synthase cDNA (Giovinazzo et al. 2005).

Similar to a number of antioxidants, consumption of resveratrol-rich foods is associated with reduced atherosclerosis and carcinogenesis (Pace et al. 1995, Jang et al. 1997). Tomato plants do not normally produce resveratrol and do not have the stilbene synthase gene. However, the precursors for resveratrol synthesis are present and are also substrates for the flavonoid enzyme chalcone synthase. Resveratrol production in transformed fruit had no significant effect on levels of the associated flavonoid naringenin or on chlorogenic acid production (Giovinazzo et al. 2005).

Phenolic Acids

Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. The hydroxycinnamic acids are phenolic acids included in the large class of secondary metabolites.
known as phenylpropanoids. Hydroxycinnamic acids in fruit tissues are typically esterfied to other polyhydroxylated compounds such as quinic acid, tartaric acid and glucose. Esters of caffeic acid predominate in Solanaceous species such as tomato, eggplant and tomato. Chlorogenic acid (5-O-caffeoylquinic acid) is typically the most abundant in these plants (Mølgaard and Ravn 1988). This abundant phenolic acid is highly bioavailable yet somewhat overlooked dietary bioactive compound. Chlorogenic and related caffeoyl esters are among the most potent free radical scavengers found in plant tissues (Sawa et al. 1999, Nakatani et al. 2000). Chlorogenic acid has been shown to act as an antioxidant in human erythrocytes and for low-density lipoproteins in vitro (Nardini et al. 1995, Lekse et al. 2001). This class of hydroxycinnamic acid esters has also been reported to have antiviral activity (Cheminat et al. 1988). Recent studies have investigated the biosynthesis of chlorogenic acid in plants. Gene silencing studies demonstrated that hydroxycinnamoyl transferase (HQT) is the primary enzyme required for chlorogenic acid accumulation in Solanaceous species (Niggeweg et al. 2004). Overexpression of HQT in tomato foliage caused plants to
accumulate higher levels of chlorogenic acid, with no demonstrable side effects on the levels of other soluble phenolics or lignin. This research offers promise for development of tomato fruit with enhanced benefits for human health.

MACRONUTRIENTS

Plants contribute to the availability of dietary macronutrients including protein, fats, and carbohydrates. A large database exists for these macronutrients in agronomic crops wherein these well-characterized constituents are significant crop yield components. Modification of plant carbohydrate composition is a research area receiving closer scrutiny by plant researchers and the health community in light of the positive effects that fiber and complex carbohydrates can have on aspects of human health such as lipid metabolism and diabetes (Anderson 1990). Dietary plant fibers are derived from plant structural components and include both water soluble and water-insoluble nonstarch polysaccharides and lignin. Genetic variability for the type and amount of soluble carbohydrates, pectin, gum, lignin, and other fiber components exists within tomato. These constituents have been studied principally for their effects on tomato
fruit quality. Little attention has been given to their contribution to fruit nutritive value.

Carbohydrates

Considerable research effort has been dedicated to improving tomato fruit solids content because of the influence of solids on fruit quality (Stevens 1986). Sugars comprise 55% to 65% of the total soluble solids fraction and approximately 50% of the total solids in tomatoes. Considerable variability for soluble solids concentration is present within the cultivated tomato and its wild relatives. Soluble solids concentration of commercial hybrid cultivars generally ranges from 4.5% to 6.0% and can approach 15% in fruit of wild tomato species (Hewitt and Garvey 1987). Transient starch accumulation, which occurs prior to fruit maturation, contributes to sink strength and solids accumulation in developing fruit by maintaining a concentration gradient for sucrose between the leaves and the fruits (Dinar and Stevens 1981). Schaffer et al. (2000) demonstrated a relationship between increased levels of starch in immature S. habrochaites fruit and ADPglucose pyrophosphorylase activity. Increased starch levels in fruits inherited from S. lycopersicum × S. habrochaites plants
were attributed to a S. habrochaites derived introgression coding for the large subunit of ADPglucose pyrophosphorylase (AGPaseL1).

Fruit of the cultivated tomato and those of red-fruited wild tomato species accumulate the reducing sugars glucose and fructose as the principal storage sugars during fruit development. Little or no sucrose is detectable in the mature fruit. In contrast, fruit of the green-fruited wild tomato species accumulate significant quantities of sucrose in addition to reducing sugars (Davies 1966b). Biochemical factors associated with sucrose accumulation in S. chmielewskii (Yelle et al. 1991), S. habrochaites (Miron and Schaffer 1991), and S. peruvianum (Stommel 1992b) have been described. Inheritance studies (Table 6.2) demonstrated that sucrose accumulation is controlled by a single recessive gene, sucr, in the green-fruited species S. habrochaites (Stommel and Haynes 1993) and S. chmielewskii (Chetelat et al. 1993). This locus maps to the pericentromeric region of chromosome 3. Transgenic tomato plants expressing a constitutive antisense invertase transgene exhibited increased sucrose and decreased hexose storage concentrations and reduced levels of acid invertase in ripe fruit (Klann
Accumulated evidence indicates that sucR encodes an inactive invertase allele.

In typical ripe fruit of *S. lycopersicum*, slightly higher amounts of fructose than glucose result in glucose:fructose (G:F) ratios of 0.8 to 1.0 (Davies 1966b). In hexose-accumulating fruit derived from interspecific crosses with *S. habrochaites*, glucose concentrations are commonly low relative to those of fructose and result in much lower G:F ratios than those typically noted in *S. lycopersicum*. Observed segregation in *S. lycopersicum* × *S. habrochaites* populations indicated that G:F ratios were controlled by at least two genes (Stommel and Haynes 1993). More recent investigations demonstrated that a major locus (FGR) located on chromosome 4 influences G:F ratios in tomato fruit and that an additional genetic factor may be involved in determining the ratio of hexose sugars (Schaffer et al. 1999, Levin et al. 2000).

FGR increases levels of fructose, relative to glucose, and exhibits an allelic dosage effect. An additional locus (FK2) located on chromosome 6 is epistatic to FGR and may decrease G:F (Levin et al. 2000). FK2 is subject to marked genotype × environment interaction.
Numerous studies have identified QTL introgressed into tomato from wild species that influence fruit-soluble solids content. Many of these QTL have a positive impact on solids content, but negatively influence fruit yield (Eshed and Zamir 1994, Tanksley et al. 1996, Chen et al. 1999, Yates et al. 2004). Additional studies identified chromosomal segments from S. chmielewskii and S. cheesmaniae that had a positive influence on fruit soluble solids while maintaining acceptable fruit size, pH, and yield (Triano and St. Clair 1995, Yousef and Juvik 2001). Collectively, these and other QTL studies underscore the contribution that multiple, non-allelic loci have on controlling soluble solids in tomato. Recent efforts to characterize a QTL that increases the hexose sugar component of soluble solids revealed a likely regulatory role for an apoplastic invertase gene (LIN5) and highlight the importance of intragenic recombination in genetic variability in soluble solids and other quantitatively inherited traits (Fridman et al. 2000).

Insoluble Solids

Tomato fruit insoluble solids contribute to fruit viscosity and firmness.
Insoluble solids comprises water insoluble solids (WIS) and alcohol insoluble solids (AIS), the former being slightly larger. A strong relationship exists between AIS and viscosity, with fruit pericarp accounting for high correlation coefficients (Janoria and Rhodes 1974). The inheritance of AIS in a cross of high and low AIS cultivars demonstrated high heritability (0.68 and 0.75), additive genetic variation, and that less than three genes influence AIS levels (Stevens 1976). Fractionation of AIS into polyuronides and polysaccharide fractions demonstrated that water soluble polyuronides and water insoluble polyuronides accounted for approximately 90% of the difference in viscosity between a high and low viscosity cultivar (Stevens 1976). Water-soluble polyuronides comprise short and intermediate length chains as found in the fruit serum fraction. Water-insoluble polyuronides represent the protopectin fraction. An increase in water-insoluble polysaccharides had the greatest potential for increasing fruit viscosity. Genetic variation for texture and factors that contribute to AIS in tomato results from the interaction of numerous QTLs. QTL associated with AIS and its constituents have been described (Fulton et al. 2000, Causse et al.)
2002, Frary et al. 2003, Yates et al. 2004). These studies have shown that a few regions on chromosomes 2 and 4 have a large influence on fruit biochemical composition and organoleptic quality as determined by both physi

Early work on the molecular genetics of fruit ripening and correspon
ing changes in fruit softening focused on polygalacturonase and its effects on ripening fruit. Antisense suppression of polygalacturonase accumula
tion demonstrated that the enzyme has only a minor effect on fruit softening, but has substantial effects on increasing viscosity of processed products and the integrity of stored fruit (Schuch et al. 1991, Kramer et al. 1992, Langley et al. 1994). Related efforts directed towards suppression of pectin methylesterase activity likewise had little influence on fruit firmness, but it increased soluble solids of juice and serum viscosity, paste viscosity, and serum separation of processed juice (Tieman et al. 1992, Thakur et al. 1996).

At least seven tomato β-galactosidase genes are expressed during to
mato fruit development, six of which are expressed during ripening and may influence fruit textural properties (Smith and Gross
2000). Antisense suppression of the tomato ß-galactosidase 3 gene did not improve fruit firmness but resulted in fruit that processed into pastes with an increased proportion of insoluble solids and slightly increased viscosity (de Silva and Verhoeyen 1998). Similar studies examining the tomato ß-galactosidase 4 gene produced fruit from antisense lines that were 40% firmer than controls. Ongoing studies of a number of tomato ripening mutants (rin, nor, Nr, Cnr) that exhibit altered fruit textural properties offer promise for further elucidation of fruit AIS constituents (Seymour 2002). These mutants are discussed in more detail elsewhere in this volume.

Lipids

Phytosterols are important structural components of plant membranes and stabilize phospholipid bilayers in plant cell membranes. More than 200 different types of phytosterols have been reported in plant tissues. Phyto-sterols have received increased attention in the last ten years because of their cholesterol-lowering properties. Moreau et al. (2002) and Piironen et al. (2000) have recently reviewed phytosterols in foods and their health promoting properties. The primary interest in tomato phyto-
tosterols has focused on ripening related changes in content, composition, and conjugation that coincide with phospholipid catabolism mediated by phospholipase D (Whitaker 1988, Whitaker et al. 2001, Pinhero et al. 2002).

Phospholipids decrease during tomato fruit ripening coincident with an increase in phospholipase D gene expression (Whitaker et al. 2001, Pinhero et al. 2002). The degradation of membrane lipids is an important feature of ethylene signal transduction pathways that take place in response to hormones, environmental stress, and senescence (Paliyath and Droillard 1992).

Transformation of tomato with an antisense phospholipase D cDNA construct reduced phospholipase D activity 30 to 40% and resulted in firmer fruit with enhanced lycopene content, vitamin C, and flavor (Oke et al. 2003). In addition, juice and sauce prepared from the transgenic fruit exhibited improved viscosity and increased levels of major flavor volatiles.

Increased membrane stability due to decreased phospholipase D activity may account for the observed enhancement in fruit quality attributes.

Related studies highlight the potential to modify the composition of fatty acids in tomato fruit and, more importantly, effect
changes in fatty acid composition that alter levels of flavor volatile compounds. Wang et al. (2001) demonstrated that expression of the yeast 9-desaturase gene in tomato resulted in changes in leaf fatty acid profiles that were accompanied by changes in volatiles derived from fatty acids. Using a similar strategy, Cook et al. (2002) expressed a 6-desaturase transgene in tomato that also modified tomato fatty acid metabolism. Transformants produced δ-linolenic acid and octadecatetraenoic acid in both leaf and fruit tissue. Fruit tissue also contained reduced levels of linoleic acid and an increased percentage of δ-linolenic acid. Additional studies suggest that a chloroplast-targeted lipoxygenase, TomloxC, can utilize both linoleic and linolenic acids as substrates to generate volatile flavor compounds (Chen et al. 2004). Gene silencing or antisense inhibition of TomloxC led to a marked reduction in levels of known flavor volatiles, including hexanal, hexenal, and hexenol. Volatile compounds in plants are derived from three different biosynthetic pathways (Croteau and Karp 1991). Enhanced levels of the aroma and flavor compound S-linalool were generated...
ated in transgenic plants by diverting metabolic flow
normally committed
to the biosynthesis of carotenoids without adverse effects
on accumulation
of lycopene or tocopherols (Lewinsohn et al. 2001).

PHYTOANTINUTRIENTS AND ALLERGENS

Genetic improvement of food crop nutritive value also
encompasses
variability present for food allergens and antinutrients. Although relatively
uncommon, allergic reactions to fruit, vegetable, and grain
crops do occur.
Numerous studies have been conducted to characterize
potential allergens
in tomato fruit (e.g. Kondo et al. 2001). Whereas
pollen-mediated allergies
are relatively common, fruit consumption induced allergies
are infrequent.
Tomatoes are a rich source of carotenoids that are valued
for their
beneficial effects on human health. Nonetheless, they may
also be considered
phytoantinutrients in special cases. Excessive dietary
intake of carotenoid
containing foods may cause various afflictions
characterized by aberrant
discoloration of the skin. Carotenemia is commonly
associated with
ingestion of excessive amounts of \( \beta \)-carotene from carrots,
but may occur
with other yellow and green vegetables (Lascari 1981).
Likewise,
lycopenaemia is a rare cutaneous disease resulting from
excessive consumption of lycopene-containing fruits and vegetables (La Placa et al. 2000).

Well publicized health studies evaluating the effects of ß-carotene supplementation demonstrated that ß-carotene does not prevent lung cancer in older men who smoke (Albanes et al. 1996) or in asbestos workers (Omenn et al. 1996). Supplementation at pharmacologic levels modestly increased lung cancer incidence in smokers, and this effect may have been associated with heavier smoking and higher alcohol intake. Whereas media reports focused upon the negatives of ß-carotene supplementation; base-line concentrations of plasma ß-carotene were inversely correlated with subsequent incidence of lung cancer. This suggests that ß-carotene that was derived presumably from foods may be protective or that ß-carotene is a marker for some unidentified protective dietary or lifestyle factor (Clevidence et al. 2000). The results of human health studies and genetic variability in food crops for potential antinutrients highlight the opportunities to breed for optimal levels of phytochemical constituents.

BIOPHARMACEUTICALS
Transgenic plants provide a novel opportunity for the development of biopharmaceuticals. Edible vaccines including those for rabies, hepatitis B, and enterotoxigenic E. coli have been expressed in tobacco, tomato, or potato and have been the focus of significant research and development (Yusibov et al. 1997, Haq et al. 1995, Mason et al. 1992). These plant derived vaccines offer the opportunity to deliver inexpensive, orally administered vaccines and thus are promising for use in developing countries where cost and efficacious administration of available vaccines are typically of serious concern. The greatest challenge in developing edible vaccines via transgenic plants is to express sufficiently high levels of the foreign protein antigens in plant tissue. In tomato, expression of the gene for the respiratory syncytial virus (RSV) antigenic protein resulted in production of antigen that was active as an oral immunogen (Korban et al. 2002). Antigen accumulation was confined to seed tissue. Likewise, Mor et al. (2001) expressed recombinant acetylcholinesterase in tomato to provide therapeutic protein for protection against organophosphate poisoning. Ruf et al. (2001) described a stable plastid transformation
system for
tomato. Transgenes of transformants exhibited maternal
inheritance as
expected for a plastid-encoded trait. Transgene expression
in chromoplasts
of tomato fruit was approximately 50% of the expression
levels observed in
leaf chloroplasts. In light of the very high foreign
protein accumulation
observed in transgenic tomato plastids (greater than 40% of
soluble
proteins), this system offers new possibilities for more
efficient production
of edible vaccines, pharmaceuticals, and antibodies in
tomato.

PERSPECTIVES

Plant breeding has been the mainstay for genetic
enhancement of
horticultural and agronomic crop nutritive value. Breeding
strategies for
improved nutritional composition are similar to those for
other traits in a
germplasm development program. Based on knowledge gained
through
investigations on the characters heritability, the mode of
inheritance, and
existing genetic variability, appropriate breeding
strategies based on
phenotypic selection of individuals or families have been
implemented to
realize improvements in crop nutritional quality.
Development of gene
specific probes and identification of markers tightly
linked to phytonutrient constituent loci enables implementation of marker-assisted selection strategies for genotypic selection of well-studied traits. Detailed knowledge of the biochemical pathways for a number of phytonutrients has assisted in identification of structural and regulatory genes responsible for metabolite accumulation. The first restriction fragment length polymorphism (RFLP) map in tomato was constructed in 1986 with only 57 loci (Bernatzky and Tanksley 1986). Many additional RFLP, and PCR-based amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites, and expressed sequence tags (EST) have since been added to tomato linkage maps (Van der Hoeven et al. 2002). These dominant or codominant markers can be used for DNA fingerprinting, gene tagging, high-density genome mapping, and positional gene cloning. The availability of S. pennellii and S. habrochaites introgression lines (Eshed and Zamir 1995, Monforte and Tanksley 2000) further facilitates rapid screening and identification of major and minor QTL present in wild tomato species that influence fruit nutritive quality.

In tomato, there is a wealth of genetic variability within modern and
heirloom cultivars, land races, and wild species for improvement of fruit nutritive value. Progress in breeding for improved tomato nutritional value is largely influenced by availability of sufficient genetic diversity and knowledge of gene action. The feasibility of a program focused on enhancement of phytonutrient content is also reliant upon methods for measuring phytonutrient constituents and evaluation of the relative importance of genotype and environment in the expression of phytonutrient content. Recent studies demonstrate renewed interest in characterizing Solanum section Lycopersicon germplasm collections for fruit phytonutrients and devising selection indices to maximize breeding efficiency (Hanson et al. 2004, Schauer et al. 2004, Willits et al. 2005). Where existing genetic variability for phytonutrient content is insufficient or difficult to introduce into adapted materials due to crossing barriers, appropriate gene constructs relevant to the biosynthesis of a specific phytonutrient or class of phytonutrients may be developed and genetically modified (GM) plants produced. The potential to introduce genetic material from unrelated organisms into tomato via these technologies provides an
important source

of new genetic variation. Unfortunately, GM plants are associated with

modern agriculture, which is also viewed negatively. Barring long-term

negative public opinion of GM crops, the wide range of products being

developed and tested in commercial and academic laboratories suggests

that the rate at which GM plants are introduced will increase. The

introduction of GM plants with enhanced nutritive properties may foster

increased acceptance of transgene technology in new value-added cultivars

(Dunwell 2002, Lindsay 2002, Mehta et al. 2002). Ironically, the tools of

modern molecular genetics that have been used to develop transgenic plants

are now enabling traditional breeding to transcend limits imposed by

conventional phenotypic selection. Although GM plant development has

generally been considered a more expedient route to develop superior

cultivars, Zamir (2001) argues that approximately ten years are required to

create a transgenic cultivar for testing and that this timeframe is similar to

that needed for development and testing of new lines developed in

traditional breeding programs using exotic germplasm.

The evidence of accumulated studies indicates a positive
link between fruit and vegetable consumption and improved health. Dietary studies in turn have stimulated interest in trying to define the constituents in fruits and vegetables that are responsible for their positive health effects.

Considerable research still needs to be done to characterize these compounds. Cataloging the demonstrated/presumed benefits of these compounds will be an important step in sustaining research to improve phytonutrient content. Investigation of potential phytonutrient properties for well characterized fruit quality constituents such as organic acids, simple sugars, and specific carotenoids that have not been previously recognized for their phytonutrient properties will add important information to this database. Such recognition will enhance the intrinsic value of the characterized nutrient constituents. In addition to phytonutrient content, knowledge of their digestibility, absorption, and utilization is important.

Whereas nutrition studies often focus on a single phytonutrient, current research clearly indicates that there are many bioactive compounds in tomato products and that it may be the combination of compounds in
tomatoes that confer the beneficial health effects described (Laquatra et al. 2005). In addition to enhancing tomato fruit nutritive value, development of novel nutritionally enhanced germplasm provides new opportunities for dietary studies to examine phytonutrient constituents in a common food matrix for characterization of phytonutrient interactions.

In the mid-1970s, and again in the late 1980s, commentaries on genetic improvement of horticultural crops concluded that nutritional improvement may be impractical because of the lack of demonstrated dietary need or availability of alternate food choices (Kelly and Rhodes 1975, Tigchelaar 1987). In developing countries, overriding demands for research that enhances crop production and thus, food availability may supersede development of locally adapted cultivars with enhanced nutritional quality.

Nonetheless, research to develop locally adapted, nutrient-dense cultivars is an important tactic in developing countries that complements related efforts to improve dietary nutrition (Hanson et al. 2004). Phytonutrient levels must be altered at the same time that numerous traits including yield, quality, disease resistance and many others are being selected for in
a cultivar development program. In addition, negative relationships may exist between phytonutrient levels and other economically important traits such as fruit size and yield. Negative correlations between soluble solids, ascorbic acid, and total antioxidant content, for example, and fruit size or yield limit genetic enhancement of nutritive value. Successful breaking of these negative linkages may be critical to the successful commercialization of a new phytonutrient-enriched tomato cultivar. Although phytonutrients are typically viewed as positive constituents, it will be important to determine if there are any liabilities associated with the consumption of phytonutrient enhanced crops, particularly the possibility of negative health consequences that result from consuming plant parts containing phytonutrients at levels far above “normal”. In collaboration with nutritionists and other health professionals, these issues should be raised early in the genetic improvement process since they too will limit successful utilization of phytonutrient-enhanced cultivars.

Consumer demand for horticultural products is a function of product price, alternative products, income, population, socioeconomic and
and Robinson 1972). A market trial conducted in 1985 demonstrated that a segment of U.S. consumers developed loyalty to a premium quality fresh tomato and purchased them frequently at premium prices (Goldman 1988).

A similar occurrence is evident in today’s market wherein premium quality cluster tomatoes sold on the vine, vine-ripened greenhouse-grown fruit, and specialty cherry tomato cultivars enjoy growing sales and command premium prices. Although often viewed as burdensome, labeling can be a positive aspect for phytonutrient-enriched crops to inform consumers of the products nutrient enhanced status. Consumer education is critical to acceptance of food products with enhanced phytonutrient value. Similarly, food producers and distributors will not be apt to produce and market a new variety without evidence that it will be saleable and profitable. Clearly, the scientific community must not work in isolation of the marketplace. As consumers gain increased knowledge of nutrients in fruits and vegetables and recognize value-added products, markets for enhanced phytonutrient enriched crops will expand.

SUMMARY
During the twentieth century, plant breeding and genetics have improved the nutritive value of horticultural and agronomic crops. Tomatoes are a major dietary source of vitamins A and C, and lycopene. In addition to these well-known vitamins and antioxidants, other compounds in tomato fruit with antioxidant properties include chlorogenic acid, rutin, plastoquinones, tocopherols, and xanthophylls. Tomatoes also contribute carbohydrates, fiber, flavor compounds, minerals, proteins, and glycoalkaloids to the diet. Considerable genetic variation exists in tomato for micronutrients with antioxidant activity. A number of these micronutrients, particularly carotenoids, have been the subject of considerable research due to their contribution to the quality of fresh and processed tomato products. Increased recognition of their health promoting properties has stimulated new research and provided added justification for investigation in this area. Plants also contribute to the availability of dietary macronutrients including protein, fats, and carbohydrates. In tomato, these constituents have been studied principally for their effects on fruit quality. Little attention has been given to their contribution to fruit
nutritive value. There is a wealth of genetic variability within modern and heirloom tomato cultivars, land races, and wild species for improvement of fruit nutritive quality. Where existing genetic variability for phytonutrient content is insufficient or difficult to introduce into adapted materials due to crossing barriers, appropriate gene constructs relevant to the biosynthesis of a specific phytonutrient or class of phytonutrients may be developed and genetically modified plants produced. Development of gene-specific probes and identification of markers tightly linked to phytonutrient constituent loci enables implementation of marker-assisted selection strategies for genotypic selection of well-studied traits. Detailed knowledge of the biochemical pathways for a number of phytonutrients has assisted in identification of structural and regulatory genes responsible for metabolite accumulation. As consumers gain increased knowledge of the nutrients of fruits and vegetables, and recognize value-added products, markets for phytonutrient enriched crops will expand.


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AN OVERVIEW OF GENETIC MARKERS IN TOMATO

For millennia we have selected plants to be more adapted to our needs, environments, and markets. These efforts were very much an art form.

Roughly a century ago, Mendel’s rediscovered pea experiments gave us an understanding of basic genetic principles and moved plant breeding from a qualitative ‘art’ towards a more quantitative science. Through most of the twentieth century, however, plant breeders heavily depended on their qualitative art skills for selection of the desired phenotype. The plethora of genes and alleles in their myriad of possible combinations mixed with environmental interactions has required a high degree of art, experience, and science to develop the most current ‘elite cultivar’ for the changing, competitive markets of today. No doubt, even without the more recent molecular tools, improvements in cultivars would continue through traditional, science/art based plant breeding methods because of the ubiquitous number of genes to reshuffle. However, most contemporary plant breeders are, to some extent, using molecular markers as aids to produce their newest, elite cultivar.

The tomato (Lycopersicon esculentum Mill.) has been in the
modern plant breeding. Hedrick and Booth (1907) reported results from tomato hybrid experiments to verify Mendel’s laws in tomato. In 1917, Jones employed Hedrick and Booth’s (1907) data to construct the first tomato genetic map. MacArthur (1934) required 10 years and more than 48,000 segregating plants to map 21 phenotypic markers on 10 of the 12 tomato (2n=2x=24) chromosomes. By 1986, Stevens and Rick estimated that 1200 monogenic markers had been identified in tomato. Only a handful of these markers could be considered to be ‘molecular,’ of which, the majority were isozymes. Tanksley and Mutschler (1990) released a comprehensive isozyme map which included 12 enzyme systems with 31 isozyme loci and 205 phenotypic markers scattered across the tomato genome. An additional 98 phenotypic markers were mapped to specific chromosomes, but their precise position was unidentified. The localization of phenotypic markers to a specific chromosomal location required crossing a mutant phenotype to a number of parents with well characterized and mapped phenotypic markers, then evaluating the progeny.
This required enormous effort along with land and/or greenhouse space, as attested by MacArthur’s (1934) monumental 10 year study.

In addition to isozyme and phenotypic marker maps, Tanksley and Mutschler (1990) published a map of 162 RFLP (restriction fragment length polymorphism) markers identified from a single interspecific cross (L. esculentum x L. pennellii (Corr.) D’Arcy). Although RFLP mapping is time consuming and costly, the resources required are trivial compared to mapping phenotypic markers. Tanksley and Mutschler (1990) required less than three years and a single segregating population of less than 100 plants to develop and map 162 RFLP markers, in contrast to well over 50 years of effort by many research groups and thousands of populations to map 205 phenotypic markers. Five years after the first tomato RFLP map, Tanksley et al. (1992) published a high-density molecular map with over 1000 RFLP markers spanning 1200 cM (centiMorgans), which covered the approximately 950 Mbp (mega base pairs) genome of tomato (Arumuganathan and Earle 1991).

Ever since Tanksley et al. (1992) published the first comprehensive mo
molecular map of the tomato genome, a number of studies have reported the
fine mapping of specific areas, and rearrangements of specific genomic
regions. For example, Haanstra et al. (1999) significantly augmented the
Tanksley et al. (1992) map with the addition of hundreds of AFLP (ampli
fied fragment length polymorphism) markers. A careful search of the current
literature would provide a listing of thousands of molecular markers that
have been positioned in the tomato genome. A comprehensive compellation
of these markers is beyond the scope of this report. The objective of this
chapter, however, is to show that the use of these molecular markers has
permanently altered our approach to tomato breeding today.

ISOZYMES AS TOMATO MOLECULAR MARKERS

Isozymes were the first forays into tomato molecular markers. Charles Rick
initiated the understanding of Lycopersicon isozyme genetics in 1973 (Rick
of L. pimpinellifolium Mill. to L. esculentum var. cerasiforme. They found that
the isozyme peroxidase (Prx) loci discriminated the accessions according
to geographic distribution and they also demonstrated tight genetic linkage
between phenotypic Ge (gamete-eliminator) and Prx-1. These findings prompted a search for isozyme diversity in tomato and its relatives, which revealed a paucity of polymorphisms within the cultivated tomato.

Conversely, the wild relatives were replete with polymorphic isozyme loci (Rick and Fobes 1975).

Out of these isozyme studies, two things became clear, both of which still impact tomato marker research today. First, the dearth of allelic variation within the cultivated tomato is due to its genetic ‘bottlenecking’ during domestication (Tanksley and McCouch 1997). This general homogeneity within the cultivated tomato was “discouraging” (Rick 1983).

However, the second finding of those studies, the cornucopia of polymorphic isozymes within Lycopersicon, laid the foundation for future molecular marker work. Interspecific crossing within Lycopersicon was well understood and utilized in tomato breeding by the early 1970’s, albeit with difficulty between the esculentum and peruvianum complexes (Rick 1979).

Examples of genes derived from L. peruvianum (L.) Mill. before 1970 include Mi, root-knot nematode (Meloidogyne spp.) resistance (Smith 1944); Tm-2
and Tm-2 2, tobacco mosaic virus/tomato mosaic virus (TMV/ToMV) resistance (Alexander 1963, Laterrot and Pecaut 1969, Yamakawa and Nagata 1975); and Sw-5, tomato spotted wilt virus (TSWV) resistance (Stevens 1964, Stevens et al. 1992). These, and a number of other exotic genes introduced into tomato, concurrently increased tomato isozyme heterogeneity due to linkage drag.

In 1980, Medina-Filho reported the isozyme allele Aps-1 (acid phosphatase) was tightly linked to Mi in cultivated tomatoes and polymorphic to Mi +. This discovery was quickly adopted in many tomato breeding programs and is still utilized today due to its ease, speed, tight linkage, and low cost. However, as Tanksley (1983b) discussed, the odds of finding other isozymes tightly linked to economically important genes would be remote. This is because most genes are not introgressed from interspecific crosses and, therefore, do not carry polymorphic isozymes as linkage drag. Nonetheless, having a simple laboratory test for a number of genes was intriguing, to the point of creativity. Tanksley et al. (1984) deliberately combined the rare Prx-2 1 isozyme allele with the nuclear male
sterile locus, ms-10, in a tight linkage of 1.5 cM on chromosome 2. This innovative approach allowed the identification of plants heterozygous at the Prx-2 1 locus, providing a high degree of confidence in maintaining ms 10 during backcrossing into an elite hybrid parent. The use of markers for more efficient introduction of male sterility into breeding material is valuable in allowing breeders the possibility of producing hybrid seed without the use of expensive, hand-labor emasculation.

Even with deliberate introgression and serendipitous discovery of linked isozymes from exotic germplasm, the ideal situation of having isozyme markers available for all characteristics of interest was impractical for four reasons. First, each isozyme required different staining procedures with a wide range of reproducibility and expense (Vallejos 1983). Second, only 15 isozyme protocols had been developed with a total of 41 alleles for tomato by 1985 (Tanksley 1985). None of the alleles were mapped to chromosome 11, and chromosomes 1 and 4 carried the maximum, each with five. Furthermore, isozyme markers were often clumped together on a chromosome, thus reducing their effectiveness in plant breeding. Third,
many of the isozyme markers were specific to tissue or environmental conditions, further complicating their full implementation (Tanksley 1980, Tanksley 1983b, Vallejos 1983, Tanksley 1985). Finally, DNA markers were beginning to show they could mitigate, to some degree, all the problems of isozymes (Tanksley 1983b).

TOMATO DNA MARKERS: THE EARLY YEARS

By 1980, a wide range of restriction endonucleases, the core of DNA-based RFLP markers, were available. Several research groups had identified the usefulness of RFLPs in monitoring genetic traits in humans (Botstein et al. 1980, Wyman and White 1980, Ruddle 1981, Kao et al. 1982, Murray et al. 1982). Tomatoes, however, were among the first crops to be utilized for RFLP markers with a plant breeding objective (Helentjaris et al. 1985). This initial study demonstrated RFLP scarcity in the cultivated tomato and an abundance of these markers in its wild relatives, comparable to the results of the isozyme research. The first DNA based tomato maps were reported in 1986 (Bernatzky and Tanksley 1986d, Helentjaris et al. 1986). Helentjaris et al. (1986) developed 50 leaf tissue cDNA probes to genotype 50 F2 inter specific plants from a 'Manpal' x L. hirsutum Humb. &
Bonpl. (PI1123317)

cross, resulting in a map with 19 linkage groups. Bernatzky and Tanksley
(1986d) used an interspecific (L. esculentum [LA1500] × L. pennellii [LA716])
backcross and F 2 population to map 57 RFLP cDNA probes, 21 isozyme
markers, and other previously mapped DNA markers, resulting in 112
mapped loci on the 12 tomato chromosomes. This single study
produced
more than twice the number of isozyme markers previously
mapped, effec
tively demonstrating that, although both marker systems are
codominant,
RFLPs have three distinct advantages over isozymes. First, RFLP markers
are much more abundant. Second, one protocol can be used instead of a
number of different staining methods. Lastly, the sampling method for
RFLPs is potentially less plant destructive compared to some of the isozyme
methods (Vallejos 1983).

In the next few years tomato mapping, marker development, and ge
nome understanding moved at an unprecedented pace using RFLPs.

Bernatzky and Tanksley (1986a,c) identified and mapped 10 actin-related
loci and quickly developed a method to detect single/low copy, noncoding
DNA sequences. They also demonstrated that most random cDNA
clones are single loci in the tomato genome (Bernatzky and Tanksley 1986b). Zamir

and Tanksley (1988) used 50 RFLP probes derived from sheared tomato DNA to learn that the tomato genome is mostly composed of “fast-evolv

ing, low copy-number sequences.” Nienhuis et al. (1987) associated RFLP markers with quantitative trait loci (QTL) linked to insect resistance de

rived from L. hirsutum. Using tomato trisomic lines, Young et al. (1987) correlated the RFLP marker map to the well-understood tomato cytogenetic

map, tying past research to the information discovered through RFLPs.

As RFLPs began to prove their worth, the late 1980’s brought reports focusing on yet another application of markers in tomato, marker-assisted

selection (MAS). Young et al. (1988) identified an RFLP marker tightly linked to Tm-2a based on the idea of linkage drag from L. peruvianum,
similar to the M1 and Aps-1 study (Medina-Filho 1980). Using Tm-2a near isogenic lines (NILs), they searched for probes present in one line and

absent in the other. This innovative study not only provided RFLP markers linked to Tm-2a, but produced evidence that multiple backcrossing did not
necessarily reduce linkage drag as quickly as traditionally thought. In theory, over 99% of the genome should be from the recurrent parent after backcrossing six generations and selecting only for the introgressed character of interest. Young and Tanksley (1989b) demonstrated that Tm 2a was introgressed into eight unique cultivars via backcrossing. ‘Craigella’ (Tm-2a/Tm-2a) still had 51 cM (the entire short arm of chromosome 9) of exotic DNA remaining after many backcross generations, while three other cultivars had as little as 4 cM of linkage drag. These results inspired the concept that RFLPs could be used to graphically illustrate genomic regions originating from specific parents (Young and Tanksley 1989a). Tanksley et al. (1989) hypothesized that by using molecular markers, it would be possible to eliminate excess linkage drag surrounding an introgressed exotic gene in no more than two backcross generations. Today, private breeding companies have, on multiple occasions, introgressed alleles from the wild species within a year or two of initiating the project by using graphical genotyping and selection for specific crossover events. Without markers, exotic gene introgression and cultivar development could take a
decade or longer.

It was abundantly clear by 1990 that DNA markers had the potential to

become one of the most powerful breeding tools since the rediscovery of

Mendel’s laws. Just six years after Bernatzky and Tanksley (1986d) and

Helentjaris et al. (1986) introduced the first tomato DNA molecular maps,

Tanksley et al. (1992) released a high-density tomato genetic map. This

map has provided the foundation for thousands of studies and hundreds

of scientific papers resulting in a dramatic evolution of the understanding

of the tomato genome. One of the more remarkable changes has been in the

area of using exotic germplasm in tomato breeding.

MOLECULAR MARKERS AND EXPANDING

THE TOMATO GERMPLASM BASE

Vavilov (1940) championed the improvement of crop genetics by utilizing

their wild relatives. When studying a specific tomato problem, especially

disease, the approach often includes the possibility of exploiting exotic

germplasm. For instance, resistance to TSWV was first reported in the wild

species L. pimpinellifolium Mill. (Samuel et al. 1930). A single L. peruvianum

gene (Sw-5) has provided the most effective resolution to this disease. It
took over two decades to introgress this gene into tomato in one program (Stevens et al. 1992) and almost a decade in another, independent program (Watterson 1993). This example is rather simplistic in that most traits of horticultural importance are controlled by QTL, rather than a single gene.

The introgression of several QTL from exotic germplasm seemed insurmountable when compared to the time it took to introgress a simple dominant gene of such importance as TSWV resistance into tomato.

Although it was generally impractical, it was well understood that QTL could be monitored with phenotypic markers. A pioneering study using phenotypes to monitor QTL was reported by Thoday in 1961. In 1983, Tanksley (1983a) proposed using isozyme markers to overcome time and linkage drag issues. However, a high-density molecular map, essential to effectively manipulate multiple loci and linkage drag, would not be ready for almost a decade (Tanksley et al. 1992). Even before the high-density molecular map was completed, Tanksley et al. (1982) and Weller et al. (1988) used isozymes and morphological markers in interspecific tomato studies demonstrating the feasibility of linking QTL to
molecular markers.

Lander and Botstein (1989) reported on statistical procedures to handle the data generated from the developing high-density molecular marker maps and QTLs. Paterson et al. (1988) utilized the expanding inventory of RFLP markers with these statistical methods to successfully determine the exotic, introgressed regions (from L. chmielewskii Rick et al.), responsible for altering soluble solids, fruit mass, yield, and pH in tomato.

By the early 1990’s, molecular markers, statistical procedures, and computers were in place and to consider innovative breeding techniques to enhance the harvesting of tomato genes from exotic germplasm (Rick 1988, Hille et al. 1989, Tanksley et al. 1989, Tanksley and McCouch 1997).

Tanksley and Nelson (1996) proposed the “advanced backcross QTL analysis” (AB-QTL) method. This strategy was to cross tomato with a wild relative, backcross the F 1 to the elite parent, randomly select 150-200 BC 1 plants, then carry out single seed decent through the succeeding BC 2 or BC 3 generations. The anticipated result was for the entire exotic genome to be represented throughout the single seed decent lines with each line mostly
containing the genetic background of cultivated tomato. With one or two selfing generations, these lines could end up being QTL-NILs. Tanksley et al. (1996) provided the data of such a project using L. pimpinellifolium as the exotic parent. They discovered that genetics of improved fruit size and shape, among other characteristics, was in complete contrast to the exotic and inferior phenotype of the parent. Thus, a succession of AB-QTL allele mining studies involving several wild species of tomato (e.g. L. hirsutum, L. parviflorum Rick et al., L. pennellii, L. peruvianum) followed (Bernacchi et al. 1998a,b,c, Fulton et al. 1997a,b, 2000, Frary et al. 2004). These studies demonstrated that multiple alleles and loci, even within different accessions of the same species, were available from exotic germplasm in most of the economically important traits in tomato. Examples of unexpected characteristics found in exotic germplasm were improved red color alleles from green fruited species, improved rooting from inferior rooting species, and many others.

Besides the AB-QTL method, which focused on detecting “additive, dominant, partially dominant, or overdominant QTLs” from exotic species
(Tanksley and Nelson 1996), other methodologies using wild species were utilized to identify genetic inheritance patterns. Using an interspecific (L. esculentum × L. pennellii) F 2 population, transgressive segregation of some QTLs was identified (deVicente and Tanksley 1993). Eshed and Zamir (1996) investigated epistatic gene action from QTL, exploiting a set of tomato NILs that contained small, overlapping introgressions from L. pennellii (prepared by Eshed and Zamir 1994). Although they determined that additive gene action predominated, clear evidence was provided for epistasis in fruit mass and total soluble solids. Molecular markers have enabled breeding programs to not only dissect quantitatively inherited traits, but have additionally allowed the introgression of these characteristics into modern cultivars. Furthermore, careful mapping of QTL have opened the door for a better understanding of the genetics in tomato and scrutinizing the physiology of economically important phenotypes.

MOLECULAR MARKERS IN GENE CLONING

Prior to the development of high-density molecular maps, gene products (proteins) were usually required previous to cloning tomato genes
responsible for a given phenotype. With efficient marker development methods and large insert genomic libraries of tomato such as yeast artificial chromosomes (YACs) (Martin et al. 1992) and bacterial artificial chromosomes (BACs) (Hamilton et al. 1999), gene cloning has become much more feasible. The first gene cloned from genetic mapping in tomato was Pto, which imparts resistance to Pseudomonas syringae pv. tomato (Martin et al. 1993b). Using more than one technique, Martin et al. (1993a) identified molecular markers so tightly linked to Pto that they “landed” on the specific YAC clone in which Pto resided. The cloning of this gene was much more efficient compared to the map-based cloning, or “chromosome walking,” efforts used in human and other organisms. As a result, the technique “chromosome landing” was suggested as a paradigm for future gene cloning efforts ( Tanksley et al. 1995). In addition to chromosome landing/walking methods, Jones et al. (1994) developed a clever scheme to clone tomato genes by using molecular markers in combination with tomato lines transgenic for the transposable elements Ac and Ds from maize (Zea mays L.). This method was used to effectively clone Cf-9, the
gene for Cladosporium fulvum resistance.

Although some genes have remained recalcitrant to cloning efforts short of sequencing the genome, a number of unique and economically important genes have been cloned through the aid of molecular markers via permutations of map-based cloning. Examples include the jointless (j) gene (Zhang et al. 1994), the TSWV resistance gene Sw-5 (Folkertsma et al. 1999, Brommonschenkel et al. 2000), the fruit size QTL fw2.2 (Frary et al. 2000), the self-pruning (sp) gene (Carmel-Goren et al. 2003) and the alleles involved with increased lycopene content known as high pigment (hp-2) and dark green (dg) (Mustilli et al. 1999, Levin et al. 2003). The impacts of cloning these and other genes on tomato breeding will be felt in transgenics for increased human nutrition, better understanding of yield, and many others. Most likely the greatest influences are yet to come.

MOLECULAR MARKERS WITHIN SOLANACEAE FOR TOMATO IMPROVEMENT

Tomatoes have led the way in developing genetic maps in Solanaceae. As could be expected, a steady ‘spillover’ effect has benefited the related genera.

Early on, tomato DNA markers demonstrated their utility in
Solanaceous crops such as peppers (Capsicum spp.) (Tanksley et al. 1988) and potatoes (Solanum spp.) (Bonierbale et al. 1988, Gebhardt et al. 1991). These studies demonstrated general conservation of gene order with broad chromosomal rearrangements between the genera of Solanaceae. Livingstone et al. (1999) provided clear evidence that tomato, pepper, and potato are essentially the same genetically, but differ in a number of chromosomal rearrangements with interesting evolutionary implications. Utilizing this understanding, Grube et al. (2000) searched for homologs of the cloned resistance genes Sw-5, Pto, N (TMV resistance from Nicotiana tabacum L.), Prf (required for Pto) and I2 (resistant to Fusarium oxysporum) among tomato, pepper and potato. They found homologs in syntenic positions in every species for all five genes tested. Moreover, these genes were mapped, in several instances, to genomic regions where previously mapped resistance genes resided within a specific species. The implications of these results will be studied for sometime to come.

One of the benefits of molecular markers and their applicability across Solanaceae has been in tomato intergeneric crosses. For
example, attempts
to introgress potato genes into tomato were monitored via molecular markers following both protoplast fusion (Jacobsen et al. 1994, Shikanai et al. 1998) and gamma-irradiation experiments (Schoenmakers et al. 1994). Other tomato intergeneric work has included eggplant (S. melongena L.) (Samoylov and Sink 1996, Samoylov et al. 1996) and the weedy species S. sitiens Johnson (Syn. S. rickii Corr.) (DeVerna et al. 1990). However, tomato x S. lycopersicoides Dun. has been the most successful intergeneric cross. Using molecular markers, Chetelat and Meglic (2000) and Ji and Chetelat (2003) examined the location and amount of S. lycopersicoides genome incorporated into specific lines of tomato. Although difficult, similar progress has been made in introgressing sizeable chromosomal sections of S. sitiens into the tomato genome (Pertuz et al. 2003). The impact on tomato of these diverse genetic elements can be expected to be significant, especially in light of the contributions from the more closely related Lycopersicon species (Rick and Chetelat 1995).

Intra-Solanaceae molecular studies have spurred interest in reexamining the systematics within the family. A sampling of some of
those studies
include discussions on the merits of the present
designation of species
within Lycopersicon (Miller and Tanksley 1990, Bretó et al. 1993, see chapter
1) and whether additional species from Solanum should be included in
Lycopersicon (Marshall et al. 2001). Due to the wealth of molecular genetic
information developed within this family, the idea of using “Solanaceae as
a model for linking genomics with biodiversity” is being considered (Knapp
et al. 2004).

MOLECULAR MARKER DRAWBACKS IN TOMATOES

With all the progress that has been made to improve the incorporation of
molecular markers into tomato breeding, there are two fundamental areas
that need to be addressed. First, we need to better employ molecular markers
in managing elite tomato germplasm. Tomato breeding has utilized more
exotic germplasm than any other cultivated crop and molecular markers
have greatly facilitated that work. However, little is understood about the
diversity of the elite alleles within L. esculentum. The second need is to
establish high throughput molecular marker systems that are economical.

At present, the cost of molecular marker analysis is usually calculated in
dollars per data point.

Due to these two limiting factors, most tomato breeding programs are using MAS for few alleles, mostly exotic disease resistant genes. The majority of the tomato breeding programs are using PCR (polymerase chain reaction) markers in proxy for disease screenings in elite germplasm breeding.

Examples of available disease resistance genes linked to PCR markers include Ol-1 (resistant to powdery mildew, Oidium lycopersicum) (Huang et al. 2000), py-1 (resistant to corky root rot, Pyrenochaeta lycopersici) (Doganlar et al. 1998), Mi (Williamson et al. 1994), Sw-5 (Stevens et al. 1995, Stevens et al. 1996), Tm-1, Tm-2, Tm-2 2 (resistance to TMV and/or TMoV) (Ohmori et al. 1996, Sobir et al. 2000, and Dax et al. 1998 respectively) and Fr1 (resistant to fusarium crown and root rot, Fusarium oxysporum f.sp. radicis-lycopersici) (Fazio et al. 1999), to name a few. Unfortunately, the effectiveness and convenience of each of these PCR markers vary from reliable to problematic.

In addressing the first drawback of molecular markers, we simply need to remember a fundamental of plant breeding. Breeding for release of the
most recent cultivar probably began with the most elite and adapted tomato

lines as parental material. The reason for this is that elite lines represent

millennia of selecting and assembling thousands of the best alleles. All

experienced breeders know that combining two, well adapted cultivars

can and do provide the underpinnings for the next ‘more elite’ cultivar.

This suggests, with foundation, that improved, elite allele combinations

are yet to be discovered solely within the narrow tomato germplasm base,

resulting in tomato improvement. Therein lies the challenge. How do we

use molecular techniques to uncover and streamline the discovery of the

untapped allele combinations to improve elite material (Yang et al. 2004)?

Both Rick (1983) and Helentjaris et al. (1985) reported the dearth of

polymorphisms in tomato and we have only made limited progress since

those early molecular discoveries.

Over the last two decades, several DNA marker systems have revealed

polymorphisms among the modern cultivars. Paran et al. (1995), Noli et al.

(1999), and others demonstrated that cultivars can be distinguished using

randomly amplified polymorphic DNA (RAPD) markers. Broun and

Tanksley (1996) reported that microsatellites, or simple
sequence repeats
(SSRs), can also be used to distinguish between tomato cultivars.

Areshchenkova and Ganal (2002) established that microsatellite markers
could be created from expressed sequence tag (EST) data available on public
databases. Nevertheless, the majority of essential horticultural characteristics
in tomato are quantitatively inherited and lack molecular markers. A few
examples include yield, flavor, shipping quality, cracking, and a number
of ripening disorders. Unfortunately, at present, our marker technology is
inadequate to address these questions.

We have made modest progress in the second deficiency of molecular
markers, the ability to develop cost-effective, high-throughput techniques.

The most outstanding improvement in marker throughput was the
development of the PCR technique (Saiki et al. 1985). PCR has reduced the
cost and increased the speed at which all DNA markers are identified and
developed. Nevertheless, genotyping a given plant sample is limited, in
most instances, to a maximum of three or four markers linked to specific
characteristics in each PCR reaction (multiplexed). Time, reagents,
equipment and supplies alone can overwhelm a breeding
program trying
to keep up with new markers linked to new traits,
especially when only
two to three markers can be examined at a time. The
pressure is steadily
increasing to utilize more markers and improve the utility
of each linked
marker.

FUTURE OF MOLECULAR MARKERS AND TOMATOES

Progress is currently being made on the two most limiting
factors in
molecular markers from: first, the rapid accumulation of
tomato DNA
sequences, second, the increasing range of single
nucleotide polymorphism
(SNP) detection methodologies, and third, from the dramatic
and steady
improvements of microarray techniques. Combinations of these
advancements with computers are preparing the way for
economical high
throughput of tomato molecular markers.

Accumulation of tomato sequences, especially EST’s (over
30,000; Fei
et al. 2004) has allowed various SNPs techniques (Rafalski
2002) to
distinguish differences between elite cultivars. Yang et
al. (2004) searched
the tomato EST databases and found one SNP for every 8,500
bases. In a
selection of 44 genes, 101 candidate SNPs were identified
between two
cultivated lines. A subset of these “in silico” SNPs were
verified in the laboratory and then genetically mapped. Labate and Baldo (2005) further verified the value of SNPs in a comparison between 15 cultivated tomato lines. Validation of the functionality of SNPs in elite x elite breeding was accomplished when these and other molecular markers were used to successfully identify the QTLs conferring field resistance to bacterial spot of tomato (Xanthomonas campestris pv. vesicatoria race T1; Yang et al. 2005).

Furthermore, to aid the tomato community in the discovery of additional SNPs a lower cost method of sequencing ten's of thousands genomic sequences within an afternoon was recently reported (Margulies et al. 2005). At present, only about 100 bases are sequenced with accuracy, however, it is anticipated this number will increase by several fold and the cost per reaction will go down! Once understanding how this pyrosequencing sequencing process is accomplished it is quickly possible to imagine studies where SNP discovery between elite tomato cultivars will be much more simplistic. These studies demonstrate that thousands of SNPs can be discovered between cultivars and provide a solution to one of the difficulties of implementing MAS in elite tomato
germplasm selection.

With a burgeoning set of EST and potential sequences data, the next obstacle is to economically handle SNP discovery and implementation to manipulate QTL in elite tomato breeding. There are already at least two techniques that approach this challenge. The first technique, known as SNP Wavetm (van Eijk et. al. 2004), has already shown 'proof of concept' in tomatoes and is commercially available. This technique is based on PCR and sequencing technology and has demonstrated the ability to monitor 100 separate Arabidopsis thaliana (L.) Heynh. loci in a single reaction and 40 loci of the more complex tomato genome. The second method is to use microarray technology, which is currently hampered by cost, but may become more available in the future.

Microarray technology presents the possibility of examining tens-of-thou sands of genomic fragments in one experiment. Array based SNP studies have already been conducted for human genetic mapping (Cutler et al. 2001, Huber et al. 2002, Shi 2001). In order to conduct similar studies, EST tomato arrays must be developed. Recently, a company (NimbleGen) has developed a photolithography based microarray chip
consisting of 15,925
ESTs derived from Fei et al. (2004). Several techniques promise to be adapt
able to EST based MAS in plant breeding of crops where vast amounts of
EST sequence data are available (Huber et al. 2002, Stears et al. 2003, Wong
et al. 2004). The deterrent to the widespread use of this technology at present
is that an individual tomato photolithography chip can cost well over a
$1000. However, each chip can provide well over 15,000 data points and
the study conducted by Yang et al. (2004) suggests that well over 5000
SNPs may exist between two tomato cultivars. If these hypotheses prove
valid, QTL studies between elite tomato lines is possible.
In addition to the use of SNPs, other techniques are being explored for
microarray technology in relationship to plant breeding. The microarray
technique was first described and utilized for monitoring the up and down
regulation of genes using ESTs in a complex genome (Shalon et al. 1996,
Winzeler et al. 1998). However, Jaccoud et al. (2001) demonstrated that
microarray technology could be utilized to identify polymorphisms from
uncharacterized genomic DNA fragments. The polymorphisms are largely
derived from diversity in restriction enzyme recognition
sites, and the technique was dubbed “diversity array technology” (DArT). Wenzl et al. (2004) demonstrated the utility of DArT for MAS in barley (Hordeum vulgare L.) breeding. In a set of experiments from an array of Fla7613 x L. pennellii (LA716 or LA2963) crosses, well over 400 markers were identified and mapped in tomato utilizing DArT. As expected, however, fewer polymorphic markers were identified differentiating 20 NILs of ‘Moneymaker,’ each with differing resistance genes (Laterrot 1996, Stevens, unpublished data). The power of DArT is the ability to quickly explore many loci in the genomes of little understood crops and species. As demonstrated in this tomato study, the throughput was phenomenal; hundreds of polymorphic markers were discovered and mapped in a wide cross in a single experiment. Furthermore, it took a matter of approximately three months to develop these markers compared to the first 50 years for approximately 200 phenotypic mapped markers ( Tanksley and Mutschler 1990) and seven years for the first 1000 RFLP markers (Tanksley et al. 1992). The colossal amount of data from this technology presents a new challenge. However, the computing power and algorithms to
manipulate

the overwhelming quantities of data are rapidly being
developed. Once the
technical aspects of DArT are established for a specific
genome, the cost is
expected to be in the neighborhood of cents per data point. Nonetheless,
the initial setup investment of both technical expertise
and equipment is
large.

Before we dismiss these advanced high-throughput
techniques, a

perspective needs to be taken. Tomato marker technologies
have evolved
from phenotypic to isozyme to the current DNA markers, and
what was
once unthinkable is now the norm. In a few years, the
current challenges of
molecular markers will most likely be overcome. Now that a
tomato EST
‘chip’ is available, research has been proposed to utilize
them for the
discovery of polymorphic ESTs between specific elite tomato
parents,
enabling the mass discovery of SNPs. With increased demand, the

cost of
these technologies will inevitably drop. New technological
adjustments,
increased understanding of the power of SNPs, and the
tomato genome
sequencing project underway, will eventually lead to
application of QTL

MAS within elite x elite crosses to create new cultivars.
Once these skills are perfected, tomato breeders will be able to make quantum leaps towards developing their latest 'elite' cultivar. Furthermore, they will be working towards resolving more recalcitrant problems such as flavor, environmental tolerances, and others utilizing combination of both exotic and elite alleles in the most precise expeditious manner.

SUMMARY

Tomatoes have a rich history of interfacing genetics and markers in germplasm development. The first tomato marker studies were reported in 1907 and the first genetic maps were developed by 1917. Over 200 phenotypic markers were mapped throughout 12 chromosomes of tomato by the early 1980’s. Isozymes, the earliest molecular markers, demonstrated a paucity of polymorphic loci within cultivated tomato, which impeded their full implementation in breeding. However, these early studies demonstrated a profusion of polymorphic isozymes among exotic relatives of tomato. Isozyme markers were replaced with DNA markers beginning with RFLPs. Utilizing cultivated by exotic tomato crosses, over 1000 molecular markers were mapped by the early 1990’s. Because cultivated by
exotic crosses were employed, the majority of tomato DNA based markers, 
even today, are located in regions associated with linkage drag from exotic 
introgressions. Historically, exotic germplasm was typically utilized for 
disease resistance gene mining. However, with the advent of DNA based 
markers, mining for QTL from exotic germplasm is altering the future of 
tomato breeding. Mining for QTL is facilitated by molecular markers as 
well as innovative statistical procedures, computers, and breeding 
methodologies which are tools for dissecting a quantitative trait into 
individual loci. These tools have aided in the identification of untapped 
exotic alleles for yield, flavor, color, fruit shape, and many other unexpected 
discoveries. Furthermore, molecular markers have facilitated the cloning of 
both cultivated and exotic genes which would have otherwise remained 
elusive to biochemical and physiological research. Although DNA based 
molecular markers have dramatically impacted tomato breeding, two 
challenges impede their broader use: first, the lack of polymorphisms within 
elite germplasm and second, the need for economical high-throughput 
marker systems. SSRs and SNPs are providing some
possibilities for working with elite x elite germplasm. However, the identification of new markers as part of these two DNA marker systems requires sequencing vast amounts of the tomato genome. Recently, considerable quantities of tomato sequence data have become available to the public. The use of sequence data, along with microarray based technologies, may resolve high throughput issues. Although microarray research is expensive and still in its infancy, the cost of this technology will inevitably subside. Microarray based technology offers the possibilities of examining thousands of markers in a single test, outperforming sequence based techniques and potentially reducing costs to a few cents per data point. SNP based marker assisted selection within tomato breeding offers unprecedented power for identifying and recombining QTL. Thus, we are possibly nearing the threshold of unparalleled advances in tomato breeding.

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INTRODUCTION

Tomato (Lycopersicon esculentum Miller, recently also called Solanum lycopersicum) is one of the most important vegetable crop all over the world.
and constitutes an important source of human diet. It is grown in practically every country in outdoor fields, green-houses, and net-houses, for fresh market and processing purposes. It ranks second (after potato) among all the vegetables and its worldwide production is approximately 97 million tons (FAO production year book 2001). Tomato belongs to Solanaceae family and its origin is from South America and Mexico. The wild species of tomato are: L. pimpinellifolium, L. cheesmanii, L. peruvianum, L. chilense, L. hirsutum, L. parviflorum, L. chmielewskii and L. pennellii. They are all diploids with 24 chromosomes (2n=24).

Tomatoes are a good source of many nutrients. They are rich in minerals, fibers, vitamins (A and C), lycopene pigment (a powerful antioxidant) and health acids. Although a variety of tomato varieties are cultivated worldwide under warm seasons, countries such as USA, several European countries, Japan and China are the most important tomato growing countries (Kalloo 1991).

Apart from being a major vegetable crop, tomato also serves as a model plant for studying fruit development and this is because of the availability of valuable germplasm, monogenic mutants (genomic resources
of tomato

such as inbred lines and mutagenized populations), enzymatic (isozyme)/

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molecular markers, and expressed sequence tags (EST) that are associated

with many useful traits, including disease resistance, plant architecture,

male sterility, fruit development, colour and ripening, and nutritional quality

(Kalloo 1991). Moreover, tomato has modest-sized diploid genome making

it amenable for tissue culture and genetic transformation studies (Bhatia et al. 2004). Further, development of resources such as a full-length cDNA

clones will expand the potential usefulness of tomato for use in genetic

and functional genomic approaches (Taneaki et al. 2005).

Tomato is highly susceptible to various pathogens (fungi, bacteria and

viruses) and pests (Table 9.1) as well as environmental stresses (e.g. chilling,

frost, heat, excessive moisture, drought and salinity), and these stresses

cause a colossal loss of tomato yield and quality (Kalloo 1991, Bhatia et al.

2004). Therefore, there is need to reduce such losses. This objective can be
achieved by various ways: i) increasing the present area under tomato
cultivation, ii) adopting better agricultural techniques, iii) developing the
improved varieties by breeding methods, and iv) producing transgenic
tomatoes with improved characteristics by deploying native and
heterologous genes. The first two approaches already being exploited
strategies, yet there is much scope of tomato improvement using these
strategies. Although plant breeding has contributed for improvement of
crop plants, including tomato, it has certain limitations regarding
improvement of this fruit crop because of the availability of limited gene
Table 9.1 Some of the important diseases and pests of tomato.

Disease Pathogen/pest
Pathogens
Fusarium wilt Fusarium oxysporum f.sp. lycopersici
Verticillium wilt Verticillium alboatrum
Damping-off Pythium aphanidermatum
Early blight Alternaria solani
Late blight Phytophthora infestans
Leaf mould Cladosporium fulvum
Anthracnose Colletotrichum coccodes
Fruit rot Phytophthora spp.
Bacterial wilt Pseudomonas solanacearum
Bacterial stem and fruit canker Corynebacterium michiganense
Bacterial spot Xanthomonas campestris
Mosaic Tobacco mosaic virus (TMV)
Leaf curl Tomato leaf curl virus
Bid-bud Mycoplasma

Pests
Gram caterpillar Heliothis armigera
Tobacco caterpillar Spodoptera litura
Leaf eating beetles Epilachna spp.
Mites Tetranychus cucurbitae
Root-knot nematode Meloidogyne spp.

pool (i.e. transfer of traits only from closely related tomato species).

Furthermore, gene transfer is through cluster of genes, which includes the gene of interest (i.e. gene transfer is not precise), and takes more time (usually 10-15 years) to develop a new variety. Thus, genetic engineering has gained relevance for crop improvement as this strategy can overcome the underlined problems.

Biotechnological approaches have in recent added a new impetus to tomato improvement programs, and good progress has been made in this direction. Efficient plant regeneration protocols now available for tomato have helped in developing transgenic tomatoes with new
traits. This chapter reviews aspects of biotechnology and its applications in tomato.

PLANT REGENERATION

Efficient in vitro plant regeneration protocol is necessary for raising transgenic crops with useful traits. As far as tomato is concerned, a good deal of tissue culture work has been done to regenerate plants from in vitro culture systems. Tomato is quite amenable and responsive to in vitro regeneration (Fari et al. 1992, Izadpanah and Khosh Khui 1992), generation of somaclonal variation, development of haploids (Gresshoff and Doy 1972, Zagorska et al. 1982, 1998, Chlyah and Taarji 1984, Shtereva et al. 1998), selection for biotic and abiotic stresses (Toyoda et al. 1984, 1985, 1989, Rahman and Kaul 1989), distant hybridization (Sink et al. 1986, Wijbrandi et al. 1988) and an efficient genetic transformation. Various cultivars and lines for resistance to various pathogens and improved quality have been released using in vitro anther culture techniques (Foroughi-Wehr and Friedt 1984, Friedt et al. 1986).

Researchers have used various types of explant sources viz. cotyledon (Schutze and Wieczorrek 1987), hypocotyl (Plastira and Perdikaris 1997,
Gunay and Rao 1980), pedicel/peduncle (Compton and Veilleux 1991),

leaf (Duzyaman et al. 1994), stem sections and inflorescence (Applewhite et al. 1994). Most tissues of tomato seem to have high totipotency, however,

the choice of the right explant may vary with the genotype.

Plant regeneration and micropropagation of tomato has been attempted through the use of various techniques, such as shoot tip culture (Novak and Maskova 1979, Padliskikh and Yarmishin 1990, Fari et al. 1992, Izadpanah and Khosh Khui 1992), somatic embryogenesis (Chen and Adachi 1994, Gill et al. 1995, Kaparakis and Alderson 2002), direct organogenesis from intact explants (El-Farash et al. 1993, Davis et al. 1994, Duzyaman et al. 1994, Ichimura and Oda 1998) or protoplast culture (Muhlbach 1980, Sink et al. 1986). Shoot-tip culture (meristem with leaf primordia) has been used as a model system to study tomato shoot development and it was noted that many factors including gibberellic acid, coconut water and kinetin (Hussey 1971). Shoot regeneration from tomato cotyledon explants were also obtained on several kinds of supporting material from polyester, ceramics, wood pulp and cotton fibres (Kazua
Ichimura et al. 1995). In tomato, adventitious shoot regeneration can be achieved either directly (Dwivedi et al. 1990) or indirectly through an intermediate callus phase (Behki and Lesley 1980, Geetha et al. 1998).

Indeed, both callus and shoots may be produced together (Bhatia 2004). A simple and efficient organogenetic mechanism of shoot regeneration via seedling decapitation method (SDM) has been reported for tomato (Fari et al. 1991). Tomato root culture dates back to 1934, when White successfully cultured them in vitro.

The success in tomato regeneration response has been found to depend largely on the genotype, explant carbon source, and plant growth regulators (PGRs) used in the culture medium (El-Farash et al. 1993). Compared to cultivated tomato, its wild counterparts such as L. pimpinellifolium, L. peruvianum and L. glandulosum show better regeneration capabilities (Lech et al. 1996). Successful attempts have been made to transfer the superior regeneration capacity of L. peruvianum into cultivated tomato through backcrossing (Koorneef et al. 1993). Furthermore, L. pimpinellifolium has been successfully used as a donor parent to introgress in vitro regeneration...
capacity in recalcitrant tomato cultivars (Faria et al. 2002). Other unconventional breeding procedures such as protoplast fusion have also been attempted to transfer regeneration capability from L. peruvianum to L. esculentum (Wijbrandi et al. 1988, 1990). Amongst the Lykopersicon species, L. peruvianum is considered to be highly organogenic species as it regenerates shoots from the roots, whereas shoot induction from roots is comparatively low in L. esculentum (Peres et al. 2001). Most researchers avoid using multifarious nutrient media for tomato tissue culture. Preferably either MS or modified MS medium is used (Kartha et al. 1976, Compton and Veilleux 1988, 1991, Chandel and Katiyar 2000, Park et al. 2001). Sucrose is almost universally used for regeneration and micropropagation purposes as carbon source, as it is readily utilisable by cells. However, others have also tried glucose, maltose, ribose, palatinose, and furanose (Locy 1995, El-Bakry 2002, Bhatia et al. 2004). It is notable that maltose followed by glucose has been found to be a better source than sucrose (El-Bakry 2002). For regeneration, a wide variety of PGRs have been used. The concentration and combination of growth regulators
employed is dependent on the cultivar being cultured and the particular

cytokinin or auxin being employed. Four major cytokinins, viz. zeatin,

N-isopentenylnamino purine (2-iP), benzylaminopurine (BAP), and kinetin,

can be used either separately or in combination with auxins for

organogenesis. Vnuchkova (1977 a, b) examined 150 different media and

concluded that combinations of kinetin and indole-3-acetic acid (IAA)

are the most suitable for meristem formation in tomato explants. In later

studies, a combination of IAA-BAP was found to be superior to IAA-kinetin

for shoot regeneration (Gunay and Rao 1980). BAP or zeatin alone induced

shoot formation from leaf callus and these were found to be superior to

kinetin (Kartha et al. 1976). Pulse treatment with cytokinin was also tried

and found to be not beneficial for shoot production (Villiers et al. 1993).

For root induction, tomato does not seem to require any exogenous PGR

because of a high endogenous auxins level in tomato explants (De Langhe

and De Bruijne 1976, Mensuali-Sodi et al. 1995).

In order to develop a very efficient and reliable procedure for tomato

regeneration, Madhulatha et al. (2006a) found that MS medium amended
with 2.5 mg/l BAP and 0.5 mg/l IAA, along with 3% maltose (in place of 3% sucrose) and 0.5 mM diamine putrescine (or 0.1 mM triamine spermidine) greatly enhanced shoot regeneration in cotyledonary explants of tomato.

GENETIC ENGINEERING

The most widely used method for transferring genes into tomato plants is Agrobacterium-mediated transformation, except a recent report on chloroplast transformation using particle bombardment (Ruf et al. 2001).

The later report demonstrated efficient transgene expression in both chloroplasts of green leaves as well as in chromoplasts of tomato fruits, demonstrating the potential of this system for biotechnological applications.

In tomato transformation, reporter and marker genes have been used and these are summarized in Table 9.2.

Since 1986, a number of reports have been published describing the use of Agrobacterium tumefaciens-mediated transformation and regeneration of different tomato cultivars (McCormick et al. 1986, Fillati et al. 1987, Chyi et al. 1987). In most cases, neomycin phosphotransferase (NPTII) has been used as plant selection marker and β-glucuronidase (GUS) as
a reporter gene. Transformation of tomato shows widely variable rates of success,

### Table 9.2 Genetic transformation of tomato using reporter and marker genes

<table>
<thead>
<tr>
<th>Variety</th>
<th>Reporter Gene</th>
<th>Marker Gene</th>
<th>Agrobacterium</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marglobe, Rutgers</td>
<td>NPT II</td>
<td>Kanamycin 100mg/l</td>
<td>Agrobacterium tumefaciens (GV3111, A208)</td>
<td>McCormick et al. (1986)</td>
</tr>
<tr>
<td>Red cherry, UC-82</td>
<td>NPT II</td>
<td>Kanamycin 50 mg/l</td>
<td>Agrobacterium rhizogenes (LBA4404)</td>
<td>Shanin et al. (1986)</td>
</tr>
<tr>
<td>Improved Pearson, Heinz 2152 &amp;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ONT 7710

UC82B GUS NPT II Kanamycin 100 mg/l Agrobacterium tumefaciens (–) Hamza and Chupeau (1993)

Moneymaker GUS NPT II Kanamycin 30 mg/l Agrobacterium tumefaciens (MOG101, Van Roekel et al. (1993) MOG301, EHA105)

Ailsa Craig _ NPT II Kanamycin 50 mg/l Agrobacterium tumefaciens (CS8C1Rif r : Lipp Jao and Brown (1993) pGSFR1161)

Moneymaker GUS NPT II Kanamycin 75 mg/l Agrobacterium tumefaciens (LBA4404) Frary and Earle (1996)

WC1, H2274

& SC2121 GUS NPT II Kanamycin 50 mg/l Agrobacterium tumefaciens (EHA105) Oktem (1998)

Moneymaker _ NPT II Kanamycin 30 mg/l Agrobacterium tumefaciens (LBA4404) Ling et al. (1998)

IAC-Santa Clara _ Aad A Spectinomycin Particle bombardment Ruf et al. (2001) 300-500 mg/l

Pusa Ruby _ NPT II Kanamycin 50-100 mg/l Agrobacterium tumefaciens (–) Patil et al. (2002) Kanamycin 30 mg/l Madhulatha et al. (2006a)

UC82B _ NPT II Kanamycin 100 mg/l Agrobacterium tumefaciens (LBA4404) Cortina and Culianez-Macia (2004)

Pixie GUS NPT II Kanamycin 100 mg/l Agrobacterium tumefaciens (EHA105) Moon and Callahan (2004)

Tomato Cultivars that have been extensively used in transformation studies via Agrobacterium are “UC 82b” (well known for its regenerating capacity: McCormick et al. 1986, Fillati et al. 1987, Hamza and Chupeau 1993), “Moneymaker” (Van Roekel et al. 1993, Frary and Earle 1996, Ling et al. 1998) and the cv “Ailsa Craig” (Bird et al. 1988,
Joao and Brown

All tomato varieties, known for their transformation competence, however, lack tolerance to TYLCV (Tomato Yellow Leaf Curl Virus). Since TYLCV attack on transgenic plants has become a severe problem for the transgenic work done in Israel, several TYLCV-tolerant tomato lines were tested for their transformation competence. In this regard, MP-1, which is named as “Ady”, has been found to be highly amenable to transformation compared with the commonly utilized tomato cultivars (Barg et al. 1997).


Regarding the choice of PGRs, IAA is generally used for tomato regeneration, but cytokinin choice is still unresolved (Ohki et al. 1978, McCormick et al. 1986, Fillati et al. 1987, Hamza and Chupeau 1993).

However, a study on shoot differentiation medium with the
combination of IAA and cytokinins - BAP, 2-iP and kinetin, showed that 2-iP gave best results on shoot formation from transformed hypocotyls and leaf segments (Ohki et al. 1978). Shoot formation on hypocotyl segments of tomato was observed in the presence of naphthaleneacetic acid (NAA) combined with BAP (Yakuwa et al. 1973). Improved results on transformed explant source have also been reported using zeatin (McCormick et al. 1986, Fillati et al. 1987, Hamza and Chupeau 1993) or zeatin riboside (Pfitzner 1998).

In vitro morphogenetic response of transformed plant tissues are also affected by the different components of the culture media and it is important to evaluate their effects on plant regeneration. MS medium (Murashige and Skoog 1962) is the commonly used medium in plant regeneration. However, a high percentage of tomato cotyledon explants have been found to develop damaging necrosis in it. Increasing the vitamin (thiamine) concentration in the MS medium decreased the expansion of necrotic lesions and promoted cell growth (Cortina and Culianez-Macia 2004). As previously mentioned (Madhulatha et al. 2006a) conditions have been
optimized for

an efficient Agrobacterium-mediated transformation for
cotyledonary

explants of tomato and were able to minimize the necrotic
problem as well

as recover more number of transformants on the improved
shoot

regeneration medium amended with 3% maltose and 0.1 or 0.5
mM

polyamines (putrescine and spermidine); this has led to the
increased

transformation frequency (Madhulatha et al. 2006b).

The most critical factors affecting the regeneration of the
number of

transformed explants recovered following co-cultivation
were, the pre

incubation period, bacterial density and co-cultivation
time. The decrease

in the transformation rate at high bacterial density and
longer than optimal

cultivation period has been reported which probably may
be due to

bacterial-induced stress and not due to a decrease in the
virulence of

Agrobacterium tumefaciens. This stress threshold may be
related to the growth

rate of bacteria, the initial inoculum of the bacteria per
explant, and the

sensitivity of the tissue used. This reflects that
co-cultivation conditions

should be optimized for each bacterial strain according to
the plant species

(Fillati et al. 1987).
Acetosyringone, a naturally occurring vir-inducing phenolic compound,

improves the transformation efficiency in plants, which naturally produce
is insufficient amounts of vir-inducing compounds
(Sheikholeslam and Weeks 1987, Joao and Brown 1993). The enhancement of the transformation fre
quency has been observed when acetosyringone is added after the pre
incubation treatment. This may be due to the accumulation of substances
that induce the vir genes or due to an increase in the rate of explant cell
division (Fillati et al. 1987). Alternatively, some authors have also used
feeder cells from species like tobacco, as phenolic source to increase trans
formation rate (McCormick et al. 1986, Fillati et al. 1987, Hamza and
Chupeau 1993).

For successful transformation using Agrobacterium, effective elimination
of bacteria from the culture is necessary as soon as their presence is no
longer required. Carbenicillin, cefotaxime and augmentin are extensively
used antibiotics for this purpose. An ideal antibiotic for inhibiting
Agrobacterium species should be highly effective, inexpensive, without a
negative effect on plant growth and regeneration (Cheng et al. 1998). Among
the antibiotics, timentin, which has recently been made available, shows a marked efficiency for eliminating Agrobacterium and also has stimulatory effects on organogenesis. Timentin has been used for eliminating Agrobacterium after explant transformation (Frary and Earle 1986, Schroeder et al. 1993, Frary 1995, Zimmerman 1995, Ling et al. 1998), but its possible effects on regeneration have not been described.

A liquid culture system for Agrobacterium-mediated transformation of tomato has been developed and found to be better for transformation, shoot regeneration and selection of transformed tomato plants as compared to solid media, because of the better distribution of the selective agent in the liquid cultures (Velcheva et al. 2005).

Tomato transformation with Agrobacterium rhizogenes has also been undertaken. This system could be used to produce transgenic tomato plants expressing the rol genes as well as the genes present in A. rhizogenes based binary vectors. Hairy roots have the potential to produce useful materials such as enzymes (Kato et al. 1991, Uozumi et al. 1991) and secondary metabolites (Flores et al. 1987). To date plant regeneration
from hairy roots has been reported for several plant species (Tepfer 1984, Ooms et al. 1985, Lambert and Tepfer 1991, Uozumi et al. 1996). In addition, the use of A. rhizogenes offers the opportunity to introduce foreign genes into plant genomes when the hairy root is induced, enabling the alteration of a given plant’s properties by genetic manipulation (Hamill et al. 1987).

Genetic manipulation via Agrobacterium has helped in increasing the plant productivity and also the nutritional content of food, which was not an easy task with traditional breeding approaches. This approach has utilized strategies applied in genetic engineering including antisense RNA technology (Stone et al. 1994), over-expression of genes (Mehta et al. 2002) and gene pyramiding (Mandaokar et al. 1999) to increase the shelf life, improve the fruit quality and nutrients, and also to develop value-added traits in tomato (Table 9.3).

Antisense RNA technology has been used successfully to manipulate the expression of several tomato ripening and softening associated genes - polygalacturonase (PG) (Sheehy et al. 1988, Smith et al. 1988, 1990; Watson et al. 1994), pectin methylesterase (PME)
(Tieman et al. 1992, Hall et al. 1993), 1-aminocyclopropane-1-carboxylic acid (ACC) synthase

(Oeller et al. 1991), ACC oxidase (Hamilton et al. 1990), sucrose synthase

(SuSy) (Marc-Andre ‘D’ Aoust et al. 1999), non-ripening gene in Nr mutant

(Hackett et al. 2000), ß-galactosidase (TBG4) (Smith et al. 2002), Rab11GTPase (Lu et al. 2001), phospholipase D (PLD) (Pinhero et al. 2003) and phytoene synthase (Bird et al. 1991). Over-expression of various genes has also been attempted for improvement of fruit quality, expansin (Le Exp1) to increase shelf life (Brummell et al. 2002), linalool synthase (LIS) (Lewinsohn et al. 2001) and carotenoid cleavage dioxygenase (LeCCD1) (Simkin et al. 2004) to enhance flavour and aroma.

To enhance the carotenoid content in tomato fruit, transgenic lines were developed by introducing bacterial carotenoid gene (crt1) encoding the enzyme phytoene desaturase, which converts phytoene to lycopene (Romer et al. 2000) and crt B gene (Fraser et al. 2002).

Fruit taste is also an important component of fruit quality. Several genes have been introduced into tomato to improve the fruit characteristics of tomato. Petunia chalcone isomerase (Petunia chi-a) gene was
introduced into tomato and the resulting transgenic tomato lines produced increased amounts of flavonols (up to 78 fold) in fruit peel, mainly due to an

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Gene</th>
<th>Gene Source</th>
<th>Marker</th>
<th>Trait</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UC 82b</td>
<td>aro A</td>
<td>Salmonella</td>
<td>NPTII</td>
<td>Glyphosate</td>
<td>Fillatti et al. (1987) typhimurium Herbicide tolerance</td>
</tr>
<tr>
<td>Rutgers</td>
<td>PME</td>
<td>L. esculentum</td>
<td>NPTII</td>
<td>Increased soluble solid content</td>
<td>Tieman et al. (1992)</td>
</tr>
<tr>
<td>Ailsa Craig</td>
<td>PG (antisense)</td>
<td>L. esculentum</td>
<td>NPTII</td>
<td>Flavr Savr (increased lycopene content)</td>
<td>Watson et al. (1994) &amp; shelf-life</td>
</tr>
<tr>
<td>Vollendung</td>
<td>Vst1 &amp; vst2</td>
<td>Vitis vinifera</td>
<td>NPTII</td>
<td>Phytophthora resistance</td>
<td>Thomzik et al. (1997)</td>
</tr>
<tr>
<td>UC82B HAL2</td>
<td>Saccharomyces</td>
<td>cereviciae</td>
<td>uid A</td>
<td>NPTII and Salt tolerance</td>
<td>Arillaga et al. (1998)</td>
</tr>
<tr>
<td>CM, L.276 iaAM</td>
<td>Pseudomonas</td>
<td>cereviciae</td>
<td>NPTII</td>
<td>Seedless fruits (parthenocarpic)</td>
<td>Ficcadenti et al. (1999) syringae</td>
</tr>
<tr>
<td>Summerset SuSy (antisense)</td>
<td>L. esculentum</td>
<td>NPTII</td>
<td>Reduced fruit setting and development</td>
<td>Marc-Andre’D’Aoust et al. (1999)</td>
<td></td>
</tr>
<tr>
<td>Ailsa Craig NR (antisense)</td>
<td>L. esculentum</td>
<td>NPTII</td>
<td>Restored ripening pattern in NR mutant</td>
<td>Hackett et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Pusa Ruby</td>
<td>Cry1Ac</td>
<td>Bacillus thuringiensis</td>
<td>NPTII</td>
<td>Resistant to fruit borer</td>
<td>Mandaokar et al. (2000)</td>
</tr>
<tr>
<td>Ailsa Craig Crt 1</td>
<td>Erwinia uredovora</td>
<td>NPTII</td>
<td>Increased levels of provitamin A</td>
<td>Romer et al. (2000)</td>
<td></td>
</tr>
<tr>
<td>Heinz 902, ACC</td>
<td>Enterobacter</td>
<td>NPTII</td>
<td>Flood tolerance</td>
<td>Grichko and Glick (2001)</td>
<td></td>
</tr>
</tbody>
</table>
Heinz 1439 deaminase cloacae UN4
UC 82b, CB3 LIS Clarkia breweri NPTII Enhanced levels of aroma and flavour Lewinsohn et al. (2001)
Ailsa Craig Rab11GTPase L. esculentum NPTII Reduced fruit softening Lu et al. (2001)
and mutants (antisense)
Alcobacca and Never-ripe (Contd.) Application of Genetic Engineering in Tomato
FM62003 chi PCR amplified product NPTII Increased levels of flavonol Muir et al. (2001)
(unilever com
mercial variety)
Ailsa Craig Le Exp 1 L. esculentum NPTII Increased shelf-life Brumwell et al. (2002)
Ailsa Craig crt B E. uredovora NPTII Increased carotenoid content Fraser et al. (2002)
CL5915-93 CBF1 Arabidopsis thaliana NPTII Tolerance to water deficit stress Hsieh et al. (2002)
D 4-1-0-3
Bailichun BADH Atriplex hortensis NPTII Salt tolerance Jia et al. (2002)
Asc/Asc,
VFNT cherry p35 Baculovirus NPTII Broad-spectrum disease resistance Lincoln et al. (2002)
L. esculentum Samdc S. cerevisiae NPTII Increased phytonutrient, carotene in fruit Mehta et al. (2002)
Mill
Rutgers TBG4 L. esculentum NPTII Decreased fruit softening Smith et al. (2002) (antisense)
___ Thaumatin Thaumatococcus. NPTII Improved fruit taste
Bartoszewski et al. (2003) daniellie Benth

Minitomato gdh A Aspergillus nidulans NPTII Improved fruit taste (increased Kisaka and Kida (2003) glutamate levels)

LEPA Cry1Ab B. thuringiensis NPTII Resistant to fruit borer Kumar and Kumar (2004)

L. 276-76, Nucleoprotein Tomato Spotted NPTII Resistant to TSWV Nervo et al. (2003)

L.149-88, gene Wilt Virus

INB777

Microtom, PLD (antisense) A. thaliana NPTII Increased fruit firmness and red colour Pinhero et al. (2003)

Celebrity

Ventura TBI-HBS Hepatitis B Virus NPTII Edible vaccine against HBV & HIV Shchelkunov et al. (2004)

M82 LeCCD1 L. esculentum NPTII Improved flavour and aroma Simkin et al. (2004)

— CtrB Vibrio cholerae NPTII Edible Vaccines Tyagi et al. (2002)

M82 LeCCD1 L. esculentum NPTII Improved flavour and aroma Simkin et al. (2004)

Moneymaker PI-II and PCI S. tuberosum NPTII Multiple insect resistance Abdeen et al. (2005) (Contd.)

Gene Improvement of Solanaceous Crops: Tomato

— Ep5c (antisense) Pseudomonas syringae -Resistant to P.s tomato Alberto et al. (2005)

Hezuo 906 ACCO (double PCR amplified modified Prolonged shelf life Xiong et al. (2005) stranded RNA) product NPTII

Pusa Ruby CP Tomato leaf Curl NPTII Resistance against TLCV Raj et al. (2005) Virus (TLCV)

Moneymaker spike SARS-corona virus NPTII Recombinant vaccine Pogrebnyak et al. (2005)

UC82B TPS1 S. cereviciae NPTII Increased tolerance of
abiotic stress Cortina et al. (2005)

L. esculentum APX Pisum sativum NPTII Tolerance to chilling and salt stress Wang et al. (2005)

L. esculentum TPS1 S. cereviciae NPTII Increased tolerance to abiotic stress Cortina et al. (2005)

L. esculentum CAX A. thaliana — Increased shelf life and Ca +2 levels Park et al. (2005)

accumulation of rutin. No gross phenotypical differences were observed between high-flavonol transgenic and control lines. The phenotype segregated with the transgene and demonstrated a stable inheritance pattern over four subsequent generations. Whole-fruit flavonol levels in the best of these lines are similar to those found in onions, a crop with naturally high levels of flavonol compounds. Processing of high-flavonol tomatoes demonstrated that 65% of flavonols present in the fresh fruit were retained in the processed paste, supporting their potential as raw materials (Shelagh et al. 2001). A double antisense construct containing ACC oxidase and ACC synthase fusion gene with NPTII as a marker gene was introduced by means of Agrobacterium-mediated transformation for longer shelf life of fruits. The transgenic lines showed remarkable delay in fruit ripening and increased shelf life (Xiong et al. 2005).
Fruits from tomato plants expressing Arabidopsis H⁺/cation exchangers (CAX) demonstrate modest increase in Ca²⁺ levels and prolonged shelf life but no detourious effects on plant growth. These findings suggest that CAX expression may fortify plants with Ca²⁺ and may serve as an alternative to the application of CaCl₂ used to extend the shelf life of numerous agriculturally important commodities (Park et al. 2005).

A gene for thaumatin (sweet tasting, flavour enhancing protein) has been introduced into tomato to improve the sweetness of fruit (Bartoszewski et al. 2003). Free amino acids are essential nonvolatile compounds involved in the overall taste of many foods and glutamate, in particular, contributes to the taste of tomatoes. (Fuke and Konosu 1991). The enzymes involved in the synthesis of amino acids during ripening of tomato fruits have been investigated. The over-expression of the gdh A gene from Aspergillus nidulans, which encodes NADP-GDH (Hawkins et al. 1989, Kisaka and Kida 2003), can modulate nitrogen metabolism in tomato, with resultant increase in levels of some free amino acids, in particular, glutamate in fruits.
RNA interference (RNAi), a new technology has also been applied for the improvement of tomato fruit characteristics. PCR amplified ACC oxidase double stranded RNA with modified NPTII, which contained the catalase intron in the coding sequence, as a marker gene was used to transform tomato. The resultant transgenic lines showed remarkable delay in fruit ripening and increased shelf life (Xiong et al. 2005). A recent study on fruit-specific RNAi-mediated suppression of DET1 (DE ETIOLATED1), a regulatory gene, which represses several signaling pathways controlled by light, enhanced carotenoid and flavonoid content in tomato (Ganga Rao et al. 2005).

Some of the genes in tomato have been cloned successfully using targeted transposon tagging (Jones et al. 1994, Bishop et al. 1996, Keddie et al. 1996, Van der Biezen et al. 1996, Takken et al. 1998). A reverse genetics approach was also developed using Ds element insertions creating a collection of 2932 families of a miniature tomato Micro-Tom (Meissner et al. 2000). An efficient reverse genetic approach to assess gene function is however still lacking.
Virus-induced gene silencing (VIGS) offers an attractive and alternative way to knockout the expression of genes without the need of genetically transforming the plants. In this method, recombinant virus carrying a partial sequence of a host gene is used to infect the plant. When the virus spreads systematically, the endogenous gene transcripts, which are homologous to the insert gene in the viral vector, are degraded by post transcriptional gene silencing (PTGS) (Baulcombe 1999). Using this system, several genes like PDS (phytoene desaturase), tCTR1 and tCTR2 (constitutive triple response 1 and 2) have been suppressed in tomato.

Several reporter genes related to ethylene responses and fruit ripening, including LeCTR1 and LeEILs genes, were also successfully silenced by this method during fruit development. In addition, silencing of LeEIN2 gene resulted in the suppression of tomato fruit ripening (Fu et al. 2005).

This system will facilitate large-scale functional analysis of tomato ESTs (expressed sequence tags) (Liu et al. 2002).

Production of ethylene has been shown to be involved in the initiation, modulation, and co-ordination of expression of many genes required for
the ripening process. Functionally and metabolically乙烯 and
polyamines (putrescine, spermidine and spermine) seem
inter-related as
they share a common precursor, S-adenosylmethionine (SAM).
Polyamines, ubiquitous, aliphatic cations, have all been
implicated in a
myriad of physiological and developmental processes,
including fruit
ripening (Rajam 1997). It has been reported that the
exogenous application
of polyamines can retard fruit ripening and prolong shelf
life of fruits
(Kumar and Rajam 2004). Transgenic approach has been
undertaken with
yeast samdc gene, which has led to increased lycopene
content, prolonged
vine life and enhanced fruit quality (Mehta et al. 2002). Over-expression
of polyamine biosynthesis genes - adc, odc, samdc and spd
syn under the
control of fruit specific promoter (2A11) was achieved and
the developed
independent tomato transgenic lines revealed delayed fruit
ripening and
parthenocarpic fruits (Rajam et al. unpublished results).
Tomato fruits are consumed either fresh or processed.
Processing
tomatoes account for most of the tomato production. So, it
is very
important to improve the fruit quality and reduce
processing costs.
Parthenocarpy (seedless fruits) is a valuable trait because
seeds are usually difficult to digest, and often their presence is undesirable. Parthenocarpic fruits are reported to have a higher percentage of soluble solids, improved yield and flavour. Phytohormonal sprays cause parthenocarpic fruit development but excess of exogenous phytohormones causes malformations of the tomato fruit (Santangelo and Soressi 1990).

Interestingly, the introduction of iaaM gene (which codes for tryptophan monooxygenase and is involved in auxin synthesis) into tomato plants has allowed for the combination of parthenocarpy (upon emasculation), high yield, and high fruit quality in tomato fruits for the fresh market (Ficcadenti et al. 1999).

A wide range of insect pests and pathogens are known to attack tomato. Hence several studies have been undertaken to confer significant resistance against such biotic stresses to remedy significant yield losses in commercial tomato via Agrobacterium-mediated transformation. Tomato is severely damaged by lepidopteran insect pest Helicoverpa armigera Hubner, also called tomato fruit borer (Atwal 1986). The introduction of the synthetic cry1Ac gene (Mandaokar et al. 2000), cry1Ab gene (Kumar
and Kumar 2004) into tomato conferred high levels of protection against H. armigera infestations (Mandaokar et al. 1999). Combined expression of defense genes such as potato protease inhibitors (PI-II) and carboxypeptidase inhibitors (PCI) was reported in tomato against multiple insect resistance (Abdeen et al. 2005). In addition to the development of a refugium strategy, pyramiding of genes encoding insecticidal proteins that differ in their mode of action might effectively curtail resistance development (Gould 1998). Genes encoding inhibitors of insect proteases and vegetative insecticidal proteins (VIP) were considered for introduction into transgenic tomatoes in conjunction with cry1Ac gene (Mandaokar et al. 1999). Broad-spectrum disease resistance in tomato has been achieved by expression of antiapoptotic baculovirus p35 gene (Lincoln et al. 2002). Likewise, the introduction of viral nucleoprotein gene into tomato conferred resistance against tomato spotted wilt virus (Nervo et al. 2003) and coat protein (CP) gene of tomato leaf curl virus (TLCV) against TLCV (Raj et al. 2005). There are very few reports of transgenic tomato for fungal resistance.

For instance, stilbene synthase (Vst1 & 2) gene (Thomzik et al. 1997) and
Thi 2.1 gene (Chan et al. 2005) have been used to create resistance against Phytophthora infestans and phytopathogens, respectively. Glyphosate (herbicide) tolerance using aro A gene has also been attempted in tomato (Fillatti et al. 1987). Bacterial speck caused by the pathogen Pseudomonas syringae pv tomato (P.s tomato) is a devastating disease of tomato plant. The inhibition of Ep5c represents a novel form of disease resistance based on a loss-of-gene function in the plants. Ep5c expression is rapidly induced by H 2 O 2 , a reactive oxygen intermediate normally generated during plant pathogen interaction (Alberto et al. 2005).

Most of the tomato cultivars are moderately sensitive to salts (Cuartero and Munoz 1999, Foolad 1999). There are very few reports published on the engineering of salt tolerance in tomatoes. Since tomato has no glycinebetaine synthesis pathway (Weretilnyk et al. 1989) to synthesize glycinebetaine (a osmoprotectant), betaine aldehyde dehydrogenase (BADH) gene from Atriplex hortensis (Xiao et al. 1995, Jia et al. 2002) has been introduced into tomato that allowed the biosynthesis of glycinebetaine to maintain an osmotic balance with the environment and
also to withstand the salinity stress (Zhang and Blumwald 2001, Robinson and Jones 1986). Other examples for salt-tolerance in tomato include introduction of HAL1 (Gisbert et al. 2000) and HAL2 (Arillaga et al. 1998) to maintain high internal K+ concentration and decreased intracellular Na+ concentration during salt-stress. Waterlogging is another stress for which ACC deaminase gene has been introduced to confer flood tolerance (Grichko and Glick 2001). Transgenic tomato that could withstand water deficit condition has also been developed by introducing C-repeat/dehydration responsive element binding factor gene (CBF1) by Hsieh et al. (2002). Tomato transgenic lines have been developed to combat abiotic stress by engineering the trehalose biosynthetic pathway. The introduction of the yeast trehalose-6-phosphate synthase gene (TPS1) resulted in pleiotropic changes such as thick shoots, rigid dark-green leaves, erected branches and an aberrant root development in transgenic plants. These plants showed improved tolerance under drought, salt and oxidative stress conditions as compared to wild type plants (Cortina and Carolina 2005).
Edible vaccine is becoming a reality as scientists have found a way to incorporate the protein gene-HIV antigen in tomatoes. This discovery has become popular when it was found that the protein needed for the vaccine could be derived from both tomato leaves and the fruit. Moreover, tomatoes are edible and immune to any thermal process, which helps retain its healing capabilities. Tomatoes producing edible vaccines were found to grow at a high rate of success in Russia, compared to bananas, which are also used to produce vaccines. A candidate edible vaccine against hepatitis B and HIV has been successfully developed in tomato by introducing TBI-HBS gene (Shchelkunov et al. 2004). Tomato-based edible vaccines against cholera have also been developed by transforming tomato plants with the gene encoding cholera toxin B subunit (ctxB) along with an endoplasmic reticulum retention signal (SEKDEI) under the control of the CaMV 35S promoter (Jani et al. 2002). The recent spread of severe acute respiratory syndrome (SARS) has heightened demand for SARS vaccine. SARS-coronavirus (CoV) spike protein (S protein) and its truncated fragments are considered to be the best candidates for
generation of recombinant vaccine in tomato and low nicotine tobacco plants (Pogrebnyak et al. 2005).

Due to decreasing public acceptance of antibiotic and herbicide resistance genes in food crops, alternative selectable markers and even complete removal of marker genes (Hohn et al. 2001, Penna et al. 2002) have been attempted. One such alternative approach utilizes mannose as a selective agent as many plants cannot utilize mannose as a carbohydrate source. Such cells will not grow when cultured on mannose containing media. However, when cells are transformed with the phosphomannose isomerase (PMI) gene, they can then survive by utilizing mannose as carbon source. Unlike standard antibiotic or herbicide selection, an efficient mannose selection protocol for tomato that has no adverse effect on ploidy levels of transgenic plants has been successfully developed. This selection does not result in a direct, acute toxic effect, but rather provides a physiological advantage to transformed cells over non-transformed cells as “positive selection” (Sigareva et al. 2004).

The major technical challenge facing the practical application of plant
transformation is the development of a method that produces a high proportion of transgenic plants without collateral genetic variations. In case of the transformed cotyledon explants of cv ATV847, only 60% of the transgenic tomato retained the diploid level (Ultzen et al. 1995). The high frequency of polyploid transgenic plants (40%) could be due to the mixoploid nature of the cotyledon tissue (Smulders et al. 1994, 1995). The confirmation of ploidy level before performing an evaluation of transgenic material is particularly important when a polysomatic tissue is to be used as an explant source (Ellul et al. 2003).

CONCLUSIONS AND FUTURE DIRECTIONS

Tomato is a major vegetable crop and serves an important source of nutrients for human consumption. As the crop is highly susceptible to various pests and diseases as well as environmental extremes, its improvement through the use of traditional breeding methods coupled with bio-technological approaches is extremely important. Besides, the improvement of nutrient quality and quantity is also very important in tomato. Tissue culture and genetic engineering of tomato has come a long way since the 1930s. A large number of publications have
Tomato biotechnology. Tomato is quite amenable for both tissue culture regeneration and genetic transformations. In general, the cotyledons are most preferred explants for plant regeneration on MS medium with BAP and IAA hormonal combination. Tomato transformation is usually done via A. tumefaciens containing binary vectors with specific selection markers.

A good number of transgenic tomatoes have been produced for introducing the traits like slow ripening, fruit softness, improvement of fruit taste, aroma, flavour and nutrient content, seedless fruits, resistance against insect pests and pathogens, herbicide tolerance, salt and drought tolerance, and antibody production. Full potential is yet to be exploited for improvement of tomato yield and quality by genetic engineering.

Further, the antisense RNA technology may be replaced with the latest and potent RNAi (RNA interference) technology for effective suppression of ethylene biosynthesis genes to achieve efficient delay of fruit ripening.

The areas that need to be strengthened in tomato genetic engineering are tolerance to various abiotic stresses (e.g. salinity, drought and cold), production of a variety of edible vaccines/antibodies.
against human and animal diseases, and stacking of transgenes for introduction of multiple and complex traits.

Acknowledgements

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INTRODUCTION

Fruits are anatomically defined as a mature ovary and include carpel tissues in part or as whole. A simple fruit develops from a single matured ovary within a single flower. However, fruit crops comprise over 30 families encompassing simple, aggregate, multiple or accessory fruits, the distinctive feature for these classes being the number of ovaries and the tissues involved in the development of the fruit. Aggregate fruits comprise a number of matured ovaries formed in a single flower and are arranged over the surface of a single receptacle; multiple fruits consist of matured ovaries of many flowers often clustered together; and the accessory fruits develop from
tissues surrounding the ovary (Coombe 1976). Differences in the
morphology of fruits in each of these classes have led to their assignment
to different groups, of which the fleshy fruits have received considerable
attention due to their economic significance, particularly to manage their
post-harvest shelf life. The succulent, flavorful and attractive tissues of the
fleshy fruit serve to disperse the seeds, features that have led to their
domestication and commercialization. Based on their respiration patterns
during development, fleshy fruits have been classified as either climacteric
or non-climacteric. In the climacteric fruits, respiration rates increase at the
onset of ripening whereas a decrease in respiration rate characterizes the
non-climacteric fruits; the respiratory climacteric is generally preceded by
an increase in ethylene, a gaseous plant hormone (Mattoo and Suttle 1991,

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Fruit development and maturation, genetically regulated complex
processes, involve interplay of plant hormones and growth regulators
with numerous biological and environmental factors. Despite varied mor-
phologies, fruits share common events and pathways in their
life cycles,

which are crucial for their induction and development. This development can be broadly separated into several distinct phases: i) initiation of the primordia; ii) development prior to pollination; iii) pollination, fertilization and initial fruit set; iv) growth after fruit set; v) ripening; and vi) senescence (Ozga and Reinecke 2003). The floral and fruit primordia initiation phase is followed by the development of the ovary and the ovule, making it ready for pollination and fertilization. The processes of pollination and fertilization initiate a slow growth phase of cell division followed by a rapid growth phase primarily involving cell expansion for both the ovary and ovule. In the absence of pollination, the developing fruit undergoes senescence and abscission. Developing seeds play an essential role in fruit development by providing phytohormones, for instance, auxins (Nitsch 1970, Ozga et al. 1992). Ripening involves changes in color, taste, aroma, flavor and texture and is initiated after the fruit acquires its maximal size and physiological maturity. The classic plant hormones, namely cytokinins (CKs), auxins (AUXs), gibberellins (GAs)
and abscisic acid (ABA) along with brassinosteroids (BRs),
jasmonates, systemins and other growth regulators like polyamines (PAs) have been
implicated to play significant roles in growth and maturation processes
at various stages of fruit development (Weyers and Paterson 2001, Ozga
and Reinecke 2003). Ethylene remains, by far, the most extensively
studied hormone with respect to tomato fruit ripening and the lack of
synthesis or perception of this hormone results in impaired ripening of
tomato fruit (Hamilton et al. 1990, Oeller et al. 1991, Fluhr and Mattoo
1996). Various aspects of tomato fruit development, maturation and ripen
ing have been reviewed (Coombe 1976, Brady 1979, Hobson and Harman
1986, Gillaspy et al. 1993, Giovannoni 2004, Tanksley, 2004). In this
chapter, the focus is on the role of plant hormones during tomato fruit set,
growth and maturation.

TOMATO FRUIT DEVELOPMENT

Tomato, a climacteric fruit, has many desirable attributes that have made
it a model plant to study development of fleshy fruits. Large populations
of well-characterized tomato mutants provide an amenable system for
analyzing molecular aspects, including hormonal regulation
of fruit
development (Table 10.1). The growth and maturation of tomato fruit follow
a single sigmoidal growth curve (Fig. 10.1), with the fruit development
being broadly divided into four phases (Tanksley 2004). Phase I represents
floral development (including ovary), fertilization and fruit set. It is during
this phase that a decision is made to either abort or proceed with cell
division leading to the fruit set. Phase II involves cell division that lasts for
7-14 days after pollination (Mapelli et al. 1978). Although during this phase
most of fruit cells are established, fruit growth is slow, reaching only about
10% of the final fruit weight (Fig. 10.1). Phase III primarily comprises of cell
expansion which, depending upon the genotype, continues for 3-5 weeks
and is responsible for attainment of the maximum fruit size (Ho and Hewitt
1986). This phase is followed by slow growth and intense metabolic changes
resulting in fruit ripening (Phase IV). On the basis of gel formation and
lycopene accumulation, the ripening phase of the tomato fruit has been
subdivided into several stages including MG1 (mature green; firm locular
tissue), MG2 (small amount of gel), MG3 (gel formation complete), MG4 or
breaker (fruit pigment detectable), turning (10-30% red fruit), pink (30-60% red), light-red (60-90% red) and red (over 90% red) (Su et al. 1984, Lincoln et al. 1987). The various structures and tissues of the fruit can be traced back to that of the ovary. Tomato fruit is composed of the flesh (pericarp walls and skin) and pulp (placenta and locular tissues including seeds), the ovary wall comprising the flesh and placental tissue expanding into locules and seeds comprising the pulp (Ho and Hewitt 1986). Life cycle of tomato fruit spans 49-70 days from fertilization to the “red ripe” stage.

Many factors such as the cultivar, position on the cluster, climatic conditions and cultural practices influence the overall fruit development. The genetic, developmental and molecular bases of fruit size and shape variation in tomato have been reviewed elsewhere (Tanksley 2004).

HORMONAL FLUX AND INTERACTIONS DURING FRUIT DEVELOPMENT

Several investigators have quantified levels of plant hormones in developing tomato fruits (Abdel-Rahman 1977, Mapelli et al. 1978, Sjut and Bangerth, 1982, 1983, Buta and Spaulding 1994). However, there are discrepancies between the reported patterns. For example, some
investigators have reported that AUXs levels peak during the cell expansion phase (Abdel-Rahman 1978, Sjut and Bangerth 1982, 1983) whereas others reported it to peak during the early phase (5-10 days after anthesis) of tomato fruit development (Mapelli et al. 1978, Buta and Spaulding 1994).

### Mutant Description

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Hormonal deviations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pat-2</td>
<td>Parthenocarpy</td>
<td>Seedless fruit set with complete High GA 20 levels in the ovaries Lin et al. (1983), Fos et al. (Russian cv. locular development and normal (2003) Severianin) fruit weight and size.</td>
</tr>
<tr>
<td>pat-3/4</td>
<td>Parthenocarpy</td>
<td>-Same as above Enhanced early 13-hydroxylation Fos et al. (2001) (German line pathway of GA biosynthesis RF75/59)</td>
</tr>
<tr>
<td>gib-1</td>
<td>GA deficient</td>
<td>Extreme dwarfism, reduced germiGA biosynthetic pathway affected. Bensen and Zeevart (1990), nation, and abnormal flower deve(Lesion in ent-copalyl diphosphate Koorneef et al. (1990) lopment. synthase, gene)</td>
</tr>
<tr>
<td>gib-3</td>
<td>GA deficient</td>
<td>Same as above GA biosynthetic pathway affected. Bensen and Zeevart, (1990), (Lesion in ent-kaurene synthase, gene) Koorneef et al. (1990)</td>
</tr>
<tr>
<td>hp/ dg</td>
<td>High pigment/</td>
<td>Exaggerated light responsiveness, Reduced GA levels Wann, (1995)</td>
</tr>
<tr>
<td>(allelic)</td>
<td>dark green high fruit and foliar mutation pigmentation.</td>
<td></td>
</tr>
<tr>
<td>RCarotenoid</td>
<td>Yellow fruit flesh and skin color, pale Reduced ABA levels, elevated GA Fraser et al. (1995), mutant deficient yellow corolla; absence of ripening levels</td>
<td></td>
</tr>
</tbody>
</table>
Fray and Grierson (1993) ripening associated carotenoid increase. mutant

sitiens ABA deficient Precocious seed germination ABA deficient Groot and Karssen (1992),

(Liu et al. 1996, 1997)


nor Non-ripening Non-climacteric, impaired ripening. Low ethylene and ABA production, Tigchelaar et al. (1978), delayed ABA accumulation. Hong and Lee, (1996) (Contd.) Hormonal Control of Fruit Maturation

Nr never ripe Failure to ripen Insensitive to ethylene. Tigchelaar et al. (1978). (ETR1) Lanahan et al. (1994)

alc alcobaca Delayed fruit ripening. Reduced ethylene, high PA contents. Dibble et al. (1986)

cnr colorless, nonRipening inhibited, fruit white at Reduced ethylene. Thompson et al. (1999) ripening maturity, turns yellow later; remains firm.


ls* lateral Absence of side shoots, lower numHigh GA and auxin levels, Schumacher (1999) suppressor ber of flowers per inflorescence, lower cytokinin levels. absence of petals, reduced male and female fertility, limited seed yield and low rate of seed germination.


flc* flacca Wilty phenotype. Reduced ABA levels. Sagi et al. (1999)

not* notabilis Wilty phenotype. Reduced ABA levels. Burbidge et al. (1999)

biosynthesis mutant)

dpy* dumpy Short stature, reduced axillary Brassinosteroid insensitive Koka et al. (2002) branching, and altered leaf (signaling mutant) morphology.

cu3* curl3 Altered leaf morphology, deBrassinosteroid insensitive. Koka et al. (2003) etiolation, and reduced fertility. (signaling mutant)


7B-I* photoperiod Photoperiod-dependent male sterility, High ABA levels. Fellner et al. (2001) sensitive reduced de-etiolation of hypocotyls in long days and increased seed size and weight.

* Effects on fruit growth and development not yet described.

Fig. 10.1 Hormonal regulation of tomato fruit development: Upper panel: Developmental phases of tomato fruit. Middle panel: Changes in hormonal levels observed during fruit development (redrawn with approval from Gillaspy et al. (1993), copyright American Society for Plant Biologists). Lower panel: Cell division as mitotic index is redrawn with approval from Cong et al. (2002). Copyright (2002) National Academy of Sciences, U.S.A.; fruit weight gain and growth rate (weight gained/day) are redrawn and calculated from Abdel-Rahman (1977) with approval. All data are normalized as percent maximum observed during fruit development.

These discrepancies need to be resolved before the correlation between endogenous levels of phytohormones and fruit development can be established. In spite of these discrepancies, some generalization can be drawn (Fig. 10.1) about the patterns of appearance and changes in the levels of the five classic plant hormones namely, AUXs, CKs, GAs, ABA and ethylene during tomato fruit development. Phase I that
comprises ovary development, anthesis, fertilization and fruit set events, witnesses increase Anthesis and fruit set Phase I Phase II Phase III Phase IV Floral development Cell division Ripening/ Senescence

Auxs
GAs
CKs
ABA

in the levels of AUXs, GAs and CKs. AUXs show steady increase through anthesis and fertilization, GAs peak during fertilization and CKs, which appear later, show a steady increase through this phase. After fertilization, as the fruit enters cell division during the Phase II, CKs levels peak, AUXs levels continue to rise and GAs levels show steady decrease. During the cell expansion phase, appearance of ABA is accompanied by a second accumulation of GAs and a peak in the levels of AUXs. The mature green stage of the fruit witnesses decrease in the levels of GAs and ABA and increase in the levels of AUXs. Towards the end of the cell expansion phase, a second accumulation of AUXs is witnessed which increases steadily through mature green stage and peaks at the
transition from

breaker to turning stage of the ripening fruit after which it steadily decreases

throughout ripening. Ethylene begins to accumulate at the on set of ripening

and peaks at the turning stage of ripening.

All classic plant hormones have been implicated as signals affecting the

fruit development and maturation (Ozga and Reinecke 2003). The

simultaneous existence of more than one hormone and their relative levels

affect many cellular processes and play decisive roles in fruit development

(Fig. 10.2), their ratios playing significant roles such as those observed in

other plant growth processes (Swarup et al. 2002). Depending upon the

molecular function of each hormone, these interactions could be synergistic

or antagonistic (Fig. 10.2). Hormones may regulate their own biosynthesis

in a feed back regulatory manner or may regulate the biosynthesis of another

hormone.

The self-regulatory mechanism of hormonal action for CKs possibly

involves auto inductive accumulation of CKs which is initiated and

promoted through increased levels of extracellular CKs (xylem or phloem

sap, exogenous application). This may increase the CKs/AUXs ratio and
may induce certain physiological and/or structural processes. Accumulated CKs [e.g., N 6 - (2-isopentenyl) adenine, iP nucleoside, zeatin nucleoside] also act as substrate inducers for CK oxidase, an enzyme which irreversibly cleaves the side chains of such CKs, leading to a complete loss of activity (see Jones and Schreiber 1997, Kaminek et al. 1997). AUXs may also control CK levels by influencing CK biosynthesis or by promoting CKs metabolic inactivation/degradation by CK oxidase (Kaminek et al. 1997, Galuszka et al. 2000). Similarly, AUXs and AUX-conjugates may play a role in regulating the metabolism of CK conjugates (Swarup et al. 2002). CK oxidase, like IAA oxidase, is possibly associated with peroxidases and is activated by certain phenolic compounds (see Galuszka et al. 2000). Tomato peroxidase is capable of oxidizing IAA during ripening of the fruit with the phenolic 3,2,0 Genetic Improvement of Solanaceous Crops: Tomato Fig. 10.2 Hormonal cross-talk during tomato fruit development. Ethylene Auxins Fruits set Cell expansion Cell division Pollination / Fertilization Parthenocarpy ? GA S CK SB Rs J A Sources sink relations ABA F loral Senescence Missed signals ? R ipening flux modulating IAA oxidase activity (Haard 1977, Thomas and Jen 1980).

IAA is considered a natural fruit ripening retardant. Increase in IAA oxidase
activity prior to respiration climacteric has been associated essentially with a decrease in IAA levels that is necessary for induction of ripening (Frenkel 1972, Thomas et al. 1981). IAA oxidation by peroxidases in tomato could also be enhanced by phosphate, oxalate, pyrophosphate, malate and citrate, and decreased by the presence of Mn 2+ (Pressey 1990). Collectively, these studies indicate that CK and AUX interact at various levels and thus affect each other’s responses in the developing fruit.

Emerging evidences indicate that GAs and AUXs affect each other’s biosynthesis (O’Neill and Ross 2002, Swarup et al. 2002). Auxin main tains bio-reactive GA levels in elongating pea hypocotyls by promoting synthesis of GA 1 from GA 20 by enhancing a GA 3 -oxidase activity and si multaneously inhibiting deactivation of GA 1 (Ross et al. 2003). AUX-spe cific regulation of gibberellin 3β-hydroxylase has been reported during pollination and development of pea fruit and seeds; however, these mecha nisms have not yet been demonstrated in tomato fruit. GA and ABA have been shown to have antagonistic effects during fruit development and seed maturation, whereas ABA has been shown to have a stimulatory effect on
ethylene production. This stimulatory effect is thought to be the result of
an enhancement in the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), a key enzyme involved in ethylene biosynthesis, rather than due to the induction of senescence (Riov et al. 1990). Ethylene synthesis is also affected by AUX levels and during the post-pollination phase. Investigations with polyamines (Mehta et al. 2002, Cassol and Mattoo 2003), brassinosteroids (Schlagnhaufer and Arteca 1985a, b; 1991) and jasmonic acids (Miyamoto et al. 1997) have also shown their possible role in regulation of hormonal activity.

Hormonal Regulation of Early Fruit Development

The signals for fruit set are generated during pollination and the decision to set fruit is dependent upon successful fertilization. The manifestations of pollination involve two diametrically opposite events: (1) Wilting and abscission of the corolla and calyx; and (2) preparation of other organs for fertilization, embryogenesis and fruit development (O’Neill 1997). Although pollination-regulated development is initiated at the stigma surface, it is hypothesized that secondary inter-organ signals amplify and transmit the
primary pollination signals to bring about a plethora of changes witnessed after pollination. Increase in ethylene production in response to pollination has been observed in many flowers (O’Neill 1997) and has been implicated in coordinating many of the pollination-associated events such as ovary growth and senescence of the perianth (Larsen et al. 1993, Woltering et al. 1994). In contrast to observations in many ornamental flowers where ethylene levels increased simultaneously or before pollen germination (see O’Neill 1997), increase in ethylene levels in tomato pistil occurs after pollen germination and penetration of the pollen tubes into the stylar tissue (Lloup Tous et al. 2000). Using dialytic (dl) and Neverripe (Nr) tomato mutants, Llop-Tous et al. (2000) reported that pollination-induced enhanced expression of LeACS1A gene, a member of the ACC synthase (ACS) gene family, is independent of ethylene, whereas the expression of LeACO1, 2 and 3, members of ACC oxidase (ACO) gene family, is ethylene dependent. In contrast, the expression of LeACO4 is ethylene independent. LeACS1A seems to be the sole ACS gene responsible for elevated ethylene levels in tomato pistils as well as the ethylene-mediated expression of LeACO1 and
LeACO3, which seems essential for the timely ethylene production in response to pollination. Since ethylene perception is necessary for the decrease in ethylene production after reaching the maximum, it has been suggested that ethylene acts as a regulator of its own biosynthesis following pollination. Ethylene is known to stimulate (auto-activation) or inhibit (auto-inhibition) its own biosynthesis (see Mattoo and White 1991).

TOMATO MUTANTS: A WINDOW TO HORMONAL REGULATION OF FRUIT DEVELOPMENT

The effect of pollination can be mimicked by hormones, in particular AUXs and GAs, resulting in fruits without seeds (Goodwin 1978, Schwabe and Mills 1981, George et al. 1984, Gillaspy et al. 1993). The phenomenon of seed-less fruit development is referred to as parthenocarpy and may occur due to lack of pollination, pollination not leading to fertilization, or embryo abortion. Parthenocarpy has provided much information about the possible roles of hormones during early fruit development (George et al. 1984). Several lines of evidences indicate that AUXs and GAs play roles in setting and development of parthenocarpic fruits. Parthenocarpic lines show higher
endogenous levels of AUXs and GAs in the ovaries as compared to the
normal tomato lines (Gustafson 1939, Nitsch et al. 1960, Mapelli et al.
1979, Mapelli and Lombardi 1982). Also, exogenous application of AUXs,
GAs, or their inhibitors, to the flower/ovary before fertilization can cause
parthenocarpy (Robinson et al. 1971). The accumulation of sufficient levels
of AUXs within the ovary effects fruit set and activation of cell division in
the absence of fertilization indicating that incorrect temporal and/or spatial
regulation of AUX synthesis may be responsible for parthenocarpy (Gillaspy
et al. 1993). GAs produced by pollens have been implied in the signal
transduction pathway leading to increased AUX synthesis during
pollination. Application of GAs to unpollinated tomato flowers causes
increased levels of AUXs in the ovary (Sastry and Muir 1963). The
developmental control of seeded tomato fruit, at least in part, by GAs is
supported by studies involving GA application to unpollinated ovaries
(Bunger-Kibler and Bangerth 1982, Sjut and Bangerth 1982, Sauheny 1984,
of GAs in parthenocarpic and seeded fruits (Bohner et al. 1988, Koshioka
et al. 1994) and the detection of transcripts of genes encoding for enzymes in GA biosynthesis in developing fruits (Rebers et al. 1999).

Genetic evidence for the role of GAs in development of fertile tomato flowers comes from studies with the dwarf, GA-deficient mutant, gib-1 (Groot et al. 1987). The floral development in this mutant is not completed in the absence of GAs but a single application of GA 3 or GA 4+7 restores its fertility resulting in seed set. The isolation and characterization of many GA-biosynthetic genes (Hedden et al. 2002) has begun to provide a new insight into the role of GAs in fruit set (Nester and Zeevart 1988, Rebers et al. 1999). Nester and Zeevart (1988) have reported that the GA-deficient tomato mutant gib-2 shows improper ovary development and degeneration of tapetal cells. Based on expression patterns and localization in anther tapetum cells and placental tissue of a GA 20 oxidase gene, namely, Le20ox 2, during flower bud and early fruit development of tomato, Rebers et al. (1999) have suggested that Le20ox-2 may be responsible for the required GA biosynthesis in these tissues. The transcript levels of Le20ox-1 were higher during anthesis, whereas the expression of copalyl...
diphosphate synthase (LeCPS) was considerably increased during flower senescence and early fruit development. Based on increased GA 20 oxidase activity in the unpollinated ovaries of tomato lines carrying pat-2 mutation, it has been suggested that the pat-2 gene induces parthenocarpy by enhancing synthesis of GA 20, the precursor of an active GA (Fos et al. 2000). Two flower specific cDNAs (tgas100 and tgas105) up-regulated by GAs have been isolated from GA deficient gib-1 mutant of tomato (van den Heuvel et al. 2002). The deduced TGAS105 polypeptide shows homology to extensin like proteins, whereas TGAS100 polypeptide is similar to a stamen specific gene from Antirrhinum. Expressions of GAD3, a short chain alcohol dehydrogenase like-gene (Jacobsen and Olszewski 1996) and H1 and H2B tomato histone (van den Heuvel et al. 1999) genes have been reported to be GA responsive in shoot and leaf tissues of tomato, respectively. Differential display amongst parthenocarpic mutants and their near isogenic wild type lines has resulted in isolation of three genes namely LeH2A-2, GAD3 and LeSPH1 (L. esculentum S protein homologue). Among these genes LeH2A-2 and GAD3
showed GA responsiveness in wild types but not in the pat lines (Testa et al. 2002).

LeH2A-2, which belongs to a small group of plant histone H2As, showed increased transcript levels in tomato ovaries and is speculated to play an important role in cell division phase of fruit growth with developmental regulation and tissue-specific expression patterns (Dong et al. 1998, Joubès et al. 1999, van den Heuvel et al. 1999). GAD3 expression is strongly up regulated in ovary at the time of pollination and is maintained throughout the phase of active ovary growth. Accumulation of gene transcripts during active cell division phase and their GA responsiveness suggest their involvement in the early fruit development (Testa et al. 2002).

Ovary growth in pat mutants initiates before anthesis (Mazzucato et al. 1998). The complete machinery for fruit set and development was found to be switched on before, and independently from pollen shedding, pollination and fertilization in these mutants. Organ identity and development was found to be affected in pat mutants with malformed male and female organs.

The androecium is typically short, irregular and the anthers are not fused.
which leave the stigma exerted; dehiscence is preferentially external and although the pollen is fertile, the number of grains was found to be reduced.

(Mazzucato et al. 1998). Since the ovule development is found to be aberrant in pat mutants, it is suggested that parthenocarpy is a secondary effect of a mutated gene whose primary function is to regulate floral organ development. It is further speculated by these authors that the anther aberrancy, especially the occurrence of adaxial carpel-like structures bearing external ovules, is indicative of pat mutation affecting a homeotic gene.

Defective cell elongation of pat organs and reversion of pat anther phenotype by application of GA 3 led Mazzucato et al. (1998) to propose that this mutation may have interactive roles with the GA metabolic pathway.

MADS-box genes have been recognized to play a central role in plant development, especially during flower development (Lohmann and Weigel 2002). At least seven MADS-box genes expressed through the first stages of fruit and seed development in tomato have been identified (Busi et al. 2003). These include three previously identified flower identity genes (TAG1, TDR4 and TDR6; Pnueli et al. 1991, 1994a, b). The other four are novel
MADS-box genes (TAGL1, TAGL2, TAGL11 and TAGL12). In view of the hypothesis that successful pollination and fertilization generates signals essential for normal fruit development, it has been suggested that induction of certain MADS box genes by these signals initiates early developmental program in the fruit (Busi et al. 2003). The proposed MADS box candidates for this process include TDR4, TAGL2, TAGL11 and TAGL1 as these are induced immediately after anthesis in the ovary wall. The antisense down regulation of TM29, a tomato SEPALLATA homolog, causes parthenocarpic fruit development and floral reversion (Ampomah-Dwamena et al. 2002).

LeMADS-RIN, a MADS box gene has been shown to play a decisive role in fruit ripening (Vrebalov et al. 2002). Further characterization of hormonal regulation of MADS box genes should help understand the roles of these genes in fruit development.

HORMONAL REGULATION IN CELL DIVISION, SEED FORMATION AND EARLY EMBRYO DEVELOPMENT

The final fruit size is dependent on the number of cells within the ovary before fertilization, the number of seeds, the number of cell divisions that occur in the developing fruit after fertilization, and the...
extent of cell expansion (Gillaspy et al. 1993). Several hormones and growth regulators including AUXs, CKs, GAs, BRs, ABA, PAs and sugar play significant roles in these processes (Dewitte and Murray 2003). Following fertilization, tomato ovary undergoes a period of active cell division (Phase II) which continues for approximately 7-14 days (Mapelli et al. 1978, Bohner and Bangerth 1988). In the very early stages of this phase, the mitotic activity is higher in outer pericarp and the placental tissue while cell division in the developing seeds is confined to the peripheral integument layers rather than the embryos (Suzuka et al. 1989, Daidoji et al. 1992). The highest CKs levels in the developing seed during Phase II correlate with cell division activity in the surrounding tissue (Abdel-Rahman 1977, Bohner and Bangerth 1988). Fruits from wild-type normal and rin mutant show little change in the endogenous CK levels during ripening (Hong and Lee 1993), indicating that although CKs play crucial role during the early cell division phase, they have a limited role in fruit ripening. CKs primarily accumulate in developing seed, with low levels in the rest of the fruit. The molecular
basis of CKs accumulation in seed is not understood. However, based on
low accumulation of CKs in parthenocarpic fruits, CKs have been
hypothesized to be transported from the site of synthesis with developing
seeds signaling this transport (Mapelli 1981, Bohner and Bangerth 1988).
The rapid seed growth during cell division phase is attributed to increased
CKs levels (Bohner and Bangerth 1988). Frequently, a positive correlation
between seed number in the fruit and fruit size is observed, with the
distribution of the seed throughout the fruit affecting fruit shape (Ho 1992).
Auxins produced in the seeds are also implied in the cell division or
cell enlargement of the tissue surrounding the seeds (Ho 1992) and are
thought to be important for the seed growth (Bohner and Bangerth 1988).
The molecular analysis of plant cell cycle progression has revealed cyclin
dependent kinases (CDKs) as one of the key regulators of this process
(Dewitte and Murray 2003). CDKs represent a highly conserved superfamily
of serine/threonine protein kinases whose activity requires binding to a
cyclin. During cell cycle, the G1-S and G2-M phase transitions in plants
are under the control of several hormones including AUX, CKs, ABA, GAs,
BRs and sugar. These growth regulators control the expression of members of CDKs and cyclins families. In plants, five distinct classes of CDKs (CDKA through CDKE) have been defined on the basis of phylogenetic, structural and functional similarities with animal and yeast CDKs (Joubès et al. 2000).

In developing tomato fruit, the differential expression of CDKA and cyclins is correlated with the temporal and spatially regulated mitotic activity. The accumulation of Lyces:CDKA1 and Lyces:CDKA2 transcripts and proteins were observed between anthesis and 5 d post anthesis (Joubes et al. 1999).

The overall expression patterns of three additional CDKs, (CDKC;1, CDKB1;1, CDKB2;1) were shown to be similar to the expression of CDKA, i.e. the expression was higher in dividing tissues (Joubes et al. 2001).

However, in contrast to that of CDKA and CDKBs, the expression of CDKC;1 in tomato cell suspension cultures and excised roots was found to be independent of sugar or hormonal supply. Taken together these results indicate a hormonal regulation of CDKs and cell division during early fruit development.

The AUX-resistant diageotropica (dgt) mutation dramatically alters early fruit development including increases in the time to
flowering and the time from anthesis to the onset of fruit, fruit weight, fruit set, and the numbers of locules and seeds, but fruit ripening remains normal (Balbi and Lomax 2003). Some of the long-term effects of CKs application to wild-type seedlings, such as stunting of root and shoot growth, reduced elongation of internodes, reduced apical dominance, and reduced leaf size and complexity, are similar to that caused by dgt mutation (Coenen et al. 2003).

Additionally, CK treatment inhibits AUX-stimulated elongation, H + secretion, and ethylene synthesis in wild-type hypocotyl segments, and thus mimics the impaired AUX responsiveness found in dgt hypocotyls. However, CK treatment inhibits the AUX-induced expression of only one of the two ACS genes that exhibit impaired AUX inducibility in dgt hypocotyls. Also, CK treatment fails to inhibit AUX induced LeSAUR gene expression, an AUX response that is exhibited by the dgt mutant. These results suggest that CKs inhibits only a subset of the AUX responses impaired in dgt hypocotyls and has led the authors to propose that CK effects on AUX responses are mediated through interactions between specific
AUX- and CK-signaling pathways rather than through global effect of CKs on active AUX levels or responses. Based on the effects of dgt mutation on fruit morphology and differential expression of subsets of LeIAA and LeACS gene family members it has been suggested that AUX- and ethylene mediated gene expression play significant roles during the early stages of fruit development. (Balbi and Lomax 2003).

REGULATION OF CELL EXPANSION AND EMBRYO DURING FRUIT MATURATION

The period of active cell division is followed by Phase-III in which the growth of the fruit is mostly due to cell expansion. In tomato, there is a 10 to 20-fold increase in the volumes of cells in placenta, locular tissue, and mesocarp tissue but cells comprising the exo- and the endocarp, which continue to divide, expand less (Gillaspy et al. 1993). There is a general consensus that AUXs play a pivotal role during cell expansion (see Hager 2003). AUXs have been implicated in the elongation of stem and coleoptile cells by promoting wall loosening via cleavage of load-bearing bonds in the cell wall (Rayle and Cleland 1992). The mode of action of the symplast located AUXs is thought to be through the release of protons from AUXs.
exposed cells into the apoplast (acid growth theory). The resulting decrease in the pH has been hypothesized to activate pH-sensitive enzymes and proteins within the wall causing cell-wall loosening and extension growth (see Hager 2003). Some of the early AUX-response genes involved in AUX signaling have been identified and grouped into four major classes viz., Aux/IAAs, SAURs, GH3s, and OsARF1 (Abel and Theologis 1996, Hagen and Guilfoyle 2002, Waller et al. 2002). Some of the cell wall loosening proteins/enzymes activated at acidic pH include expansins (Cosgrove et al. 2002), xyloglucan hydrolase (XGH) and xyloglucan endotransglycosylase (XET) (Fry et al. 1992) and yieldins (Okamoto Nakazato 2002). Expression of an expansin gene (LeExp2), xyloglucan endotransglycolase (LeEXT1) and endo-1, 4-β- glucanase (Cel7) are correlated with the increases in AUX levels during tomato fruit growth with negligible expression in ripening fruit (Catala et al. 2000). LeExp2 was isolated from AUX-treated etiolated tomato hypocotyls whereas LeEXT1 and Cel7 have been reported to be AUX-regulated in etiolated hypocotyls (Catala et al. 1997). The transcript levels of both LeExp2 and LeEXT1 peaked
during the stages of higher rates of fruit growth, while Cel7 expression increased and remained high during later stages of fruit expansion. The
down regulation of these genes coincides with the cessation of the cell expansion phase and entry of the fruit into the ripening phase. The pat
terms of LeEXP2, LeEXT1 and Cel7 expression during the period of rapid growth and increased AUX levels have been interpreted to indicate their roles in developmental and/or hormonal signal transduction network con
trolling cell expansion in fruit. On the other hand, expression of ripening related endo-1, 4-β-glucanases, XET, and expansin genes have been shown to be ethylene up-regulated in tomato fruit (Lashbrook et al. 1994, Arrowsmith and de Silva 1995, Rose et al. 1997). These data suggest that different hormones regulate expression of the cell wall modifying proteins/
enzymes and thereby coordinate the growth and ripening processes.
The exogenous application of AUXs causes delayed tomato fruit ripening (Vendrell 1985, Cohen 1996) indicating a role for AUXs in regulating the capacity of fruit to ripen. AUXs have been shown to modulate plant growth and development through transcriptional regulation of
specific genes

(Ulmasov et al. 1999). The functional analyses of the promoter regions of AUX-regulated genes have identified a conserved AUX-responsive cis element (AuxRe) such as TGTCTC (Ulmasov et al. 1995) and TGTCCCAT (Oeller et al. 1993). AUX response factors (ARFs) that bind to these cis elements have been characterized and shown to regulate transcription in an AUX-dependent manner and to interact with short-lived nuclear proteins belonging to the Aux/IAA family of AUX-responsive transcription factors (Abel et al. 1994, Kim et al. 1997, Ulmasov et al. 1997, 1999). Differential screening of gene expression during fruit development has resulted in isolation of four developmentally regulated cDNAs (DR1, DR3, DR4 and DR8), which show homologies to Aux/IAA family and one cDNA (DR12) belonging to ARF transcription factor family (Jones et al. 2002). Ethylene regulates accumulation of the Aux/IAA like transcripts in tomato fruits but not in leaves, suggesting that these ARFs participate in the ethylene dependent, developmentally regulated gene expression. DR12 shows nuclear localization and its transcripts are most abundant in ripening
fruit. Antisense and sense co-suppressed DR12-inhibited transgenic tomato lines display pleiotropic phenotypes that include dark-green immature fruits, unusual cell division in pericarp, blotchy ripening and enhanced fruit firmness, upward curling of leaves and increased hypocotyl and cotyledon growth (Jones et al. 2002). These authors have suggested that the observed phenotypes of DR12 transgenic lines could be due to increased responsiveness of tissues to AUXs or alternatively, the inhibition of DR12 affects response to the endogenous CKs and ethylene. CKs are involved in regulation of cell division and biological processes such as active growth, metabolism and plant development, all of which show enhanced carbohydrate demand. Because of this association, CKs have been linked (Balibrea et al. 2004) to regulation of assimilate partitioning (Brenner and Cheikh 1995), sink strength (Kuiper 1993), and source-sink relations (Roitsch and Ehness 2000). The extracellular invertases have been implicated in supplying carbohydrate to various sink tissues (Koch 2004). GAs, AUXs and ethylene are also known to regulate extracellular invertases (Roitsch et al. 2003). Amongst the four tomato
invertase genes identified (Lin5, Lin6, Lin7 and Lin8), Lin6 transcripts accumulate in actively growing sink tissues such as seedlings, roots, flower buds and tumors (Godt and Roitsch 1997). Since the expression of Lin6 is induced by zeatin, it has been suggested that CKs regulate the expression of extracellular invertases in sink tissues. Extracellular invertases also play an essential role in CK-mediated delay of senescence. Tobacco transgenic plants harboring an invertase inhibitor gene under the control of CK inducible Lin6 promoter failed to show a delay in leaf senescence in the presence of CK (Balibrea-Lara et al. 2004). Lin6 has also been shown to be induced by BRs (Goetz et al. 2000). Lin7 expression is localized in tapetum and pollen and Lin5 expression is higher in non-mature green tomato fruit than mature or red tomato fruits (Proels et al. 2003). The Lin5 promoter sequence contains GA-, AUX- and ABA-response motifs that confer hormone inductivity to a truncated nos promoter-GUS fusion. Lin5 induction by AUX has been suggested to mediate a higher carbohydrate supply to developing flower and fruit tissue, especially in the early developmental stages. The inductivity of Lin5 promoter with GAs further suggests that
GAAs affect the extracellular invertase activity during flower development.

During Phase III, the seed growth does not parallel cellular expansion of fruit. As the embryo develops from globular to bilateral structure it shows well-developed cotyledons and an established root-shoot axis (Smith et al. 1935). AUX concentration that is higher in the seeds than in the surrounding fruit cells has been interpreted to play a role in the cell wall extensibility and establishment of fruit tissue as the sink. Polar auxin transport (PAT) has been implicated in determination of pattern specificity and embryological studies in Arabidopsis mutants. These results suggest roles for elements involved in AUX perception and distribution during transition from globular to heart shape and the ultimate appearance of the cotyledons, marking the establishment of the bilateral symmetry (see Al-Hammadi et al. 2003). The polycotyledon (poc) mutant of tomato, which has multiple cotyledons and displays several abnormalities during vegetative development, shows enhanced PAT (Al-Hammadi et al. 2003). The poc is located on the 9th chromosome of tomato and has been implicated in the separation of the two cotyledons (Hadfi et al. 1998).
Abscisic acid (ABA) is known to play important roles in seed maturation and dormancy. Developing seeds in contact with the sheath and the locular tissues do not germinate inside the fruit (Gillaspy et al. 1993). However, the removal of tomato seeds even at an early stage of fruit development does not prevent their germination in water, suggesting that seed germination is suppressed within the fruit. Osmotic potential, along with ABA, which induces dormancy to the seed, has been suggested to prevent precocious seed germination (Berry and Bewley 1991). Characterization of gib-1 and ABA-deficient sit w tomato mutants indicates that the precocious seed germination is prevented by the action of the fruit’s osmotic environment and ABA on the seed tissues surrounding the embryo and not the embryo itself (Liu et al. 1996). It has been proposed that the combined action of these two components prevents early germination by preventing endosperm weakening which is an essential feature in germination as it facilitates water uptake by the embryo. ABA and sugars show similar or antagonistic effects on diverse physiological processes during seed development, germination, and
seedling growth. A number of Arabidopsis mutants that exhibit germination ability and seedling development on inhibitory concentrations of sugars have been identified in Arabidopsis, and interestingly, are found to be allelic to known mutations in ABA synthesis (Bradford et al. 2003). These and other observations indicate interactions between sugar and hormonal signaling (Finkelstein and Gibson 2002). Sucrose non-fermenting 1 (SNF1) related kinase (SnRK1) complex has been implicated in both sugar and ABA sensing (Himmelbach et al. 2003, Lunn and MacRae 2003). The central component of the sugar sensing and response mechanism is thought to be the SNF1-related kinase (SnRK1) complex which was first identified in yeast (Halford and Hardie 1998, Halford et al. 2000, 2003). cDNAs corresponding to the kinase (LeSNF1), regulatory (LeSNF4), and localization (LeSIP1 and LeGAL83) subunits of the SnRK1 complex were identified and their expression characterized during seed development in tomato (Bradford et al. 2003). These studies revealed that LeSNF4 expression is influenced by ABA and GA levels, along with other factors influencing seed germination. These authors have suggested that during the seed
maturation binding of LeSNF4 to LeSNF1/LeGAL83 (or other SIP proteins) alters the kinase activity of the complex, thereby promoting metabolic pathways involved in the accumulation or maintenance of storage reserves and blocking those involved in the mobilization or utilization of stored reserves. After imbibition, expression of LeSNF4 was found to be reduced in seeds that were not dormant or stimulated by GA, which potentially altered LeSNF1 kinase activity to de-repress genes encoding enzymes required for reserve mobilization and metabolism (Bradford et al. 2003). This study provides an insight into the possible mechanism of sugar and hormonal regulation of seed maturation and germination.

ROLES OF OTHER PLANT GROWTH REGULATORS

Brassinosteroids (BRs), a group of plant steroid hormones, play diverse roles in plant growth and development (see Bishop 2003). Exogenous BRs application to tomato pericarp discs leads to elevated levels of lycopene, sugars (reduced and total) and ethylene, and decreased ABA levels (Vardhini and Rao 2002). BR regulated genes including cell wall modifying xyloglycan endotransglycosylases (Zurek and Clouse 1994, Oh et al. 1998)
are implied in BR-induced cell elongation. BR induced growth responses

have been correlated with increased carbohydrate supply brought about

by the increased levels of the extracellular invertase, Lin6 (Goetz et al. 2000). Characterization of tomato extreme dwarf (d x ) and dumpy (dpy)

mutants have revealed genes that are involved in the biosynthetic pathway

of BRs (Bishop et al. 1999, Koka et al. 2000). Tomato homologue of BRI1, an essential component of the BR receptor complex that was isolated from the signaling mutants curl3 (cu3) and abs (altered brassinolide sensitivity 1),

showed over 99% identity with the SR160 systemin receptor (see Szekeres 2003). Systemin is a polypeptide hormone known to regulate wound inducible genes in tomato (Pearce et al. 1991) and activates a lipid-based signaling cascade, which causes release of linolenic acid from the membrane which is then converted to oxylipins and jasmonic acids (Bergey et al. 1996). Both BRI1 and SR160 are implicated in BR and systemin signaling (see Szekeres 2003).

Jasmonic acid (JA) and its methyl ester (methyl jasmonate, MeJA) are derivatives of linolenic acid and are known to modulate aspects of fruit
ripening, pollen viability, root growth and resistance to insect and pathogen

attack (Creelman and Mullet 1997). JA-induced defense responses in tomato have profound effect on their reproductive fitness. Treatment of plants with higher levels of JA produce fewer but larger fruits with fewer seed per unit of the fresh weight (Redman et al. 2001). Leucine aminopeptidase (LapA) expression increases under the influence of systemins, MeJA, ABA, ethylene and stress conditions such as water deficit and salinity in tomato. LapA1 promoter:GUS transgenic tomato plants revealed that this promoter is active during floral and fruit development (Chao et al. 1999). However, at present little is known about the role of JA in fruit growth and maturation.

Polyamines (PAs) are ubiquitous organic cations that affect a large number of developmental and physiological responses in a number of organisms, including plants (see Walters 2003). They have also been implicated to influence early fruit development and ripening. In tomato, both ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) pathways for PA biosynthesis are active (Alabadi and Carbonell 1998).

Application of PAs to wild type unpollinated ovaries
results in partial

parthenocarpy. The higher PA levels in unpollinated pat-2 ovaries are

correlated with the activation of the ODC pathway, which in turn is

influenced by elevated GA levels found in these ovaries (Fos et al. 2003).

Decrease in arginase levels and ODC activity after tomato fruit set is

hypothesized to indicate that the ADC pathway is involved in cell

expansion and the ODC pathway in cell division during early fruit growth

of tomato (Cohen et al. 1982, Alabadi et al. 1996). Developmentally regulated

increases in spermidine and spermine in transgenic tomato overexpressing

a yeast SAM-decarboxylase enhanced lycopene and ethylene levels and

increased fruit juice viscosity in tomato fruit (Mehta et al. 2002). PAs, along

with salicylic acid, which is an inhibitor of wound-responsive genes in

tomato, have been suggested to regulate ethylene biosynthesis at the level

of ACC synthase transcript accumulation (Li et al. 1992).

FUTURE PERSPECTIVE

Fruit development has emerged as an important area of research in plant

development. Much progress has been made in identifying the growth

regulators involved in fruit development and ripening and the gene and
protein receptor players. Most of these studies have implicated cross talks and signaling among the classic hormones. However, there seem to be other dimensions to these processes, involving other plant growth regulators such as polyamines, brassinosteroids and methyl jasmonates.

Only recently, molecular work with other plant growth regulators such as polyamines and its role in these processes has begun (Mehta et al. 2002).

The hormonal regulation of plant development is a complex process, especially because of the interactive nature of plant hormones. Likely, many more signaling compounds play role in fruit set, development and ripening. The primary question regarding fruit growth and development is not only which genes regulate these processes but how the genetic and molecular circuitries determine the differential expression of the genes underlying the development of fruit phenotype. In addition to genes that are induced during fruit ripening, several groups have cloned and characterized genes that are differentially expressed during fruit cell division, expansion and ripening phases (Tieman and Handa 1996, Lemaire-Chamley et al. 2000, Alexander and Grierson 2002, Seymour et al. 2002, White,
2002, Testa et al.

2002, Giovannoni 2001, 2004). The DNA microarray analyses, proteomics and functional genomics should, in not-too-distant-future, reveal genes whose expression is intimately associated with fruit development. We will also understand how the temporal and spatial expression of these genes is regulated by various developmental cues, including hormones, other growth regulators, and by environmental extremes. Such an information base will begin to provide specific information that will ultimately translate into the development of designer fruits with enhanced quality and longer shelf-life.

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INTRODUCTION

Fruit ripening is an especially significant aspect of plant biology because of the importance of fruit in the human diet. Plant species devote vast
amounts of energy and resources to the maturation of fruit organs (Gray et al. 1992). The ripening of fruit can be generally defined as the summation of changes in texture, flavor, aroma, and color marking the complete maturation of the organ in terms of attraction for seed dispersing organisms (Brady 1992, Grierson et al. 1992, Giovannoni 2001, Adams et al. 2004, Giovannoni 2004). Lipid metabolism, fiber content, and vitamin levels are also affected over the course of ripening, ultimately impacting the nutritional quality of mature fruit tissues. It has also been shown that levels of antioxidant compounds, capable of modifying enzyme activities and detoxifying potentially damaging free radical compounds, can be altered substantially during ripening, with effects on both fruit color and nutrient quality (Ronen et al. 1999, Verhoeyen et al. 2002).

Ripening has also been defined as a “functionally modified, protracted form of senescence” as it is the final stage of fruit development and often terminates in tissue decay and death (Huber et al. 1987, Grierson 1987) thereby leading some to surmise that ripening represents in its essence a degradative process. In our view, ripening can more accurately be compared
to other active developmental processes involving de novo modification of
gene expression (Brady 1992, Grierson and Schuch 1993).

From a structural perspective, fruits are typically either fleshy
(non-dehiscent) or dry (dehiscent) with tomato receiving the most scientific
attention as a model for fleshy fruit development, while Arabidopsis
(siliques) are the best studied dehiscent fruit at the molecular level (reviewed
in White 2002).

Arabidopsis has been used as a model genetic system for plants due in
part to its small stature, compact genome, short life cycle, simple genetics,
and efficiency of transformation. Tomato has been used as a model system
for fleshy fruit development and ripening for many of the same reasons
mentioned for Arabidopsis, especially as compared to other species yielding
fleshy fruit. In addition, numerous tomato mutations have been identified
in which the normal ripening program is altered. These mutations include
Never-ripe (Nr), ripening-inhibitor (rin), non-ripening (nor) (Tigchellar et al.
1973) and Colourless non-ripening (Cnr) (Thompson et al. 1999) which have
universal effects on ripening. Nr, rin, nor, and Cnr
display the most complete inhibition on ripening although numerous additional mutants alter subsets of ripening phenotypes, most notably pigment accumulation, have been described (see Gray et al. 1994, Giovannoni 2001, Giovannoni 2004, Barry et al. 2005).

Human manipulation of fruit ripening dates back thousands of years as greater molecular understanding of the ripening process will allow for improvement in fruits with respect to increase in yield, quality, nutrition and storage attributes. A number of attempts have been made to modify fruit quality and ripening characteristics via biotechnological approaches at the level of “proof-of-concept”, and in some instances, in pursuit of commercial objectives.

CLIMACTERIC VS NON-CLIMACTERIC RIPENING

Ripening fleshy fruits are classically divided into two physiological categories, climacteric and non-climacteric, based on respiration patterns associated with ripening (Biale et al. 1981). Climacteric fruit displays a spike of respiration and ripen on or off the plant if mature when picked. Climacteric fruits are also typified by increased ethylene production at the
onset of ripening (Abeles et al. 1992). Ethylene is required for the ripening of climacteric fruit and the addition of exogenous ethylene is a window just prior to the climacteric typically induced ripening. The necessity of ethylene in climacteric ripening has been shown through the action of a number of ethylene biosynthesis and action inhibitors (Yang 1985).

Silver thiosulfate (STS or AgSTS) retarded ripening of these sectors of tomato fruit to which it had been infiltrated (Hobson et al. 1984).

Aminoethoxyvinylglycine (AVG) is an inhibitor of 1-aminocyclopropane 1-carboxylic acid synthase (ACS), a limiting step in ethylene synthesis (Boller et al. 1979, Yu and Yang 1979). 1-methycyclopropene (1-MCP) inhibits ethylene action by competitively binding ethylene receptors (Sisler and Serek 1997) and has been shown to retard ripening in many agriculturally important fruit species including apple (Watkins et al. 2000) and banana (Jiang et al. 2002). Examples of important climacteric fruits include tomato, apple, pear, avocado, melon, most stone fruits, and many tropical fruits including papaya, mango and banana.

Non-climacteric fruits include strawberry, grape, cucumber, pineapple,
cherry, and citrus. Fruits that are non-climacteric do not display the burst of respiration or the increase in autocatalytic ethylene characteristic of their climacteric counterparts (McMurchie et al. 1972), and typically do not ripen as fully as climacteric fruit after harvest. While these fruits generally do not require ethylene for ripening, some non-climacteric fruits do respond to exposure to exogenous ethylene. Non-climacteric fruit, with the exception of cherries, display a rise in respiration upon addition of exogenous ethylene; however this increase reverts upon removal of the hormone (Lelievre et al. 1997). In strawberry fruit, addition of exogenous ethylene causes softening and enhances color development (Tian et al. 2000). In non-climacteric pepper, ethylene has been shown to induce biosynthesis of carotenoids (Ferrarese et al. 1995, Harpster et al. 1997). Exogenous ethylene also causes increased sensitivity to chilling injury, enhanced senescence, and a higher susceptibility to pathogen infection in non-climacteric fruit (Kader 1985). It is important to note that the ripening classification of a number of species is not completely clear. While some varieties of melon and pepper are clearly climacteric, others behave physiologically as non-
climacteric fruits. It remains unclear as to whether the non-climacteric
varieties display a truly different ripening program or simply produce and/
or perceive less ethylene than their climacteric counterparts.

MODEL RIPENING SYSTEMS

Strawberry

Strawberry has become one of the most important model systems for non
climacteric fruit ripening. The true fruit (ovaries) of the strawberry are the
achenes lining the outside of a fleshy receptacle (Seymour et al. 1993).

Strawberry fruits have a rapid growth cycle reaching full size approximately
30 days post anthesis, but time to ripening can vary considerably depending
on temperature (Seymour et al. 1993). The process of ripening for a
strawberry consists of accumulation of anthocyanins, sucrose, hexoses,
voltiles, and the concomitant loosening of cell wall integrity leading to
tissue softening (Manning 1998). Auxin released from the achenes has
been shown to regulate maturation and development of the receptacle and
its loss in later fruit development is associated with ripening (Given et al.
1988, Civello et al. 1999). Much like its climacteric counterparts, strawberry
ripening involves a complex group of processes, which rely on numerous changes in the expression of ripening related genes (Manning 1998).

Wilkinson et al. (1995) reported a cDNA (RJ4), discovered through differential display of unripe and ripe strawberry fruit, with homology to the annexin super-family. Annexins are described as phospholipid-binding proteins which are calcium dependent and have been associated with voltage-gated ion channels, signaling molecules, and regulation of enzyme activities (e.g. callose synthase in cotton; Andrawis et al. 1993, Wilkinson et al. 1995, Verma and Hong 2001). Annexins may contribute to changes in cell wall structure and membrane properties, both of which are affected by calcium (Ferguson 1984, Brett and Waldron 1996). Wilkinson et al. (1995) also identified a cDNA (RJ5) with homology to chalcone synthase (CHS).

CHS catalyzes conversion of 3 molecules of 4-coumaroyl-CoA and 1 molecule of p-coumaroyl to naringenin chalcone, representing the first committed step in flavanoid biosynthesis, leading to anthocyanins responsible for ripe strawberry color (Clegg and Durbin 2000, Hadacek 2002).

Manning (1998) used differential screening of two cDNA libraries con
structured from either white (unripe) or red (ripe) strawberry to isolate additional ripening related genes. Of the gene families recovered, six were related to expression of enzymes responsible for phenylpropanoid synthesis of the red pigmentation of ripe strawberry fruit, present in low amounts in the white stage while elevated in immature and ripening fruit (Manning 1998). Manning (1998) also isolated an enzyme involved in cell wall metabolism (EGase). EGases (endo-Beta-(1,4)-glucanase) are enzymes which hydrolyze (1,4)-Beta linkages, flanking un-substituted glucose residues, and thought to act on xyloglucan (a component of plant cell walls; Brummell and Harpster 2001) which may contribute to softening of strawberry and other fleshy fruit.

A small strawberry micro-array was constructed containing 1701 randomly selected strawberry cDNAs, plus 480 cDNAs from corollas of petunia (Aharoni et al. 2000). This array was probed with RNA from multiple stages consisting of green, turning, white with red, and red strawberry fruit. A number of cDNAs were shown to display differential expression when compared to the different stages with the largest differences.
occurring between red versus green stages. From these clones one cDNA

was identified as the SAAT (strawberry alcohol aclytransferase) gene

impacting flavor biogenesis in ripe strawberry fruit (Aharoni et al. 2000).

The expression pattern of SAAT showed a 16-fold increase in red versus

green fruit and was limited to receptacle tissue (Aharoni et al. 2000). These

results correlate well with the belief that AATs are involved in ester volatile

formation by catalyzing their production from acyl CoA and alcohol

(Aharoni et al. 2000).

Aharoni et al. (2002) performed a second microarray analysis to gain

knowledge regarding large scale gene expression of maturing and ripening

strawberry fruit, specifically in response to oxidative stress and auxin

treatment. In response to oxidative stress, 20 ripening-related cDNAs were

recovered including chalcone synthase (Aharoni et al. 2002). Auxin

treatment resulted in observation of ripening-related genes involved in

flavonoid metabolism which are repressed. In addition, expression of auxin

independent genes, such as annexin, was also observed thereby suggesting

a complex regulatory arrangement involved in non-climacteric ripening
and an entry point for further elucidation of this process. A key question to be addressed in continuing molecular analysis is “how and at what level are climacteric and non-climacteric ripening regulatory mechanisms related?” The continued discovery of genes involved in ripening of both non-climacteric and climacteric fruit will allow for cross-comparison among species of different ripening types. The recent cloning of a MADS-box transcription factor necessary for tomato ripening at the rin locus, indicates a well conserved regulator of ripening acting upstream of ethylene with an apparent homologue expressed in fruit from the strawberry genome (Vrebalov et al. 2002). Efforts are underway to repress the strawberry RIN homologue in transgenic strawberry to test for function in ripening (K. Manning, G. Seymour, J. Giovannoni, unpublished). Conservation of RIN homologues across species representing diverse ripening types suggests a more universal (ethylene-independent) mechanism of ripening control. The characterization of RIN homologues from other fruit species will provide possible tools for controlling fruit ripening in a variety of agriculturally important plant species in a manner that does not focus
on ethylene synthesis or responses (Vrebalov et al. 2002).

Charentais melons (a climacteric variety) were transformed with
antisense 1-aminocyclopropane-1-carboxylic acid oxidase
(ACO) which
resulted in melon fruit displaying reduction of ethylene
production to <1%
of wild-type (Ayub et al. 1996). These antisense fruit also showed inhibition of ripening regardless of whether they were on or off the vine. When crossed to lines of agronomic importance, the aroma-related volatile ester content of the resulting fruit was shown to be reduced (Bauchot et al. 1998). These results indicate ethylene has an important regulatory role in many aspects of climacteric ripening, and while one desirable trait may be achieved via manipulation of ethylene, another equally important trait may suffer. Molecular tools, such as RIN gene, for comprehensive regulation of ripening at a step upstream of ethylene will be both more universal in application to both climacteric and non-climacteric species resulting in climacteric fruit with better quality than those whose ripening is regulated only at the level of ethylene.

Tomato

Tomato belongs to the Solanaceae, or nightshade family, and
is native to South America and Mexico (Kalloo and Bergh 1993). The importance of
tomato as a crop plant can be readily seen in the vast amount of land
under cultivation for commercial tomato production amounting to over
2,000,000 hectares in recent years (Kalloo and Bergh 1993). Declining con
sumer satisfaction with the flavor/appearance/spoiling of many fruits and
vegetables, including tomato, has prompted considerable interest in the
field of ripening and additional emphasis on this model system.

Tomato possesses a diploid genome comprised of 12 chromosomes and
is enhanced by a number of tools that contribute to its molecular and
 genetic characterization. Mutants involved in various aspects of tomato
growth and development, including ripening (as mentioned above), have
been isolated and characterized (Giovannoni 2001) along with numerous
Quantitative Trait Loci (QTL) identified in tomato, including QTLs respon
sible for variation in time of maturation to ripening (Doganlar et al. 2000).

Over one thousand molecular markers have been mapped in the
tomato genome spacing at an average less than 2cM (Tanksley et al. 1992). These
Markers have contributed to chromosome walks to a number of important loci including fw2.2 (Frary et al. 2000), ovate (Liu et al. 2002), jointless-1 (Mao et al. 2000), Pto (Martin et al. 1993), and rin (Vrebalov et al. 2002).

Eshed and Zamir (1995) produced a series of 50 ordered introgression lines (IL) from a cross between Lycopersicon esculentum and the wild tomato species Lycopersicon pennellii which represents a library of the complete L. pennellii genome through ordered and overlapping introgressions. Each introgression line contains a small portion (avg. 10 - 50 cM) of the L. pennellii genome in the background of the M82 L. esculentum parent, resulting in abundant phenotypic variation including fruit ripening and color traits that are linked to variation at the corresponding introgressed L. pennellii chromosomal segment. Recently, additional IL lines have been isolated to define smaller introgressions for a total of 76 IL lines (Liu and Zamir 1999).

These ILs have been used to map quantitative trait loci (QTLs) for important fruit quality traits, such as fruit brix, or total soluble solids like fructose and sucrose (Fridman et al. 2002).

Tomato is also benefiting from a NSF-funded expressed sequence tag...
(EST) development and utilization project (Van der Hoeven et al. 2002, Alba et al. 2004, Fei et al. 2004). Numerous cDNA libraries were constructed and sequenced from a comprehensive selection of tissues and physiological treatments to yield an extensive EST database (Moore et al. 2002 and Van der Hoeven et al. 2002). The EST collection has been annotated into a unigene set based on combining multiple copy ESTs into intersecting contigs totaling 27,274 unigenes (Van der Hoeven et al. 2002). This EST collection along with a set of sequenced bacterial artificial chromosome (BAC) clones has allowed the prediction of the number of genes within the tomato genome (Van der Hoeven et al. 2002). The Arabidopsis Genome Initiative (2000) has shown the estimate of Arabidopsis genes based on an EST unigene set was 35% overestimated. Therefore when this percentage was applied to tomato, it was determined that the unigene set would stand for 17,500 genes. This number represents half of the number of predicted genes in the BAC clones resulting in an estimate of 35,000 genes in the tomato genome (Van der Hoeven et al. 2002).

Finally, microarrays are being employed to explore the expression profile
of tomato developmental processes such as fruit ripening (Moore et al. 2002). A microarray has been constructed using the above mentioned EST libraries enriched with genes implicated in ethylene biosynthesis and fruit ripening including representative of a wide range of pathways and processes (Moore et al. 2002). Tomato microarrays are available to the research public at http://bti.cornell.edu/CGEP/CGEP.html. A comprehensive expression profile for ripening fruit based on a 10-stage time course from 7 days post anthesis to 15 days post breaker is currently under development (R. Alba, S. Moore, P. Payton and J. Giovannoni, unpublished; Moore et al. 2002, Alba et al. 2005).

BIOSYNTHESIS AND SIGNALING OF ETHYLENE

Ethylene is synthesized via the Yang cycle (Fig. 11.1) employing methionine as the initial substrate (reviewed in Kende 1993, Yang and Baur 1969). The pathway proceeds by conversion of methionine to S-adenosine-L-methionine (SAM). The enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) synthesizes 1-aminocyclopropane-1-carboxylic acid (ACC) among other products. ACC oxidase converts ACC to ethylene.

ACS is 1 of the enzymes involved in the biosynthesis of ethylene. The enzyme catalyzes the conversion of SAM to ACC. ACS is a key enzyme in the biosynthesis of ethylene, and its expression is regulated by various factors, including ethylene itself. The regulation of ACS expression is tightly controlled, and changes in ACS expression can lead to changes in ethylene levels, which in turn can affect plant growth and development.

The enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACS) catalyzes the conversion of S-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is the immediate precursor of ethylene, and the conversion of ACC to ethylene is catalyzed by ACC oxidase.

The ethylene biosynthesis pathway is illustrated in Fig. 11.1. The pathway begins with the conversion of methionine to S-adenosyl-L-methionine (SAM) by the enzyme SAM synthase. SAM is then converted to ACC by the enzyme ACC synthase (ACS). ACC is then converted to ethylene by the enzyme ACC oxidase.

The signal transduction cascade for ethylene (ET) involves several key components, including the ethylene receptor (ETR1), the EIN2 MAP kinase, and the EIN3 EIL1 transcription factors. Ethylene binds to the ETR1 receptor, which activates the EIN2 MAP kinase cascade, leading to the activation of EIN3 EIL1 transcription factors and downstream responses.

The regulation of ethylene biosynthesis and signal transduction is complex and involves multiple genes and pathways. Understanding these processes is crucial for developing strategies to manipulate ethylene production in crop plants, which has potential applications in crop improvement and agriculture.
considered to be a rate-limiting step and is encoded by multigene families varying in size among species and whose members demonstrate differential expression and include ripening-related ACS genes (Yip et al. 1992). The final step in the synthesis of ethylene is generation of ethylene from ACC via the enzyme ACC oxidase (ACO, reviewed in Fluhr and Mattoo 1996). A multigene family consisting of four tomato genes, ACO1 to ACO4 (Nakatsuka et al. 1998) display variation in expression both temporarily and spatially (Barry et al. 1996, Nakatsuka et al. 1998). ACO has also been found to represent a second rate-limiting step in ethylene biosynthesis (Barry et al. 1996). Repression of ACS or ACO results in decreased ethylene synthesis and inhibition of ripening in tomato (Theologis et al. 1993, Fray and Grierson 1993). Genes encoding both ACS and ACO have been cloned from a number of additional plant species such as rice (ACS: Zhou et al. 2002), apple (ACS: Lay-Yee and Knighton 1995), apple (ACO: Ross et al. 1992, Atkinson et al. 1998), banana (ACO: Lopez-Gomez et al. 1997), and melon (ACO: Balague et al. 1993).
Arabidopsis has proven to be of exceptional importance in elucidating mechanisms of ethylene signal transduction (Fig. 11.1). This is due in large part to the ease of screening for seedling-triple response phenotypes in mutagenized seeds which revealed key steps within the signaling pathway (reviewed in Ecker 1995, Kieber 1997, Chang and Shockey 1999). A number of ethylene signaling genes identified in Arabidopsis have been shown to have homologues in crop species including tomato (reviewed in Watkins 2002). These include homologs to the CTR1 kinase, the EIN3 family of transcription factors, as well as members of the ethylene receptor family (Leclercq et al., 2002, Tieman et al. 2001, Yen et al. 1995). While Arabidopsis has been used to study many aspects of general ethylene signaling, it clearly can not be used to study the role of ethylene signaling in fleshy climacteric fruit development.

The Nr mutation was originally characterized as a dominant mutation displaying delayed and incomplete ripening and reduced softening (Rick and Butler 1956, Hobson 1967). Lanahan et al. (1994) found the Nr mutant fruit is unable to ripen in response to either endogenous or exogenously
applied ethylene. Nr seedlings exposed to exogenous ethylene failed to show the “triple response” (Fig. 11.2) suggesting a greater perception of effects of ethylene (Lanahan et al. 1994, Yen et al. 1995). In support of this hypothesis, the Nr mutant also demonstrated delayed floral abscission and impaired senescence of leaves and petals, in addition to reduced expression of ethylene regulated genes (Lanahan et al. 1994, Yen et al. 1995).

The NR gene was isolated and is homologous to ethylene receptors found in Arabidopsis, including ETR1, though with most similarity to ERS (Wilkinson et al. 1995, Hua et al. 1995, reviewed in Lashbrook et al. 1998a).

The ETR1 gene encodes a protein homologous to bacterial two component regulators (Chang et al. 1993), which is sufficient to confer dominant ethylene insensitivity when mutated and transferred to the Arabidopsis or other plant genomes (Wilkinson et al. 1997). Unlike bacterial two-component systems, ETR1 contains a sensor and a response-regulator.
domain in one protein. The ERS1 and ERS2 genes are distinguished by structural variation compared to ETR1 in that they do not contain the receiver domain (reviewed in Stepanova and Ecker 2000). It is thought receptors missing this domain may utilize the receiver domain of other receptors such as ETR1 (Stepanova and Ecker 2000). Homologues for the ethylene receptors have also been cloned from non-climacteric fruit including an ERS homologue from Citrus sinensis (Li et al. 1998). The presence of ethylene receptor homologues in non-climacteric fruit indicate either use of ethylene in processes other than ripening or ethylene presence influencing processes that are exerted with lower ethylene concentrations. Along with Nr (also known as LeETR3), additional genes (LeETR1, 2, 4, 5) belonging to the ethylene receptor family have been isolated from tomato (Lashbrook et al. 1998a, Tieman et al. 1999, reviewed in Klee and Tieman 2002). NR and LeETR4 are distinct among others in this gene family from their sharp induction during fruit ripening (Tieman et al. 2000). NR ripening induction is due in part to its own responsiveness to ethylene (Wilkinson et al. 1995), suggesting multiple levels of ripening
influence by this hormone.

Transgenic lines showing repression of LeETR4 exhibited a wide range of phenotypes including extreme epinasty of leaves, increased senescence of flowers, and a reduction in ripening time (Tieman et al. 2000), all of which are consistent with increased sensitivity to ethylene. Conversely, lines having reduced expression of NR showed normal sensitivity to ethylene.

While counter-intuitive in isolation, this result accounted for a concomitant increase in accumulation of LeETR4 mRNA (Tieman et al. 2000). The converse proved true in transgenic lines engineered to be deficient in LeETR4 expression, suggesting that NR and LeETR4 are redundant in function (Tieman et al. 2000). This result is distinct from observations in Arabidopsis, where single gene receptor knock outs result in no discernible phenotypes and only multiple knockouts of at least three receptor family members display a phenotype consistent with constitutive response to ethylene (Hua and Meyerowitz 1998). Comparative functional and expression analyses of the tomato and Arabidopsis ethylene receptor families suggest similar gene/predicted peptide structures under regulatory constraints that likely
reflect differential selective pressures on the Solanaceae versus the Brassicaceae.

In addition to Nr, a spontaneous tomato mutation called epinastic (epi) is believed to be involved in the ethylene signaling pathway. This single gene mutation was originally described as a semi-dominant mutation (Ursin 1987), but is now thought to behave as recessive (Barry et al. 2001). This mutation leads to profound epinasty of leaves, thickening of petioles and stems, a shorter yet highly branched root system, overproduction of ethylene in vegetative tissues, and normal ripening (Ursin 1987, Fujino et al. 1988). Constitutive seedling triple response in the absence of ethylene is also a characteristic of the epi mutant (Fig. 11.2; Lee 1999) and is comparable to the constitutive triple response (ctr1) mutant of Arabidopsis (Kieber et al. 1993). The CTR1 gene encodes a MAP kinase kinase kinase (MAP KKK) through which all measured ethylene responses must flow in Arabidopsis (Kieber et al., 1993). Although homologs to Arabidopsis CTR1 have been found in tomato (Leclercq et al., 2002; Adams and Giovannoni unpublished), epi does not appear to map to any SlCTR loci (Fig. 11.3; Barry,
Adams, and Giovannoni unpublished).

To further characterize epi, and to help elucidate the mechanism of its effects, it was crossed with the ethylene insensitive receptor mutant Nr to create a Nr/Nr:epi/epi line (Barry et al. 2001). Wild-type seedlings displayed a typical triple response with the addition of ACC synthase while Nr showed characteristic insensitivity to ethylene. Nr/Nr:epi/epi exhibited a typical epi response regardless of treatment (Barry et al. 2001). In the presence of the ethylene action inhibitor 1-MCP the triple response of wild-type seedlings was inhibited while Nr seedlings remained insensitive. epi and Nr/Nr:epi/epi were not reverted to wild-type (Barry et al. 2001). Nr/Nr:epi/epi plants displayed constitutive ethylene response phenotypes following 1-MCP treatment in leaves as well (Barry et al 2001). Conversely, petal senescence and ripening of Nr/Nr:epi/epi was ethylene insensitive similar to the Nr parent (Barry et al. 2001) suggesting epi, unlike ctr1, is involved in only a subset of ethylene responses (Barry et al. 2001). Alternatively, epi may represent a step in a separate pathway involved in cross-talk to the ethylene signal transduction pathway (Barry et al. 2001). Microarray analysis
of epi versus its NIL normal control could provide insights into the role of epi in tomato ethylene signal transduction.

RIpening-related Genes and mutants

A variety of tomato genes related to ripening have been identified in recent years. For example, the pTOM series of clones were isolated Genetic Control of Fru-ripening 355 T G 3313.54.011.010.58.29.77.99.57.04.511.39.518.4 CT 106 A C T 2 0 S C T 2 S S T G 5 5 4 T G 4 5 1 T T G 3 0 8 T G 3 5 3 C T 7 5 T G 4 2 6 C T 2 2 9 C T G 5 3 7 T G 6 7 (A C S 3) T G 1 5 4 C H R O M O S O M E 2 T G 4 7 9 1 0.010.111.913.011.32.710.410.2 7.98.110.48.42.96.4 CT 31 AT G 5 8 5 T G 5 6 4 C T 1 7 1 T G 3 6 6 T G 2 2 2, (E 8 B) T G 4 2 T G 3 5 9 T G 3 7 7 T G 4 1 1 T G 2 1 4 T G 5 4 9 T G 2 4 4 C H R O M O S O M E 3 T G 4 5 7 P 1 8 0 6.413.03.8 3.87.58.65.94.58.97.85.912.06.47.910.6 T G 1 5 0 T G 3 7 0 T G 4 8 3 T G 4 7 4 T G 5 0 6 T G 2 0 8 T G 5 1 9 T G 6 2 T G 6 5 T G 5 7 4 T G 1 5 5 C T 5 0 C D 3 9 T G 2 2 T G 4 6 4 C H R O M O S O M E 4 T G 6 2 3 4.87.16.19.310.44.513.55.41 2.92.89.4 C T 5 3 T G 4 4 1 C T 1 6 7 C D 6 4 T G 5 0 3 T G 3 7 9 C T 1 1 8 A T G 4 1 9 T G 3 5 1 C D 7 8 T G 6 9 T G 2 3 8 (r i n ) 4.98.9 T G 3 1 8 C H R O M O S O M E 5 T G 2 9 7 4.83.42.337.09.214.40.6 9.93.48.79.119.74.1 C T 1 1 9 T G 1 7 8 T G 2 3 2 T G 5 0 0 T G 1 5 3 T G 3 6 5 T G 4 4 4 T G 2 9 2 T G 1 6 2 T G 5 4 8 T G 9 9 T G 4 8 2 T G 2 2 1 C H R O M O S O M E 6 C T 2 3 3 1 0.04.78.710.814.814.59.312.95.25.710.810.68.2 T G 1 8 4 C D 1 5 T G 5 1 T G 2 7 3 T G 5 9 T G 6 5 0 T G 6 3 T G 4 6 5 T G 4 4 0 T G 3 3 3 T G 1 7 T G 2 6 7 T G 2 7 C H R O M O S O M E 1 y , N r 2 a t (A C S 2) T O M 7 5 T C T R 2 J 4 9 B E R T S 2 (P SY ; r ) (E 4) E R T D 1 E R T 1 6 D D T F R B D D T F R 1 8 B P G (A C S 4) A C S 5 (h p 1) ( h p 2) L e E T R 4 T O M 6 6 (T O M 5 B) E I N 3 1 0 E I N 3 8 (B) E I N 3 1 3 S E N U 5 D D T F R 1 0 A A C S 8 A C S 7 C n r e p i (Contd.) 3 5 6 G e n e t i c I m p r o v e m e n t o f S o l a n a c e o u s C r o p s : T o m a t o T G 4 9 9 2.010.87.28.97.23.610.34.99.814.6 C T 1 9 5 C T 1 1 3 A T G 2 0 T G 2 1 6 T G 5 7 2 C T 8 4 T G 2 0 2 T G 2 5 2 T G 1 6 6 T G 6 1 T G 4 1 0 A 1 2 .3 C H R O M O S O M E 7 T G 1 7 6 1 1.84.91 2.59.14.43.89.613.09.04.14.7 C T
Fig. 11.3 Genetic map of tomato showing location of ripening-related loci.

by differential screening of mature green versus ripening fruits (Slater et al. 1985) and number well over 100 independent ripening-related tomato genes

(reviewed in Gray et al. 1992, Grierson and Schuch 1993, Gray et al. 1994; Table 11.1). The pTOM clones were either present or absent in unripe fruit,

but displayed a marked increase as fruit ripened. A number of the pTOM

series proved to be important in key aspects of tomato fruit ripening.

pTOM6 corresponded to polygalacturonase (PG) which catalyzes hydrolitic cleavage of alpha-1,4 linkages of polygalacturonic acid (pectin)

whose solubilization has been associated with
ripening-related changes in fruit texture (Grierson and Schuch 1993). Antisense expression of PG, under CaMV 35s, did not result in significant delay effects in softening of ripening fruit but did limit supply and action of the enzyme (Smith et al. 1988, Sheehy et al. 1988, Carington et al. 1993), suggesting PG is not the only significant player in cell wall breakdown during ripening (Smith et al. 1988, reviewed in Theologis et al. 1992).

pTOM13 was shown as ACC oxidase in antisense technology used to functionally define the activity of a plant gene (Hamilton et al. 1990, 1991). pTOM13 was also shown to be regulated at the mRNA level by ethylene in both fruit and leaves (Maunders et al. 1987, Smith et al. 1988). Several additional clones from the pTOM series of note include TOM5 phytoene desaturase (Bird et al. 1991), TOM66 low M.Wt. heat-shock protein (Fray et al. 1990), and TOM75 membrane-spanning solute channel (Fray et al. 1994). Lincoln et al. 1987 also identified a series of clones through differential screens based on ethylene induction including E4, E8, and E17. E4 was shown to have homology to a methionine sulphoxide reductase, though the role of this gene in ethylene response remains
mysterious (Montgomery et al. 1993). E8 encodes a member of a small multi gene family that shares homology with dioxygenase (Kniessel and Deikman 1996). E8 is itself transcriptionally activated by ethylene (Lincoln et al. 1988), and on analysis of antisense lines has shown to play a significant regulatory role in the synthesis of ethylene during fruit ripening (Penarrubia et al. 1992).

Although E8 is activated by ethylene, it has been shown that along with PG and ACOE8 is expressed in transgenic plants characterized for suppressed biosynthesis of ethylene, thus, further supporting the existence of non-ethylene mediated regulatory factors controlling aspects of climacteric ripening (Oeller et al. 1991 and Theologis et al. 1993).

There are a substantial number of mutations that impact ripening of tomato fruit (Table 11.1). Included are the previously mentioned Nr (Rick and Butler 1956), rin (Robinson and Tomes 1968), nor (Tigchelaar et al. 1985 Genetic Improvement of Solanaceous Crops: Tomato Mutant Chromosome Identification of Gene and/or Mutant Fruit Phenotype

Ripening

1. ripening inhibitor (rin) S Mads-box (Vrebalov et al. 2002) yellow and firm
2. non ripening (nor) 10 Tigchelaar et al. (1973) severely delayed ripening

3. Never-ripe (Nr) 9 Ethylene receptor (Wilkinson et al. 1994) orange

4. Never-ripe-2 (Nr-2) 1 Rick and Butler (1956) yellow-green

5. alcobaca (alc) 10 Kopeliovich et al. (1981) delayed ripening

6. Colorless non-ripening (Cnr) 2 Thompson et al. (1999) yellow skin/non-pigmented pericarp

7. Green-ripe (gr) Barry and Giovannoni Kerr (1958) green unpublished

8. high-pigment 1 (hp 1) 2 Peters et al. (1998), Liu et al. (2004) deep red

Carotenoid

1. apricot (at) 5 Jenkins and Mckinney (1955) yellow-pink hue

2. green-flesh (gf) 8 Clayberg et al. (1967) green

3. tangerine (t) 10 Carotenoid isomerase (Issacson el. al. 2002) orange

4. old gold crimson (ogc) 6 Clayberg et al. (1966) dark red

5. uniform (u) 10 Rick and Butler (1956) darker green shoulder

Ripening-Related Genes Gene ID Reference

1. pTOM5 phytoene synthase Bird et al. (1991)

2. pTOM6 polygalacturonase Grierson et at. (1986)

3. pTOM13 ACC oxidase Hamilton et al. (1990)

4. pTOM66 heat-shock protein Fray et al. (1990)

5. pTOM75 solute channel Fray et al. (1994)

6. E8 dioxygenase homology Kniessel and Deikman (1996) fruit specific
21973) and Cnr (Thompson et al. 1999) mutants, in addition to Green-ripe (Gr) (Kerr 1958), Never-ripe2 (Nr2) (Kerr 1982), and alcobaca (alc) (Kopeliovich et al. 1981). Many of these mutations have been placed on the tomato genetic map (Fig. 11.3) (Giovannoni et al. 1999). The mutation Nr is a lesion in one of the genes in the family of ethylene receptors which results in the inability of the receptor to bind ethylene (Klee 2002). Some of the remaining mutations appear to impact non-ethylene signaling aspects of ripening control. For example, the phenotype of the spontaneous recessive and non-ripening rin mutation includes the inability of fruit of this mutation to ripen when supplied with exogenous ethylene, yet ethylene responsive genes are induced in such ethylene treated fruits (Giovannoni et al. 1989).

Furthermore, rin seedlings display the triple response to exogenous ethylene, indicating that non-fruit ethylene responses also remain intact (Yen et al. 1995). These phenotypes of rin have been interpreted to indicate a lesion in a regulatory component that impacts both ripening-related ethylene
production and non-ethylene mediated components of climacteric fruit

ripening (Vrebalov et al. 2002). As stated above, RIN encodes a MADS-box

transcription factor which, in plants, have been primarily associated with

floral development (reviewed in Soltis et al. 2002). A floral development

function is consistent with the predicted developmental ripening regulation

implied by the rin phenotype (Vrebalov et al. 2002).

The single rin mutant allele, in addition to ripening impaired fruit, also

results in enlarged sepals (macrocalyx) and both traits map in perfect link

age on tomato chromosome 5 (Fig. 11.3; Giovannoni et al. 1995). A separate

recessive mutation bearing large sepals, termed macrocalyx (mc), was also

mapped to a similar location on chromosome 5 (Fig. 11.3; Rick and Butler

1956), suggesting the possibility that the enlarged sepal phenotype dis

played by the rin mutation was a result of a mutation in an adjacent (MC)
gene (Robinson and Tomes 1968). MC, like RIN, was also cloned and shown

to be a MADS-box gene (Vrebalov et al. 2002).

Another mutation closely resembling rin in phenotype is the recessive

non-ripening (nor) mutation (Tigchelaar et al. 1973). The latter mutation
displays ethylene synthesis/response phenotypes of the whole fruit and
the level of gene expression is similar to that described for rin (Giovannoni et al. 1995). This suggests that both gene products may participate in the same or similar regulatory circuits. NOR gene isolation is now complete and functional characterization is in progress (J. Vrebalov and J. Giovannoni, unpublished).

LIGHT SIGNAL TRANSDUCTION AND RIPENING-RELATED PIGMENTATION

All photosynthetic organisms depend on light as their source of energy. Plants are also extremely dependent on their respective light environment for cues impacting optimal growth and development. At the molecular level, plants discern various wavelengths of light using photoreceptor molecules which impact plant development as a result of quality, quantity, direction, and period of light (Alba et al. 2000). Photoreceptors that absorb red or far-red light are phytochromes, while blue-light receptors are termed cryptochromes. Phytochromes in particular are known to regulate the accumulation of a number of pigments in various plant tissues (Kendrick and Kronenberg 1994), and phytochromes localized to ripening tomato
fruit influence the accumulation of lycopene (Alba et al. 2000).

The change in fruit pigmentation from green (chlorophyll) to red (lycopene and beta-carotene) is a hallmark of tomato fruit ripening and is influenced by light. Genes representing steps involved in biosynthesis of certain pigments have been cloned from tomato and in some cases through screens for ripening-related genes. Indeed this was precisely the case for phytoene synthase-pTOM5 (Fray and Grierson 1993).

A number of fruit color mutants in tomato have been shown to reflect lesions in various steps of the carotenoid biosynthesis pathway. Many of these mutants have been mapped (Fig. 11.3) and most of the corresponding genes recently been cloned (Table 11.1). Practical use of a number of caroteneoid mutants in tomato breeding confirms the potential for manipulating fruit carotenoid levels for fruit quality and nutritional impact (reviewed in Hirschberg 2001). Carotenoids are antioxidant pro-vitamin A compounds capable of quenching oxygen radicals produced as a consequence of photosynthesis. As such, carotenoid synthesis, including that associated with fruit ripening, can be strongly influenced
by light quality and intensity (Alba et al. 2000).
Manipulation of light perception and response may serve as an additional avenue through which carotenoid accumulation may be enhanced in fruit color and nutrient quality in crop species. High levels of dietary lycopene have been associated with lower prostate cancer cases in men (reviewed in Giovannucci 2002).

The tomato high-pigment (hp1) mutant is reported to represent lesion in a repressor of light signal transduction resulting in light hypersensitivity and elevated carotenoid accumulation in hp1/hp1 fruit (Peters et al. 1989). The mutation is a single recessive gene mutation (Reynard 1956) displaying an increase in the amount of hypocotyl anthocyanin, reduced hypocotyl elongation, and increased leaf chlorophyll content, in addition to elevated ripe fruit carotenoids (Kerr 1965, Mochizuki and Kamimura 1985, Peters et al. 1989, Jarret et al. 1984, Thompson 1962). The seedling phenotypes of hp1 have been shown to be red light enhanced, and far-red reversible, serving as the basis of the hypothesis of a lesion resulting in hyper-activity of phytochrome responses (Kerckhoffs and Kendrick 1997). hp1 also
specifically alters expression of genes encoding proteins involved in
photosynthesis (CAB chlorophyll a/b-binding protein and
RBCS Rubisco
small subunit) as might be predicted for a hypersensitive
light response
mutant (Peters et al. 1998). In further support of a role
in phytochrome
hyper-signaling, over-expression of oat phytochrome (phyA)
in tomato
resulted in phenotypes similar to those observed in hp1
(Boylan and Quail
1989), indicating responses mediated by phytochromes are
expressly
affected by the hp mutations (Mustilli et al. 1999).
Although to date there
have been no reported mutants in Arabidopsis corresponding
directly to
hp1 at the phenotypic level, Arabidopsis cop, det, and fus
light signal
transduction mutants share some similar seeding
characteristics (reviewed
in Hardtke and Deng 2000, Schwechheimer and Deng 2000). The
gene for
HP1 has been mapped to chromosome 2 in tomato (Yen et al.
1997) and is
currently a target for a chromosome walk (Y. Liu and J.
Giovannoni,
unpublished).
A second mutation phenotypically similar to hp1 and termed
hp2-mu
tant has also been isolated (Soressi 1975). HP-2-wild type
gene was cloned
and determined to be the tomato homolog of the Arabidopsis DET1 gene (Mustilli et al. 1999). DET1 encodes a nuclear localized protein implicated in regulation of gene expression and development in response to light (Pepper et al. 1994). This was despite the fact hp-2 mutants (Mustilli et al. 1999) did not display many of the phenotypes which characterize the mutant det1 (e.g. no discernable seedling phenotypes in the dark). The differences between hp2 and det1 mutants are postulated to represent the possibility that tomato and Arabidopsis contain modifications in regulatory pathways which control photomorphogenesis (Mustilli et al. 1999).

Changes in fruit color and carotenoid levels may also be achieved by influencing levels of polyamines (PAs) (Mehta et al. 2002). Polyamines are organic cations of low molecular weight derived from amino acids via decarboxylation and associated with numerous physiological processes across diverse taxa (Kakkar and Sawhney 2002). Two PAs in particular, spermidine and spermine, are synthesized by adding aminopropyl groups removed from decarboxylated S-adenosyl methionine (SAM) to the precursor putrescine (Tiburcio et al. 1997, Kakkar and
Sawhney 2002). In plants, polyamines are said to be involved in cell division, formation of tubers, embryogenesis, flower development, root initiation, and ripening though the precise relationship to carotenoid accumulation in fruit remains unclear (Mehta et al. 2002, reviewed in Kakkar and Sawhney 2002).

In Mehta et al. (2002), a yeast S-adenosylmethionine decarboxylase (ySAMdc) gene was introduced into tomato under the fruit-specific E8 promoter (Deikman et al. 1992). The resulting transgenic plants displayed increased levels of spermine and spermidine throughout fruit ripening, with corresponding reduction in levels of putrescine (Mehta et al. 2002).

Ripening fruit on transgenic lines also appear to have extended “vine life” (Mehta et al. 2002). An increase of 50% in precipitate weight ratio (PPT), a gauge of the quality of processing tomatoes (Takada and Nelson 1983), was seen in juice and enhanced juice quality (Mehta et al. 2002). Lycopene was also increased by 200-300% yielding deeper red transgenic fruit (Mehta et al. 2002). Lastly, the transgenic fruit displayed elevated levels of ethylene but with no effect on time of ripening, suggesting the increase in polyamines
may countermand the effects of amplified ethylene levels (Mehta et al. 2002).

These results highlight new avenues of possible manipulation of fruit pigmentation and associated nutritional properties in crop plants and downstream products.

CELL WALL MODIFICATIONS AND RIPENING

Fruit ripening and cell wall metabolism are clearly interconnected processes. Cell walls in tomato consist of cellulose microfibrils, a hemicellulose matrix (primarily xyloglucan in dicots), and a pectin network (reviewed in Carpita and Gibeaut 1993, Cosgrove et al. 2000b). Cell wall related changes occurring in ripening fruit include: reduction of turgor, decrease in cell-cell adhesion, decline in apoplast pH, depolymerization/solubilization of pectins and hemicelluloses, and increase in cell wall porosity (Brett and Waldron 1995, Brummell and Harpster 2001). Decline of cell wall internal integrity, and loss of attachments to adjacent cells, governs the structural integrity and texture of many ripening fruit (Redgwell and Fischer 2002). During ripening, cell wall compounds including polyuronides are solubilized resulting in cell wall destabilization and loss of side chain sugars-galactan and arabinan (Gross 1984,
Seymour et al. 1990). This loss in cell wall integrity results in swelling of the wall space and loss of cell to cell adhesion leading to softening of the pericarp and locule liquefication upon ripening. A key enzyme long implicated in cell wall degradation and associated textural changes during tomato ripening is polygalacturonase (PG). There is a family of tomato genes encoding PG enzymes involved in various developmental processes including fruit ripening (Sitrit and Bennett, 1998). The PG involved in ripening is known to be expressed only in fruit with transcription limited to the ripening process (Bird et al. 1988, Della-Penna et al. 1989, Montgomery et al. 1993).

It had been thought previously that PG alone was responsible for the majority of cell wall degradation and associated softening in ripening fruit (Bennett and Della-Penna 1987). However, repression of PG resulted in degredation of pectin, but did not affect softening (Sheehy et al 1988).

Furthermore, PG coding sequence was also fused to a fruit specific promoter E8 (Deikman et al. 1992) in the sense orientation and transformed into a cultivar homozygous for the rin allele to determine whether PG activity
was sufficient to restore softening in the mutant (Giovannoni et al. 1989).

The resulting transgenics displayed accumulation up to 60% of PG activity,

but did not restore softening to any appreciable degree (Giovannoni et al.

1989, reviewed in Brummel and Harpster 2001). This result, in concert

with data from PG antisense experiments, provided the first evidence that

cell wall modifications leading to tomato softening are more complicated

than activation of a single cell wall hydrolytic enzyme.

Additional cell wall metabolizing enzymes have been cloned and char

acterized in ripening tomato fruit, revealing a more complex view of cell

wall ultrastructural changes associated with ripening than simple pectin

degradation (reviewed in Brummell and Harpster 2001).

Pectinmethylsterase (PME) has been found in a variety of plant species as

well as bacteria and fungi that are pathogenic to plants (Rexova-Benková

and Markovic 1976, Huber 1983, Collmer and Keen 1986). PME is involved

in the removal of methyl groups from pectin (Grierson and Schuch 1993)

allowing pectins to form Ca 2+ crosslinked gel structures and providing

additional susceptibility to PG activity (reviewed in Brummell and Harpster

2001). Antisense PME tomato lines with <10% normal PME
activity yielded
the anticipated higher MW pectins but had no other notable impact on
softening or other aspects of ripening (Tieman et al. 1992). These results
indicate that like PG, PME is not sufficient for softening of ripening tomato
fruit (Carington et al. 1993).

Expansins are a group of integral cell wall proteins originally identified
as contributing to cell wall extension of cucumber hypocotyls (McQueen
Mason et al. 1992). Expansins have been shown to be capable of loosening
and stretching of isolated cell walls (reviewed in Cosgrove 1999) and two
distinct families (α-expansins and β-expansins) have been identified
(Cosgrove 2000a). α-expansins are tightly associated with cell elongation
and wall loosening (Cosgrove 2000a), while β-expansins are among the
glass pollen allergens (Cosgrove 1999).

The tomato α-expansin family has been extensively characterized
(Reinhardt et al. 1998, Brummell et al. 1999a, reviewed in Brummell and
Harpster 2001). LeEXP1 is the primary member expressed in ripening fruit
(Rose et al. 1997), and homology to LeEXP1 has been employed to isolate
homologous genes from melon and strawberry (Rose et al. 1997). LeEXP1
expression is regulated by ethylene and found at 1-2% of wild-type levels in rin and nor (Rose et al. 1997). Also, LeEXP1 was not induced upon treatment of mature green (MG) rin and nor fruit given exogenous ethylene (Rose et al. 1997). These results correspond well with the decrease in fruit softening characteristic of both mutations and indicate an influence on LeEXP1 by the pathway involving rin and nor (Rose et al. 1997). In the receptor mutant Nr, expression of LeEXP1 was observed at comparable levels to wild-type, but did not increase upon addition of exogenous ethylene (Rose et al. 1997). These results may be due to leakiness of the Nr allele in the Alisa Craig background or may represent compensation by other members of the tomato ethylene receptor family. Rose et al. (2000) defined a subclass of \( \beta \)-expansins based on a dendrogram constructed from amino acid sequence consisting of LeEXP1, LeEXP4 present in growing flowers and fruit (Brummell et al. 1999a), and LeEXP18 expressed primarily in meristematic tissues (Reinhardt et al. 1998). These alignments were used to isolate antibodies for LeEXP1 to detect expansin activity in actively growing and ripening fruit as well as to indicate
characteristic expansin activity along with the hydrolytic capabilities of expansin proteins (Rose et al. 2000). The function of LeEXP1 in ripening fruit has been studied via over-expression under a 35sCaMV promoter. The results displayed increased softening at all stages of ripening including typically firm MG fruit. Antisense suppression of the gene displayed inhibition of breakdown of polyuronide and an increase in fruit firmness during the course of ripening, but was insufficient in impeding breakdown of hemicelluloses (Brummell et al. 1999a).

The endo-1,4-Beta-Glucanases (EGases or Celluases-CEL), mentioned above are implicated in a number of physiological processes such as abscission of organs as well as ripening in both climacteric and non-climacteric ripening fruit. EGases have been isolated from strawberry (Harpster et al. 1998, Manning 1998, Llop-Tous et al. 1999, Trainotti et al. 1999, Spolaore et al. 2003), pepper (Ferrarese et al. 1997, Harpster et al. 1997), and tomato (Brummell et al. 1997, Lashbrook et al. 1994; reviewed in Rose and Bennett 1999). Ethylene has also been found to either be required or induce accumulation of EGases (Brummell et al. 1999b). Two
EGases, Cel1
(Lashbrook et al. 1994) and Cel2 (Gonzalez-Bosch et al. 1996), increase in accumulation upon ripening pointing to a possible role in cell wall disassembly during ripening.

In Lashbrook et al. (1998b), Cel1 was placed under CaMV 35s promoter in antisense orientation to determine the effects of suppression in tomato. Transgenic lines displayed a decrease in Cel1 expression to 0.1% of wild type levels in fruit resulting in no measurable decrease in softening during ripening, but a decrease of 5-6% in abscission zones caused reduction in abscission of transgenic flowers (Lashbrook et al. 1998b). A second suppression study using Cel2 also under 35s resulted in transgenic tomatoes whose expression was reduced by greater than 95% in fruit and ~80% in abscission zones (Brummell et al. 1999b). Transgenics exhibited no discernable changes in softening of ripening fruit, but did display an increase in break-strength (e.g. the amount of pressure required to remove abscission zone 4 days post breaker) (Brummell et al. 1999b). Taken together, these studies indicate that during ripening Cel1 and Cel2 may compensate for absence of the other. Harpster et al. (2002a) showed...
suppression of a ripening-related EGase (CaCel1) in pepper did not cause a decrease in the softening of pepper fruit upon ripening and did not affect depolymerization of matrix glycans. CaCel1 was also placed under CaMV 35s promoter and transformed into tomato (Harpster et al. 2002b). Transgenics did not show any increases in depolymerization of xyloglucans or an increase in softening of ripening fruit. These results once again indicate that suppression/over-expression of only one EGase is not sufficient for significant changes in cell wall softening, suggesting either compensation by other family members and/or involvement of other enzymes.

A third enzyme believed to contribute to cell wall breakdown in ripening fruit is xyloglucan endotransglycosylase (XET), or XTH (Rose et al. 2002). This enzyme was purified originally from nasturtium seed due to its capability to hydrolyze xyloglucan in the absence of supplemental xyloglucan sugars (Edwards et al. 1986, Faik et al. 1998). XHTs catalyze cleavage of xyloglucan backbone internal linkages in the (1à4) Beta-D orientation and shift the xyloglucan molecule to another xyloglucan with a non-reducing end (Steele et al. 1999). Several genes
encoding XHTs have been isolated from tomato (tXET-B1, tXET-B2, and LeExt2, down-regulated by auxin in hypocotyls; Arrowsmith and de Silva 1995, Catala et al. 2001), kiwi (AdXET1-6, linked with ripe kiwi fruit, Schroder et al. 1998) and grape (VXET1, coupled with grape softening; Ishimaru and Kobayshi 2002).

The results suggest a role of these genes in ripening of both climacteric and non-climacteric fruit. LeEXRTB1 (expressed in ripening fruit) and LeEXGT1 (expressed in green fruit during cell expansion) have both been expressed in transgenic tomato (de Silva et al. 1994). In the over-expression of LeEXGT1, abundance of the mRNA transcript increased the size of fruit along with higher levels of sugars, indicating an important role for LeEXGT1 in young expanding fruit for affecting the final size of the fruit (Asada et al. 1999).

de Silva et al. (1994) showed suppression of LeXETB1 under a fruit specific PG promoter, but found no parallels between suppression and softening. These results would indicate possible redundancy of other family members while reiterating the belief that multiple enzymes must be involved in the breakdown of cell walls during ripening.

SUMMARY AND FUTURE PROSPECTS
The growth and maturation of fruits is a unique aspect of plant development which serves as a significant component of human diets with associated impacts on nutrition and health. Progress has been made in recent years in both biochemical and molecular genetics of fruit ripening leading to enhanced understanding of the molecular basis of fruit development and ripening control. Various model systems have greatly contributed to the understanding of fruit ripening of both climacteric and non-climacteric fruits and recent efforts have demonstrated molecular connections between these physiologically distinct two ripening types. Extrapolation of rapid molecular advances in ethylene and light signaling in Arabidopsis has led to important insights pertaining to how these signaling pathways impact maturing fruit. Advancing molecular genetic, genomic and biotechnological approaches have yielded additional insights into universal control of fruit ripening besides specific regulatory and biosynthetic pathways impacting distinct quality characteristics. This chapter summarizes recent developments pertaining to the molecular and genetic characterization of maturation and ripening of fleshy fruits.
Fruit ripening is a complex process which includes changes in physiology, biochemistry, color, flavor, and nutritional content. Many fruit species are of great economic importance, and thereby are targets for extensive study and manipulation at both the molecular and biochemical levels. While the division between fruits that ripen climacterically and those that are non-climacteric is clear at the physiological level, current work on genes present in both types of fruit is beginning to piece together possible universal regulation mechanisms for non-ethylene mediated ripening. Recent technical advances such as microarrays and EST databases are allowing further comparisons between ripening profiles of model systems in both climacteric tomato and non-climacteric strawberry.

Numerous homologues of genes involved in ethylene biosynthesis have been cloned in several species allowing a broader understanding of ethylene biosynthesis. Comprehensive knowledge of ethylene signal transduction has greatly benefited from studies on both Arabidopsis and tomato. Cloning of genes encoding enzymes thought to be involved in ripening has contributed to better understanding of cell wall breakdown.
during this

process. These enzymes have become useful in the quest to manipulate

softening of many fruits. Understanding of the function of light during

fruit ripening has been advanced through considerable innovations in the

field of light signaling and color development. Several genes involved in

light signaling and fruit pigment accumulation have provided targets for

manipulation of ripening color as well as nutritional content of fruit. Further

elucidation of the biosynthesis of ripening pigments, light signaling, and

pathways discussed in this chapter will increase the capacity for nutritional

value and attractiveness for the consumer.

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INTRODUCTION

Numerous fungal pathogens attack tomato (Lycopersicon esculentum Mill.) causing crop loss directly by reducing yields, or indirectly by reducing marketable yield as a result of defoliation and exposure of fruit to the elements. Profits for growers are also reduced by the costs of chemical sprays or fumigants used to prevent the diseases. These diseases, as well as bacterial and viral tomato diseases, are discussed by Watterson (1986).

The reader is also referred to the chapter of Lukyanenko (1991), where resistances to fungal and other diseases are discussed. This chapter will focus only on diseases that have been subjected to conventional plant breeding.
formation. To date all disease resistance traits in commercial tomato varieties have been derived from conventional plant breeding. Multiple disease resistant varieties generally derive their resistance from single major genes that usually have dominant inheritance, and of these fungal resistances are the most common (Laterrot 1997, Scott 2005). The dominant inheritance of these disease resistant genes is one of the major reasons that hybrid varieties are so prevalent, other reasons for the prevalence of hybrids are discussed by Scott and Angell (1998). A summary of fungal diseases and resistance genes used to control them is presented in Table 13.1.

A map showing approximate locations of resistance genes is depicted in

Fungal


Anthracnose ripe rot Anthracnose (Q) y Colletotrichum coccodes L. esculentum Different chr.s. (Stommel and Zhang 1998)

Blackmold Blackmold (Q) Alternaria alternate L. cheesmanii L. cheesmanii 2, 3, 9, 12 (Robert et al. 2001)

Corky Root py-1 Pyrenochaeta lycopersici L. peruvianum 3 (Doganlar et al. 1998)

Early Blight EBR (Q) Alternaria solani L. esculentum, L. hirsutum, Different chr.s. (Foolad et al. 2002, Zhang L. pimpinellifolium et al. 2003), Foolad et al. (unpubl.),
Fusarium Crown Fr1 Fusarium oxysporum f. L. peruvianum 9 (Vakalounakis et al. 1997).

and Root Rot sp. radicis-lycopersici


Gray Leaf Spot Sm Stemphylium spp. L. pimpinellifolium 11 (Behare et al. 1991)

Late Blight Ph-1, Ph-2, Ph-3 Phytophthora infestans L. pimpinellifolium 7, 9, 10 (Chunwongse et al. 2002, Moreau et al. 1998, Pierce 1971).


Leaf Mould Cf-1, Cf-2, Cf-4, Cladosporium fulvum L. hirsutum, L. 1, 6 (Balint-Kurti et al. 1994, Jones Cf-5, Cf-9 pimpinellifolium et al. 1993, Lauge et al. 1998).

Powdery Mildew Lv Leveillula taurica L. chilense 12 (Chunwongse et al. 1994).


Powdery Mildew Ol (Q)-1, Ol (Q)-2, Oidium lycopersici L. parviflorum 6, 12 (Bai et al. 2003) Ol (Q)-3

Verticillium Wilt Ve Verticillium dahliae L. esculentum 9 (Diwan et al. 1999, Kawchuk et al. 1998).

Bacterial

Bacterial Canker Cm 1.1-10.1 (Q) Clavibacter michiganensis L. peruvianum 1, 6, 7, 8, 9, 10 (Sandbrink et al. 1995).

Bacterial Canker Rcm2.0 (Q), Calvibacter michiganensis L. esculentum, L. hirsutum 2, 5 (Coaker and Francis 2004, Rcm6.1(Q) subsp. michiganensis Kabelka et al. 2002).
Bacterial Canker Three (Q) Calvibacter michiganensis L. peruvianum 5, 7, 9 (van Heusden et al. 1999). subsp. michiganensis

Bacterial Speck Pto Pseudomonas syringae L. pimpinellifolium 5 (Martin et al. 1993). pv. tomato Prf
Required for Pto/Fen L. pimpinellifolium 5 (Salmeron et al. 1996).

Bacterial Spot Bs4 Xanthomonas compestris L. pennellii 5 (Ballvora et al. 2001). pv. vesicatoria

Bacterial Wilt Bw-1, Bw-3, Ralstonia solanacearum L. pimpinellifolium 4, 6, 10 (Danesh et al. 1994, Thoquet Bw-4, Bw-5 (Q) et al. 1996).

Bacterial Wilt Two (Q) Ralstonia solanacearum L. peruvianum 6 (Mangin et al. 1999).

Viral

Cucumber Mosaic Cmr CMV L. chilense 12 (Stamova and Chetelat 2000).

Virus

Potyviruses Pot-1 Potyviruses L. hirsutum 3 (Parrella et al. 2002).

Tobacco/Tomato Tm-1, Tm-2 a TMV/ToMV L. hirsutum, 2, 9 (Levesque et al. 1990, Vakalounakis

Mosaic Virus (allelic to Tm-2) L. peruvianum et al. 1997, Young and Tanksley 1988).

Tomato Mottle Virus Two genes ToMoV L. chilense 6 (Griffiths and Scott 2001).


Tomato Yellow Ty-1, Ty-2, TYLCV L. esculentum, L. chilense, 6, 11 (Chagué et al. 1997, Hanson et al.

Leaf Curl Virus two (Q) L. pimpinellifolium, 2000, Zamir et al. 1994) L. hirsutum
Nematode

Potato Cyst Hero Globodera restochiensis L. pimpinellifolium 4 (Ganal et al. 1995)

Nematode

Root-Knot Mi, Mi-1, Meloidogyne spp. L. peruvianum 6, 12 (Ammiraju et al. 2003, Veremis et al.

Nematode Mi-3, Mi-9 1999, Williamson et al. 1994, Yaghoobi et al. 1995)

y Q in parentheses indicates quantitative trait loci (QTLs).

(Contd.)

Fig. 12.1 of the bacterial resistance chapter and another is in the paper of Foolad and Sharma (2005) and chapter 16 of this book.

SOIL-BORNE DISEASES

Alternaria stem canker. This disease caused by Alternaria alternaria (Fr.) Keissler f.sp. lycopersici was first described in California (Grogan et al. 1975). Infected plants have dark brown to black cankers with concentric zonation on the stems near the soil line or above ground. Tissue beneath the cankers has a brown dry rot in the pith area and discontinuous brown streaks may emanate in the xylem near the cankers. The lesions can girdle the plants destroying them by harvest time. Foliar symptoms include spotting and eventual necrosis of leaflets on one or both sides of the midrib. Susceptible varieties had ‘Pearson’ in their
pedigree while varieties 
bred in Florida such as ‘Florida MH-1’, ‘Walter’, ‘Homestead’ and ‘Manalucie’ were resistant. Grogan et al. (1975) postulated that resistance was conferred by a single dominant gene. This was later confirmed by Vakalounakis (1988a), who also tested 105 varieties for resistance and found that 103 of them were resistant. Clouse and Gilchrist (1987) showed that resistance in isogenic lines was controlled by a single dominant gene at the Asc locus and that Asc was incompletely dominant to a host specific pathotoxin. There was complete co-segregation of simultaneously tested plants to both toxin and the pathogen. Witsenboer et al. (1989) found Asc was linked 10-17.8 cM from the solanifolium (sf) gene on chromosome 3. Later it was reported that Asc was tightly linked to RFLP markers TG 134 and TG 442 on chromosome 3 (Van der Biezen et al. 1995, Mesbah et al. 1999). While inoculations can be done by several methods, the method of Grogan et al. (1975) will be described. The pathogen is cultured on PDA or CMA (cornmeal agar) where it maintained virulence for at least six weeks. Spores and mycelia were scraped from the plates and water was
added to obtain a suspension concentration of $2.5 \times 10^5$ spores/ml. The suspension was sprayed on plants at the 2 leaf stage or older, since younger plants at the cotyledon stage were not infected. The inoculated plants were kept in a mist chamber for 36-48 h at 21-24 °C and then removed to a greenhouse. Susceptible plants develop stem lesions and are girdled in 10-15 days while resistant plants remain healthy. A toxin can also be used in a detached leaf bioassay which is described by Clouse and Gilchrist (1987).

Corky root rot. This disease is caused by Pyrenocaeyta lycopersici Schn. & Ger. and has caused problems in European greenhouse production and is referred to as “old land disease” in Florida. Infected plants form root lesions that eventually become furrowed and develop a corky texture causing a progressive deterioration of the root system. Resistance has been reported from the wild species L. hirsutum and L. peruvianum (Hogenboom 1970). A single recessive gene py-1 was introgressed from L. peruvianum (Laterrot 1983). However, greenhouse inoculation is unreliable and field tests are required. Thus, the transfer of resistance has
been tedious and slow. Doğanlar et al. (1998) grew segregating populations in California, USA and Italy and used RAPD and RFLP marker analysis to map the py-1 gene in a 8.8 cM region between TG 40 and CT 31 on the short arm of chromosome 3. They were unable to obtain recombinants in this region from 100 F 2 plants and concluded recombination was suppressed in the region. CAPS markers were derived from three RFLP markers and the one derived from TG 324 R/F should facilitate future breeding efforts. Stamova (2004) reported on a tomato line called Pirelly 38 with resistance derived from the subgenus Eriopersicon that appears to have resistance from a single dominant gene when tested with infested soil from California farms. The py-1 gene was not effective for the isolate(s) tested (Stamova, personnel communication). More inheritance work needs to be done with Pirelly 38 and its effectiveness against other isolates should be tested. Recalcitrant sporulation of the pathogen has made it difficult to work with. McGrath and Campbell (1983) reported on methods to improve sporulation. V8 agar was superior to 18 other media they tested and sporulation and pathogenicity could be maintained for at least two years.
Sporulation was enhanced: by 3-4 day incubation of isolates on water agar before transfer to V8 agar, by constant light from cool white fluorescent lamps over 12 or 8 hr photoperiods, by media at 5.5 pH, and with incubation temperatures of 20-24 o C. Doganlar et al. (1998) inoculated plants by growing the pathogen on V8 agar, then 20 10 mm 2 pieces were added to 100g of sterilized millet seed in 120 ml water. After 30 days the millet seed was mixed in soil (50% sand and 50% peat moss) at a 1:20 dilution. The soil was placed in seedling trays and the tomato seeds directly planted in this soil. At the sixth true leaf stage the plants were transplanted to a field infested with the pathogen. When plants had 15% red fruit they were dug and the root systems rated for disease symptoms on a 1-5 scale where 1= no disease symptoms and 5 = extensive corking of the root system.

Foot and stem rot. This disease is a problem in western European greenhouses. The pathogen, Didymella lycopersici Kleb., causes dark brown sunken lesions on the stem that can girdle the plant. Resistance from L. hirsutum f. glabratum accession 61292 was reported to be dominant and controlled by more than one gene (Boukema 1982).
Heritability was high suggesting selection efficiency, but no resistant cultivars are available and there does not appear to be much breeding work with this disease.

Inoculation procedures were reported by Van Steekelenburg (1981).

Pathogen isolates, which maintain virulence for at least five years of subculturing, are grown on cherry decoction agar at 18-20° C and exposed to near UV-light (Philips TL 20W F20 T12 bulb) to induce sporulation.

Fourteen-day-old cultures (9 cm) are either homogenized with water in a blender or the conidia are washed off and the suspension adjusted to 10^6 conidia per ml. Four-week-old plants are inoculated either by dipping the roots for 5 sec in the suspension or 4 ml of the suspension is applied at the base of the plants with a syringe. Final scoring is done three weeks later.

The inoculation was better with soil temperatures of 15° C than at higher soil temperatures. Wounding roots by transplanting was beneficial in inducing disease. Two-week-old plants showed as much infection as four week-old plants, but the younger plants were prone to Pythium sp. infection, so the latter stage was recommended.

Fusarium wilt. The causal organism is Fusarium oxysporum
f.sp. lycopersici

(Sacc.) Snyder & Hansen. The disease has a world-wide distribution and can cause major crop losses. There are presently three races of the pathogen commonly referred to as races 1, 2, and 3 which are analogous to races 0, 1, and 2, respectively using the nomenclature of Gabe (1975). The first vertical gene for resistance (I) was reported by Bohn and Tucker (1939) in L. pimpinellifolium accession PI 79532. This gene was later assigned to chromosome 11 (Paddock 1950). Recently it was found to be linked to RFLP TG523 (P. Lindhout pers. comm.). A second race was discovered before 1940 (Alexander and Tucker 1945) but was not reported again until 1961 when the pathogen caused serious crop losses in Florida (Stall and Walter 1965). A gene for resistance to the second race (I-2) was discovered in a L. pimpinellifolium-L. esculentum F 1 accession, PI 126915 (Stall and Walter 1965).

The I-2 gene was mapped to chromosome 11 by morphological markers (Laterrot 1976) and by RFLP marker TG105 (Sarfatti et al., 1989). The I-2 gene has been cloned and found to be a complex locus (Ori et al. 1997, Simons et al. 1998). Stall and Walter (1965) originally reported PI 126915
resistant to race 1, but it was not clear if this was due to I-2 or to a linked gene, either I or another gene. Cirulli and Alexander (1966) reported that resistances to race 1 and race 2 from PI 126915 were controlled by separate genes. Later, Laterrot and Philouze (1984) reported recombination between the genes when they obtained a line that was resistant to race 2, but not race 1, although it was less susceptible to race 1 than the susceptible control line. It was concluded that the I-2 gene gave a reduced susceptibility to race 1. Meanwhile, a third race of fusarium wilt was discovered in Australia (Grattidge and O’Brien 1982) and has now spread to the southeastern and western USA, Mexico and Japan. Resistance to race 3 (I-3 gene) was discovered in L. pennellii accessions PI 414773 (McGrath et al. 1987) and LA 716 (Scott and Jones 1989). In fact, all accessions of L. pennellii carry resistance to all three races of the Fusarium wilt organism (Scott and Jones 1990). The gene conferring race 3 resistance from LA 716 was determined by Bournival et al. (1989) to be linked to an allozyme of Got-2 on chromosome 7. PI 414773 also had the same Got-2 allozyme (Scott, unpublished data). This marker could also be used to select for race 1 and 2 resistance derived
from LA 716 (Bournival et al. 1990). From this work, it was not clear whether the resistances to races 1 and 2 were conferred by I-3 or genes linked to I. 

3. Tanksley et al. (1992) reported I-3 was linked to RFLP markers TG128, TG217, and TG170. Sarfatti et al. (1991) reported gene I1, derived from LA 716, conferred race 1 resistance and was linked in a 22 cM region between RFLP markers TG20 and TG128 on chromosome 7. They reported this gene not to be an allele of I-3 presumably due to differences in linkage estimates from Got-2. Scott et al. (2004) obtained four lines that were resistant to races 2 and 3 but susceptible to race 1. Two other lines resistant to race 3 had partial resistance to race 2 and were susceptible to race 1. All six had L. esculentum alleles distal to TG 639 which reduces the possible I1 region by 13 cM from that of Sarfatti et al. (1991). However, Scott et al. (2004) speculated that I1 was located near CT113 6.5 cM beyond TG20 which bordered the region reported by Sarfetti et al. (1991). It is evident that race 2 resistance is more tightly linked to I-3 than race 1 resistance (Scott et al. 2004). The likely location of a putative race 2 locus is distal to the I-3 locus, but further research is needed to verify the existence of such a locus.
The I and I-2 genes have been two of the most widely deployed genes in tomato cultivars. It is difficult to find any modern cultivars without these genes, especially the I gene. Scott and Jones (1995) released two race 3 resistant breeding lines, and cultivars such as 'Floralina', 'Sunguard', 'Solar Fire' and 'Escudero' are now available that have the race 3 resistance conferred by the I-3 gene with numerous other cultivars soon to be released.

Delta Contender is a race 3 resistant cultivar that has been grown in Australia for a number of years. Numerous other accessions have resistance to various races of fusarium wilt (Scott and Jones 1986, Bournival and Vallejos 1991, Huang and Lindhout 1997). Some of these might also be resistant to new races of the pathogen that may emerge in the future. Screening for fusarium wilt is done with seedlings and is quite routine.

The pathogen is grown on potato dextrose agar at 28 o C for 1 week and comminuted in a blender with dH2O and adjusted to 6 x 10^7 spores/ml. Seedlings at the cotyledon stage are root dipped in the slurry and transplanted to flats under temperatures of 28 o C or more with a soil media at 6.5 pH. High (>7) pH soils suppress fusarium wilt and should be avoided.

Symptoms can be read 3 weeks later. Susceptible plants may
die or express

stunting, wilting, yellowing of foliage, epinasty, and/or have enlarged

stems. When plant stems are cut longitudinally, susceptible plants have

vascular browning whereas resistant plants do not. The penetrance of the

I gene is sometimes reduced with this test (Alon et al. 1974) but varieties

with the I gene are healthy under field conditions. The race 2 test is the

most straightforward as the penetrance of I-2 is close to 100% and there are

few susceptible escapes. The I-3 gene has penetrance similar to I-2, but the

susceptible plants show symptoms a few days later than with race 2 and

overall the symptoms are not as severe. Although dead plants are evident,

there are more susceptible plants with lesser symptoms for race 3 than

with race 2. Thus, more plants require cutting to verify if they are suscep

tible with vascular browning.

Fusarium crown and root rot. The causal organism of this pathogen is

Fusarium oxysporum Schlecht. f. sp. radicus-lycopersici, Jarvis and Shoemaker.

This disease has been found in both greenhouses and field production.

Plants wilt similarly to those infected with fusarium wilt, but the vascular

browning is confined to the crown of the plant whereas
browning in plants

infected with fusarium wilt can also be found in the apices. Fusarium
crown rot thrives at cooler temperatures whereas fusarium wilt thrives
under high temperature conditions. Resistance is conferred by the single
dominant gene Frl (Berry and Oakes 1987a, Vakalounakis 1988b) that likely
was introgressed from L. peruvianum (Yamakawa and Nagata 1975). The
Frl gene has been mapped to chromosome 9 at 5.1cM from the Tm-2 locus
(Vakalounakis et al. 1997). Several researchers have found that germplasm
selected for the Tm2 2 gene also carry resistance to fusarium crown rot
gene was introgressed from L. peruvianum accession PI 128650 (Alexander
1963). Resistance has also been identified in several accessions of L. pennellii
(Scott and Jones 1990). Development of breeding lines with resistance from
LA 1277 has been pursued but it is not clear how it is inherited despite
two attempted genetic studies (Scott, unpublished data). Stamova (1996)
reported resistance was found in lines derived from L. pennellii, L. chilense,
and L. pimpinellifolium. The first greenhouse tomato cultivar with resistance
was Ohio CR6 (Scott and Farley 1983). Since then numerous resistant varieties have been developed for greenhouse or field production. A disease screening method has been described based on parameters defined by Jones et al. (1990). The pathogen is grown on PDA as with fusarium wilt. Plants at the cotyledon stage or with 2 true leaves are inoculated by dipping into a suspension with $2 \times 10^7$ spores/ml of the pathogen and transplanted to a soil amended to a pH of 5.0 in a growth chamber at 20 °C with 12 hr days. Results can be evaluated in a week when some plants have died. Living plants are dug and roots are rinsed. Susceptible plants are often stunted and have some brown roots while the roots of resistant plants remain white.

Phytophthora root and crown rot. This disease, caused primarily by Phytophthora parasitica Dast. and secondarily by P. capsici Leonian, has been a problem in processing tomatoes in California, especially in wet soils. High salts also exacerbate symptoms of this disease (Swiecki and MacDonald 1991). Seedlings can be killed, but the most severe damage occurs late in the season when fruit are ripening. The entire canopy can collapse and die leaving fruit exposed to sunburn (Blaker and Hewitt 1987).
Moderate resistance was reported in the cultivar CX8303 and *L. esculentum* var. cerasiforme accessions LA 1312, 11-2A, 13-1A, and 27-1A (Blaker and Hewitt 1987a). Blaker and Hewitt (1987b) concluded that at least two types of resistance exist in tomato when they compared LA 1312, 27-1A, and CX8303 to susceptible genotypes. Both mechanisms resulted in less colonization of roots. The former two Lycosperium esculentum var. cerasiforme genotypes inhibited lesion extension and hyphal growth from the point of infection. CX8303 had fewer propagules per gram of root tissue than susceptible genotypes suggesting less extensive colonization with possibly only the outer cortical layers being infected, although this was not tested.

The ability to regenerate roots after infection is another possible secondary resistance mechanism as 27-1A and CX803 regenerated more roots than Peto 343, the susceptible control. Inheritance of resistance from LA 1312 was determined to be quantitative (Kozik et al. 1991). The F1 was intermediate to the parents and skewed slightly toward resistance. Additive effects were 96% but dominance and epistasis were also detected. Broad sense heritability was 36% and narrow sense heritability
was 22%. The effective factor estimate suggested five genes control resistance. Since heritability was low, family selection rather than individual plant selections should be utilized. This resistance will have to be incorporated into both parents of a hybrid and this coupled with the low heritability has been a hindrance to developing commercial cultivars. Bolkan (1985) described an inoculation technique that correlated well with field results (r=0.77). Other studies use close adaptations of this method. The method of Kozik et al. (1991) will be described here. Cultures are maintained on V-8 agar and renewed monthly. Mycelia are incubated at 24 °C for three days to form zoosporangia. Cultures are flooded with distilled water and transferred to 8 °C for 30 min to induce zoospores. Discharge of zoosporangia is facilitated within 45 min by transfer to room temperature (20 ±3 °C). The mycelial suspension is filtered through two layers of cheesecloth and zoospores quantified with a haemocytometer to a concentration of 10³ zoospores /ml. Seedlings at the 2 to 3-leaf-stage are dipped into the inoculum suspension for 1 min and then transferred to 50 ml beakers with 20 ml distilled water and held at 20 °C.
under fluorescent lights and 10 h days. Disease symptoms were read 11 days after inoculation on a 0-4 scale: 0= no symptoms; 1= superficial browning of tap root and/or lateral; 2= moderate browning of roots with lesions > 2 cm and discoloration not penetrating pith; 3= all root surface with confluent brown lesions, pith discolored with rotted roots; 4= stem and canopy collapsed, seedling dead.

Southern blight. The causal organism for this tropical disease is Sclerotium rolfsii Sacc. This pathogen thrives in warm (>29° C) acidic soils (Sherf and MacNab 1986). Symptoms include dark brown or black lesions near the soil surface, lesions are covered with white mycelium when soil moisture is high. Later 1-2 mm sclerotia are formed. Plants are killed or often stunted and wilted. Fruit can also be infected. Resistance was reported in L. pimpinellifolium accessions PI 126932 (Mohr and Watkins 1959) and PI 126432 (Phatak and Bell 1983). Resistance was attributed to the precocious development of secondary periderm on the basal stem (Mohr and Watkins 1959). However, it seems that this resistance has not been deployed in tomato. Six breeding lines were released from Paul Leeper’s
breeding program at Texas A & M, but the source of resistance was not specifically known (Leeper et al. 1992). All six had 100% resistance as did PI 126432 (Leeper et al. 1992). They were all derived from a cross made in the 1950’s of STEP 54 from the University of Hawaii and ‘Southland’ from the USDA-ARS at Charleston, SC. One or both of these lines may be the source of resistance. ‘Southland’ has L. pimpinellifolium in its background (Scott 1983). No inheritance studies have been conducted. Phatak and Bell (1983) described a field inoculation procedure. Isolates were grown for 85 days on whole crimped oat grains in Fernbach flasks containing 300 g oats and 300 ml water. The oats and water had been previously autoclaved at 121 o C for one hour on two successive days before the isolates were added. The oat grains with mycelia and sclerotia were passed through a 7- mm diameter sieve to break up clumps. Ten percent (w/w) fresh media was mixed with the sieved inoculum and stored at 3 o C for 16 h before use. Ten to 15 g of inoculum per 30 cm of row was mixed into the top 5 cm of soil with a rake immediately after transplanting. Verticillium wilt. This disease, which has a broad host range, is caused by the soil fungi Verticillium dahliae (Kleb) and V.
Resistance to race 1 is conferred by the single dominant gene Ve that was discovered in 1932 in the accession Peru Wild (Schaible et al. 1951). The first cultivars with resistance were released in 1952 (Cannon and Waddoups 1952) and most modern cultivars have this resistance. There has been considerable confusion in mapping the Ve gene as various Tomato Genetics Cooperative reports have linked it to chromosomes 4, 7, 9 or 12 (see Diwan et al. 1999). Diwan et al. (1999) presented strong evidence based on recombinant inbred, F2, and introgression line populations that Ve is linked to RFLP GP39 on the short arm of chromosome 9. A co-dominant SCAR marker linked less than 0.67 cM from Ve has been developed that should be useful for tomato improvement (Kawchuk et al. 1998). Strains of the fungus that are virulent on cultivars with Ve, collectively called race 2, have been reported in California and North Carolina, USA; Canada, Australia, Brazil, Morocco, and Crete (see Baergen et al. 1993).

Race 2 was also found in greenhouses in Ohio, USA (Berry and Oakes 1987b) and Africa according to Stamova (2005). However, there may in fact be several different strains of the fungus that are virulent on race 1 resis
tant cultivars. Baergen et al. (1993) tested six lines with reported resistance
to race 2 against four isolates: two from North Carolina, one from Spain,
and one from Brazil. They found that resistance sources provided partial
resistance, primarily to the isolate for which they were tested originally,
but not to most other isolates. The resistance was not as great as that
provided by the Ve gene for race 1. Thus, race 2 does not fit a gene for gene
system. Instead, several strains that overcome Ve are different from each
other and are controlled to some extent by different genotypes. Recently,
Stamova (2005) reported a high level of resistance to verticillium wilt race
2 against 11 California isolates. The resistance, in a line called VEDA,
derived from the subgenus Eriopersicon appeared to have a dominant in
heritance when tested against 2 of the isolates in a genetic study. The
VEDA resistance needs to be tested against isolates from other regions. Inoculum is grown on PDA for a week as with Fusarium cultures.
Screening is done with a seedling dip technique where plants at the
cotyledon stage are inoculated with a slurry of the pathogen at 10^7 or more
spores/ml and disease symptoms are rated about three weeks later before
field transplanting. Symptoms include stunting, yellowing of foliage, tan
discoloration of vascular bundles, wilting, and death. Symptoms are more
severe when day length is reduced to 4 hours (Jones et al. 1975). Although
verticillium wilt is considered to be a cool weather pathogen, Jones et al.
(1977) found that symptoms increased on the Ve resistant cultivar ‘Tropic’
as temperature was increased from 20 to 32 °C under short day (4 hour)
conditions.

FOLIAR DISEASES

Cladosporium leaf mold. This foliar disease most often causes problems
in greenhouse production under high humidity conditions. The pathogen
is Cladosporium fulvum (Fulvia fulvum) Cke. Much basic research has been
done on this host-pathogen interaction (Joosten and DeWit 1999). Resistance was discovered in the 1930’s when the first dominant gene, Cf-1,
was identified (Langford 1937). After Langford, much resistance research
was done by Ernie Kerr and coworkers in Canada, who discovered 24
resistance genes (Kanwar et al. 1980a,b, Table 13.2). However, Haanstra
(2000) found five of the genes were identical in their specificites using a
potato virus X (PVX) expression system. The genes were
renamed accord

...ing to the elicitor or to the avirulence protein which they recognize (Table 13.2). Haanstra (2000) found two new genes and thus concluded there are 21 Cf genes (Table 13.2). When cultivars with single resistance genes are deployed, a virulent race generally appears rather quickly. Cladosporium resistance genes are at the opposite end of the durability spectrum from the Stemphyllium resistance gene discussed below. Thus, it has been suggested that at least two genes that have not yet been overcome by current isolates be incorporated into varieties (Lindhout et al. 1989). The race situation can

Cf-4 allele. LA 3271 L. esculentum. Cf-ECP5 G1.1161 L. esculentum 1 Derived from L. pimpinellifolium CGN 15529.

Also found in L. pennellii (Stamova 1985).

Also found in L. peruvianum (Lukyanenko 1991).

be rather confusing and it does not appear that anyone has done a thorough characterization of the resistance genes against all the pathogen races.

Kerr (1977) presented results for genes Cf1 through Cf-11 for 5 common Canadian races, and this summary has been presented elsewhere (Lukyanenko 1991, Stevens and Rick 1986). Lindhout et al. (1989) tested 11 resistant lines for 12 races and found Cf-6 was resistant to all of them.

Theoretically there could be $2^n$ races of the pathogen where $n$ is the number of resistance genes (Day 1956). The highest number of reported races has been 18 (Lukyanenko 1991). There are Canadian and European nomenclature systems for races that are cross referenced in Table 13.3. The Canadian system is based on the order of appearance while the European system is based on the gene-for-gene relationships between the host and the pathogen. Thus for the latter, a race called 2.4 would be virulent on hosts with resistance genes Cf-2 and/or Cf-4. Breeders use genes Cf-4 and Cf-5 according to Lukyanenko (1991), but there are races that are virulent on this gene combination.
Some seed companies use Cf-2 and an unknown gene (van den Bosch personal comm.). Given the data of Lindhout et al. (1989), the unknown gene might be Cf-6. According to Beek et al. (1991) Cf-9 was still effective in the Netherlands and was being used in cultivar development. Jones et al. (1993) showed that Cf-2 and Cf-5 were allelic or tightly linked on chromosome 5, while Cf-4 and Cf-9 were allelic or tightly linked on chromosome 1.

Balint-Kurti et al. (1994) reported Cf-4 and Cf-9 were allelic and located on chromosome 1.2.6 cM distal to CP46 and 3.7 cM proximal to TG236.

Haanstra et al. (1999) reported Cf-ECP2 was 11.3 cM proximal to Cf-4 and had no recombination with CT116. Haanstra (2000) mapped Cf-ECP5 3.5 cM proximal to Cf-4 and 3.9 cM distal to TG236. Inoculation (Lindhout et al. 1989) is done on plants at the 1- or 2-leaf stage using a mixture of common pathogen races, usually five in number.

A suspension of 10^6 spores/ml is sprayed on the plants and they are kept in a chamber at 100% RH and 20 °C for 48 h. The plants are
then kept in a greenhouse at 20 °C for 2 weeks with the RH at >70% with a humidifier.

Plants with no or arrested symptoms are considered resistant while those with extensive disease development are susceptible. Kerr (1977) used the following scale: 1= lesions 10 mm diameter, sporulating freely, surface whitish; 2= delayed sporulation, extensive chlorosis; 3= chlorotic spots 8 mm diameter, sporulation under low light and high humidity; 4= chlorotic flecks 2-3 mm diameter, no sporulation; 4.5= necrotic flecks 1 mm diameter; 4.8= pale flecks 1 mm diameter; and 5= immune, no visible infection.

Early Blight. Early blight of tomato, caused by the fungus Alternaria solani (Ellis and Martin) Jones and Grout, occurs worldwide in areas with frequent rainfall and/or heavy dew. Symptoms of early blight include stem lesions or cankers, referred to as collar rot in tomato seedlings, leaf spotting and blighting, and sunken lesions at the stem end of the fruit. Lesions are dark brown with concentric rings, producing a chlorotic halo as they expand on leaves to the blighting phase. Early blight is more severe on plants that are stressed nutritionally or bear a heavy fruit load (Barratt and
It is particularly severe on early maturing varieties with a small, determinate plant type. Varieties with the curled foliage trait are more susceptible to early blight, and many other foliar fungal and bacterial diseases, than varieties with non-curl foliage. Varieties with potato leaf foliage are reportedly less affected by early blight than are varieties with normal, serrated leaves. Later maturing varieties, particularly of the indeterminate type, are generally less affected by early blight than the determinate varieties, probably because of less stress resulting from lower fruit to foliage ratio. Highly vigorous sterile plants with little or no fruit generally develop little early blight in field plantings where plants with normal fruit load are severely affected by the disease (Gardner, personal observations). Early breeding of resistant varieties was focused on incorporation of collar rot resistance because of the severity of this phase of the disease in field grown transplants before the advent of highly effective fungicides (Andrus et al. 1942a). The sources of resistance used for collar rot, primarily ‘Campbell 1943’, also confer a moderate level of leaf spot resistance and resistance to the fruit lesion phase of the disease (Andrus et al. 1942b,
Barksdale and Stoner 1977, Gardner 1990). The breeding lines NC EBR-2, -3, and -4 and the hybrid ‘Mountain Supreme’ have resistance derived from ‘Campbell 1943’ (Gardner 1988, Gardner and Shoemaker 1999). In addition to stem lesion resistance, more recent breeding has included foliar resistance. The early blight resistant processing tomato, 71B2, developed by Barksdale and Stoner (1973, 1977), has moderate foliar resistance but is susceptible to the stem and fruit lesion phases of the disease. Recently developed fresh market plum tomatoes derived from the 71B2 resistance source include NC EBR-5, -6, -7, and -8 and the hybrids ‘Plum Dandy’ and ‘Plum Crimson’ (Gardner 2000). Numerous small-fruited accessions of L. hirsutum, L. pimpinellifolium and other wild species with indeterminate growth habit and a high foliage to fruit ratio have been identified as highly resistant to early blight (Foolad et al. 2000). The high level of early blight resistance from these sources may be lowered as the resistance genes are advanced into an acceptable background. A good example of this is the resistance in NC EBR-1, derived from the L. hirsutum selection PI 126445 (Gardner 1988, Nash and Gardner 1988a, b). The original PI 126445 plants were free of early
bight symptoms

in the field and in greenhouse inoculations of seedlings as
was the F 1

hybrid of L. esculentum × PI 126445. With additional
backcrosses to L.

esculentum and selection for determinate growth habit with
a heavy set of

large fruit, the resistance level became lower. Screening
of early backcrosses

to L. esculentum was possible in the greenhouse, but in
more advanced

backcrosses as the resistance level became lower, field
screening had to be

used because of the failure to clearly distinguish
resistance in greenhouse
tests. The lower level of resistance to early blight in NC
EBR-1 compared to

the original L. hirsutum selection is likely to have
resulted from the additional

stress imposed on the plant by advancing to a determinate
background

and earlier maturity with a heavy set of large fruit as
well as the possible

loss of resistance genes linked to undesirable traits. This
situation is typical

of the challenge repeatedly facing breeders in transferring
quantitative traits

from a highly vigorous wild background to commercially
acceptable

varieties. Screening for stem lesion resistance can be done
efficiently in a simple

plastic-covered growth chamber in the greenhouse (Gardner
1990). The
method developed by Barksdale (1969), or modifications thereof, are used
to produce early blight spores. This method consists of
growing the fungus
in petri dishes of lima bean agar, scraping the mycelium to
flatten it, and
exposing the inverted, uncovered dishes to an alternating
light and dark
period to induce sporulation. Spores are washed from the
dishes into
distilled water and the inoculum concentration adjusted to
10,000 spores/ml. Spores are atomized in late afternoon onto the stems of
6-7-week-old
plants using a plastic household spray bottle. For the
development of
disease, plants need to be kept wet during the night. This
is easily
accomplished by misting the plants in late evening prior to
closing the
chamber and running a humidifier to introduce moisture into
the chamber
during the night. During the day the chamber is opened and
the foliage
allowed to dry. Disease ratings can be made approximately
one week
following inoculation. Resistance from sources such as
‘Campbell 1943’,
which is highly resistant to stem lesions, is expressed as
minute necrotic
flecks on the stems compared to large expanding lesions on
the stems of
susceptible plants (Gardner 1990). Attempts to screen for
moderate levels
of foliar resistance in the greenhouse have been less successful than the stem lesion resistance because leaf spot development on resistant plants is not consistently limited enough to clearly distinguish susceptible from resistant plants. Screening for foliar resistance in the greenhouse, however, is useful for determining a high level of early blight resistance in wild tomato selections (Foolad et al. 2000). Some believe that early blight resistance is associated with low fruit yield and late maturity. Yield trials with ‘Mountain Supreme’, an early blight resistant hybrid, showed that its yields over several seasons were not less than other commercial varieties (Gardner and Shoemaker 1999).

However, attempts to produce early maturing tomato cultivars with an acceptable level of early blight resistance have not been successful, presumably because of the stress resulting from a heavy fruit load early in development of the plant. Sources of early blight resistance incorporated into acceptable cultivars have been moderate, but appear to be durable. This resistance is useful in reducing the frequency of fungicide applications needed for control of early blight, but generally does not eliminate the need for chemical control (Shoemaker and Gardner 1986, Gardner and Shoemaker 1999).
Early blight resistance in tomato follows the disease development pattern seen with other moderate, horizontal plant disease resistances. Under conditions of low inoculum pressure, the resistance is highly effective but can be overcome under conditions of high inoculum pressure and environmental factors favorable for the disease. Resistance is most effective when resistant cultivars are not grown in close proximity to susceptible cultivars and in fields where there is not a heavy carryover of inoculum from a previous tomato crop. Because of the additive nature of genes for early blight resistance (Maiero et al. 1989, Nash and Gardner 1988a) both parents need to carry the resistance to achieve a useful resistance level in hybrids. Most disease resistances in commercial tomato hybrids are conferred by single dominant genes in heterozygous condition. This simplifies the breeding, allows for using genes that are associated with deleterious effects when they are homozygous but not heterozygous, and makes it easier to combine multiple disease resistance genes in hybrids. From a seed company standpoint, a further limitation of varieties with moderate horizontal resistance is that
the resistance is subject to being overcome under severe conditions. As a result, the company has to be more cautious about claiming disease resistance because of possible liability issues. QTLs associated with early blight resistance in wild tomato species have been identified (Table 13.1; Zhang et al. 2003). Accordingly, Graham et al. (2005) did not find much resistance in any of the introgression lines developed from L. hirsutum accession LA/777 (Monforte and Tanksley 2000).

If useful markers can be developed for these QTLs, the transfer of early blight resistance into cultivated backgrounds should be facilitated. Because single dominant genes conferring a high level of resistance to early blight have not been identified, the breeder is challenged with transferring and accumulating quantitative genes into a useful horticultural background.

Based on the undesirable linkages and pleiotropic effects generally encountered in such breeding approaches, continued development of acceptable early blight resistant cultivars, particularly early maturing determinate types with high fruit to foliage ratio, will remain a challenge.

Gray leafspot. This warm weather foliar disease can be caused by four species of Stemphyllium: S. solani Weber, S. floridanum
S. botryosum Wallr. and S. vesicarum (Waller.) Simmons (Bashi et al. 1973).

The circular lesions on the foliage are shiny, smooth and gray on the underside of the leaf. Under dry conditions, the lesions become tan in color. When the disease is severe, plants can be defoliated as lesions coalesce. Andrus et al. (1942b) identified resistance in L. pimpinellifolium PI 79532, which was the source of fusarium wilt race 1 resistance (I).

Resistance is conferred by a single incompletely dominant gene Sm (Hendrix and Frazier 1949). The Sm gene is linked to the I gene with a crossover value of 36% (Dennett 1950). Behare et al. (1991) mapped Sm on chromosome 11 between RFLP markers T10 and TG110 at 4.1 and 6.8 cM from the markers, respectively. The distance of Sm from TG105, the marker linked to I-2, was 30.2 cM. The approximate distance to TG523 which marks the I gene is 31.1cM ( Tanksley et al. 1992). These linkages coupled with preferential fertilization for I (Kedar et al. 1967) and Sm (Behare et al. 1991) have contributed to the prevalence of this resistance in tomato varieties. The Sm gene has been widely deployed in cultivars since 1950.
and there are no reports of resistant fungal races overcoming the resistance.

It is a rather rare example of a non-viral, durable, single gene (Parlevliet 2002). A screening method originally developed by Blanchard and Laterrot (1986) was described by Behare et al. (1991). The pathogen is grown on a solid medium consisting of 200 ml of V8 vegetable juice, 2 g of CaCO₃, 18 g of gelose, and 800 ml of dH₂O. Seedlings three weeks past germination are sprayed to runoff with a suspension of 10⁴ conidia/ml. The plants are transferred to a moist chamber at 24 °C, sprayed twice a day with water, covered with a plastic sheet the first four days after inoculation, and illuminated for 12 h with fluorescent light at 4,000 lx. After 10 days plants are assessed for disease severity on a 1-5 scale where 1= coalescence of lesions, 2= numerous lesions, 3= few lesions, 4= rare lesions, and 5= no lesions.

Gray mold. This disease, named for the color of its fluffy mycelial growth, is most common in greenhouse culture under cool conditions. The necrotrophic causal organism Botrytis cineraria Pers.:Fr. affects all above ground plant parts with fruit rots often causing the most serious losses.
However, if stems are infected, often from pruning wounds, girdling can occur and whole plants can be lost. This is especially serious for the intensive production of greenhouse tomatoes. Despite considerable interest in greenhouse tomato breeding, no resistant varieties have been developed due to a lack of resistant sources. Lobo et al. (1986) reported L. esculentum × Lycopersicon esculentum var. cerasiforme hybrid PI 119214 had fruit resistance after puncture inoculation of fruit. Suppression of botrytis fruit rot has also been reported in rin and nor genotypes (Lavy-Meir et al. 1989). Hybrids heterozygous for nor also suppressed symptoms and this appears to be one advantage of nor hybrids in the market. Chetelat et al. (1997) inoculated stems of an intergeneric L. esculentum × Solanum lycopersicoides LA2951 hybrid and found it had resistance. Later, the same hybrid was also found resistant when detached leaves and entire seedlings were inoculated (Guimaraes et al. 2004). In this work the lesion size and spore production were reduced and hyphae were killed in the resistant germplasm. Egashira et al. (2000) tested six tomato cultivars, 44 wild tomato accessions, and S. lycopersicoides accession LA2386 for resistance using leaf and stem assays.
They found no correlation between the assays, but *L. peruvianum* LA2745, *L. hirsutum* LA2314 and *L. pimpinellifolium* LA1246 showed high resistance in both leaflets and stems. Nicot et al. (2002) also found no correlation between stem and leaf reactions. *L. chilense* accession LA1969 and *L. chmielewskii* accession 731089 were in the most resistant categories for both stem and leaf assays of the 19 wild species accessions tested. In this study, LA2745 had no lesions two weeks after inoculation. It was suggested that this was a promising source of resistance in breeding. Although several possible sources of resistance have been reported, much of their breeding application remains to be accomplished. Little is known about how strain affects on resistance. Inoculation methods need to be evaluated against performance in the field and an optimal screening technique adopted. The introgression lines derived from *S. lycopersicoides* LA2951 (Chetelat and Meglic 2000) should be tested for resistance to move the breeding forward. The reported resistance sources should be compared and those with useful resistance need to be introgressed and possibly combined.

Late Blight. Late blight, caused by *Phytophthora infestans*
Mont.) de Bary, is a destructive disease of potato and tomato occurring worldwide. Wet, cloudy periods with cool temperatures are most conducive to development of the disease, and under such conditions, the disease develops rapidly, destroying all above ground plant parts. Single dominant resistant (R) genes conferring a high level of resistance to late blight in potato and tomato are relatively common in wild species.

Late blight resistance in potato follows the typical gene for gene interaction between host plant and disease organism (Al-Kherb et al. 1995). A major shortcoming of the R genes is the ability of the late blight organism to quickly overcome the resistance mechanisms making them ineffective. In potato in particular this is a severe problem since strains capable of overcoming R gene resistances singly or in combinations can readily carry over in potato tubers to infect subsequent crops. The airborne nature of late blight over long distances makes single gene resistance of little value because of the potential for rapid and widespread dissemination of strains capable of overcoming a particular resistance gene. Occurrence of both the A-1 and A-2 mating types of the late blight fungus outside Mexico makes
possible sexual recombination and overwintering of oospores

independent

do live plant tissue. Moderate, or horizontal, resistance
to late blight is of

questionable value because of the short disease cycle,

heavy spore

production of the late blight organism, and the ability of

the disease to be

quickly spread by airborne spores over long distances.

Named genes currently being used in tomato breeding include

Ph-1

(Bonde and Murphy 1952, Gallegly 1952), Ph-2 (Turkensteen

1973, Laterrot

1975), and Ph-3 (Black et al. 1996). Because of the high

level of resistance

conferred by these genes, it is easy to screen for them in

breeding. So far, no

linkage to undesirable traits or adverse pleiotropic

effects have been reported

with the Ph genes. The Ph-1 gene has been of limited value

because of the

rapid occurrence of strains capable of overcoming it

(Conover and Walter

1953). Ph-2 is considered to represent incomplete

resistance (Turkensteen

1973, Laterrot 1975), showing good resistance in early

season but sometimes

failing in late season. Ph-3 provides a very high level of

resistance but like

Ph-1 and Ph-2 has been overcome (Brusca 2003). Varieties

with Ph-1 and

Ph-2 have been developed (Walter 1967, Laterrot 1997) but

do not occupy

an important role in tomato production. All three Ph genes
have been mapped (Fig. 12.1 – bacterial resistance Chapter). Ph-1 was mapped to the distal end of chromosome 7 (Pierce 1971). Ph-2 is flanked by CP105 and TG 233 on chromosome 10 (Moreau et al. 1998). Ph-3 was mapped to TG S91A on chromosome 9 (Chunwongse et al. 2002). A co-dominant PCR marker for Ph-3 has been developed (M. Mutschler, personal communication). Numerous selections from the species L. hirsutum have been identified as highly resistant to late blight. Studies with the accession LA1033 using traditional breeding methods and quantitative trait analysis indicate that its resistance is based on two or more genes (Lough 2003) and that the resistance in an L. esculentum background is not as high as that conferred by the Ph genes (Gardner, unpublished). QTLs were identified for late blight resistance for the L. hirsutum accession LA2099 but were found to be linked to undesirable horticultural traits (Brouwer and St. Clair 2003, Brouwer et al. 2004), indicating a need to develop closely linked molecular markers for the QTLs to efficiently transfer resistance to an acceptable background. Breeding in North Carolina is currently focused on combining late blight resistance with early blight resistance and on investigating the effects of
combining various late blight resistance genes in an effort to increase durability of resistance. Hybrids with a combination of Ph-2 and Ph-3 appear to be useful in this breeding effort (Brusca 2003). However, under ideal environmental conditions for late blight, heavy inoculum pressure, and presence of other lines with the single genes in the same plantings; lines with resistance conferred by the combination of the two genes in both heterozygous and homozygous condition were overcome by late blight at two field locations in North Carolina in the summer of 2004 (Gardner, unpublished). In plantings in North Carolina, a hybrid combining heterozygous Ph-2 and Ph-3 when grown in the absence of lines carrying the single genes, resistance held up throughout the season. Late blight resistance screening techniques using detached leaves or leaflets have been developed. Also, it is easy to select strains of late blight that differentially overcome the known Ph genes. In the North Carolina breeding program, strains specifically overcoming Ph-2 and Ph-3 are main tained by weekly transfers onto live tomato leaves of breeding lines that carry the specific gene overcome by the late blight strain. Whole detached leaves from 6 to 8-week-old plants are inoculated by
atomizing leaves with

a water suspension of 5000 sporangia/ml and incubating the leaves in

clear sealed plastic containers in a growth chamber with 12 hours

nating light (21 °C) and dark (16 °C). Disease ratings are made 5-7 days

after inoculation. By inoculating an F2 population derived from a hybrid

heterozygous for Ph-2 and Ph-3 combined, plants with a very high level of

resistance to combined isolates were identified in detached leaf inocula

tion tests. Progeny testing of F3 lines in the field and F4 lines detached leaf

 inoculations, verified homozygosity for Ph-2 and Ph-3 in some selections. Because the genomes of potato and tomato are closely related and at

least 15 R genes are present in potato (Umaerus et al. 1994, Van der Plank

1971), it is likely that there are numerous other R genes in tomato,

particularly in L. pimpinellifolium from which the three currently identified

Ph genes were derived. Although the use of R genes in potato breeding has

met with limited success because of the lack of durability of resistance

(Black and Gallegly 1957, Ross 1986), the potential usefulness of

combinations of R genes in tomato should still be investigated. Breeding

for late blight resistance in tomato presents a dilemma often faced in the
development of useful resistance to plant diseases. The R genes confer a high level of resistance, are easy to screen and incorporate, and do not appear to be linked to genes with deleterious effects. Their usefulness, however, is limited by the lack of durability of resistance. Horizontal resistance to late blight is likely to be of limited value because the moderate level of resistance conferred is not sufficient to be of value with a disease as aggressive as late blight. In addition, the quantitative resistance sources are more difficult to breed because it is more difficult to develop simple, clearcut screening techniques and because of the increased chances for linkage to undesirable traits or deleterious pleiotropic effects associated with multiple genes. Pyramiding of resistance genes has been proposed for many years as a method of providing more durable disease resistance, and breeders have been criticized for using vertical genes, which can be easily overcome. Therefore, single dominant resistance genes continue to be used almost exclusively for major disease resistances in tomato because of the difficulty of developing commercially acceptable varieties with adequate levels of multiple gene resistance. Marker-assisted
The selection may be of value in developing improved late blight and other disease resistances based on multiple genes if markers tightly linked to QTLs can be identified that facilitate combining multiple resistance genes and reducing linkages to undesirable traits.

Powdery mildew. Historically, the causal organisms of the diseases referred to as powdery mildew were Oidium lycopersici and Leveillula taurica, the latter occurring in subtropical regions. Recently the nomenclature of the former species outside Australia has been changed to O. neolycopersici (Bai 2004). Morphologically the mycelium of L. taurica grows into the leaf mesophyll on the underside of the leaf, while O. neolycopersici grows mainly on the upper side and does not penetrate into the mesophyll (Lindhout et al. 1994). Powdery mildew has spread around the world often in greenhouse production but also in the field in some instances. Resistance to Oidium species has been found in several wild species (Lindhout et al. 1994, Ciccarese et al. 1998, Kozik 1999, Mieslerova et al. 2000). Incompletely dominant genes O1-1 and O1-3 were introgressed from L. hirsutum accessions G1560 and G1.1290, respectively, and they are probably
allelic (Huang et al. 2000). They are flanked by RFLP markers TG153 and TG164 on the long arm of chromosome 6 near the Mi gene. SCAR markers are available and are used for breeding purposes (Huang et al. 2000). The recessive ol-2 gene was derived from L. esculentum var. cerasiforme and has been mapped to chromosome 4. CAPS and AFLP markers are available for marker-assisted selection (De Giovanni et al. 2004). Recently, Bai et al. (2003) found three QTLs linked to quantitative genes derived from L. parviflorum accession G1.1601 that could be added to other resistance genes to provide more durable resistance. One was on chromosome 6 in the same region as Ol-1 and Ol-3 and two were on chromosome 12 with one being in the region of Lv (see below). Bai et al. (2003) also mapped a gene (Ol-4) from L. peruvianum accession LA2172 to chromosome 6 in the region near markers Aps-1, TG153, and TG25 and close to Ol-1. Another gene (Ol-5) from L. hirsutum PI 247087 was mapped to chromosome 6 near marker TG 25 and between Ol-4 and Ol-1/Ol-3 (Bai 2004). Dominant Ol genes conferred isolate dependent hypersensitive resistance that was whole the recessive Ol-2
gene associated with papillae formation (Bai et al. 2005). A resistance gene to L. taurica (Lv) was first introgressed from L. chilense accession LA 1969 by Yordanov et al. (1975). Later this gene was named Lv by Stamova and Yordanov (1987). More recently it was fine mapped to chromosome 12 in a 0.04 cM interval between RFLP markers CT121 and CT129 with CT 121 being only 0.16 cM from the gene (Chunwongse et al. 1997). PCR markers are presently being used by tomato breeders to incorporate this resistance gene into tomato varieties (Barten, pers. comm.). Disease screening is generally done by washing spores from infected leaves and adjusting the suspension to 2x10^4 spores/ml. Plants to be tested are about one month old and have 3-4 true leaves. They are sprayed with the spore suspension and grown in greenhouses at about 20 o C ± 3 with 30-70% RH. Fungal growth is rated at intervals from 10-20 days later on a scale where 0= no sporulation; 1 = slight sporulation with less than 5% of the foliage affected, 2= moderate sporulation with 5-30% of the area affected; and 3 = abundant sporulation with over 30% of the area affected (Bai 2004).

Septoria leaf spot. This disease caused by Septoria lycopersici Speg. infects tomatoes in eastern Canada and the United States. Barksdale
and Stoner (1978) reported resistance in L. pimpinellifolium accession PI 422397 that was controlled by a single dominant gene. Later, it was reported that this resistance was not incorporated into cultivars because the resistance was moderate and associated with small fruit size and lateness (Poysa and Tu 1993). To date we are not aware of any resistant cultivars. Screening for resistance can be accomplished by a brushing method (Tu and Poysa 1990). Spore suspensions were prepared from 3-wk-old colonies on PDA; 5 ml of sterile distilled water was added to each plate and the surface of the culture was scraped to dislodge the spores. Conditions considered optimal for inoculation were an inoculum concentration of 10^6 spores/ml, temperature at 24 o C, moisture period of 48 h, and a photoperiod of 14 h. The brushing of adaxial and abaxial leaf surfaces of plants at the three-leaf stage with a camel hair brush was superior to spraying or dipping methods tested. Disease scoring was done 8 to 15 days after inoculation on a 0-9 scale where 0= <10% leaf area with symptoms, 1 = 10-20%, 2= 20-30% to 9=90-100%. However, in a later report, the same authors used the method of Gardner (1990) to inoculate over 500 breeding
lines and over 200 wild species to find resistance (Tu and Poysa 1993).

Presumably this method was used because it is easier (see the early blight section for this procedure.). All tomato varieties and accessions tested were susceptible. Highest levels of resistance (<3) were found in L. hirsutum accessions LA 2100, LA 2650, LA 2204, and PE-36; in L. peruvianum accessions LA 1675, LA 1360, PI 251307, PE-33, PI 270435, PI 390655; and L. pennellii accession PE-44. High resistance (3-3.9) was found in L. hirsutum accessions LA 1366, LA 2124, and LA 255; and in L. peruvianum accessions LA 1910, LA 1292, LA 1304, LA 1365, PI 128654, PI 390671, PI 365951, and PE-32. Poysa and Tu (1993) also found inter specific hybrids from resistant parents sensitive parents were resistant, indicating dominant inheritance of this trait.

Target spot. This foliar disease is caused by Corynespora cassicola (Berk & Curt.). It can be controlled by fungicides if detected early, but if not, it can cause serious problems with defoliation and fruit rots. Bliss et al. (1973) tested 242 accessions and reported high resistance from L. esculentum PI 120265 and L. pimpinellifolium PI 112215. Slight resistance was reported in
12 other accessions of the above two species. The high resistance lines have been effective in Florida (Scott, unpublished data). The resistance from PI 120265 and PI 112215 was conferred by the same incompletely dominant gene (Bliss et al. 1973). No highly resistant varieties have been developed, but Blazquez (1972) reported ‘Homestead 24’ was less susceptible than ‘Bonny Best’ indicating that some partial resistance may be present in some varieties. Disease screening has been done in greenhouses by inoculating plants at the four-leaf stage with a suspension of 80,000 spores/ml mixed with one drop/100 ml Tween 80 as a wetting agent. The plants were sprayed until wet and placed in a mist chamber at 20-24 °C for 24 h. The disease reaction could be scored 36 h later. Inoculum was prepared by washing canisters from 5-day old cultures on PDA plates exposed to continuous, white fluorescent light at 2,690 lux. Inoculum was then filtered through cheesecloth (Bliss et al. 1973).

FRUIT

Anthracnose. Anthracnose of tomato is caused by several species of the genus Colletotrichum, including C. coccodes (Wallr.), C. dematium (Pers.) Grove,
and C. gloeosporioides (Penz.) Penz. & Sacc. C. coccodes is most commonly associated with fruit anthracnose symptoms which are sunken, dark, circular lesions. Symptoms express on ripe fruit and the disease is primarily a problem on processing tomatoes that are being ripened on the vine in humid regions such as the Northeast and Midwestern US. It is also found in Asia, Europe, Africa, and the East Indies. Screening for fruit rot is done by placing a suspension droplet of the pathogen at 5,000 spores/ml on red ripe fruit with a hypodermic needle and then pricking the epidermis under the droplet with the needle (Robbins and Angell 1970a). This can be done at room temperature and humidity. Lesion size is measured nine days after inoculation. Susceptible lines have larger lesions and resistant lines have smaller or no lesions. The method overcame the problem of other methods where lines selected for resistance turned out to be susceptible in the field. Resistant fruit using this technique apparently have an internal environment that is not conducive to growth of the pathogen. Resistance to anthracnose caused by C. coccodes was first reported in L. pimpinellifolium PI 127833 and L. esculentum × L. pimpinellifolium PI 129027, in 1964, according to Robbins and Angell (1970b).
Resistance reported in 

L. esculentum accession PI 272636 (Barksdale 1971) and PI 272636 was also

found to be resistant to five other Colletotrichum spp. fungi that cause

anthracnose (Barksdale 1972). Robbins and Angell (1970b) found resistance

from PI 129027 and PI 127833 was partially dominant and controlled by

six genes. All other genetic studies have been based on resistance derived

from PI 272636. Miller et al. (1983) studied this resistance in a diallel using

C. dematium as the pathogen and found general combining ability (GCA)

was highly significant while specific combining ability (SCA) was smaller

but also significant. There was partial dominance for susceptibility. Narrow

sense heritability was 70% over the 2 locations in the experiment. A study

comparing C. coccodes and C. dematium had similar but not identical results

as SCA was not significant; narrow sense heritability was 78% for the

former and 64% for the latter (Miller et al. 1984). Later Ng et al. (1990) did

a generation means analysis using C. dematium as the pathogen and found

inheritance was primarily additive but there was also dominance and

homozygous x homozygous epistasis. Broad-sense heritability was 0.57
while narrow-sense heritability was 0.42. Stommel and Haynes (1998) did a genetic study using an advanced breeding line, 88B147, inoculated with C. coccodes. They found partial dominance for resistance. An additive dominance model with an additive x additive (homozygous x homozygous) epistatic interaction best explained the data. Broad sense heritability was 0.42 and narrow sense heritability was only 0.004. One gene or linkage group was estimated to control resistance. However, Stommel and Zhang (1998, 2001) found RAPD’s linked to resistance in an F 2 population derived from 88B147 and reported at least 3 regions were associated with resistance.

Later, Stommel (2001) studied inheritance using three breeding lines at various stages of horticultural advancement, 88B147 being the most advanced line. Heritability and gene number estimates declined as the level of resistance declined in more horticulturally advanced lines.

Inheritance was primarily additive. This may help to explain some of the discrepancies between studies. It has been difficult to transfer resistance from the small fruited wild species into advanced breeding lines and varieties because of the polygenic nature of the resistance where each gene
has a small effect (Stommel 2001). This is like an example of linkage drag
problems that hamper the development of varieties with quantitative resistance as discussed by Scott (2005) for early blight.

Black mold and Phoma. As indicated for anthracnose, breeding for resis
tance to fruit rots is difficult, varieties with such resistances are lacking.

There has been some work done with black mold incited by Alternaria
alternata (Fr.) Keissler (Cassol and St. Clair 1994, Robert et al. 2001) and
Phoma andina (Lobo et al. 1987, 1988). Readers interested in resistance to


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INTRODUCTION

As with other tomato disease resistances, breeders have developed cultivars with virus resistances that are conferred by single dominant genes. In the previous chapter, Table 13.1 provides a summary of the virus resistance genes that have been utilized in breeding. Cultivars with tomato mosaic virus resistance are common, especially in greenhouse tomatoes. Some varieties with spotted wilt virus resistance have been developed although virulent races have overcome many of the genes deployed in the past. More recently, cultivars with resistance to tomato yellow leaf curl virus (TYLCV) have been developed and the best of these utilize major vertical genes as opposed to horizontal genes. More details are available in the sections for each of these diseases. There has
been considerable work done on tomato virus resistance using genetic
transformation and the potential for commercial impact from these
approaches may be nearer than is the case for other types of pathogens.

But cultivars have yet to be deployed with genetically engineered
mentioned but an adequate treatment of this topic is beyond the scope of
this writing. Several book chapters have information on tomato virus
and other resistance(s) (Dixon 1981, Tigchelaar 1986, Watterson 1986,
Lukyanenko 1991). Herein, the primary goal will be to relay new
information and provide reasonably thorough coverage of virus diseases
where genetic information on resistance has been reported. Information
on sources of resistance, inheritance, virus strains, inoculation techniques,
and the status of breeding efforts will be given for each disease that are
presented alphabetically.

Alfalfa mosaic virus (AMV). This virus is grouped in the genus Alfamovirus
and through natural processes infects over 600 plant species of 250 genera
belonging to 70 families (Parrella et al. 1997a). It is not considered a major
tomato pathogen although infections have been reported in several countries
and usually connected to growing tomatoes in the presence of nearby alfalfa crops (Zitter 1991). Recently, necrotic strains have emerged that have caused more serious disease outbreaks (Parrella et al. 2000). The disease is spread by 22 species of aphids. Symptoms range from bright yellow mosaic on leaves with necrotic patches and deformation of fruit for non-necrotic strains to severe necrosis of the leaves, and fruits in the case of necrotic strains. Early infection with necrotic strains can result in plant death. Strains have been grouped into two phylogenetic groups, subgroup I and subgroup II, based on coat protein sequences which reflect mainly a geographic structure (Parrella et al. 2000). Resistance was identified in three accessions of L. hirsutum PI 134417, LA 1777, and Bruinsma (Parrella et al. 1997a). A single dominant resistance gene, Am, from PI 134417 was described against necrotic strain LYH-1 (Parrella et al. 1998). Recently Am was mapped to the short arm of chromosome 6 near the centromere at 1.1 cm from RFLP markers CT21 and TG 232 (Parrella et al. 2004). The location of Am is in a major disease resistance hot spot (Fig. 12.1, bacterial resistance chapter). Preliminary indications in this work showed Am was
resistant to 16
diverse strains of AMV, but did not provide protection to unrelated viruses
CMV, TSWV, TMV, or PVY when co-inoculated. The mechanical inoculation procedure was described by Parrella et al. (1997a). Virus strains are propagated in Nicotiana tabacum ‘Xanthi n.c.’ for 15 to 20 days before inoculation to obtain a high titer. Inoculum is prepared by grinding 1g of young tobacco leaves in 4 ml of a solution containing 0.03M Na 2 HPO 4 with 0.2% sodium diethyldithiocarbamate.
Before inoculation, 75 mg/ml of both carborundum and activated charcoal was added to avoid virus inactivation. Plants are grown in a growth chamber with a 16 h photoperiod and temperatures of 24 o C day and 16 o C night for 16 days. Inoculum is then rubbed on cotyledons. Symptoms can be rated about two weeks later on susceptible lines while there are no symptoms on the resistant plants. Symptomless plants can also be checked by ELISA (Parrella et al. 1997a). The resistance was reported to be of the extreme resistance (ER) type and not a hypersensitive type (Parrella et al. 2004).
Cucumber mosaic virus (CMV). The type member of the Cucumovirus group (Murphy et al. 1995), CMV is an RNA virus with some
strains that have satellite RNA that modulates CMV symptoms. This aphid transmitted disease has a very broad host range and many strains. The strains have been divided into subgroups I and II based on nucleic acid or protein sequence composition and on serological studies (see Kaniewski et al. 1999).

Strains from both subgroups infect tomato. Symptoms in tomato range from severe stunting, fern leaf, filiform leaves, and necrosis. Resistance or tolerance has been reported in many wild tomato species including L. pimpinellifolium, L. peruvianum, L. hirsutum, L. cheesmanii var. minor, L. chilense, and Solanum lycopersicoides (Phillis et al. 1977, Gebre et al. 1990, Nitzany 1992, Stoimenova et al. 1992, Parella et al. 1997b, Cillo et al. 2005). Inheritance of resistance from these species is largely unknown and no cultivars have yet been developed. L. hirsutum PI 247087 expressed resistance most clearly in plants that were 45 days old when inoculated and grown at 20°C. Resistance appeared to be controlled by a single gene (Parella et al. 1997b). More recently, a single dominant resistance gene Cmr was introgressed from L. chilense accession LA458 and mapped to
chromosome 12 (Stamova and Chetelat 2000; Fig. 12.1
bacterial resistance

chapter). Seven RFLP markers spanning 24.7 cM were
associated with

resistance. There was no clear indication of where in this
region the

resistance gene was and possibly there could even be two
linked genes in

the region. Nevertheless, CMV resistance was most strongly
associated

with markers TG68 and CT79 that are 4.3 cM apart.
Interestingly, the

powdery mildew resistance gene Lv, also introgressed from
L. chilense, is

linked to markers in the same general region indicating
presence of a

resistance gene cluster in this region. Stamova and
Chetelat (2000) reported

that there was recombination between the two resistances
indicating the

linkage was not very tight. Cmr provided resistance that
was not always

complete and the virus was isolated from resistant plants
indicating

immunity was not involved. Pathogen-derived resistance has
shown considerable promise for

developing broad spectrum resistance to CMV. Approaches
using coat

protein genes appear to be the most effective. There is
considerable

literature in this area that is beyond the scope of this
chapter. Two coat

proteins from subgroups I and II with a single transgene
copy worked
well in providing resistance to multiple isolates via mechanical or aphid transmission (Kaniewski et al. 1999). Fuchs et al. (1996) developed lines with one coat protein gene that provided immunity to a range of isolates in field tests. An inoculation procedure was described by Stamova and Chetelat (2000).

The pathogen was maintained on small pumpkin seedlings, at the cotyledon to second leaf stage, by rub inoculation. The leaves were first dusted with Celite abrasive powder. The inoculum was a 1:10 (w/v) ratio of young symptomatic leaves in freshly prepared ice-cold grinding buffer (1:1-0.03 M potassium phosphate buffer, pH 7 and 0.1% sodium sulfite). Seedlings of tomato were inoculated at the first to second true leaf stage, followed by a second inoculation a week later to reduce the chance of escapes. Disease symptoms were scored 2-3 weeks after the last inoculation.

Curly top virus (CTV). This is a member of the Geminiviridae genus and has also been known as beet curly top virus (BCTV). It is vectored by leafhoppers (Circulifer tenellus Baker). If tomato plants are infected early they are severely stunted, epinastic, and often die so no fruits are
produced. This disease has been a problem in semiarid regions from Mexico to Canada and in the Mediterranean basin (Zitter 1991). Resistance work started in 1930 in Utah with Loran Blood and then was carried on by his successor Oscar Cannon (Martin et al. 1971). Thereafter, Mark Martin of the USDA at Washington State University pursued this work. The resistance that was developed is based on an ability to escape infection from the leafhopper as opposed to a tolerance since once plants become infected the symptoms in resistant lines are as severe as in susceptible lines. The resistance was derived from crosses involving several wild species including L. peruvianum var. dentatum PI 120660, L. peruvianum var. humifusum PI 127829, L. hirsutum PI 127826 and L. pimpinellifolium Utah 45 (Martin 1963, Martin and Clark 1966, Martin et al. 1971). Breeding lines CVF4 and C5 from this program were released in the late 1960’s (Martin 1966, Martin and Thomas 1969) and factors involved in the utility of this resistance were discussed in a later paper (Martin and Thomas 1986). The ability to escape infection is multigenically inherited and closely linked with undesirable characters (see Martin and Thomas
Younger plants are more susceptible than older plants. Useful resistance for commercial production can be overwhelmed by severe seedling tests that have greater exposure than that normally seen in the field. Thus, field testing was suggested with an early and late rating of disease incidence. Once infected, plants generally die and thus the need for the early rating. Since the mechanism is escape rather than tolerance, rating disease severity is not useful. Repeating plantings at different times in a season was suggested to elucidate useful, moderate levels of resistance.

It was suggested that a range of resistance levels from the cultigens C5, C27, C193, CVF4, C22, ‘VF145’, and ‘VR Moscow’ would provide resistance to tomato mottle virus (ToMoV) and they were all very susceptible (Scott and Schuster 1991). Resistance from L. chilense accessions have been shown to have resistance to several other tomato viruses as indicated in this writing. It would be interesting to test L. chilense accessions and/or early generation lines derived from them.
for CTV

resistance, either of the escape type or for actual
tolerance. If tolerance
could be found, combining it with the ability to escape
infection might

provide superior resistance most. Also Martin (1962)
reported resistance

in *Solanum pennellii* (later=*L. pennellii*) and this may well
relate to the

insect resistance of that species. Thus, tomato germplasm
derived from

*L. pennellii* for resistance to other insects (Liedl et al.
1995) might also

have some resistance to CTV as would insect resistant lines
derived from

*L. hirsutum* (Muigai et al. 2003). Martin (1962) also
reported that *S.

lycopersicoides* Dun. had a high level of CTV resistance and
thus it would

be worth testing the introgression lines now available at
the Tomato

Genetic Resource Center for resistance (Chetelat and Meglic
2000).

Pepino mosaic virus (PepMV). This member of the Potexvirus
genus in the

family Potexviridae was first reported on pepino (*Solanum
muricatum*) in

Peru in 1980 (Jones et al. 1980). It was first reported on
greenhouse tomatoes

in the Netherlands and the U.K. in 1999 (Van der Vlugt et
al. 2000). Since

then it has rapidly spread to greenhouse tomato crops in
much of Europe
(Verhoeven et al. 2003) and, North and South America (French et al. 2001, Soler et al. 2002). The rapid spread is likely due to the ease of mechanical transmission and the ability of the virus to survive in soil and on planting tools for extended periods. There is a high sequence similarity (96-97\%) between the Peruvian pepino strain and European isolates, but there is greater sequence variability in tomato isolates from the United States (Maroon-Lango et al. 2003, Ling, unpublished data).

Symptoms on tomato vary depending on the age of infection, the cultivar, and environmental conditions. Often symptoms do not appear until up to six weeks after infection and could be most severe on plants that are under some type of growth stress. Infected plants grown under optimal conditions often become symptomless after showing symptoms earlier. Generally young plants develop yellow mosaic that may lead to bright yellow spots or patches with severe leaf distortion or blistering on older leaves. Sometimes the infected leaflets become narrower and pointed so the growing point resembles a nettlehead. Mature fruits may show uneven ripening that resembles marbling. Yield losses of 15\% have been reported in the U.K.
PepMV is also associated with the collapse syndrome recently reported by Soler-Aleixandre et al. (2005a). There has been little published information on resistance to PepMV mosaic virus. Picó et al. (2002) indicated partial resistance to Spanish isolates existed in L. chilense accession LA1963 and L. peruvianum accessions PI 126944 and LA1708. Little is known about the total number and type of accessions that have been tested and of the importance of strain effects on resistance. Recently, Soler-Aleixandre et al. (2005b) reported some resistance in several accessions with the best resistance in L. chilense accession LA470. Undoubtedly more information will emerge on resistance to this disease since there is much current concern over it.

In 2000, it was put under quarantine in the European Community and seedling transplants must be tested by ELISA before shipment. In 2003, it was put on the European and Mediterranean Plant Protection Organization Alert list which monitors diseases considered a serious risk to crop production. For the mechanical inoculation procedure (Ling, personnel communication), the virus isolate is maintained and propagated on susceptible tomato plants or N. benthamiana. Virus inoculum is prepared.
by grinding the leaf tissue 1:10 (w/v) in phosphate buffered saline.

Inoculum is rubbed gently on carborundum-dusted cotyledons and the first true leaf with a cotton swab. Plants are then grown in a greenhouse at 25-30 oC and fertilized weekly (20:20:20) to promote symptoms for up to a six week period. Plants are rated on a scale where 0= no symptoms; 1= light yellow mosaic symptoms; 2= strong yellowing patches with leaf curling; and 3= leaf curling and puckering, plant stunting. ELISA tests with PepMV specific antiserum can be performed on symptomless plants.

Potato virus X (PVX). This Potexvirus is a disease that is often latent in potato where it causes mosaic symptoms. In tomato it causes mosaic symptoms and slight stunting. When cultivars are infected with both ToMV and PVX whereby a more serious disease than either alone called double streak results (Watterson 1986, Zitter 1991). There has been very little reported on PVX resistance. It has been a troublesome disease when tomatoes and potatoes are grown at the same time or sequentially in a region. Such is the case in Pakistan where cultivars were tested and three: Tobol, Turquesa, and Parana had moderate resistance (Rashid
Lower virus titers were found in plants of these cultivars than in plants of susceptible cultivars. A high level of resistance to a transformed PVX was shown in some intriguing experiments reported by Tobias et al. (1999). The bacterial speck Pseudomonas syringae pv. tomato resistance gene Pto provides resistance to bacterial strains (see chapter 12) that have the avirulence gene avrPto. The avrPto gene was cloned to a PVX vector and it was shown that when this derivative was inoculated onto tomato plants only those without the Pto gene were infected. The recognition of the avirulence gene by plants with Pto prevented the PVX virus from being expressed.

This work is of interest from a pathogen recognition/plant defense standpoint but will not be helpful for all the wild PVX strains that do not have the avrPto gene. In some related work, gene Prf, which recognizes a bacterial pathogen, provides resistance to both bacterial and viral pathogens (Oldroyd and Staskawicz 1998). Inoculation is done mechanically. Rashid et al. (1989) maintained the pathogen on Datura stramonium plants. Leaves were mixed in a 0.02 M phosphate buffer, with pH 7.0, in a 1:1 w/v ratio. Plants were inoculated
at the 4 to 5 leaf stage by rubbing one leaf with the inoculum. Symptoms were expressed 5-6 days later. Tobias et al. (1999) used a phosphate buffer with other ingredients and inoculated plants by gently rubbing the upper surface of cotyledons with a gloved finger.

Potato virus Y (PVY). This Potyvirus is also known as vein banding mosaic. It causes problems primarily in tropical and subtropical regions, has a narrow host range, and is transmitted in a non-persistent fashion by aphids (Watterson 1986, Zitter 1991). Various sources of resistance have been reported and many appear to be strain specific (Legnani et al. 1997). Nagai et al. (1992) incorporated resistance from PI 126410 into ‘Santa Cruz’ and developed the resistant cultivar 'Angela' that was widely grown in Brazil. They reported resistance was conferred by two recessive genes and that these were the same genes as in L. pimpinellifolium accession NAV 1062. PI 128887 which was resistant in Florida was susceptible to the Brazilian isolate tested. Thomas (1981), using greenhouse and field inoculations, tested 14 accessions with reported resistance to potyviruses (PVY, Peru tomato virus, tobacco etch virus) and found uniform resistance only in L.
hirsutum accession PI 247087. Later, Thomas and McGrath (1988) tested PI 247087 against 10 Australian isolates and it was resistant to all of them. In an interspecific inheritance study it appeared the resistance was controlled by a single recessive gene, but there were fewer resistant plants than expected in the backcross to the resistant parent. This could be related to disturbance in gametes due to the interspecific nature of the study. In another study, Legnani et al (1995) determined that PVY resistance from PI 247087 was conferred by one or two recessive genes depending on the strain used. Parrella et al. (2002) used AFLP markers and a set of L. hirsutum introgression lines (Monforte and Tanksley 2000) to map the recessive gene pot-1 from PI 247087 to a 10cM interval on the short arm of chromosome 3 near the corky root rot resistance locus py-1. The pot-1 gene was flanked by RFLP markers TG135 and CT31 at distances of 2.1 and 4.6 cM, respectively. Green and Hanson (1996) reported PI 247087 was resistant to Taiwan and Australian isolates but was susceptible to isolates from Hawaii and Thailand. In addition they screened 169 accessions from several Lycopersicon species and found that L. hirsutum L3683 (PI 365904)
was resistant (no disease symptoms and ELISA negative). Furthermore,

L3683 was resistant to isolates from Australia, Hawaii, California,

and Thailand. The F1 was susceptible suggesting resistance was

recessive. Inoculation is the same as that described for tobacco etch

virus.

Tobacco etch virus (TEV). This aphid transmitted Potyvirus causes losses

in tobacco, pepper, and tomato. It has been found on tomato in Florida,

South America, Cuba, the Philippines, Taiwan, Thailand, and Turkey

(see Legnani et al. 1996). Both leaves and fruits are mottled and plants

are stunted. There are no reports of cultivars with resistance although

several reports have identified sources of resistance. These include PI

183692 (Walter 1956); PI 166989 and L. hirsutum accessions PI 134417,

and PI 127827 (Alexander and Hoover 1955), and L. hirsutum PI 247087,


The latter report indicated that after inoculation the virus was undetectable

by ELISA in PI 247087 but virus was detectable in LA 1478 and LA 716.

They also reported PI 134417 was susceptible possibly meaning that the
reported resistance from this PI is strain specific. PI 247087 was resistant
to all four strains that were tested. Even with PI 247087, virus was
recovered by back inoculations to tobacco plants. Thus, the resistance
mechanism does not provide immunity but impairs virus
multiplication
and/or virus migration from cell to cell and therefore prevents systemic
spread of the virus. Inheritance of resistance from PI 247087 was conferred
by a single recessive gene in an intraspecific L. hirsutum
population (Legnani et al. 1996). PI 247807 also had resistance to PVY and CMV
as discussed
in those sections of this chapter. Susequently, Parrella et al. (2002) found
the pot-1 gene from PI 247807 conferred resistance to both PVY and TEV.
Further information on the pot-1 gene is presented in the section on PVY.
The author is not aware of work indicating if pot-1 confers resistance to
CMV. The inoculation procedure was described by Legnani et al. (1996). Virus
isolate(s) are inoculated to Datura stramonium 15 days before inoculation
of tomato plants. One gram of young leaves of Datura were ground in 4
ml of 0.03 M Na 2 HPO 4 containing 0.2% sodium
diethyldithiocarbamate.
Carborundum and charcoal each at 75 mg/ml were added to the
solution before inoculation. The inoculum was rubbed on the first three leaves of 21 day old plants. After inoculation the plants were grown at 25 °C with 16 h days. Symptoms were evident before 15 days and correlated well with ELISA data at 45 days after inoculation. Thirty days after inoculation may give similar results to 45 days.

Tomato mosaic virus (ToMV). This virus is closely related to tobacco mosaic virus (TMV) and it used to be commonly referred to as the latter. These Tobamovirus species can be distinguished from each other by differences in their serological affinities and protein compositions. ToMV has been shown to be the predominant virus in tomato crops around the world (Brunt 1986). Historically ToMV has caused considerable crop damage especially under the intensive culture systems used in greenhouse tomato production where it can easily be spread mechanically by pruning and training operations. The disease can also be spread by infected seed transmission where the virus is on seed coats. The virus can be removed by treating seed with tri-sodium phosphate during the seed extraction process. Resistance to ToMV or TMV was first reported in
1939 (Porte 1939) and the early work was reviewed by Pelham (1966). Since then ToMV resistance work has been summarized by Lukyanenko (1991) and the reader is referred to these publications as it is not the intent of this writing to repeat much of the information reported in these publications.

Numerous cultivars with ToMV resistance have been released including all modern greenhouse cultivars and a number of field and home garden cultivars. Three dominant resistance genes have been named and used in the development of resistant cultivars, Tm-1, Tm2, and Tm2 2 (Tm2 a). The Tm 1 gene was named in 1960 and was made available in PI 235673, an L. esculentum accession. The source of resistance from the breeding program at Hawaii was not clear as the pedigree involved several wild species (Pelham 1966). Later evidence strongly supported L. hirsutum as the source since the ribosomal DNA sequence of an iso-line with Tm-1 was similar to that of two L. hirsutum accessions (Levesque et al. 1990). Tanksley et al. (1992) mapped Tm-1 to chromosome 2 (Fig. 12.1 bacterial resistance chapter). Japanese researchers later developed two co-dominant SCAR
markers linked to Tm-1 that would be useful for marker assisted breeding

(Ohmori et al. 1996). The Hawaiian program of Frasier was also the source

of the Tm-2 gene that was named by Clayberg et al. (1960). This gene was

tightly linked to the deleterious netted virescent (nv) gene that causes

stunting and yellowing when homozygous. Breeding work with this gene

was not successful because the linkage could not be broken. However,

Laterrot and Pecaut (1969) developed a line called Perou 2, derived from L.

peruvianum PI 126926, that had the Tm-2 allele without nv. This is the

source of many cultivars that possess the Tm-2 gene. The Tm-2 gene was

also incorporated with the Fusarium crown rot resistance gene Frl that

originated from IRB-301 (Vakalounakis et al. 1997; fusarium crown rot

section of Chapter 13). In 1963, another resistance gene was discovered

that was introgressed from L. peruvianum PI 128650 (Alexander 1963).

Pecaut (1965) determined that this gene was allelic to Tm-2 and named it

Tm2 2 although Cirulli and Alexander (1969) later suggested Tm2 a was more

appropriate because it might be a different gene linked to Tm2 and not an

allele. They cite its more broad resistance against pathogen strains as
another reason for Tm2 a designation. Young et al. (1988) used isogenic
lines with and without Tm2 a to map the locus in a 0.4 cM
region of
chromosome 9 near RFLP markers TG101 and TG79. McRitchie
and Alexander (1963) reported there were four strains of
the virus, designated I, II, III, and IV, based on their
symptoms on host
genotypes. Pelham (1969) later indicated they had four
races designated
0, 1, 2, and 1.2 based on reactions to the resistance
genes. Merging the
two systems, I and II = 0, III = 1, IV = 2 while 1.2 is a
new race. Cirulli
and Alexander (1969) reported a fifth strain (V) in Ohio
that was later
determined to be the same as strain 1.2 (Pelham 1972). The
Pelham strain
designations refer to the resistance genes they are
virulent on. Thus strain
1 isolates are virulent on Tm-1, strain 2 isolates are
virulent on Tm-2, and
strain 1.2 isolates are virulent on Tm-1 and Tm-2. Systemic
necrosis is a deleterious resistance reaction that causes
more
severe symptoms than does a susceptible reaction. There is
considerable
literature on the systemic necrosis response of tomato
genotypes and the
reader is referred to the paper of Hall (1980) for more
information on this
topic. Reactions of tomato lines with various resistance
genes against the
strains of the virus are summarized in Table 14.1. It can be seen that

genotypes heterozygous for Tm-2 or Tm-2 2 can exhibit systemic necrosis

for some virus strains. The occurrence of systemic necrosis is contingent on several factors; temperature, virus isolate and strain, plant age,

resistance genotype including cytoplasmic factors, and the number and combinations of resistance alleles (Hall 1980). Systemic necrosis occurs more commonly under high temperatures (>26 o C) and with genotypes heterozygous for Tm-2 or Tm-2 2 . Kopeliovich et al. (1978) found heterozygotes with resistance on the seed parent side of the cross had more necrosis than their reciprocals. Thus, if cultivars are heterozygous for the above two genes the susceptible parent should be used as the seed parent and the resistant parent as the pollen parent. However, several researchers advise against using heterozygous resistant cultivars because of the potential problem. Many cultivars today are homozygous for resistance at the Tm-2 locus as years of breeding has eliminated the undesirable traits that were linked to the resistance genes early on (Alexander 1971, Laterrot 1971). It should also be noted that systemic
necrosis can occur in homozygous resistant genotypes but this occurred with less frequency and under more severe conditions than with heterozygotes (Hall 1980). To insure against this it is suggested that resistance genes be combined. Thus, when cultivars are homozygous at

Table 14.1 Disease reactions z for tomato genotypes against various tomato strains. (Modified from Hall and Bowes 1980: as presented by Lukyanenko 1991) Resistance genotypes ToMV Tm-1/+ Tm-2/+ TmTm2 z /+ Tm2 z /Tm2 z Tm1/+Tm2/+ Tm1/+Tm strain y 2/Tm-2 2/Tm2 z 0 T R* R R* R R R 1 S R* R R* R R 2 T S S R* x R R 1.2 S S S R* R S R* y Strains 0, 1, 2, and 1.2 are equivalent to Ohio strains 1 & II, III, IV, and V, respectively, see z S = susceptible – severe mosaic symptoms and stunting T = tolerance – mild mosaic symptoms, little effect on growth. R = resistant, no symptoms. R* = A hypersensitive, deleterious, systemic necrosis reaction can occur under high temperatures. x Cirulli and Alexander (1969) did not find systemic necrosis for this genotype with this strain. the Tm-2 locus adding Tm-1/+ will prevent systemic necrosis from being caused by any isolates controlled by Tm-1 (Hall 1980). The Tm-2 2 gene has proven to be a very durable resistance gene effective against all pathogen strains. It has been widely deployed in cultivars since
1970 and there are no published reports of virus strains that have become virulent on it and persisted in the environment. Hall (1980) theorizes that if Tm-2 2 mutated from the Tm-2 allele, it would take at least two steps toward virulence for the pathogen to overcome it. Another option for resistance breeding is via development of transgenic tomatoes expressing the ToMV coat protein gene (Sanders et al. 1992). Mechanical inoculation is used to select for resistance in breeding programs using strains controlled by the gene(s) being used. Tobacco plants Nicotiana tabacum n n (systemic) are inoculated with the pathogen when they have 5-6 true leaves. Once the plants begin flowering and all leaves are infected, leaves are removed, placed in plastic bags, and frozen until needed. Five to 10 leaves are blended in a Waring blender with enough dH 2 O to make 50 ml. The mixture is strained through cheesecloth, diluted 1:5 with dH 2 O and a pinch of carborundum (600 mesh) is added. If not used right away the inoculum can be stored in a refrigerator. Tomatoes to be inoculated are at the first true leaf stage. A cotton swab is dipped in the inoculum and rubbed over the cotyledons or the true leaves. Symptoms can be evaluated in three weeks. To
systemic necrosis of heterozygous plants the screen should be done under high temperatures (>30 °C) in a growth chamber.

Tomato Spotted wilt virus (TSWV). This is the type species of the Tospovirus genus in the Bunaviridae family and it has a broad host range attacking at least 1090 plant species from over 92 botanical families from both the monocots and dicots (Parrella 2003). It is transmitted by at least seven thrips species, Thrips tabaci (Pittman 1927), T. palmi (Iwaki et al. 1984), T. setosus (Kobatake et al. 1984), Scirtothrips dorsalis (Amin et al. 1981), Frankliniella schultzei (Samuel et al. 1930), F. fusca (Sakimura 1963), and F. occidentalis (Gardner et al. 1935). Various strains have been identified and are categorized by their symptoms and severity. The most stable are TB (tip blight), N (necrotic), R (ringspot), M (mild), VM (very mild) (Dixon 1981). Today pathogens are categorized as serial groups species based on DNA sequences-and there are now 13-15 species. Some of the early resistance work was reviewed by Stevens et al. (1992) and the genes were derived from L. pimpinellifolium. Finlay (1953) tested resistance sources against 10 strains in Australia and
identified two dominant resistance alleles at the Sw-1 locus (Sw-a, Sw-1 b) and three recessive resistance genes: sw-2, sw-3, and sw-4. According to the gene list published in 1959 the cultivar ‘Pearl Harbor’ has Sw-a and ‘Rey de los Tempranos’ has Sw-1 b and the three recessive genes (Clayberg et al. 1959).

These genes were strain specific in their effects. Paterson et al. (1989) tested the resistant sources of Finlay and found them to be susceptible to isolates in Arkansas. In 1986 resistance was reported in the South African cultivar ‘Stevens’ with resistance being introgressed from L. peruvianum (van Zijl et al. 1986). The resistant accession used in ‘Stevens’ is not known for sure but it may be PI 128654 (see Stevens et al. 1992). Stevens et al. (1992) determined that the resistance from ‘Stevens’ was conferred by a single dominant gene, named it Sw-5, and found it not to be race specific. Boiteux and Giordano (1993) found Sw-5 also provided resistance to the tospoviruses chlorotic spot virus (TCSV) and groundnut ring spot virus (GRSV). The Sw-5 gene has been mapped to the long arm of chromosome 9 between CT71 and CT220 (Stevens et al. 1995, Brommonschenkel and
A co-dominant SCAR marker linked tightly to the Sw-5 locus was developed from RAPD primer UBC#421 (Stevens et al. 1996). It is presently in wide use by tomato breeders around the world and Sw-5 has now been deployed in a number of cultivars. However, under high disease pressure the resistance from Sw-5 can be partially overcome (Roselló et al. 1997, 1998). Furthermore, strains virulent on Sw-5 cultivars have been reported in Hawaii (Cho et al. 1996), South Africa (Thompson and van Zijl 1996), and Australia (Latham and Jones 1998). If such strains appear in tomato production regions the resistance in cultivars based on Sw-5 could be rendered useless. Thus, it is important to have other sources of resistance available. Roselló et al. (1999) used mechanical and thrips inoculation to screen L. peruvianum and L. chilense accessions and found useful resistance in L. peruvianum accessions PI 126935, PI 126944, CIAPAN 16, PE-18, and CIAPAN 17. Earlier, Roselló et al. (1998) reported resistance gene Sw-6 was introgressed from L. peruvianum accession PE-18. The level of resistance of Sw-6 was not as high as that provided by Sw-5. In later work, the Sw-6 gene present in line UPV 1 was...
determined to be allelic to Sw-5, and the former in heterozygous condition

had a higher resistance level than the latter indicating an advantage in

developing hybrid cultures. (Roselló et al. 2001). Other sources of resistance

have been reported including a recovery type of resistance derived from L.

chilense accession LA 1938 (Canady et al. 2001). Furthermore, this resistance

hold up against the Hawaiian strain that overcomes Sw-5 (Stevens, personal

communication). Recently, advanced breeding lines derived from this

source have been obtained that are homozygous resistant to a strain

controlled by Sw-5. (Scott et al. 2005). Resistance appeared to be controlled

by 1 or 2 dominant genes. The inheritance of this resistance to strains that

do and do not overcome Sw-5 needs to be elucidated and the development

of molecular markers linked to the resistance gene(s) will greatly facilitate

breeding progress with this material. A genetically engineered possibility for spotted wilt resistance was

demonstrated in tomato and tobacco plants transformed with the nucleo

capsid protein (N) gene from the virus inserted either in sense or antisense

orientations (Kim et al. 1994). Another approach to provide broad spec

trum resistance was to combine transgenic resistance with
Sw-5 resistance (Gubba et al. 2002). As mentioned a SCAR marker is the method of choice for the incorporation of the Sw-5 gene into elite germplasm due in part to difficulties encountered with inoculation procedures. Resistance is verified by field tests or artificial inoculation methods. The two artificial methods used are mechanical and thrips inoculation and these two methods were compared for an array of germplasm (Kumar et al. 1993). Thrips inoculation is cumbersome involving maintenance of viruliferous thrips colonies and infection of susceptible controls is often less than 100% due to erratic behavior of thrips. For mechanical inoculation repeated mechanical passes can result in defective isolates (Ie 1982). Mechanical inoculation does not always reflect field resistance and with some germplasm field resistance could be deemed susceptible if only mechanical inoculation is used. If the resistance mechanism was based on vector resistance it would not be detected by mechanical inoculation for instance. Escapes can be a problem with mechanical inoculation methods as well. To detect the presence of the virus in plants that may be symptomless after inoculation
tion, an ELISA method has been developed (Gonsalves and Trujillo 1986) and a kit is now available (Agdia Inc. Mishawaka, Indiana, USA). Resistance conferred by Sw-5 is detectable by mechanical inoculation. The method of Stevens et al. (1992) will be described. TSWV isolates are maintained in young (four-leaf) N. rustica plants by rubbing 600 mesh carborundum-dusted leaves with sterile pads dipped in cold buffer (0.1 M phosphate buffer, pH 7.4, containing 0.01 M sodium sulfite) containing homogenized symptomatic tissue. This was done every two weeks.

Tomato plants 10-15 cm tall were inoculated with a touch-up paint sprayer at 3.56 x 10^5 N/m². Infected N. rustica leaves were homogenized in a blender in cold inoculation buffer (10% w/v) followed by filtration through sterile cheesecloth. Carborundum (600 mesh) was added at 1% w/v. The sprayer nozzle was held 3-5 cm from the apices of the plants with inoculation done with 0.4-0.8 second bursts of the sprayer. Inoculations are repeated 6-8 days later to prevent escapes. Symptoms can be evaluated in two weeks and ELISA can be done in that time frame as well.

Temperatures above 30 °C should be avoided. The thrips inoculation method
Tomato yellow leaf curl virus (TYLCV). This virus of the family Geminiviridae is transmitted by the tobacco or sweetpotato whitefly Bemisia tabaci Genn. biotype B, which has also been classified as the silverleaf whitefly B. argentifolii Bellows and Perring. The type species of this whitefly-transmitted group of viruses is bean golden mosaic virus and thus they are members of the Begomovirus genus. Over 100 begomoviruses are transmitted to over 20 plant species of economic importance and the list of viruses is expanding rather dramatically. TYLCV is a monopartite virus that originated in the old world and has spread to the Western hemisphere in tropical and subtropical zones. It is one of the most serious diseases presently affecting tomato crops in these regions (Picó et al. 1996). TYLCV is a heterogeneous complex of geminiviruses (Czosnek and Laterrot 1997) and eight species were proposed based on their country of origin (Fauquet et al. 2000). All have the TYLCV acronym followed by a hyphen with a code for the country of
origin as follows: -CH (China), -IS (Israel), -NG (Nigeria), -Sar (Sardinia) [also called -SR], -SA (Saudi Arabia), -TZ (Tanzania), -TH (Thai land), and -YE (Yemen). Other species have evolved and have now been added to this list. Accotto et al. (2000) reported that TYLCV-IS was identified in Portugal and Spain while TYLCV-Sar was found in Italy and Spain. No resistance was found in early testing of L. esculentum accessions.

Resistance was found in several accessions of L. pimpinellifolium. Pilowsky and Cohen (1974) reported resistance from LA 121 was controlled by a single incompletely dominant gene. In India, Banerjee and Kalloo (1987) also found control by a single incompletely dominant gene from ‘A1921’.

However, Hassan et al. (1984) found resistance from LA 121 and LA 373 was controlled quantitatively with partially recessive gene action. Kasrawi (1989) studied resistance from L. pimpinellifolium accessions hirsute-INRA and LA 1478 and both were controlled by a single dominant gene. The gene symbol Tylc was proposed for this gene although complementation tests to prove that two sources had the same gene were not carried out.

Later, a resistance gene, referred to as a major QTL that accounted for
27.7% of the resistance from hirsute-INRA was mapped to chromosome 6 between RFLP markers TG153 and CT83 (Chague et al. 1997 see Fig. 12.1).

In Sudan, it was reported that resistance from LA 1478 and LA 1582 was each conferred by a single dominant gene (Geneif 1984). More recently, Hassan and Abdel-Ati (1999) reported resistance from three L. pimpinellifolium accessions was each conferred by single genes with dominance in PI 407543 and PI 407544 and partial dominance in PI 407555.

Laterrot (1995) used only two L. pimpinellifolium accessions, hirsute INRA and LA 1478, in the development of his pimpertylc populations that were based on crosses from selections made in different countries. Because breeding lines derived from LA 121 had reduced plant vigor and yield in the field, Pilowsky and Cohen (1990) started a new breeding program in 1977 based on resistance from L. peruvianum accession PI 126935. The tolerant cultivar TY-20 was released from this program with tolerance conferred by five recessive genes. Young TY-20 plants had to be protected from viruliferous whiteflies for best results. In later work at the Volcani Center in Israel, highly resistant lines TY 172 (and TY 197)
were derived from L. peruvianum (Friedmann et al. 1998). The exact source of resistance was not indicated but PI 126906, PI 126930, PI 390681, and LA441 were used in the crossing. Plants of these lines carry a low level of viral DNA after being inoculated, but do not suffer the yield reductions of some commercial cultivars derived from other sources of resistance (Lapidot et al. 1997, 2001). Thus, they show promise as resistance sources.

Hassan et al. (1984) showed two plants of L. cheesmanii LA 1401 were resistant and they recovered only one of 118 F 2 plants that was resistant with no symptoms and no virus graft transmission. The same authors found L. hirsutum accession LA 386 and the F 1 with a susceptible parent were resistant, but low plant numbers precluded an in depth genetic analysis. Later, Kasrawi et al. (1988) reported a single plant of LA 386 was free of disease symptoms but did harbor the virus after inoculation. Resistance was also reported in L. hirsutum accession LA 1777 (Ioannou 1985, Fargette et al. 1996). Vidavski and Czosnek (1998) crossed LA 386 and LA 1777 and developed an inbred, 902, that had good resistance to TYLCV and to a mixture of bipartite begomoviruses in Guatemala (Mejia...
et al. 2002). Picó et al. (2000) reported that the resistance from LA 386 was superior to that of LA 1777. Furthermore, Momotaz and Scott (2005) were unable to find a good level of resistance to TYLCV or tomato mottle virus (ToMoV) in any of the recombinant inbred lines that were derived from LA 1777 (Monforte and Tanksley 2000). Thus, although breeding work has been done with LA 1777, it does not appear to be a good source of resistance to TYLCV. Pico et al. (2000) also report on L. pimpinellifolium and L. hirsutum accessions that have improved levels of resistance after agroinoculation than many sources reported above. In India, Banerjee and Kalloo (1987) found resistance from L. hirsutum f. glabratum ‘B6013’ was conditioned by two epistatic genes. Later, Kalloo and Banerjee (1990) developed a line, H24, from this accession. H24 showed good resistance in Taiwan which led to the mapping of a resistance gene on chromosome 11 in a 14.6 cm region between RFLP markers TG36 and TG393 (Hanson et al. 2000). A second gene was not detected and might have been lost in the development of H24. Plants of H24 were free of virus in Taiwan and this gene appeared to be completely to partially dominant
to TYLCV

TW. In India, there were some virus symptoms in H24 where the virus isolates were different and this could account for the difference in the plant responses between the two locations. L. chilense derived resistance is also being used in several breeding programs around the world. Zakay et al. (1991) reported L. chilense accession LA 1969 had higher level of resistance than that of several other accessions from other species. Later, Zamir et al. (1994) mapped a partially dominant resistance gene from LA 1969, Ty-1, to chromosome 6 in a 4 cM region between RFLP markers TG97 and TG297 (bacterial resistance chapter, Fig. 12.1). The Ty-1 gene has been widely used in breeding programs and has in some regions provided good resistance but in others virulent strains have overcome the resistance with Spain being one example (Barten, personnel communication). A larger introgression on chromosome 6 from LA 1969 is being used by Seminis Seed Company in their cultivars and it may contain two linked genes with Ty-1 being one of the two genes. This resistance has held up better than Ty-1 alone in some regions (Mercier, personnel communication). Scott et al. (1995) found
six other L. chilense accessions bred for ToMoV had resistance to TYLCV in the Dominican Republic. This will be discussed further in the section on ToMoV. Resistance to TYLCV-SR was demonstrated in the lines of Scott et al. (1995) but lines selected specifically for this virus strain were made to obtain higher levels of resistance (Picó et al. 1999). Transgenic approaches have also been used to develop TYLCV resistance. Readers interested in this work are referred to the paper of Yang et al. (2004) where the replication associated protein (Rep) sequence from the pathogen has been altered and inserted into tomato to provide resistance and no detectable viral DNA. Owing to the devastating nature of this disease in a number of important tomato production regions, there have been intensive breeding efforts to develop cultivars with TYLCV resistance. A number of cultivars have been developed using various resistance sources and some of these have been inoculated with TYLCV and evaluated for virus accumulation, symptoms, and yield reductions (Lapidot et al. 1997, Picó et al. 1998, Vidavsky et al. 1998). To date conventionally bred cultivars often have reduced virus titers compared to susceptible cultivars, but none are
immune to TYLCV. Private companies have done much of the breeding,

but information as to how the resistance being used is generally not published. The heterogeneous nature of the pathogen requires testing of resistant germplasm in the regions of interest to determine the utility of the resistance. Often a genotype that appears resistant in one location may not be as resistant in another region. Combining resistance genes is an approach to provide adequate and more broad-based resistance. However, it is often difficult to obtain resistant cultivars with horticultural characteristics comparable to existing susceptible cultivars and this problem is compounded as the number of resistance genes increases. The insecticide imidocloprid has been effective in many regions in limiting TYLCV damage and growers in such regions are reluctant to grow resistant cultivars due to their horticultural limitations. Significant challenges remain for tomato breeders to develop TYLCV resistant cultivars for some production regions. Whitefly mediated inoculation is the primary course to screen for resistance. Researchers use various methods for screening disease resistance and some comparisons have been made (see Picó et al.
1998). Some details of a typical method will be described in the next section on ToMoV.

Other methods use an inoculation time of 48 hours and in such situations high densities of whiteflies (20-50/plant) are used to avoid escapes.

Agroinoculation procedures (Navot et al. 1991) have also been used, but these procedures resulted in symptom development in LA 1969 and LA 1777, genotypes that were essentially virus free when whitefly mediated virus inoculation was used (Kheyr-Pour et al. 1994). This demonstrated that agroinoculation circumvented natural resistance mechanisms which prevent the replication, spread and expression of symptoms.

Tomato mottle virus (ToMoV) and other bipartite begomoviruses. The new world geminiviruses have bipartite genomes as opposed to the monopartite genome of TYLCV. There are numerous bipartite begomoviruses that have been reported in the Western hemisphere with new ones still being documented. These viruses can cause devastating crop losses and some regions have mixtures of several begomoviruses. For instance, six have been reported in Guatemala (Nakhla 2005). Although losses from early infections can be total, the bipartite disease symptoms are
generally not as severe as the symptoms of TYLCV. There has been far less

resistance work on these viruses since they have not been around as long

as TYLCV and none of them are as widespread. ToMoV was the first

begomovirus to attack tomato crops in Florida in 1989 and a breeding

program was started in 1990. An initial assay of germplasm, including many accessions with reported resistance to other viruses, revealed six accessions of L. pimpinellifolium and one each of L. hirsutum and L. peruvianum had significantly less disease severity than the susceptible control (Scott and Schuster 1991). No symptoms were present in many L. chilense accessions and 12 were selected for breeding based on their resistance and larger leaf size. Introgression with this material was described and tomato lines with ToMoV resistance were also found resistant to TYLCV in the Dominican Republic (Scott et al. 1995). Griffiths (1998) used RAPD markers and found three resistance regions on chromosome 6 using lines derived from accessions LA 1938, LA 2779, and LA 1932. Breeding lines from the former two accessions had L. chilense introgression in a region that overlapped the Ty-1 region.
A second region present in LA1938 and LA1932 derived lines was closely
linked to the self pruning (sp) locus that confers plant
habit. The third
region present in LA 1932 derived lines was about 30 cM
from the sec
ond region (Griffiths and Scott 2001). The marker work from
LA1932
derived lines was supported by a genetic study which
estimated two
genes conferred resistance. The data fit an
additive-dominance model
with a high degree of additive gene action. Thus, to
provide acceptable
resistance to ToMoV requires resistance in both parents of
a hybrid. Ji
and Scott (2005) have assayed the RAPD markers from the
work of
Griffiths (1998) and are in the process of developing
co-dominant SCAR
markers for the resistance genes. In addition to the three
genes on
chromosome 6 there appeared to be another gene in lines
derived from LA
2779 that was previously not detected. These lines were
susceptible to gray
leafspot (Stemphyllium sp.) and it was thought that a
begomovirus resis
tance gene was linked in repulsion to Sm, a gene present in
the recurrent
parents being used. However, preliminary tests did not
detect a L. chilense
introgression in this chromosome 11 region (Ji, unpublished
Once SCAR markers are found for all the resistance genes, lines with all combinations of the genes will be synthesized and tested for various begomoviruses around the world to discern what gene combinations are effective against the various viruses and strains thereof. Incorporation of the genes for breeding can then be done using marker assisted selection. As mentioned, ToMoV resistant lines had resistance to TYLCV in the Dominican Republic. Hundreds of breeding lines with resistance from LA 1932, LA 2779, and LA 1938 combined with Tyking have been screened for both ToMoV and TYLCV since 1997 when TYLCV was found in Florida. Lines with high levels of resistance to ToMoV also have had high levels of resistance to TYLCV (Scott, unpublished data). However, most lines bred for TYLCV resistance have been susceptible to ToMoV and these include Ty-52 (Ty-1 gene), Fiona, H24, Gempride, Lignon, Avinash 2, Tycoon, Ty 20, Ty 202, pimpertylc-1 to -5, 8 chepertylc populations, 4 chiltylc populations, hinseptylc -1 to -3 populations, pertylc -1 and -2 populations, 10 Cln lines from AVRDC, Peto 150535, HA 3057, and 'Tygress' (Scott, unpublished data). TYLCV resistant lines that have shown some
resistance to
ToMoV are Tyking, Ty 197, Ty 34, and chiltyle -2 & -3 where
the latter three
of the five segregated. Several lines with L. chilense
derived resistance have
been tested for TYLCV and bipartite geminiviruses around
the world. Some
of the lines have had resistance in every test but no one
genotype has
emerged that is universally resistant, so it is apparent
that there is no
simple solution to begomovirus resistance breeding.
However, ToMoV re
sistant lines have had good field resistance in Guatemala
where there are
mixed infections of up to six different viruses as
mentioned. Line 902
(Vidavski and Czosnek 1998) has also shown good resistance
in Guatemala
and Ty 197 has shown fair resistance. It does appear
that resistance
genes selected for ToMoV and some genes selected for TYLCV
resistance
confer resistance to a broad array of these viruses and
this does at least
make the breeding for resistance feasible. If the
resistance genes were spe
cific for one, or only a few viruses, breeding of resistant
cultivars would be
impossible. Little breeding work has been reported for
bipartite viruses other than
ToMoV. Piven et al. (1995) reported resistance to tomato
yellow mosaic
virus (ToYMV) in L. chilense accessions LA 1963 and LA 1969, L. hirsutum accession LA 1353, and L. peruvianum var. glandulosum accession LA 1292. Inoculation is whitefly-mediated and has been described (Griffiths and Scott 2001). ToMoV viruliferous colonies are maintained of the dwarf tomato 'Florida Lanai' in a controlled temperature room with a 14-h photoperiod under cool white fluorescent lights with irradiance of 50 mmol m⁻² s⁻¹ at 25 °C. Six weeks before inoculation, viruliferous whitefly numbers are increased by adding additional non-infected 'Florida Lanai' plants (with 7 to 10 leaves growing in hanging baskets) to screened cages with the infected plants. Plants to be inoculated are grown in styrofoam trays (cell size = 3.8 cm²) for approximately 20 days past the cotyledon stage when they are at the third leaf stage. They are moved to benches in whitefly proof greenhouses along with the infected 'Florida Lanai' plants that are hung above them so that there are approximately 512 seedlings (four trays) for each infected source plant. Source plants are shaken periodically to distribute whiteflies and a dowel is rubbed over the plants to disperse the whiteflies for more uniform distribution. Inoculation is for 14 d or 21 d if there do not seem to be many whiteflies.
Tomato yellow top (TYTV) and Potato leaf roll virus (PLRV). These Luteoviruses are closely related and are persistently transmitted by several species of aphids. They have narrow host ranges, mostly Solanaceae species. The former causes more severe symptoms than the latter. A search for TYTV resistance was conducted using a number of accessions from several Lycopersicon species. This led Hassan and Thomas (1988) to inoculate L. peruvianum PI 128655, and its hybrids, with 9 isolates of TYTV and one isolate of PLRV. They found very strong resistance, with little virus recovery, in some plants with some isolates. However, there has been virtually no breeding work done with these diseases.

SUMMARY

The difficulties in developing horticulturally acceptable disease resistant cultivars have been recently discussed (Scott 2005). Much earlier, in a review well worth the reading by anyone interested in tomato disease resistance breeding, Walter (1967) quotes a 1953 paper by Andrus who concluded that “if a new disease-resistant variety is to be acceptable to farmers, it must yield fully as well as the old, susceptible variety in all
features that influence the net value of the crop even when
disease is absent”. Molecular markers tightly linked to resistance
genes would greatly facilitate combining resistance genes in new
cultivars. However, few “breeder friendly” markers are presently available
that, despite impressive advancement in identifying and locating resistance
genes, many challenges remain for future researchers.


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INTRODUCTION

The cultivated tomato, Lycopersicon esculentum (L.) Miller, is attacked by a number of arthropod pests, which are capable of causing devastating losses (Lange and Bronson 1981). The pest complex includes species that feed almost exclusively on foliage (e.g. spider mites, dipterous leafminers), species that feed on both foliage and fruit [such as the lepidopterans Helicoverpa zea (Boddie) and Spodoptera exigua (Boddie)] and species such as aphids and whiteflies that feed on plant sap. Because tomato is a high value crop, which must meet rigorous market standards that preclude even minimal damage to the harvested fruit, populations of pests that attack the fruit must be maintained at very low levels. Consequently, management of insect pests on tomato has relied heavily on chemical control measures. Arthropod resistant cultivars and hybrids play an important role in pest management on many crops, including some vegetable crops, and their use holds considerable promise in tomato. During the last three decades, considerable research has been directed towards
identifying
and developing a mechanistic understanding of traits within the genus
Lycopersicon that confer resistance to arthropod pests. It is noteworthy
that research has resulted in the identification of numerous resistance
traits that have potential utility in the development of pest resistant tomato
cultivars. It has also revealed levels of detail and complexity in plant
arthropod interactions that are unique among well-studied systems
involving crop plants and their close relatives. This chapter provides an
overview of the occurrence, mechanisms, and genetics of arthropod
resistance traits in the genus Lycopersicon. Additional, detailed information
on resistance mechanisms in Lycopersicon can be found in reviews by
Duffey (1986), Duffey and Bloem (1986), Kennedy (1986), Kennedy et al.
(1987), Farrar and Kennedy (1991a), and Kennedy (2003) In practice, arthropod resistance or susceptibility in crops is typically
defined in relation to a commonly grown cultivar. Resistance traits, which
are genetically controlled, result in lower levels of attack, lower pest
populations, and/or lower levels of damage to the crop than observed
on plants lacking the resistance trait(s) under conditions of comparable
pest pressure (Painter 1951). Arthropod resistance that is of value in crop protection can be categorized as: antibiosis (pest development delayed and/or survival reduced), antixenosis (=non-preference - normal pest behavior is adversely affected in a way that interferes with its ability to utilize the resistant plant), or tolerance (reduced level of damage under a given level of pest infestation), although these categories are not mutually exclusive (Painter 1951, Kogan and Ortman 1978). Most resistance traits identified in Lycopersicon involve antibiosis. The genus Lycopersicon is characterized by great diversity within and among its nine species (Miller and Tanksley 1990, Kalloo 1991). Although all species have been examined for resistance to at least some arthropod pests, the highest levels of resistance to the greatest number of arthropod species are found in L. hirsutum f. typicum (=hirsutum) Humb & Bonpl., L. hirsutum f. glabratum C.H. Mull and L. pennellii (Corr.) D'Arcy. There is extensive variation among accessions of these species in the spectrum and level of arthropod resistance. A diverse array of traits, including the physical and chemical properties of glandular trichomes as well as con
stitutively expressed and wound-induced chemical defenses present in the leaf lamella, have been associated with resistance.

OCCURRENCE AND MECHANISMS OF RESISTANCE

Aphids

The potato aphid, Macrosiphum euphorbiae (Thomas), and to a lesser extent the green peach aphid, Myzus persicae Sulzer, are major pests of tomato. Dense populations developing on the young foliage can cause severe yield reductions (Walgenbach 1997). Resistance to M. euphorbiae has been reported from L. esculentum, L. esculentum var. cerasiforme, L. hirsutum f. typicum, L. hirsutum f. glabratum, and L. pennellii (Gentile and Stoner 1968a, Stoner et al 1968b, Clayberg and Kring 1974, Clayberg 1975, Quiros et al. 1977). Type VI trichomes, which have a 4-cell glandular head, have been implicated in the entrapment of aphids and other small arthropods on several Lycopersicon species, including L. esculentum, L. hirsutum f. typicum, L. hirsutum f. glabratum and L. pennellii (Duffey 1986, McKinney 1938, Kennedy unpub.). In most cases entrapment accounts for only a low level of resistance to aphids. The chemical mechanisms responsible for entrapment are well documented in Lycopersicon and Solanum berthaultii
Hawkes. Phenolic substrates and the enzymes polyphenol oxidase and peroxidase are compartmentalized within the trichome tip until an insect discharges the trichome tip and the contents are mixed. The ensuing enzymatic reaction (known as the browning reaction) oxidizes the phenolic substrates to quinones, which polymerize. The product of the browning reaction entangles small arthropods or collects on their appendages and mouthparts, inhibiting their ability to move, cling to the plant and feed (Duffey 1986, Duffey and Isman 1981, Gregory et al. 1986). There is extensive variation both within and among Lycopersicon in the level of browning reaction associated with type VI trichomes. This variation reflects differences in polyphenol oxidase and peroxidase activity as well as density of type VI trichomes (Duffey 1986). In L. esculentum, and L. esculentum var. cerasiforme, resistance to the pink biotype of M. euphorbiae has been associated with the density of non-glandular trichomes, the presence of quinic acid in the foliage, and the poor nutritional quality of the foliage as a source of food for the aphids (Quiros et al. 1977). Definitive proof of the specific mechanisms
underlying this resistance is lacking. Resistance to the
pink biotype of M. euphorbiae is conditioned by the
gene Mi, which also confers resistance to root knot
nematodes. Mi was
introgressed into L. esculentum from L. peruvianum to
confer nematode
resistance and has been widely used in tomato breeding to
produce nema
tode resistant cultivars. Its role in aphid resistance was
only recently
documented (Rossi et al. 1998, Vos et al. 1998). Mi is
simply inherited
and the aphid resistant phenotype is dominant under field
conditions
but incompletely dominant under greenhouse conditions
(Kaloshian et
al. 1995). The precise mechanism conditioning aphid
resistance is not
known, nor is it known whether the same mechanism
conditions both
aphid and nematode resistance (Rossi et al. 1998).
Resistance in L. hirsutum f. glabratum to the aphids M.
euphorbiae and
Myzus persicae (Sulzer) has been reported from accessions
PI134417 and
PI126449 (Musetti and Neal 1997, Leite et al. 1999). The
high level of
resistance to M. euphorbiae is manifested as altered
feeding behavior
(reduced number and duration of probing and a decrease in
total time
spent probing plant tissue with mouthparts), elevated
mortality and a
high rate of dispersal by apterous aphids from resistant
foliage (Musetti and Neal 1997). This resistance has been associated with the presence of 2-tridecanone in the tips of type VI glandular trichomes (Musetti and Neal 1997a and b, Kennedy and Yamamoto 1979). 2-Tridecanone is toxic to a number of insect species including M. euphorbiae and M. persicæ but is both repellent and toxic to M. euphorbiae at concentrations associated with resistant foliage. The repellent and deterrent effects may play a more important role than toxicity in the resistance to M. euphorbiae (Williams et al. 1980, Kennedy 1986, Musetti and Neal 1997 a, b, Kennedy 2003). Aphid resistance has been studied most intensively in L. pennellii accession LA716, which exhibits a high and stable level of resistance to M. euphorbiae (Goffreda and Mutschler 1989). On LA716, the normal probing and feeding behavior of M. euphorbiae and M. persicæ is disrupted such that fewer aphids feed, and probing and feeding times of aphids are significantly less than on L. esculentum (Goffreda et al. 1988, Rodriguez et al. 1999). This difference is related to the presence of acylsugars in the tips of type IV trichomes of LA716, which deter both settling and feeding by aphids, although other factors appear to contribute to aphid resistance
as well (Goffreda et al. 1990, Hartman and St. Clair 1999a). Acylsugars, primarily 2,3,4-tri-O-acylated glucose esters possessing C4 to C12 fatty acids, constitute approximately 90% of the exudate of type IV trichomes of LA716 (Forbes et al. 1985). Goffreda et al. (1990) reported a negative relationship between acylsugar levels on the foliage and abundance of M. euphorbiae in segregating F2 populations from crosses between L. esculentum and L. pennellii. L. esculentum does not possess type IV trichomes and does not accumulate acylsugars (Goffreda et al. 1990). The inheritance of aphid resistance is complex and involves epistatic effects (Goffreda and Mutschler 1989). The occurrence of type IV trichomes is simply inherited and controlled by two dominant genes in crosses between L. esculentum and L. pennellii. In F1 hybrids, the presence of either gene confers the presence of type IV trichomes (Lemke and Mutschler 1984). The genetic control of acylsugar synthesis and accumulation in type IV trichome tips is more complex. L. esculentum X L. pennellii hybrids produce both glucose and sucrose esters with a different fatty acid composition than the glucose esters of L. pennellii, which
produces primarily glucose esters (Goffreda et al. 1990). An RFLP/QTL analysis revealed 5 genomic regions on 4 chromosomes associated with acylsugar production; 2 regions on chromosome 2 and one region on each of chromosomes 3, 4, and 11. The regions on chromosome 2 and the region on chromosome 3 account for 11-16% and 7-12% of the total variation in acylsucroses and total acylsugars, respectively. The alleles from L. esculentum are partially dominant to the alleles from L. pennellii in both regions of chromosome 2, whereas the L. pennellii allele is at least partially dominant to the L. esculentum allele on chromosome 3. The region on chromosome 4 is associated with increases in acylglucoses and accounts for 7 to 9% of the variability in levels of acylglucoses. The L. esculentum allele on chromosome 4 is partially dominant or co-dominant to the allele from L. pennellii. The region on chromosome 11 accounts for 17.7 to 22.2% of the variation in molar percentage of acylglucose and likely affects the levels of acylglucose directly. The allele from L. pennellii is dominant to that from L. esculentum (Mutschler et al. 1996). RAPD markers for 3 of the 5 QTLs have been identified and a genomic map
consisting of 111 RAPD and 8 acylglucose transferase-related markers has been added to an existing framework of 150 RFLP markers (McNally and Mutschler 1997). RFLP/PCR-based marker assisted selection through 3 backcross generations for the 5 QTL regions associated with acylsugar accumulation successfully transferred acylsugar accumulation traits from L. pennellii to L. esculentum but indicated that additional QTLs are likely involved in the accumulation of higher levels of acylsugar accumulation (Lawson et al. 1997). A QTL analysis by Blauth et al. (1998) of an F2 population generated by an intraspecific cross between L. pennellii accessions exhibiting high (LA716) and low (LA1912) acylsugar accumulation revealed that the relative proportion of acylglucoses and acylsucroses are largely controlled by a one QTL on chromosome 3. A QTL on chromosome 10 was associated with total acylsugar levels and a QTL on chromosome 4 was associated with leaf area. Leaf area is important in resistance because acylsugars are confined to the tips of type IV trichomes and there is a significant association between acylsugar levels and type IV density. Despite this association, the genetic control of acylsugar accumulation
is such that
merely selecting for increased density of type IV trichomes would likely
be ineffective in raising acylsugar levels (Blauth et al. 1998).

Whitefly

Research on resistance to whiteflies has emphasized two
important pest
species, Trialeurodes vaporariorum (Westn.), the greenhouse
whitefly, and
Bemisia argentifolii Bellows and Perring, the silverleaf
whitefly (=strain B
of B. tabaci). Both species have piercing sucking
mouthparts and feed on
phloem sap. T. vaporariorum is primarily a pest on
glasshouse-grown
tomatoes. On susceptible cultivars, it is capable of
developing large popu
lations, which reduce yields and contaminate the fruits and
plants with
honeydew. B. argentifolii is primarily of concern in the
field because it is
a vector of geminiviruses that affect tomato and because
heavy infesta
tions can cause irregular ripening of tomato fruits.
Resistance to T. vaporariorum has been reported from L.
hirsutum
f. typicum, L. hirsutum f. glabratum and L pennellii.
Entrapment of whitefly
adults in the tips of glandular trichomes has been
implicated in resis
tance (Gentile et al. 1968, Clayberg and Kring 1974, Ponti
et al. 1975,
Breeding lines developed by Ponti and Stienhuis (1984) apparently express different mechanisms of resistance, although both reduce survival and reproductive rate of *T. vaporariorum*. On breeding line 82207, whitefly contact the phloem more frequently as they feed but spend less time ingesting phloem sap and more time salivating in the phloem than on the susceptible cultivar ‘Moneymaker.’ No such differences in feeding behavior are observed on another resistant breeding line, 82216, which also reduces survival and reproductive rate of *T. vaporariorum* (Lei et al 1999). A QTL analysis of *T. vaporariorum* resistance and glandular trichome densities in F2 population of *L. esculentum* x *L. hirsutum* f. *glabratum* provided no evidence for an association between whitefly resistance and density of type IV glandular trichomes. Two QTL’s, which mapped to chromosomes 1 and 12, were associated with reduced oviposition rate.

Two QTL’s affecting type IV glandular trichomes were identified and mapped to chromosomes 5 and 9. One QTL affecting type VI trichome density was also identified, and mapped to chromosome 1 (Maliepaard et al 1995). Resistance of *L. pennellii* to *T. vaporariorum* has been reported in
olve entrapment of adults in the exudate of foliar glandular trichomes (Gentile et al. 1968, Clayberg and Kring 1974, Ponti et al. 1975). Other traits may be involved, however, and resistance has been assumed to be polygenically inherited (Berlinger et al. 1991). Because Encarsia formosa, the primary parasitoid used in biological control of T. vaporariorum in glasshouse tomato production, is also entrapped by the foliar glandular trichomes, Ponti et al. (1983) considered the resistance incompatible with biological control and abandoned efforts to incorporate the glandular trichome mediated resistance of L. pennellii into tomato varieties adapted for glasshouse production. Resistance to B. argentifolii has been reported from L. pennellii, L. peruvianum (L.) Mill., and L. hirsutum f. typicum. Resistance in L. pennellii accession LA716 greatly reduces oviposition. A major portion of the genetic basis for this resistance is associated with chromosome 6 although additional minor components appear to be associated with chromosomes 2, 3, 8, and 11 (Heinz and Zalom 1995). The resistance appears to be independent of trichome density but may be conditioned at least in part by high concentrations of acylsugars in the glandular tips.
of type IV trichomes of L. pennellii (Liedl et al. 1995, Nombela et al. 2000). Channarayappa et al. (1992) reported resistance to B. tabaci (presumably argentifolii) in L. peruvianum, which they attributed to factors other than foliar trichomes. Some preliminary results suggest that the Mi gene from L. peruvianum may contribute to this resistance (Nombela et al. 2000); however, additional studies are needed to confirm this. High levels of resistance to B. argentifolii (=tabaci) reported from L. hirsutum f. typicum are conditioned, in part by high densities of type IV trichomes, which are associated with reduced attractancy to adults and reduced oviposition (Snyder et al. 1998). Type VI trichomes, which entrap adults, may also play a role in the resistance (Channarayappa et al. 1992).

This resistance has been associated with a reduction in the incidence of leaf curl virus transmitted by B. argentifolii (Channarayappa et al. 1992).

The photophase under which L. hirsutum f. typicum is grown influences the densities of type IV and type VI trichomes and the expression of resistance to B. argentifolii. Type IV trichome densities and resistance to B. argentifolii are higher and type VI trichome densities are lower when plants are grown under a photophase of 8 h than 16 h.
This sensitivity to photophase may limit the utility of L. hirsutum f. typicum as a source of resistance.

Thrips

The western flower thrips, Frankliniella occidentalis Pergande, is of concern as a pest of tomato primarily because it transmits tomato spotted wilt virus (TSWV), although feeding in blossoms by high populations of thrips can result in external scarring of fruit. Kumar et al. (1995a) reported high levels of resistance to foliage feeding by nymphs in L. hirsutum f. glabratum (accession PI 134417), and L. pennellii (accession LA716), and a moderate level of resistance in L. chilense (accession LA1782). They also reported a high level of resistance to adult feeding on L. hirsutum f. typicum (accession LA1353). They characterized this resistance as antixenosis and demonstrated that feeding by adults on antixenotic lines was limited to the epidermal cells, whereas feeding on susceptible foliage caused severe damage to epidermal, palisade and spongy parenchyma cells. In a related study, Kumar et al. (1995b) reported significant differences among Lycopersicon accessions in flower infestations of F. occidentalis. After
accounting for flower number, and flowering period, L. pennellii (LA716) and the L. esculentum cultivar ‘Ray de los Tempranos’ supported the lowest floral infestations, but there was also significant variation in floral infestations among L. esculentum cultivars. L. pennellii shows resistance to thrips transmission of TSWV (Kumar et al 1993). The value of this resistance in breeding thrips resistant cultivars to reduce the incidence of TSWV has yet to be demonstrated.

Diptera

Resistance has been reported to the fruit fly or vinegar fly, Drosophila melanogaster Meigen, and the agromyzid leafminers Liriomyza trifolii (Burgess) and L. sativae Blanchard (=L. munda Frick). D. melanogaster oviposit on fruit and larvae develop within damaged and ripe tomato fruits. Potentially useful levels of variation in resistance to D. melanogaster have been reported among cultivars and breeding lines of L. esculentum but the genetics and underlying mechanisms have not been elucidated (Stoner et al. 1969). Larvae of the agromyzid leafminers L. sativae and L. trifolii feed on mesophyll tissue and produce serpentine leaf mines. Adults oviposit in
leaf tissue and feed on sap exuding from leaf cells punctured by their ovipositor. High levels of resistance to L. trifolii and L. sativae have been reported from Lycopersicon pennellii (accessions LA1735 and LA716), L. cheesmanii (accession LA1401) and L. hirsutum f. glabratum (accession PI126449) (Webb et al. 1971, Laterrot et al. 1987, Erb et al. 1993). Resistance in these L. pennellii and L. cheesmanii accessions is apparently inherited with a high degree of dominance in crosses with L. esculentum because F 1 progeny from crosses with L. esculentum express high levels of resistance. This resistance involves larval antibiosis, (apparently associated with the mesophyll tissue), adult antibiosis, and antixenosis, which is due in part to glandular trichomes (Erb et al. 1993). The resistance of L. pennellii to oviposition and feeding by adult L. trifolii has been attributed to the presence of acylsugar esters in the type IV trichome tips (Hawthorne et al. 1992). Eigenbrode et al. (1993) reported that Lycopersicon accessions with higher densities of nonglandular trichomes on the foliage were generally less damaged by Liriomyza spp. than those with low densities.

Beetles
Resistance in tomato has been reported to two beetle pests, tobacco flea beetle, *Epitrix hirtipennis* (Melsheimer), and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). *E. hirtipennis* adults feed on the older foliage of plants causing a “shot hole” type of injury and are typically most severe early in the season, when the plants are young.

Resistance to *E. hirtipennis* has been reported from several accessions of *Lycopersicon hirsutum* f. *typicum* and *L. hirsutum* f. *glabratum*. This resistance has been associated with repellant(s) produced by glandular trichomes on the foliage (Gentile and Stoner 1968b).

*L. decemlineata* is a severe pest of tomato in some production areas of the eastern United States. Geographically separated populations of *L. decemlineata* differ in their ability to use *L. esculentum* as a host (Kennedy and Farrar 1987, Lu et al. 1997, Lu et al. 2001). Populations adapted to *L. esculentum* are capable of causing severe damage to tomato crops through defoliation as well as direct feeding on fruit (Schalk and Stoner 1979, Kennedy et al. 1983). In general, infestations tend to be most severe on young plants. Resistance to tomato adapted populations of *Colorado potato beetle* has been reported from *L. hirsutum* f. *typicum* (accession
PI126445 (Carter et al. 1989a), L. hirsutum f. glabratum (PI134417 and
134418; Kennedy et al. 1985, Schalk and Stoner 1976) and L. pennelli
(Carter and Schurig, cited in Farrar and Kennedy 1991a). The expression of resistance in L. hirsutum f. typicum accession PI126445
as measured by survival of Colorado potato beetle varies seasonally in
response to photoperiod and temperature. When plants are
grown in
spring, potato beetle survival is comparable on PI 126445
and L. esculentum
but when plants are grown in autumn, survival on PI126445
is very low.
This resistance has been associated with the acute toxicity
of the sesquit
erpen zingiberene, which is present in the tips of type VI
glandular
trichomes (Carter et al. 1989 a, b). The accumulation of
zingiberene in the
tips of type VI trichomes is influenced by temperature and
photoperiod,
such that the quantities zingiberene associated with the
foliage of PI126445
plants are acutely toxic to Colorado potato beetle larvae
during autumn
but not during spring and summer (Gianfagna et al. 1992). The presence
of zingiberene is controlled at a single locus. An allele
from L. hirsutum f.
typicum, which controls expression of zingiberene, is
recessive to an al
ele from L. hirsutum f. glabratum but dominant to an allele from L. esculentum (Rahimi and Carter 1993). Although inherited simply, zingiberene-mediated resistance to Colorado potato beetle is likely to be of limited utility unless the effects of temperature and photoperiod on expression of resistance can be mitigated (Raimi and Carter 1993). Two genetically distinct mechanisms of resistance to Colorado potato beetle have been documented from L. hirsutum f. glabratum accession PI134417 (Kennedy et al. 1985, Kennedy and Sorenson 1985, Sorenson et al. 1989). One mechanism is associated with type VI glandular trichomes, which abound on the foliage and stems. The other is associated with the leaf lamella. The glandular trichome-mediated resistance results from the presence of high concentrations of the methyl ketone, 2-tridecanone, in the trichome tips. 2-Tridecanone is acutely toxic to young larvae at concentrations associated with the foliage of PI134417. When young larvae move about on the foliage, they discharge the trichome tips, contact lethal quantities of 2-tridecanone, and die within 72 hours. Because 2-tridecanone is contained in the tips of type VI glandular trichomes, the level of resistance is related to both the average amount of
2-tridecanone per trichome tip and the density of type VI trichomes.

Consequently, there is substantial variation in resistance expression. Both

2-tridecanone level and type VI trichome density are under separate
genetic control but there are epistatic effects (Fery and Kennedy 1987,
Nienhuis et al. 1987). Expression of both traits is strongly influenced by
environmental conditions, including daylength, light intensity, and plant
nutrient status (Kennedy et al. 1981, Snyder and Hyatt. 1984, Barbour et
al. 1991). High levels of 2-tridecanone are conditioned by at least three
major genes inherited in a recessive manner (Fery and Kennedy 1987).

Restriction fragment length polymorphism (RFLP) analyses of QTLs as
sociated with 2-tridecanone levels in F2 progeny of crosses between L. esculentum and PI134417 identified three different linkage groups associ
ated with the expression of 2-tridecanone. One of the RFLP loci having
the highest correlation with 2-tridecanone levels is primarily associated
with expression of type VI trichome density (Nienhuis et al. 1987). Zamir
et al. (1984) reported a pleiotropic effect of the gene for determinant
plant growth, which caused low levels of 2-tridecanone expression in
plants having a determinant growth form. As indicated elsewhere in this chapter, the presence of high levels of 2-tridecanone contributes to the resistance of L. hirsutum f. glabratum to a number of arthropod species. F 1 progeny from crosses between L. hirsutum f. glabratum accession PI134417 and L. esculentum do not express the trichome mediated resistance attributable to 2-tridecanone, but express resistance that causes high levels of mortality during the later larval stages. Factors responsible for this resistance are inherited in a dominant or semi-dominant fashion mainly associated with the leaf lamella rather than the trichomes (Kennedy 1986, Sorenson and Kennedy 1989). The glycoalkaloid \( \alpha \)-tomatine is common among species of Lycopersicon acting as a potent feeding deterrent and growth inhibitor for Leptinotarsa decemlineata larvae and adults (Sturkow and Low 1961, Schreiber 1968, Hsiao and Frankel 1968b, Roddick 1974, Sinden et al. 1978, Juvik et al. 1982a, Mitchell and Harrison 1985, Hare 1987). Sinden et al. (1978) demonstrated that \( \alpha \)-tomatine content of foliage of L. esculentum and L. hirsutum f. glabratum (PI134417) varied with plant age and the daylength under which the plants were grown. They observed significant negative
correlations between feeding by adults and foliar concentration of tomatine for both species. The correlation was stronger for L. esculentum

\( r = -0.897, P = 0.01 \) than for L. hirsutum f. glabratum

\( r = -0.613, P = 0.05 \). However for plants containing comparably low levels of \( \beta \)-tomatine, foliage consumption was less on L. hirsutum f. glabratum than on L. esculentum, suggesting other factors contributed to the reduced feeding on L. hirsutum f. glabratum. Other in-depth studies have failed to detect any evidence that \( \beta \)-tomatine levels contribute to the lamella-based resistance of PII34417 to L. decemlineata (Barbour and Kennedy 1991). It remains possible that \( \beta \)-tomatine contributes to the relatively poor suitability of L. esculentum as host for many populations of L. decemlineata. Its value as a resistance mechanism appropriate for use in the development of tomato cultivars resistant to L. decemlineata is likely to be limited because its activity appears to depend on concurrent levels of phytosterols and pro tease in the foliage (Hare 1987).

Lepidoptera

Resistance in Lycopersicon has been reported for more species of Lepidoptera than any other taxon of arthropods (Farrar and
Kennedy 1991a). The high level of interest in screening germplasm for resistance to Lepidoptera reflects their importance as fruit-feeding, defoliating and leaf mining pests of tomato. Resistance to one or more of the following species of the family Noctuidae has been reported from at least one accession of all Lycopersicon species: Helicoverpa armigera (Hubner), H. zea (Boddie), Spodoptera exigua (Hubner), S. eridania (Cramer), S. littoralis (Boisduval), and Plusia chalcites (Esp.) (Kashyap et al. 1990, Farrar and Kennedy 1991a, Eigenbrode and Trumble 1993, Eigenbrode et al. 1993).

In addition, resistance to the foliage and fruit feeding noctuid Trichoplusia ni (Hubner) has been reported in L. hirsutum f. typicum and L. hirsutum f. glabratum (Sinha and McLarin 1989). Resistance has also been reported to sphingids, Manduca sexta (L.) and M. quinquemaculata (Haw.), and to gelechiids, Keiferia lycopersicella (Wals.), Phthorimaea operculella (Zell.), and Tuta (=Scrobipalpula) absoluta (Meyerich) (Kennedy and Henderson 1978, Juvik et al. 1982b, Lin et al. 1987, Farrar, et al. 1994, Maluf et al. 1997, Leite et al. 1999). In many cases the levels of resistance are modest and
the underlying mechanisms and genetic basis are not known, but some resistances are of a very high level and their mechanisms have been well studied. Some resistance mechanisms are shared by more than one Lycopersicon species and affect more than one insect pest, whereas others are more limited in scope and spectrum of activity. Although no extremely high levels of resistance to lepidopteran pests of tomato have been reported from L. esculentum, there is significant variation in the suitability of L. esculentum accessions as hosts of some important species, including H. zea, H. armigera, S. exigua, K. lycopersicella, T. absoluta, and S. eridania (Eigenbrode et al. 1993, see also Farrar and Kennedy 1991a). Resistance factors are present in the tips of type VI glandular trichomes, in the leaf lamellae, and in green fruit. In general, these factors act to slow larval growth either by deterring larval feeding, intoxicating the larvae or by interfering with the utilization of nutrients. Detailed information about these mechanisms is presented in several reviews (Duffey 1986, Duffey and Bloem 1986, Kennedy, 1986, Kennedy et al. 1991, Kennedy 2003). Glandular trichomes are important contributors to Lepidoptera resistance in a number of Lycopersicon species. Removal of
glandular trichomes

(primarily type VI) from L. esculentum foliage increased its suitability as a food source for H. zea larvae. Larvae reared on foliage from which trichomes were removed grew faster and suffered 10 to 17% less mortality than those reared on foliage containing the normal complement of glandular trichomes (Duffey 1986, Farrar and Kennedy 1987). These effects were associated with a 27% reduction in the relative consumption rate (= dry wt. of foliage ingested per initial dry wt. of larva) in the 2nd instar and a 22% reduction in the efficiency of conversion of ingested foliage (= dry wt. gained per dry wt. of foliage ingested) in the 5th instar (Farrar and Kennedy 1987). The specific factors causing these effects have not been determined. The catecholic phenolics, rutin and chlorogenic acid, present both in the leaf lamella and the tips of type VI trichomes of L. esculentum, upon incorporation into artificial diet cause a dose-dependent growth inhibition of H. zea and S. exigua larvae (Elliger et al. 1981, Isman and Duffey 1982a, Duffey and Bloem 1986). However, no correlation was found between total phenolic content of foliage and growth rate of H. zea larvae.
on L. esculentum cultivars, despite consistent differences among cultivars in larval growth rate over 2 years (Isman and Duffey 1982, Duffey 1986).

An explanation for this lack of correlation lies in the fact that the growth inhibitory effects of phenolics are dependent on protein levels in the diet (Duffey and Bloem 1986). The abundance of both phenolics and proteins in tomato foliage are known to be influenced by nitrogen fertilization, water stress, and light intensity (English-Loeb et al. 1997, Stout et al. 1996, Wilkens et al. 1996). The levels of phenolics and protein also vary greatly and independently among plants of the same and different L. esculentum cultivars (Stamp and Horwath 1992). The phenolic rutin has also been shown to prolong development of M. sexta larvae at cool but not at warm temperatures, in studies involving artificial diet, apparently by interfering with physiological processes involved in the initiation of molting (Stamp 1990, Horwath and Stamp 1992, 1993). The effects of rutin on M. sexta have not been documented for larvae fed Lycopersicon foliage and the effect may be of little consequence in the context of host plant resistance for crop protection. The glycoalkaloid γ-tomatine, present in the leaf lamellae and green fruit, but not the tips of glandular trichomes, has been
hypothesized as another growth inhibiting compound for H. zea and S. exigua in Lycopersicon (Elliger et al. 1981, Duffey and Bloem 1986). \(\beta\)-Tomatine is also a potent inhibitor of larval growth in both species when incorporated into artificial diets at concentrations commonly found in L. esculentum cultivars (Elliger et al. 1981, Duffey 1986, Bloem et al. 1989). Foliar levels of \(\beta\)-tomatine vary within and among Lycopersicon species, with the highest levels found in L. esculentum var. cerasiforme and L. pimpinellifolium (Juvik et al. 1982a). \(\beta\)-tomatine levels of progeny from crosses between L. esculentum var. cerasiforme and L. pimpinellifolium express high and low levels of this glycoalkaloid, its expression being controlled by segregation of 2 co-dominant alleles at a single locus (Juvik and Stevens 1982a). In L. esculentum fruit \(\beta\)-Tomatine content was positively correlated to development time and mortality of H. zea larvae fed on different aged fruit and negatively correlated with larval growth rate and adult weight. However, for S. exigua, there were no correlations between \(\beta\)-tomatine content of the fruit and growth or survival rate of larvae but there was a
significant correlation between cuticular toughness of the fruit and larval mortality in S. exigua (Juvik and Stevens 1982b). Like the phenolics, the growth inhibitory effects of \( \beta \)-tomatine on H. zea and S. exigua are dependent on the composition of the dietary milieu in which the larvae experience them. The toxicity of \( \beta \)-tomatine to these species is dependent on the relative concentrations of 3-\( \beta \)-hydroxy-sterols present in the diet.

\( \beta \)-Tomatine is non-toxic to H. zea and minimally toxic to young S. exigua larvae in the presence of equimolar concentrations of dietary sterols (Bloem et al. 1989). It is likely that both phenolics and \( \beta \)-tomatine provide significant but variable levels of background resistance to lepidopterous species in L. esculentum as well as other Lycopersicon species. However, as is the case with \( \beta \)-tomatine and Colorado potato beetle, the potential to select for high and stable resistance to Lepidoptera based on phenolic or \( \beta \)-toma
tine concentrations in the foliage or fruit appears to be very limited. The impact of growth inhibiting resistance mechanisms such as toma
tine and phenolics under field conditions has not been well documented and definitive field studies are needed. It is difficult or impossible to predict population level consequences for a pest species or
utility in crop protection of resistance traits that reduce larval growth. Farrar and Kennedy (1990) found that the commonly used procedure of measuring inhibition of larval growth by plant foliage or plant chemicals exaggerated the effects. Because of the sigmoid shape of larval growth curves, a 50% reduction in growth after a fixed time interval does not necessarily translate into a 50% change in either developmental time or final pupal weight. For example, in studies involving \( \alpha \)-tomatine, a concentration that caused a 47% reduction in larval size at 10 days caused only a 4.6% reduction in pupal weight and a 15% increase in development time. In addition to the constitutively expressed resistance mechanisms already discussed, \( L. \) esculentum and very likely other \( Lycopersicon \) species, possess an array of inducible defenses that act to significantly reduce the suitability of induced plants as hosts for insect pests and pathogens.

These induced defenses are important in reducing the damage potential of many pests. They provide a critical base level of resistance in tomato.

Induced defenses of \( L. \) esculentum affecting \( H. \) zea, \( S. \) exigua, and \( M. \) sexta
have been intensively studied. Minor injury to L. esculentum foliage by
insects, pathogens, or mechanical wounding elicits a systemic response
that leads to synthesis of numerous defense-related proteins and alters
the suitability of foliage for some insects and plant pathogens at the
wound site or elsewhere on the plant (Ryan 2000). The inducible defenses
of L. esculentum consist of an array of sets of defensive compounds that
are differentially inducible by different insects and pathogens acting
individually or in combination (Stout et al. 1999). Injury to a plant by
different agents may cause different patterns of resistance to any given
array of insects and pathogens because individual insect and pathogen
species vary in their sensitivity to each set of defensive compounds. Feeding on L. esculentum by caterpillars induces
proteinase inhibitors, peroxidase, polyphenol oxidase and lipoxygenase. The resulting elevated
levels of proteinase inhibitors and to a lesser extent polyphenol oxidase
result in reduced growth and delayed development in H. zea, M. sexta
and S. exigua, and increased mortality of S. exigua feeding on the induced
foliage (Broadway et al 1986, Stout et al. 1998a, b, Stout and Duffey
1996). The induction of proteinase inhibitors and
polyphenol oxidase is systemic, occurs within hours and lasts for at least 21 days. Induction is not uniform throughout the plant. Consequently, some portions of the plant manifest higher levels of resistance than others (Stout et al. 1998a, Stout and Duffey 1996). Levels of induction of proteinase inhibitors and polyphenol oxidase in response to feeding vary among L. esculentum cultivars and generally decline with plant age (Stout et al. 1998a, Cipollini and Redman 1999). The tomato genes coding for proteinase inhibitors I and II have been sequenced and cloned into tobacco (Johnson et al. 1989).

Growth of M. sexta larvae was reduced by 50 to 67% over controls on transgenic tobacco plants expressing tomato proteinase inhibitor II. In contrast, larval growth was only slightly inhibited on transgenic plants expressing tomato proteinase inhibitor I. The production of jasmonic acid via the octadecanoid pathway in response to caterpillar feeding serves as the signal for expression of inducible proteinase inhibitors, polyphenol oxidase and peroxidase in tomato foliage (Ryan 2000, Thaler et al. 1999, Fidantsef et al. 1999). Mutants that block jasmonic acid biosynthesis and or inhibit the response to
jasmonic acid have been identified in tomato. The resistance of plants possessing either of these mutants is compromised (Bergey et al. 1996, Howe et al. 1996, Li et al. 2002, Howe and Schilmiller 2002). Given the level of understanding that exists for induced defenses at the biochemical and genetic level, there would seem to be tremendous potential to enhance and stabilize the expression of these defenses throughout the plant to achieve higher levels of resistance to key insect pests. Resistance to H. zea, S. exigua and S. littoralis has been reported from various accessions of L. pennellii. These species typically oviposit on the foliage and the larvae feed initially on the foliage before boring into fruit in later instars. Because young larvae feed on foliage, resistance mechanisms in the foliage but not in the fruit can be effective. L. pennellii accessions LA1277, Atico 716 and Sisicaya 751, possess high levels of resistance to Spodotera littoralis - the cotton leafworm (Berlinger et al. 1997). Numerous first instar S. littoralis become entrapped in the exudate of foliar glandular trichomes on these accessions and die. Entrapment on all accessions involves physical entanglement but not toxicity. Both Sisicaya 751 and LA1277 also possess additional resistance.
mechanisms associated with the leaf lamella, which cause reduced larval survival, increased larval development time and an elevated incidence of deformed adults. In addition, the adult males that develop on resistant foliage exhibit anomalous sperm development. S. littoralis larvae fed on artificial diet containing foliar extracts from the resistant accessions exhibited similar effects. Based on the solubility characteristics of the extracted compounds, Berlinger et al. (1997) hypothesize at least three compounds contribute to the lamella-based resistance to S. littoralis. Resistance of L. pennellii accession LA716 to H. zea and S. exigua is due at least in part to the acylglucoses produced by the type IV glandular trichomes (Juvik et al. 1994). Application of acylglucose to L. esculentum foliage fed to H. zea and S. exigua larvae caused a reduction in growth rate and survival in both species. When given a choice of acylglucose treated or non-treated L. esculentum foliage, both species preferred the non-treated foliage. When fed synthetic diet containing acylglucose, weight gain by H. zea larvae was significantly reduced but survival was unaffected. In contrast, both survival and growth of S. exigua larvae were significantly reduced. Interestingly, acylglucoses appear
to stimulate egg laying by H. zea moths (Juvik et al. 1994). The genetic control of acylsugar production has been described previously in the context of resistance to aphids. Factors in addition to acylsugars apparently contribute to resistance to H. zea and S. exigua because inbred backcross populations from crosses between L esculentum and L. pennellii, which produced acylsugar levels comparable to L. esculentum, expressed significant levels of resistance to both species. Further, an additional inbred backcross line expressing acylsugars did not exhibit higher levels of resistance to H. zea and S. exigua. Resistance to both species was associated with smaller fruit size, delayed maturity and lower fruit yield (Hartman and St. Clair 1998). A multiple trait selection procedure enabled the selection of an inbred backcross line that did not exhibit a negative association between S. exigua resistance and fruit size or yield. However, there were differences in general combining ability among elite inbreds for percent S. exigua damaged fruit (Hartman and St. Clair 1999b). In these studies, resistance was measured as the proportion of insect damaged fruits, so it is difficult
to compare results to those of studies reporting putative resistance mechanisms on the insects. Resistance in L. hirsutum f. typicum accessions LA1777, LA2329 and PI126445 to S. exigua causes reduced larval growth and survival, relative to L. esculentum. Toxins contained in the tips of type VI glandular trichomes are primarily responsible for the resistance of LA1777 and LA2329, but contribute less to the resistance of PI126445. Factors associated with the leaf lamella are primarily responsible for the resistance of PI126445 but also contribute to the resistance of LA1777 and LA2329 (Eigenbrode and Trumble 1993, Eigenbrode et al. 1994, Eigenbrode et al. 1996). The specific lamella-based factors have not been identified. The sesquiterpene carboxylic acids (+)-(E)-\(\beta\)-santalen-12-oic, (-)-(E)-endo-\(\beta\)-bergamoten-12-oic, and (+)-(E)-endo-\(\alpha\)-bergamoten-12-oic acid, produced in the type VI trichomes of LA1777, have been implicated as the causal mechanism for resistance to S. exigua and H. zea, and to other Lepidoptera as well (Frelichowski et al. 2001). Resistance to Lepidoptera in L. hirsutum f. glabratum has been studied extensively and reviewed in detail elsewhere (Kennedy et al. 1991, Kennedy 2003). L. hirsutum f. glabratum accessions possess high levels of

Resistance to *M. sexta*, which is manifested by mortality of young larvae on the foliage, is due to the presence of lethal concentrations of the methyl ketone 2-tridecanone in tips of type VI glandular trichomes (Kennedy and Yamamoto 1979, Williams et al. 1980). 2-tridecanone contributes to the resistance to numerous other species. It is toxic to a broad range of insect pests and acts as a feeding and oviposition deterrent for some species such as *T. absoluta* (Maluf et al. 1997). Resistance to *S. littoralis*, *T. absoluta* and *P. operculella* was correlated with 2-tridecanone concentrations in segregating F2 populations (Ben-David 1983 cited in Zamir et al. 1984, Maluf et al. 1997). 2-Tridecanone plays only a minor role in the resistance to *H. zea* because exposure of eggs and neonates to sub-lethal concentrations of 2-tridecanone vapors surrounding resistant foliage induces the cytochrome P450 detoxification system in the insect, which then detoxifies the methyl ketone (Kennedy et al. 1988).
feeding on the foliage by induced larvae exposes them to unknown resistance factors present in the leaf lamellae, which reduce growth and survival of the larvae. Only a small percentage of larvae survive to pupation and most of those die as pupae due to exposure to a second methyl ketone, 2-undecanone, present in the tips of type VI trichomes (Farrar and Kennedy 1988). The methyl ketone based resistance is controlled by at least 3 recessive genes although the lamella-based resistance to H. zea is inherited as a dominant or semi-dominant trait (Fery and Kennedy 1987, Farrar and Kennedy 1991b). Resistance in L. hirsutum f. glabratum to H. armigera, which significantly reduces the incidence of fruit damage in the field, is inherited in an additive manner and may involve the same mechanisms as resistance to its close relative H. zea (Kalloo et al. 1989). The lamella-based factors responsible for resistance to H. zea are inherited independently from the lamella-based factors conditioning resistance to Colorado potato beetle, thus indicating that separate mechanisms contribute to the lamella-based resistances to
H. zea and L. decemlineata (Farrar and Kennedy 1991b). Although 2-tridecanone and 2-undecanone are acutely toxic to K. lycopersicella and S. exigua, in contact toxicity assays, other factors are primarily responsible for the resistance. Resistance of L. hirsutum f. glabratum to S. exigua is not correlated with concentration of either methyl ketone (Eigenbrode and Trumble 1993). K. lycopersicella larvae avoid lethal exposure to the methyl ketones contained in tips of type VI trichomes because they are leafminers (Lin et al. 1987). The mechanisms responsible for resistance to these species are not presently known. 2-Tridecanone has also been implicated in resistance of L. hirsutum f. glabratum to T. absoluta (Maluf et al. 1997). However, it is likely that other factors are involved because production of 2-tridecanone is inherited as a recessive trait (Fery and Kennedy 1987), yet F 1 offspring from L. esculentum x L. hirsutum f. glabratum crosses appeared resistant to T. absoluta, on the basis of damage assessments (Maluf et al. 1997).

Mites

Spider mites (Acari: Tetranychidae), primarily the twospotted spider mite, Tetranychus urticae Koch, and the carmine spider mite, T. cinnabarinus
(Boisd.), are important pests of tomato, especially in glasshouse production and under hot, dry conditions in the field. Resistance to either or both of these closely related species has been reported in L. esculentum, L. esculentum var. cerasiforme, L. hirsutum f. typicum, L. hirsutum f. glabratum, L. pennellii and L. pimpinellifolium (Gilbert et al. 1966, Stoner and Stringfellow 1967, Stoner and Gentile 1968, Stoner et al. 1968a, Gentile et al. 1969, Aina et al. 1972, Rodriguez et al. 1972, Cantelo et al. 1974, Gilbert et al. 1974, Patterson et al. 1975a,b, Chiavegato and Mischan 1981, Weston et al. 1989, Fernandez-Munoz et al. 2000). Resistance to another related spider mite, T. marianae McGregor, has been reported from L. hirsutum f. typicum and L. peruvianum. Resistance levels in L. esculentum are generally low to moderate and generally lower than levels in L. hirsutum f. typicum, L. hirsutum f. glabratum and L. pennellii. In most cases, glandular trichomes contribute significantly to the resistance through entrapment of mites and through the repellent or toxic qualities of their tip contents (e.g. Gentile et al. 1969, Knavel et al. 1972, Snyder et al. 1993, Goncalves et al. 1998). Resistance in L. hirsutum f.
Typicum accessions to T. urticae causes reduced mite survival and is associated with high densities of type IV trichomes (Snyder and Carter 1984, Carter and Snyder 1986). Foliage of resistant accessions is also repellent to T. urticae due to the presence of 2,3, dihydrofarnesoic acid in the type IV tips (Guo et al. 1993, Snyder et al. 1993). Resistance in L. hirsutum f. glabratum involves both toxicity and repellency of the foliage due in part to the presence of 2-tridecanone in the type VI trichome tips, but other factors also appear to be involved (Chatzivasileiadis and Sabelis 1998, Goncalves et al. 1998). L. pimpinellifolium accession T0-937 is highly resistant to T. urticae. The resistance is inherited as a completely dominant trait reportedly controlled by 2 to 4 genes (Fernandez Munoz et al. 2000).

Transgenic Resistance

Expression in tomato plants of one or more modified insecticidal protein genes from Bacillus thuringiensis Berliner (Bt) has resulted in high levels of resistance to several lepidopterous pests including M. sexta, H. virescens, H. armigera, and S. exigua (Fischhoff et al. 1987, Delannay et al. 1989, Reynaerts and Jansens 1994, van der Salm et al. 1994, Mandaokar et al. 2000). Transgenic maize and cotton, and to a much less
extent potato,

expressing Bt insecticidal crystal protein toxins have been widely and

successfully grown for crop protection. Consumer resistance to transgenic
crops in the USA and Western Europe has severely limited the use of

transgenic potato cultivars expressing Bt genes but had a lesser impact

on the use of transgenic maize and cotton cultivars (Shelton et al. 2002). Although Bt genes offer tremendous potential for the production of
tomato cultivars resistant to most if not all lepidopterous pests and

Colorado potato beetle, such cultivars have not been commercialized
due largely to concerns over consumer resistance to transgenic food crops.

Transgenic technology offers tremendous potential to readily achieve
high levels of resistance to major insect pests in elite plant types thereby
greatly simplifying insect management and reducing the quantities of
synthetic insecticides required to produce high quality fruit. It is highly
likely that consumer resistance to this technology will wane over time

and transgenic insect resistance based on Bt insecticidal crystal toxins

and other novel resistance mechanisms (e.g. Dowd et al. 1998, Gatehouse

et al. 1997, Gatehouse et al. 1999) will become widely used in tomato.
NON-TARGET EFFECTS OF RESISTANCE

A number of resistance mechanisms in tomato exert adverse effects on parasitoids and predators of tomato insect and mite pests. These effects have been reviewed recently by Kennedy (2003). Trichome mediated resistance traits have been most extensively implicated in negative effects on parasitoids and predators in both the field and laboratory. Effects range from reduced searching efficiency, resulting from physical interference with movement, to increased mortality rates due to entrapment and contact with lethal contents of glandular trichome tips.

predators depend on the specific attributes of the trait and the details of the interaction between the natural enemy, its host or prey, and the plant. Effects can be mediated directly by contact between a parasitoid/predator and the plant or indirectly through the host or prey of the natural enemy. The consequences of any such effects on biological control and pest population levels may be positive, negative or neutral and are difficult to predict based on observed effects on individual parasitoids or predators (Hare 1992). The diversity that exists in parasitoid and predator biology with respect to the details of these interactions makes it difficult to predict such effects and their implications for crop protection.

It seems clear, however, that glandular trichome-mediated resistance mechanisms are more likely than non-trichome-mediated resistance traits to exert general adverse effects on parasitoids and predators of tomato pests (Kennedy 2003). However, the simple demonstration of a negative effect on parasitoid or predator, either in the field or in the laboratory, should not automatically be construed to mean that the effect will have significant negative effects on biological control that
mitigate the benefit of moderate levels of resistance in protecting the crop from damage.

Field experiments specifically designed to characterize the net effects of resistance and biological control should be conducted before conclusions are reached regarding the potential value of a resistance trait for crop protection.

VARIATION IN THE EXPRESSION OF RESISTANCE

glandular trichomes of Lycopersicon can influence tritrophic interactions and biological control (Nihoul 1993). Variation in the expression, whether associated with plant development or environmentally induced, is an important determinant of the general utility of individual resistance traits in pest management. Such variation is common among resistance traits in Lycopersicon and should be fully understood before any commitment is made to breeding for a particular resistance trait.

IDENTIFYING RESISTANCE TRAITS

Identifying potentially useful pest resistance traits requires a knowledge of the biology of the target pest and its relation to crop damage. For pests, such as whitefly and spider mites, which do not feed on fruit and pass many generations within the crop, low pest populations do not cause reductions in yield and quality. For such species low to moderate levels of resistance (that reduce population growth rate by prolonging development time, reducing fecundity and/or increasing mortality) can cause dramatic reductions in population levels in the crop and have great value in managing the pest. In contrast, for species such as fruit...
feeding Lepidoptera, such as H. zea, H. armigera and S. exigua, much
higher levels of resistance are needed to protect the crop. For such pests,
there has generally been little incentive to develop tomato cultivars with
only moderate levels of resistance because the resistance would not
dramatically alter insect management practices and significantly reduce
insecticide use. However, even moderate levels of resistance to such pests
can complement and thereby enhance the effectiveness of other pest
management measures, including application of conventional insecticides
and, in the absence of adverse tritrophic effects, biological control. Identifying useful sources of resistance presents a significant challenge,
especially in wild relatives of tomato that have distinctly different plant
characteristics that may affect pest resistance but which are incompatible
with commercially acceptable plant types. Such characteristics as vine
size, growth habit, and number and size of fruit may affect incidence of
insect damaged fruit (Fery and Cuthbert 1973, Zamir et al. 1984,
plant lines based on differences in incidence of damaged fruit without
accounting for potential effects of such characteristics
and without additional information on insect populations have a high potential for missing potentially valuable resistant plants and for misclassifying otherwise susceptible plants as resistant. Other characteristics also may obscure the existence of valuable resistance traits. For example, Juvik et al. (1988) documented significant variation among Lycopersicon accessions in the occurrence of phytochemical oviposition stimulants for H. zea. They found extracts of L. hirsutum f. typicum accession LA1777 with much more stimulants than extracts of L. esculentum (UC82-1-8). The oviposition stimulants in LA1777 were identified as three structurally similar sesquiterpene carboxylic acids produced by the trichomes (Coates et al. 1988). The occurrence of phytochemically mediated variation in attractiveness for oviposition by H. zea has the potential to mask the occurrence of meaningful levels of larval antibiosis. It also invites further study to determine the feasibility of selecting for resistance due to reduced levels or absence of oviposition stimulants for key lepidopterous pests. In the field, egg populations of H. zea averaged 3.2 times greater on resistant L. hirsutum f. glabratum accession PI134417 than on tomato (L. esculentum). Additionally because of adverse effects of
high densities of
2-tridecanone-containing type VI trichomes, egg parasitism by

Trichogramma on PI134415 averaged less than 1% compared to
43% on L. esculentum. Despite these differences, the populations of
large larvae of

H. zea were similar on PI134415 and L. esculentum plants
because the

effects of higher oviposition and reduced parasitism on
PI134417 were

offset by higher larval mortality in the resistant PI134417
(Farrar et al. 1994). In this case, a simple comparison of larval
populations would not

reveal the potent resistance of PI134417 to H. zea. The
full intensity of the

resistance is only revealed by detailed life table studies or field

observations guided by the results of well-designed laboratory bioassays.

When working with exotic plant types and novel resistance
traits, a

combination of laboratory and field experiments is recommended to

ensure that valuable traits are not overlooked. Complete
documentation

of carefully designed laboratory or greenhouse experiments on the effects

of resistance traits and the life history traits of the
target pest can prove

extremely valuable in designing optimal selection protocols for use in
resistance breeding efforts (Romanow et al. 1991).

SUMMARY AND CONCLUSIONS

Resistance to insect and mite pests of tomato is common but highly variable both within and among Lycopersicon species.

Resistance to numerous important pests has been reported from L. esculentum Mill., but the levels of resistance are generally low. High levels of resistance to major pest species are most common in L. hirsutum f. typicum Humb and Bonpl., L. hirsutum f. glabratum C.H.Mull and L. pennellii (Corr.) D’Arcy,

but significant levels of resistance to insect pests have been documented from L. cheesmanii Riley, L. chmielewskii Dun., L. peruvianum (L.) Mill., and L. pimpinellifolium (Jusl.) Mill. There is considerable variation among accessions within each of these species in the levels of resistance to individual pest species and the array of pest species resisted. The majority of research on insect resistance in Lycopersicon has focused on identifying sources of resistance to selected pest species and elucidating the mechanisms responsible for resistance.

In only a few instances has the genetic basis for resistance been investi gated. In even fewer cases have the effects of resistance traits on pest populations and crop damage been systematically
characterized under field conditions to assess potential utility in crop protection. There is a clear need for systematic field studies of candidate resistance traits to characterize these effects. The chemical/physical mechanisms responsible for insect resistance are, in most instances, complex. In many cases, multiple mechanisms are involved such as physical entrapment by foliar trichomes, presence of chemical toxins, feeding detersants, and growth inhibiting compounds.

Foliar glandular trichomes are important contributors to resistance to a number of important pest species. In some cases resistance traits associated with the leaf lamella are expressed constitutively by the plants whereas in others resistant expression is induced by insect feeding or mechanical damage. Even in instances where the mechanisms are relatively simple, as in the case of 2-tridecanone/type VI trichome-mediated resistance of L. hirsutum f. glabratum, the biological interactions involving the resistance characters can be extremely complex affecting both pests and their natural enemies. In most instances, expression of resistance is conditioned by multiple genes, and in a number of cases, is subject to significant
environmental influence. Nonetheless, there are a number of traits that condition resistance to multiple insect pests, such as those found in L. pennellii and L. hirsutum, which hold considerable promise for incorporation into commercial tomato cultivars using conventional plant breeding techniques. The mechanics and genetic basis for resistance in Lycoperscon and tomato to aphids, white fly, thrips, diptera, beetles, caterpillars, and mites are summarised, including non-target effects of resistant traits. The relative ease with which tomato can be genetically transformed and the success with transgenic insect resistant cotton and maize suggest that, with time, transgenic insect resistant tomato cultivars will become commonplace.


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INTRODUCTION

Current world agricultural production is largely limited by environmental
stresses (Boyer 1982). It is estimated that only 10% of the world’s arable

land may be free of stress (Dudal 1976). Extreme temperatures, drought,

soil salinity, and nutrient imbalances (including mineral toxicities and
deficiencies) are among the major environmental constraints
to crop

production worldwide. Most plant breeding programs have focused on the
development of cultivars with high yield potential in favorable (i.e.,
nonstress) environments. Such efforts have been extremely successful in

improving the efficiency of crop production per unit area thus resulting in

significant increase in total agricultural production (Duvick 1986).

However, with the rapid increase in human population there is a greater

need for food production, and with the increasing diminution in natural

resources, and arable lands, greater efforts must be made to increasing
crop productivity under stressful agricultural environments and bringing

marginal lands under cultivation. Marginal agricultural environments
could be enriched by growing stress tolerant plants to increase yields,

which is an important step toward solving the problem of feeding the

world’s growing population. The genetic improvement of plant for stress tolerance can be an
economically viable solution for crop production under stressful environments (Blum 1988). The progress in breeding for stress tolerance depends on an understanding of the physiological mechanisms and genetic basis of stress tolerance at the whole plant and cellular levels.


Accumulating evidence suggests that plant response to abiotic stress is generally complex; it is often controlled by more than one

and highly influenced by environmental variation (Ceccarelli and Grando 1996, Richards 1996). The quantification of stress tolerance often poses serious difficulties. Direct selection under field conditions is generally difficult because uncontrollable environmental factors adversely affect the precision and repeatability of such trials (Richards 1996). In addition, stress tolerance appears to be a developmentally-regulated, stage-specific phenomenon; tolerance at one stage of plant development is generally not correlated with tolerance at other developmental stages (Greenway and Munns 1980, Shannon 1985, Maas 1986, Lauchli and Epstein 1990, Johnson et al. 1992, Foolad and Lin 1997a, Foolad 1999). Specific ontogenetic stages, such as seed germination and emergence, seedling survival and growth, and vegetative growth and reproduction should be evaluated separately for assessment of tolerance and the identification, characterization, and utilization of useful genetic complements. The knowledge gained from developmental and physiological aspects of stress tolerance will positively
facilitate a better understanding of its genetic basis and the development of stress-tolerant plants. Recent advances in molecular genetic techniques, including genetic transformation, analysis of gene expression, marker mapping, and quantitative trait loci (QTLs) analysis have contributed greatly to better understanding of the genetic and biochemical basis of plant stress-tolerance and, in some cases, also led to the development of plants with enhanced tolerance to abiotic stress. For example, significant progress has been made in the identification of genes, enzymes or compounds with significant effects on plant stress tolerance at the cellular or organismal level (Morgan 1991, Bohnert and Jensen 1996, Shinozaki and Yamaguchi, Shinozaki 1997, Shen et al. 1997, Winicov 1998, Allakhverdiev et al. 1999, Apse et al. 1999, Bohnert and Shen 1999, Grover et al. 1999, Seki et al. 2003, Wang 2003, Yardanov et al. 2004.). Furthermore, manipulation of the expression or production of the identified genes, proteins, enzymes, or compounds through transgenic approaches have resulted in the development of plants with enhanced stress tolerance in different plant species (Apse et al. 1999, Lilius et al. 1996, Rathinasabapathi 2000,
Molecular marker technology also has allowed the identification and genetic characterization of QTLs with significant effects on plant stress tolerance during different developmental stages (Ellis et al. 1997, Foolad and Chen 1999, Foolad et al. 1998a, Foolad et al. 2001, Forster et al. 1997, Mano and Takeda 1997) and has facilitated the determination of the genetic relationships among tolerance to different stresses and tolerance at different developmental stages (Foolad 1999). Such advancements are expected to contribute significantly to the development of plants with tolerance to different stresses in near future (Flowers 2004). The cultivated tomato, Lycopersicon esculentum Mill., is moderately or highly sensitive to many abiotic stresses, including salinity, drought, extreme temperatures, excessive moisture, nutrient imbalances, and environmental pollution. However, within the genus Lycopersicon, there are several wild species that represent a rich source of useful genetic variation (Rick 1976, 1979). Such variation has been extensively utilized in
tomato breeding programs for improving desirable agricultural characteristics, especially disease resistance. More recently, this variation has also been used to characterize the physiological and genetic basis of abiotic stress tolerance in tomato and to develop stress-tolerant plants. In this chapter, the available variation in relation to abiotic stress tolerance and recent advancements in genetics and breeding of tomato for stress tolerance are reviewed. The potential and limitations of different approaches for developing commercial tomato cultivars with improved stress tolerance are also discussed.

TOLERANCE TO SALT STRESS

Background Information

Salinity is an increasingly important environmental constraint to crop production mostly in the arid and semi-arid regions of the world (Boyer 1982, Tanji 1990, Rowley 1993). Regardless of the suspected physiological cause (ion toxicity, water deficit, and/or nutritional imbalance), high salinity in the root zone severely impedes normal plant growth and development, resulting in reduced crop productivity or total crop failure. Of the total 14 billion ha of land available on earth, 6.5 billion...
ha are estimated to be arid and semi-arid and about 1 billion ha are natural saline soils. It is estimated that worldwide about 20% of cultivated lands and 33% of irrigated agricultural lands are afflicted by high salinity (Epstein et al. 1980, Flowers et al. 1986, Francois and Maas 1994, Tanji 1990, Szabolcs 1992).

These estimates do not account for the lands that are considered not arable due to the very high concentrations of salts, including areas along the seashores in temperate regions of the world and millions of hectares of desert lands of Africa, the Middle East, Asia, and North America. Such marginal lands could be agriculturally productive if more salt tolerant species or cultivars were available. Furthermore, the salinized areas are increasing at a rate of 10% annually. Low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water, and poor cultural practices are among the major contributors to the increasing salinity (Kalaji and Pietkiewica 1993, Syverstein et al. 1989, Tanji 1990, Szabolcs 1994). In some coastal areas, such as those of the United States, the gradual intrusion of seawater into the fresh-water aquifers threatens water sup
plies for agricultural, industrial, and municipal users
(Cole 1993). The presence of saline lands and saline
agricultural water has been

apparent to farmers for centuries. However, it has only
been since the

beginning of the 20th century that, because of the
rapidly growing

population and the need for greater food production,
cultivation under

saline conditions has been considered. Two major approaches
have been

proposed to minimize the deleterious effects of high
soil/water salinity

(Epstein et al. 1980) and these must be considered
simultaneously to

achieve a sustainable crop production. Implementing large
engineering

schemes for reclamation, drainage and irrigation with high
quality water

have been one of the first technological approaches used
effectively.

Although this approach has been successful in some areas,
the associated
costs are high and it often provides only a temporary
solution to the

problem. The second approach entails biological strategies
focused on

the exploitation or development of plants capable of
tolerating high soil/

water salinity. This approach includes: (a) diversifying
cropping systems

to include crops that are known to be salt tolerant (e.g.,
crop substitution),

(b) exploiting wild or feral species that are adapted to
saline environments
(e.g., domestication), and (c) genetically modifying
domesticated crops by
breeding and selection for improved salt tolerance (ST).
Crop substitution
has been largely practiced since the beginning of
agriculture, and is one of
the most practical strategies to deal with salinity when
growing field crops
(Shannon 1996). However, most vegetable crops, including
tomatoes, are
generally more sensitive to salinity than field crops.
Thus, the idea of
developing salt-tolerant vegetable crops, which can produce
economic yields
under salinity stress, has drawn more attention (Shannon
1979, Rush
et al. 1981). Salt tolerance (ST) is generally defined as
the inherent ability of the
plant to withstand effects of high salts in the root zone
or in the plant’s
leaves without significant adverse effect on plant
productivity. During the
past several decades considerable research has been done on
the
physiological and metabolic aspects of plant ST at the
whole plant and
cellular levels and the subject has been reviewed
extensively (Levitt 1980b,
Bohnert and
Ashraf and Mc
Neilly 2004). However, efforts devoted to the genetic characterization of
plant ST at the cellular or organismal level, and the integration of whole
plant response in a developmental context, are more recent and rather
incomplete (Blum 1988, Ashraf 1994, Foolad 1997, Jain and Selvaraj 1997,
et al. 2001). Accumulating evidence suggests that plants respond to salt
stress (SS) through numerous quantitative traits involving many genes or
proteins whose expression is influenced by external environmental
variation and plant developmental stage (Levitt 1980a, Blum 1988, Chaubey
and Senadhira 1994, Richards 1996). The quantification of plant ST poses
serious difficulties. Direct selection in the field is often difficult because of
the spatial and temporal variation in soil salinity (Hajrasuha et al. 1980,
Richards and Dennett 1980), and the influence of other environmental
factors, including variations in rainfall, nutrient availability, and
temperature (Richards 1996). There is no reliable field screening technique
that could be used year after year or generation after generation. Thus,
breeders have been in search of more effective approaches for screening
and improving crop ST. Commercial cultivars of tomato are sensitive to salinity at all stages of plant development, which include seed germination, vegetative stage, and reproduction. Consequently, the growth and economic yield is substantially reduced under SS (Maas 1986, Jones et al. 1988, Bolarin et al. 1993). However, genetic resources for ST have been identified largely within the related wild species of tomato. Attempts to identify sources of genes for ST in tomato were first made by Lyon (Lyon 1941), who suggested that ST of the cultivated tomato might be improved by introgression of genes from L. pimpinellifolium (Jusl.) Mill., the most closely related wild species of tomato. Later investigations resulted in the identification of a few other salt-tolerant accessions within L. esculentum and the related wild species, including L. pimpinellifolium, L. peruvianum (L.) Mill., L. cheesmanii Riley, L. hirsutum Humb. and Bonpl., and L. pennellii (Corr.) D’Arcy (Foolad and Lin 1997b, Jones 1986a, Phillips et al. 1979, Rush and Epstein 1976, Sarg et al. 1993, Tal 1971, Tal and Shannon 1983). It is expected, however, that more salt-tolerant accessions can be identified within the wild species of tomato with a more thorough
evaluation of the available germplasm. By using the identified salt-tolerant germplasm and various genetic tech

iques, notable progress has been made during the past few decades in characterizing the genetic basis of ST in tomato. A significant finding has been that ST at each stage of plant development in tomato is genetically not correlated with tolerance at other developmental stages (Asins et al. 1993a, Foolad 1999, Foolad and Lin 1997a, Jones and Qualset 1984), similar to findings in other plant species (Ashraf and McNeil 1988, Johnson et al. 1992). Furthermore, it has been demonstrated that, in tomato, ST generally increases with plant age (Bolarin et al. 1993), again similar to the findings in other plant species such as barley (Hordeum spp.), corn (Zea mays L.), rice (Oryza sativa L.) and wheat (Triticum spp.) (Maas 1986).

Therefore, it has been suggested that ST at each developmental stage must be evaluated separately for the assessment of tolerance and for the identification, characterization, and utilization of useful genetic compo

ents. In this section, the recent advancements on tomato ST during different developmental stages are presented and the potential for
developing salt-tolerant cultivars using different genetic approaches
discussed.

Salt Tolerance during Seed Germination and Seedling Emergence

The commercial cultivars of tomato are most vulnerable to salinity stress at
the seed germination (SG) and early seedling growth stages (Cook 1979,
Foolad and Jones 1991, Jones 1986b, Maas 1986). At these stages, tomato
exhibits sensitivity even to low concentrations of salts (~75 mM NaCl)

However, surface soils may have salinities several fold that of the subsoil,
presenting a serious problem at the germination stage. High salinity delays
the onset, reduces the rate and final percentage germination, and increases
the dispersion of germination events. This sensitivity has important
biological and applied significance. The costly operation of greenhouse
seedling production and transplantation into the field has encouraged
many tomato producers to grow direct-seeded crops (Liptay and Schopfer
1983). Furthermore, the dependence upon mechanization in modern
cultivation systems, and the use of costly hybrid seed, requires rapid,
uniform and complete germination. Improving the uniformity and rapidity of SG responses under saline conditions would contribute significantly to the efficiency of stand establishment in tomato. Genetic resources for ST during SG have been identified within the cultivated and related wild species of tomato, including L. pennellii, L. pimpinellifolium, and L. peruvianum (Cuartero and Fernandez-Munoz 1999, Foolad and Lin 1997b, Jones 1986a). Some of the identified salt-tolerant accessions have been used to investigate the genetic basis of ST during SG in tomato, as briefly described below.

Inheritance

Using parental, filial and backcross populations (total of 10 generations) of a cross between a salt-sensitive tomato breeding line (UCT5) and a salt tolerant L. esculentum plant introduction line PI174263, Foolad and Jones (1991) determined that the ability of tomato seed to germinate rapidly under SS was genetically controlled with a narrow-sense heritability ($h^2$) of $0.75 \pm 0.03$. Parent-offspring regression analyses, using $F_2:F_3$ and $F_3:F_4$ progeny of the same hybrid (UCT5 $\times$ PI174263), provided similar estimates of $h^2$ for this trait (Foolad and Jones 1992). Both studies indicated that
tomato ST during SG was genetically controlled with additivity being the major genetic component and that it could potentially be improved by directional phenotypic selection. In a later study, the effectiveness of phenotypic selection to improve tomato SG under SS was examined using $F_2$, $F_3$ and $F_4$ progeny populations of a cross between UCT5 and PI174263 (Foolad 1996b). Analysis of response to selection indicated that ST during SG in tomato was improved by directional phenotypic selection; estimates of realized $h^2$ for rapid SG under SS ranged from 0.67 to 0.76 (Foolad 1996b). The overall results from these studies suggested that ST during SG in tomato was most likely controlled by a few major genes with additive effects, and therefore it could be improved by directional phenotypic selection. A better understanding of the genetic control of ST during SG in tomato, however, was achieved using molecular marker technology as mentioned in below.

QTL Mapping

An approach for a better understanding of the genetic basis of, and improving the selection efficiency for, complex traits, including plant ST, is to discover molecular genetic markers that are associated,
either through linkage or pleiotropy, with genes or QTLs that control the trait(s) of interest.

Molecular marker technology can facilitate precise determination of the number, chromosomal location, and individual and interactive effects of QTLs that control the trait. Following their identification, useful QTLs can be introgressed into desirable genetic backgrounds by marker-assisted selection (MAS) (Tanksley et al. 1989; see also Chapter 7). During the past several years, a few QTL mapping experiments were conducted to determine the number, genomic location and individual effects of QTLs affecting ST during SG in tomato. In one experiment, seed of an F2 population of a cross between a salt-sensitive tomato breeding line (UCT5) and a salt-tolerant accession (LA716) of L. pennellii was subjected to SS and the two extreme tails (salt-tolerant and salt-sensitive) of the response distribution were selected (Foolad and Jones 1993). The selected individuals in each tail were subjected to marker analysis, and marker allele frequency differences between the two tails were determined. A trait-based marker analysis (also known as selective genotyping or distributional extreme analysis),
which determines the significance of the marker allele frequency differences between selected tails of a response distribution (Lebowitz et al. 1987, Darvasi and Soller 1992), was used to identify marker-linked QTLs. Five genomic locations were identified on chromosomes 1, 3, 7, 8 and 12 bearing QTLs for ST during SG in this population (Foolad and Jones 1993). The validity of these QTLs was examined in a few subsequent investigations using various populations of the same (UCT5–LA716) or different interspecific crosses, including BC 1, BC 1 S 1 and recombinant inbred line (RIL) populations of a cross between a L. esculentum breeding line (NC84173) and a salt-tolerant accession (LA722) of L. pimpinellifolium (Foolad et al. 1997, Foolad and Chen 1998, Foolad et al. 1998a, Foolad et al., unpublished data). In all of these studies, larger populations and larger number of molecular markers were used compared to the original study. In addition, different mapping strategies, including trait-based and marker-based (standard interval mapping) analyses, were employed. These studies validated most of the previously identified QTLs and detected a few new QTLs on chromosomes 2 and 9. The combined results supported the previous
suggestion that ST during SG in tomato was a quantitative trait controlled by more than one gene. However, in all of these studies it was demonstrated that ST during SG in tomato was controlled by a few QTLs with major effects and several QTLs with small effects (Foolad and Jones 1993, Foolad et al. 1997, Foolad and Chen 1998, Foolad et al. 1998a). Further more, these studies indicated the presence of limited or no epistatic interactions among the identified QTLs. The demonstration that a few QTLs with major and independent effects determined most of the variation in ST during SG in tomato was consistent with the previous observation that this trait could be improved by directional phenotypic selection (Foolad 1996b). The overall results suggested that the prospect for improving this trait by MAS was good. Comparison of QTLs identified for ST during SG in different interspecific populations of tomato, including those derived from L. esculentum × L. pennellii (Foolad et al. 1997, Foolad and Chen 1998) and L. esculentum × L. pimpinellifolium crosses (Foolad et al. 1998a), indicated that some QTLs were conserved across species whereas others were species-specific.

However, because in most cases more than one genetic
resource is utilized
during the life of a breeding project, it may be possible
to incorporate ST
QTLs from different resources using MAS. Furthermore,
comparison of QTLs
in different populations (e.g. BC 1 S 1 and RILs) of the
same L. esculentum ♀ L.
pimpinellifolium hybrid indicated that some QTLs were
stable across
populations/generations while others were
population-specific. The
combined results suggest that it should be feasible to
improve ST during
SG in tomato by either phenotypic selection or MAS.
However, because
tolerance genes are often found within the wild species of
tomato, a
combination of phenotypic and marker-assisted selections
might be more
advantageous.

Response to Different Levels of Stress

It has been argued that in many saline soils the
concentration of salts
varies across the soil horizon, ranging from low to
moderate and high
(Richards and Dennett 1980). A successful cultivar would be
one which
exhibits ST at a wide range of SS and whose performance
would not decline
in the absence of salts. Selection under different levels
of SS, however, may
not always be practical in a breeding program. Thus, it is
essential to
examine relationships among responses to different levels of salts and
determine whether there is a critical salt concentration at which selections
should be conducted. Several investigations have examined relationships
among germination responses under different levels of SS in tomato. For
example, evaluation of 56 tomato genotypes (commercial lines, plant
introductions and wild accessions) for SG at different levels of SS, including
75 mM (low), 150 mM (intermediate) and 200 mM (high) salt concentrations,
indicated genotypes that generally germinated rapidly at the low salt
congestion also germinated rapidly at the moderate or the high salt
concentration (Foolad and Lin 1997b). Linear correlation analysis indicated
the presence of a phenotypic relationship ($r = 0.90, P < 0.01$) between
germination at 75 mM and 150 mM salt. The results suggested that the
same genes control the rate of tomato SG under different levels of SS. This
suggestion was later confirmed genetically by an analysis of response and
correlated response to selection for ST, where selections were made
separately under 100 mM (low), 150 mM (intermediate) and 200 mM (high)
salt and their progeny responses also examined at all three
salt concentrations (Foolad 1996b). In this study, selection for rapid SG at any SS level resulted in progeny with enhanced germination rate at all three (100, 150 and 200 mM) SS levels, regardless of which salt concentration was used during the selection process. A genetic correspondence (genetic correlation) of up to 100% between germination at different SS levels was observed. The results suggested that similar or identical genes with additive effects contributed to rapid SG at different SS levels. This suggestion was also consistent with the finding that in F2 populations of a cross between a salt-tolerant accession (LA716) of L. pennellii and a salt-sensitive tomato cultivar (UCT5) identical QTLs contributed to ST during SG at different SS levels (Foolad and Jones 1993). The combined results indicated that the development of tomato cultivars with improved SG at diverse SS levels could be accomplished at a single SS level. However, because the rate of tomato SG at a moderate level of SS (150 mM salt) was highly correlated with that at both a low (100 mM salt) and a high SS level (200 mM salt), it was suggested that selection and breeding for ST should be performed at
an intermediate SS level (Foolad 1996b).

Physiological Genetics

Although QTLs for ST during SG in tomato have been identified and verified,

the genetic nature of the QTLs or the physiological mechanism(s)

that they modulate has not been determined. However, based on the

current genetics and physiological knowledge of ST during SG, some

inferences as to the role of these QTLs can be made. ST during SG is a

measure of the seed’s ability to withstand the effects of high concentrations

of salts in the medium. Excessive salt depresses the external water

potential, making water less available to the seed. Slower SG under SS

compared to nonstress conditions, however, could be due to osmotic

and/or ionic effects of the saline germination medium. Physiological

investigations to distinguish between these two effects have been limited.

However, accumulating evidence in different crop species suggests that

low water potential of the external medium, rather than ion toxicity

effects, is the major limiting factor to germination under SS (Kaufman


1995), although a few reports have indicated otherwise
In a recent investigation, germination responses of eight tomato genotypes, including salt-tolerant and salt-sensitive accessions of L. esculentum and L. pimpinellifolium, were evaluated in iso-osmotic (water potential ~ -700 kPa or ~15 dSm⁻¹) media containing either NaCl, MgCl₂, KCl, CaCl₂, sorbitol, sucrose, or mannitol (J.R. Hyman and M.R. Foolad, unpublished data). Comparison of germination rates in the SS treatments (NaCl, MgCl₂, KCl, and CaCl₂) with those in osmotic-stress treatments (mannitol and sorbitol) indicated that all genotypes responded similarly to these two types of stresses.

Furthermore, comparison of germination rates among the various SS treatments indicated that the different types of salt generally affected germination similarly. The overall results suggest that ST during SG in tomato is an adaptation to germinate rapidly under low water-potential and that there is little or no ionic toxicity effects. Anatomically, the tomato seed comprises a seed coat (testa) that encloses a curved filiform embryo surrounded by an endosperm, which practically fills the lumen of the seed not occupied by the embryo (Esau...
For germination to occur, the hydraulic extension force of the embryo must exceed the opposing force of the seed coat and the living endosperm tissues at the placental end of the seed (Hegarty 1978, Bradford, Liptay and Schopfer 1983, 1986, Groot and Karssen 1987). Thus, embryo was suggested to play a major role in determining the time to germination of tomato seed (Liptay and Schopfer 1983). According to this hypothesis, differences in salt sensitivity of tomato seeds during germination reside either in the osmotic potential or pressure potential of the germinating embryo. However, external osmotic stress can also negatively affect seed imbibition, and thus retard (or prevent) weakening of the restrictive forces of the endosperm and testa, resulting in reduced rate (or inhibition) of germination (Liptay and Schopfer 1983, Groot and Karssen 1987, Dahal et al. 1990). Hence, the rate of SG in tomato may be influenced by the physical, chemical, and thus, the genetic composition of the embryo, endosperm and/or the testa. The identified QTLs for ST during SG in tomato could therefore affect germination rate by affecting the vigor of the germinating embryo, the variation in the thickness of the
endosperm,
the physical and permeability properties of the endosperm
cell walls,
the time of onset or rate of activity of enzymes which
modify the properties
of the endosperm cell wall, the release of gibberellin by
the embryo (which
is necessary for endosperm weakening), the base water
potential required
for seed germination, the hydrotome constant (Bradford
1995), the rate of
metabolic activities in the embryo or endosperm under
osmotic stress,
osmoregulation during germination, or any other
physiological or
metabolic processes which are essential for the initiation
of germination.
However, isolation, characterization and comparison of
functional genes
which facilitate rapid SG under SS would be necessary to
determine the
actual role(s) of the identified QTLs. Nonetheless,
irrespective of the
physiological mechanism(s) of ST during SG in tomato, the
identified QTLs
could potentially be useful for improving tomato ST using
MAS and
breeding.
Salt Tolerance during Vegetative Growth and Reproduction

For tomato production under saline conditions, ST during
the vegetative
stage (VS) is more important than ST during SG because most
tomato crops
are established by seedling transplantation rather than direct seeding in the field. Furthermore, ST during VS may also be more important than ST during reproduction (i.e., flowering and fruit set) because tomato tolerance of salinity generally increases with plant age so that plants are usually most tolerant at maturity (Bolarin et al. 1993). For example, during flowering and fruiting stages, tomato plants can withstand salt concentrations that can kill them at the seedling stage. Furthermore, there is a positive correlation between tomato yield and plant size under SS (Pasternak et al. 1979, Bolarin et al. 1993), indicating the importance of ST during the VS. Most commercial cultivars of tomato are moderately sensitive to SS during VS (Tal and Shannon 1983, Maas 1986, Foolad and Lin 1997b). At low concentrations of salt (electrical conductivity (EC) = 3-5 dSm⁻¹), tomato growth is mainly restricted by nutritional imbalances, as nutrients become the limiting factor under such conditions (Cuartero and Fernandez Munoz 1999). At moderate to high levels of salt (EC = 6 dSm⁻¹), in addition to nutrient imbalances, osmotic effects and ion toxicity contribute to reductions in growth. Phenotypic variation for ST during VS
in tomato has been identified within the cultivated (Curatero et al. 1992, Sarg et al. 1993, Foolad 1997) and related wild species, including L. peruvianum (Tal and Gavish 1973), L. pennellii (Dehan and Tal 1978, Saranga et al. 1991, Perez-Alfocea et al. 1994, Cano et al. 1998), L. cheesmanii (Rush and Epstein 1976, Asins et al. 1993a), and L. pimpinellifolium (Bolarin et al. 1991, Curatero et al. 1992, Asins et al. 1993a, Foolad and Chen 1999). Although much of this phenotypic variation has not been genetically verified, it can be potentially useful for developing tomato cultivars with enhanced ST. Comparatively, less research has been conducted on tomato ST during reproduction than earlier stages. Limited effort has been devoted to determine tomato pollen viability or stigma receptivity, and/or the ability of the plant to produce flowers and set fruit under SS. This may be due in part to a higher ST generally observed during reproduction than earlier stages in tomato. Adams and Ho (1992) reported that increasing salinity to 10-dSm did not significantly affect fruit set in tomato, which was reduced only at 15-dSm -1 . Grunberg et al. (1995) reported that salinity does not affect to
mato pollen viability, however, the number of pollen grains per flower decreases with the duration of salinity, ca. 30% of the normal in 70 days after the beginning of salinization. The suggestion of a higher ST during reproduction, however, is in contrast with a report that several tomato genotypes that grew adequately under saline conditions failed to produce any fruit (Asins et al. 1993a). In a recent study, 13 accessions from three different tomato species were grown under both saline (300 mM NaCl + 30 mM CaCl₂; equivalent to ~100 mM NaCl) and control (no salt) conditions, and their pollen production and in vitro pollen germination examined (S. Prakash and M.R. Foolad, unpublished data). For most accessions, there was no significant reduction in pollen production (per flower) in response to SS.

Pollen from both salt-grown and control-grown plants was cultured at different salt concentrations, including 0, 0.2, 0.4 and 0.8% NaCl and evaluated for percentage germination after 4 or 8 h of incubation. In all accessions, pollen germinability was decreased under salt compared to the control (no salt) treatment, and the reduction was greater at higher than lower salt concentrations. However, in most accessions, in vitro pollen
germinability for salt-grown plants was generally higher than that for the control-grown plants at high salt concentrations (0.4 and 0.8% NaCl), suggesting that pollen ST in tomato was increased by acclimating plants under SS. The cultivated tomato is considered “moderately sensitive” to salinity considering the reduction in fruit yield under SS compared to normal conditions (Maas 1990). Fruit yield of tomato cultivars starts decreasing when the EC of the saturated soil extract exceed 2.5 Sm⁻¹ (Maas 1990, Saranga et al. 1991), although there are reports of higher thresholds for yield reduction in tomato (Adams 1991). A 10% reduction in fruit yield is expected per additional dSm⁻¹ beyond the threshold level (Saranga et al. 1991). The major cause of yield reduction in tomato under low to moderate levels of salinity (EC = 3-9 dSm⁻¹) is the reduction in average fruit size, and not a reduction in fruit number (van-Ieperen 1996). A 10%, 30%, and 50% reduction in fruit size is caused following irrigation with 5-6, 8, and 9 dSm⁻¹, respectively (Cuartero and Fernandez-Munoz 1999). Thus, small-fruiting cultivars, including cherry tomatoes, may be better than large fruited cultivars for growing under low to moderate levels
of SS. This is because the relative reduction in fruit size is less in smaller fruits (Caro et al. 1991). However, at higher levels of salinity, or prolonged exposure to salinity, a reduction in the total number of fruits per plant, mainly due to a reduction in the number of trusses per plant, is the major cause of yield reduction in tomato (Cuartero and Fernandez-Munoz 1999, van-Ieperen 1996). Thus, under high SS levels, significant yield reduction is expected for both large-fruited and small-fruited cultivars. Furthermore, because upper inflorescences are more sensitive to salinity (Cuartero and Fernandez-Munoz 1999), when breeding for ST in tomato, it would be better to develop cultivars with a compact plant type and early maturity, in which only 4-6 trusses are produced and harvested. The potential of wild tomato species as sources of ST during reproduction (i.e., flower and fruit set and fruit yield) has not been assessed critically, mainly because most of the wild accessions are self-incompatible and/or produce very small fruits that cannot be easily compared with fruits of the cultivated species. However, progenies derived from interspecific hybrids of the cultivated and wild tomatoes have been used
Inheritance

Most of the earlier research on tomato ST during VS was focused on the physiology of plant response to SS. During the past few decades, however, research has also been conducted on genetics of ST in tomato with the goal of facilitating the development of cultivars with enhanced ST. In 1976, Rush and Epstein (1976) proposed the exploitation of genetic resources within the wild Lycopersicon species to increase ST of the cultivated tomato.

However, at that time there was little information regarding the genetic control of ST in tomato to warrant such an endeavor. Rush and Epstein (1981b) hybridized a “salt-tolerant” L. cheesmanii accession (LA1401) with a salt-sensitive tomato cultivar (Walter) and produced filial (F 1 and F 2 ) and backcross (BC) progeny. They reported that selections for ST in the F 2 and BC generations resulted in progeny with enhanced ST compared to Walter, suggesting that ST of LA1401 was heritable and could be transferred to the cultivated tomato by hybridization and selection. However, recent studies did not confirm the presence of ST in LA1401 or its interspecific progeny (author’s unpublished data). Saranga et al. (1992)
developed BC 1

and BC 1 S 1 (self-progeny of BC 1) populations of a cross between a salt-sensitive tomato processing line (M82) and a “salt-tolerant” L. pennellii accession (LA716) and evaluated them for ST under field conditions.

Evaluations were based on total dry matter and total fruit yield under saline conditions as well as total dry matter under salt relative to control (no salt). Estimates of h2 for these traits were moderate (0.30-0.45), suggesting that ST of the cultivated tomato could be improved by selection for dry matter and yield parameters under salinity using LA716 as a genetic resource. By evaluating F 2 progeny of a cross between a salt-sensitive cultivated tomato and a “salt-tolerant” accession of L. pimpinellifolium under SS, Asins, et al. (1993b) concluded that total fruit yield and total fruit number were useful selection criteria for improving tomato ST; estimates of broad sense h2s for these two traits were 0.53 and 0.73, respectively. In a greenhouse hydroponics study, using parental, filial and backcross generations (total of 10 populations) of a cross between a salt-sensitive tomato breeding line (UCT5) and a salt-tolerant primitive cultivar (PI174263),
Foolad (1996a) determined that growth under SS relative to control (the most widely used index in physiological investigations of ST in tomato) was under additive genetic control and could be an excellent selection criterion for improving tomato ST. However, in none of these studies was any empirical selection made to verify that ST of the tomato could be improved by directional phenotypic selection under saline conditions.

Nonetheless, these and other studies (Bolarin et al. 1991, Foolad 1996a) suggest that shoot growth under salinity relative to control (also known as relative growth under SS) is the best indicator of ST in tomato. It is generally agreed that direct selection for ST under field conditions is difficult because of the confounding effects of numerous other environmental factors (Richards 1983, Tal 1985, Yeo and Flowers 1990).

A suggested approach to improve the efficiency of selection for ST has been the adoption of new selection criteria based on genetic knowledge of physiological processes which limit crop production under saline conditions (Tal 1985). Physiological criteria which have been suggested as potential indicators of ST in tomato include tissue water potential, tissue ion content, K+/Na+ ratio, osmoregulation, succulence,
and water use

efficiency (WUE). Whether these physiological parameters are valid

indicators of ST in tomato, or if there is genetic variation within Lycopersicon

for these responses, must be determined before the question of their

genetic controls can be addressed. Several researchers have investigated

the relationship between tomato ST and various physiological responses

and have commented on their utility as indirect selection criteria


studies and their findings are reviewed below. Physiological investigations indicate that most of the salt-tolerant

genotypes within the cultivated tomato and the closely-related wild

species L. pimpinellifolium exhibit a glycophytic response to salinity, that

is, exclusion of toxic ions (in particular Na+) at the root or shoot level and

the synthesis and accumulation of compatible organic compounds (e.g.,

sugars and amino acids) for osmoregulation (Caro et al. 1991, Cuartero et


Santa-Cruz et al. 1990). In contrast, salt-tolerant accessions within the
tomato wild species L. pennellii, L. cheesmanii and L. peruvianum generally
exhibit a halophytic response to salinity, in which osmotic adjustment is
achieved by uptake of inorganic ions from the soil and compartmentalizing
them in the vacuoles of the leaf or other plant organs (Sacher et al. 1983, Tal and Shannon 1983, Bolarin et al. 1991, Perez-Alfocea et al. 1994).
However, differential accumulation of ions in the leaf tissue has not
always been determined as a major factor affecting tomato ST or salt
sensitivity. Analysis of BC 1 and BC 1 S 1 populations of a cross between a
salt-sensitive L. esculentum (M82) and a “salt-tolerant” L. pennellii (LA716)
indicated that tissue ion content was not likely to provide an efficient
selection criterion for ST, as no direct relationship was observed
(Saranga et al. 1992). Paradoxically, analysis of the relationship between
ST and leaf ion compositions in the cultivated and three wild species
of tomato prompted Saranga et al. (1993) to conclude that dry matter
production under SS was positively correlated with K+/Na+ ratio in the
stem and negatively correlated with the Cl concentration in leaves and
stems. The authors suggested that tissue ion content and
ion selectivity were good selection criteria for breeding for ST in tomato. Potassium selectivity over Na+ was also reported as a good indicator of ST in an investigation of several genotypes of the cultivated and wild species of tomato (Curatero et al. 1992). Studies in wild species of tomato, such as L. peruvianum (Tal 1971), L. cheesmanii (Rush and Epstein 1981a), and L. pimpinellifolium, L. hirsutum, and L. pennellii (Bolarin et al. 1991), demonstrated elevated concentrations of Na+ in the leaf tissue in relation to plant ST. In contrast, no relationship was observed between tissue ions content and plant ST (as determined by survival under SS) in BC 1 and BC 1 S 1 populations of a cross between a moderately salt-sensitive tomato breeding line (NC84173) and a salt-tolerant accession (LA722) of L. pimpinellifolium (Foolad and Chen 1999). Other studies also suggested that the ability to regulate Na+ concentration in the leaf tissue was more closely correlated with ST than Na+ concentration per se (Sacher et al. 1983) and that the distribution of Na+ in young and mature leaves was an important part of such regulation (Shannon et al. 1987). The overall conclu
sion that can be drawn from the various investigations is that tissue
ion content per se may not be a universal indicator of ST across tomato
genotypes. In salt-tolerant genotypes of tomato exhibiting a glycophytic response
to SS, increase in ion concentration beyond a threshold results in failure
of the exclusion mechanism at the root and/or shoot level. Consequently,
further increases in ion concentration in the root zone leads to declining
Thus, salt-tolerant tomato genotypes bearing an exclusion mechanism
may be useful for cultivation only in regions with low to moderate levels
of salinity. At higher salinity levels, genotypes that exhibit a halophytic
type response to SS may be more advantageous. Unfortunately, many
salt-tolerant wild accessions of tomato exhibiting a halophytic response to
SS often have the undesirable characteristic of slow growth (Foolad 1996a,
Tal 1997). Such accessions may survive high levels of salinity, but they
often grow extremely slowly—a trait that is highly undesirable for
tomato cultivation. Whether this association is due to pleiotropic
effects of the same genes or linkage between different genes affecting
growth rate and ST is unknown. It has not been determined whether this
association can be eliminated in segregating populations derived from
crosses between slow-growing salt-tolerant wild accessions and fast
growing salt-sensitive cultivated types of tomato. If possible, the salt-tolerant
wild accessions would be potentially useful for ST breeding in tomato.

Several studies in tomato and in other plant species, however, have
suggested that genes contributing to plant vigor are different from those
conferring ST (Foolad 1996a, Forster et al. 1990). When breeding tomatoes
for efficient production under saline conditions, genes for both plant vigor
and ST are important. Research to determine the genetic basis of ion accumulation/exclusion
in tomato plants grown under SS has been very limited. In one study,
analysis of the parental, filial and backcross generations of a cross be
tween a salt-sensitive tomato breeding line (UCTS) and a salt-tolerant primitive cultivar (PI174263) indicated that growth under SS was positively
correlated with leaf Ca 2+ content and negatively correlated with leaf Na +
content (Foolad 1997). Generation Means Analysis (GMA) of these popula
tions indicated that, under SS, accumulation of both Na +
and Ca\textsuperscript{2+} in the leaf was genetically controlled with additivity being the major genetic component. Thus, tissue ion concentration was suggested as a useful selection criterion when breeding for improved ST of tomato using PI174263 as a genetic resource (Foolad 1997). In spite of these and other studies, there is no consensus on what might be the best physiological or morphological trait(s) used as indirect selection criteria for ST breeding in tomato. Most likely a combination of different traits must be considered if salt-tolerant genotypes with commercial value are anticipated. This, by itself, indicates the complexity of ST and the need for identifying better approaches for characterizing genetic basis of tolerance components and facilitating breeding for ST. QTL mapping, MAS, and genetic transformation could be some promising approaches.

**QTL Mapping**

Significant progress has been made in mapping QTLs for ST during the vegetative and reproductive stages in tomato. In one study, a BC 1 S 1 population of a cross between a moderately salt-sensitive tomato breeding line (NC04173) and a salt-tolerant accession of *L. pimpinellifolium*...
(LA722) was evaluated for survival under SS using an aerated hydroponics system (Foolad and Chen 1999). The two parents were distinctly different in ST: while 80% of LA722 survived two weeks after the final salt concentration (700 mM NaCl + 70 mM CaCl₂; equivalent to ~64 dSm⁻¹) was reached, only 25% of NC84173 remained alive within that period.

The BC 1 S 1 population (consisting of 119 families) exhibited continuous variation for ST, with a survival rate ranging from 9% to 94% across families and a mean of 51%. Interval mapping identified five putative QTLs for ST on tomato chromosomes 1, 3, 5 and 9, with individual effects ranging between 5.7% and 17.7% and with combined effects of 46% of the total phenotypic variation under SS (Foolad and Chen 1999). All QTLs had the positive QTL alleles from the salt-tolerant L. pimpinellifolium parent. The results supported the hypothesis that ST during VS in tomato was controlled by more than one gene (Foolad 1996a, 1997). However, the involvement of only a few major QTLs, which accounted for a large portion of the total phenotypic variation, suggested the utility of MAS for improving tomato ST using LA722 as a genetic resource. Analyses of leaf ion
content (including Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻, NO₃⁻, SO₄²⁻ and PO₄³⁻) in the BC 1 S 1 population indicated the absence of a correlation between ST and tissue ion content in this population. Furthermore, despite the presence of significant variation among BC 1 S 1 families in concentration of the various ions, no major QTL was identified for tissue ion content under SS. Using a different BC 1 population of the same L. esculentum × L. pimpinellifolium cross, a selective genotyping approach was employed to verify the previously identified QTLs and possibly identify new QTLs for ST during VS (Foolad et al. 2001). From a population of 792 BC 1 plants, 37 (4.7% of the total) exhibiting the highest ST were selected, grown to maturity and self-pollinated to produce BC 1 S 1 seeds. The 37 selected BC 1 S 1 families and 119 nonselected (random) BC 1 S 1 families were evaluated for ST and their performances compared. A realized $h^2$ of 0.46 was obtained for ST during VS, consistent with a previous estimate of $h^2$ for this trait obtained from an intraspecific cross of tomato (Foolad 1996a). The 37 selected BC 1 plants and the 119 nonselected BC 1 plants were subjected to RFLP analysis using 115 markers, and marker allele
frequencies determined. A comparison of marker allele frequencies between the selected and nonselected populations detected five genomic regions on chromosomes 1, 3, 5, 6 and 11 bearing QTLs for ST (Foolad et al. 2001). Except for one, all QTLs had positive alleles contributed by the salt-tolerant L. pimpinellifolium parent. Three of the five QTLs were at the same locations as those identified in the previous study (Foolad and Chen 1999). Only one of the major QTLs that was identified in the previous study was not detected in this study (Foolad et al. 2001).

The high level of consistency of the results of the two studies indicated the genuine nature of the detected QTLs and their potential usefulness for ST breeding using MAS. In each of the two studies described, a few BC 1 S 1 families were identified with most or all of the identified QTLs and with a ST comparable to that of the salt-tolerant L. pimpinellifolium accession. In a more recent study, 145 F 9 RILs of the same L. esculentum × L. pimpinellifolium cross were evaluated in replicated trials for ST during VS (plant survival under SS). The RILs were genotyped for 129 RFLP and 62 resistance gene analog (RGA) markers. Interval analysis identified 7 QTLs.
for ST during VS on tomato chromosomes 3, 4, 5, 7, 8, 9 and 12 (M.R. Foolad et al., unpublished data). The QTLs detected on chromosomes 3, 5, and 9 were the same as those identified in the previous studies (Foolad and Chen 1999, Foolad et al. 2001) and exhibited larger effects than the newly identified QTLs on chromosomes 4, 7, 8 and 12. The overall results from these three studies indicate that the stable QTLs on chromosomes 3, 5 and 9 should be useful for introgression into the cultivated tomato via MAS to improve tomato ST during VS. Very limited research has been conducted to identify QTLs for ST during reproduction in tomato. Using an F 2 population of a cross between a salt-sensitive L. esculentum cultivar and a salt-tolerant accession of L. pimpinellifolium, and by using only 14 genetic markers, Breto et al. (1994) identified a few QTLs which appeared to be associated with fruit yield, fruit number and/or fruit size under SS. However, because of the extreme difference in fruit size between the parents of this F 2 population, QTL identification was most likely confounded by the effects of genes controlling fruit size, and thus, the identified QTLs should be considered with caution and should be verified in advanced generations.
before use

for MAS. Similar studies were also conducted using F2 populations of
different crosses between L. esculentum and either L. pimpinellifolium or
L. cheesmanii. Several other QTLs were identified for the same fruit-related
traits under SS (Monforte et al. 1996, 1997, 1999). However, large
morphological and physiological differences between parental lines of these
populations, including differences in flowering habits, maturity times, fruit
size and number, and total fruit yield, could have adversely affected the
power of experiments in detecting genuine QTLs for ST. These QTLs also
should be validated in advanced populations where such confounding
effects are eliminated or minimized.

Transgenic Approaches

Plant’s response to SS involves the functions of many genes that lead to a
wide variety of biochemical and physiological changes. These include
expression of genes that facilitate compartmentalization of toxic ions in
the vacuole, activation of detoxification enzymes, synthesis of late
embryogenesis-abundant (LEA) proteins, and accumulation of low
molecular weight organic compounds (collectively known as compatible
solutes or osmolytes). During the past several years, genetic engineering approaches have been employed to produce transgenic plants with enhanced tolerance to various abiotic stresses, including SS, by overexpression of genes controlling different tolerance-related physiological mechanisms (Bajaj et al. 1999, Serrano et al. 1999, Apse and Blumwald 2002, Rontein et al. 2002). For example, plants have been engineered with genes encoding enzymes that enhance the synthesis of compatible solutes, such as mannitol (Thomas et al. 1995), glycine betaine (Lilius et al. 1996), proline (Zhu et al. 1997), and polyamines (Galston et al. 1997), that contribute to osmotic adjustment and improving plant stress tolerance (Rathinasabapathi 2000, Rontein et al. 2002). Compatible solutes may also contribute to stress tolerance through other functions such as protection of enzyme and membrane structure and scavenging of radical oxygen species (Shen et al. 1997, Bohnert and Shen 1999, Rathinasabapathi 2000).

Transgenic plants also have been produced with overexpression of different vacuolar antiports, which facilitate exclusion of toxic ions from the cell cytosol (Apse et al. 1999, Serrano et al. 1999, Zhang and
Furthermore, transgenic plants have been developed with increased expression of detoxification enzymes, which reduce oxidative stress (Tanaka et al. 1999). Although in almost all cases plant growth and stress treatments were conducted in controlled conditions, and in many cases the increased tolerance has been rather marginal, the transgenic approach has provided opportunities for a better understanding of mechanisms leading to stress tolerance. The preliminary results have been particularly encouraging for enabling scientists to better understand the effects of single-gene transfers to plants. Notwithstanding the efforts to develop tomatoes with enhanced ST using transgenic approaches have been rather limited, a significant advancement was recently reported by Zhang and Blumwald (2001) who developed transgenic tomato plants overexpressing AtNHX1, a single gene controlling vacuolar \( \text{Na}^+ / \text{H}^+ \) antiport protein, introduced from Arabidopsis thaliana. The overexpression of this gene was previously shown to increase ST in Arabidopsis (Apse et al. 1999). Transgenic tomato plants overexpressing this gene were able to grow, produce flower and set fruit.
in the presence of 200 mM NaCl in greenhouse hydroponics, whereas the
nontransgenic (control) plants did not survive the saline conditions (Zhang
and Blumwald 2001). The transgenic plants acquired a halophytic response
to SS, that is, accumulated salts in the cell and sequestered them in the
vacuole. As indicated earlier, the normal response of the cultivated tomato
to salinity is of a glycophytic type, that is, excluding salt from the cell at
the plasma membrane in the root and/or in the shoot. This was the first
reported example of a single-gene transformation in any crop species that
resulted in such a significant enhancement in plant ST. According to
this report, under high salinity conditions, transgenic tomato plants
accumulated very high concentrations of Na + and Cl in the leaves. The
overproduction of the vacuolar Na + /H + antiport protein enhanced the
ability of the transgenic plants to sequester Na + in their vacuole, averting
its toxic effects in the cell cytosol. At the same time, Na + was used to
maintain an osmotic balance to drive water into the cells and thus use
saline water for cell expansion and growth. Furthermore, there were only
minimal increases in concentrations of Na + and Cl in the
fruit, a great horticultural advantage for commercial production of such transgenic plants. The low Na+ content of the fruit was attributed to the ability to maintain a high cytosolic K+/Na+ concentration ratio along the symplastic pathway in the transgenic plants. The results indicated that the enhanced accumulation of Na+, mediated by the vacuolar Na+/H+ antiport, allowed the transgenic plants to ameliorate the toxic effects of Na+. More recently, the transfer and overexpression of the same AtNHX1 gene into canola (rape seed), Brassica napus, resulted in salt-tolerant transgenic plants that were able to grow, flower, and produce seeds in the presence of 200 mM NaCl (Zhang et al. 2001). Although the transgenic tomato and canola plants are yet to be evaluated for ST under field conditions, these findings suggest the potential for producing salt-tolerant plants using the transgenic approach. It should be noted that transformation technology for improving plant stress tolerance has just begun. There is no report to date of any field studies testing the performance of transgenic plants under SS conditions. Much more work on transgenics is needed to gain a better understanding
of the genetics, biochemical and physiological basis of plant ST.

Future knowledge of tolerance components along with identification and cloning of responsible genes may allow transformation of plants with multiple genes and production of highly stress-tolerant transgenic plants.

It is possible that transferred multiple genes may act synergistically and additively to improve plant stress tolerance.

Comparison of Salt Tolerance during Different Stages of Plant Development

Knowledge of the genetic relationship between ST at different plant stages is necessary to expedite breeding efforts to develop cultivars with enhanced tolerance throughout the ontogeny of the plant. Early studies in different plant species had suggested the absence of phenotypic relationships among different developmental stages with regard to plant ST (Abel and Mackenzie 1963, Greenway and Munns 1980, Johnson et al. 1992). Recently, however, systematic approaches were taken to examine the phenotypic and genetic relationships between ST during SG and later stages in tomato. In one study, an F 4 population of a cross between a tomato breeding line (UCT5), with salt sensitivity during both SG and
VS, and a primitive cultivar (PI174263), with ST during both of these stages,

was evaluated for ST independently during SG and VS. Although there

were significant variation among F 4 families in ST during both stages,

there was no significant correlation ($r = -0.10$, $P > 0.05$) between the ability

of the seed to germinate rapidly and the ability of the plant to grow under

SS (Foolad and Lin 1997a). In a second approach, to examine the genetic

correlation between ST during SG and VS, selection was made for rapid

SG under SS in an F 2 population of the same cross, and the selected F 3

progeny were evaluated for ST separately during SG and VS. Selection for

ST during SG significantly improved germination ST of the F 3 progeny,

indicating that the selection was effective. However, the selection for ST
during SG did not affect ST of the F 3 progeny during VS, suggesting that

the genetic and physiological mechanisms that contributed to ST during

SG in these genetic materials were different from those conferring ST during

VS (Foolad and Lin 1997a). In a more robust approach to determine the genetic relationship

between ST during SG and VS, QTLs for ST at these two developmental

stages were identified in a BC 1 S 1 population of a cross
between a tomato breeding line (NC84173, salt sensitive during both SG and VS) and a
L. pimpinellifolium accession (LA722, salt tolerant during both stages).

Comparison of QTLs indicated that in most cases the locations of QTLs for ST during SG were different from the locations of QTLs for ST during VS (Foolad 1999). This study clearly indicated the involvement of different genes (QTLs) controlling ST during SG and VS in these genetic materials.

A similar study was recently conducted using 145 F9 RILs of a cross between NC84173 and LA722 (M.R. Foolad et al., unpublished data). The RILs were evaluated in replicated trials for ST during SG and vegetative growth (plant survival under SS). There was no significant phenotypic correlation between the rate of SG and the plant survival under SS across the RILs. The RILs were genotyped for 129 RFLP and 62 RGA markers, covering 1,505 cM of tomato genome with an average marker distance of 7.9 cM. Marker analysis identified QTLs for ST during SG on chromosomes 2, 3, 4, 8, 9 and 12 and QTLs for ST during vegetable stage on chromosomes 3, 4, 5, 7, 8, 9 and 12. Different QTLs were detected for ST during these two
stages of plant development, suggesting the involvement of different genes controlling ST during SG and VS in this population. The overall results from these investigations indicate that, in tomato,

ST during SG is generally independent of ST during VS. This conclusion is consistent with earlier reports that ST of young tomato plants did not correlate with that of mature plants (Shannon et al. 1987) and that ranking of salt-tolerant tomato genotypes based on vegetative characteristics in mature plants differed from the ranking based on fruit yield (Caro et al. 1991). Absence of genetic relationships among different developmental stages with regard to ST have also been reported in many other plant species, including alfalfa (Medicago sativa L.) (Johnson et al. 1992), wheat (Triticum aestivum L.) (Ashraf and McNeilly 1988), triticale (Triticale hexaploide Lart.) (Norlyn and Epstein 1984), and slender wheatgrass [Elymus trachycalus spp. Trachycalus (Link) Malte] (Pearen et al. 1997).

These findings indicate that when breeding for improved ST, each stage of plant development may have to be evaluated separately for the assessment of tolerance and the identification, characterization and utilization of useful genetic components. However, the
identification of QTLs for ST at different developmental stages would facilitate simultaneous or sequential introgression of QTLs for tolerance and the development of cultivars with improved tolerance at all desirable stages.

Furthermore, the finding that in tomato only a few major QTLs account for a large portion of the total phenotypic variation for ST at each plant stage suggests that MAS for ST should be feasible, providing the opportunity to develop germplasm with enhanced ST at various developmental stages.

Future Prospects for Developing Tomato Cultivars with Enhanced Salt Tolerance

Although no commercial cultivar of tomato with proven field tolerance to salinity has yet been released through the use of either the conventional protocols of plant genetics and breeding, MAS or transgenic approaches, the prospect for such development in the near future is very good. Limited progress in developing salt-tolerant tomatoes during the past few decades has been in part due to the complexity of the trait, complex interactions of ST with other agronomically important traits, insufficient understanding of the basic physiological mechanisms as well as genetic
controls of
tolerance-related traits, lack of efficient selection
criteria, and limited effort
devoted to the identification and characterization of
genetic resources that
could be used for breeding for ST. With the current
knowledge of the
physiological and genetic basis of ST, the availability of
molecular markers,
QTLs, MAS, and genetic transformation technologies applied
in developing
salt-tolerant genotypes, it would not be unexpected to
witness tomato
cultivars with field ST in near future. It is anticipated
that, similar to that
in several other major crop plants, the importance of
breeding tomatoes for
ST will increase in near future and no bably a few research
groups in the
United States and worldwide are currently attempting to
produce salt
tolerant tomato cultivars.

TOLERANCE TO DROUGHT STRESS

Background Information

Drought, defined as the occurrence of a substantial water
deficit in the soil
or in the atmosphere, is an increasingly important
constraint to crop pro
ductivity and yield stability worldwide (Ceccarelli and
Grando 1996). It is
by far the leading environmental stress in agriculture. The
worldwide losses
in yield due to drought probably exceed the losses from all other causes.

combined (Kramer 1980, Blum 1988, Schonfeld et al. 1988). In the U.S., for example, up to 45% of the land surface is subject to continuous or frequent water stress (Boyer 1982, Tanji 1990) and a drought occurs somewhere in the country every year, costing billions of dollars in damage to crops and businesses (Ross and Lott 2000). Most crop plants, including tomato, are sensitive to drought stress (DS) throughout the ontogeny of the plant, from SG to harvest (Hsiao 1973).

Plant response to DS can be generally classified into three categories, drought escape (or avoidance), dehydration avoidance (or postponement), and dehydration tolerance (Kramer 1983, Blum 1988). Drought escape includes situations where plants with short growth cycle and early maturity avoid experiencing the drought. Breeding for drought escape should therefore be directed toward developing cultivars with early maturity so that by the time drought occurs the plant has already completed its life cycle. Advantages and disadvantages of plants with a short growth cycle under conditions of water stress and under normal conditions have been reviewed elsewhere (Reitz 1974, Jordan et al. 1983, Saeed and Francis 1983,
Blum 1988). Extremely late maturing cultivars may also escape drought damage. Dehydration avoidance is defined as the ability of the plant to retain a relatively higher level of “hydration” during the period of water stress (Blum 1988). In this situation, the plant protects its various growth related physiological, biochemical, and metabolic processes from the external water stress. A common measure of dehydration avoidance is the maintenance of a higher tissue water or turgor potential under conditions of water stress.

Thus, osmotic adjustment, as a means for retaining a higher turgor at a given tissue water potential, is an example of dehydration avoidance at the cell level. Osmotic adjustment is usually obtained by the production and accumulation of compatible organic solutes, such as amino acids, glycine betaine, sugars, proline and ectoine in the cytoplasm. Various mechanisms employed by different species to avoid dehydration have been described elsewhere (Levitt 1980a). When the tissue is not protected by any of the avoidance mechanisms, cells lose turgor and dehydrate, resulting in various cellular physicochemical injuries (Hsiao and Bradford 1983). Complete loss of free water will result in desiccation or dehydration. While our
understanding on the effects of dehydration at the cellular level and various processes at higher

levels of plant organization (e.g. photosynthesis, respiration, etc.) has been improving (in consideration of the application of new molecular techniques), it is far from complete, and there are many unanswered questions (Blum 1988, McCue and Hanson 1990, Bohnert et al. 1995, Bohnert and Jensen 1996, Richards 1996, Shinozaki and Yamaguchi-Shinozaki 1996, 1997, Bray 1997, Zhu et al. 1997, Lutfor-Rahman 1998). What is known, however, is that different genotypes exhibit different responses to cellular

and whole plant stresses caused by dehydration, and that there are different levels of dehydration tolerance. It should also be noted that characteristics of the three categories of plant response to DS (drought escape, dehydration avoidance, and dehydration tolerance) are not generally independent of each other, and some plants may exhibit characteristics for more than one category (Blum 1988). A complementary approach in agricultural methods currently followed is to minimize losses incurred by water stress and develop, via genetic means, “drought tolerant” cultivars with the ability to escape, avoid and/
or tolerate effects of water stress. Development of such plants would have
a lasting economic impact on crop production worldwide. Despite many
decades of research on drought tolerance (DT), so far drought stress
continues to be a major challenge to plant breeders. This is in part due to
the complexity of the trait. Accumulating evidence suggests that plant
response to DS is controlled by the function of many genes and physiological
2001) and varies depending on the influence of other environmental factors
(Ceccarelli and Grando 1996, Richards 1996). Selection and breeding for
DT is also difficult because tolerance appears to be a developmentally
regulated, stage-specific phenomenon (Blum 1988, Ludlow and Muchow
1990, Richards 1996, Mitchell et al. 1998). Each stage may be considered as
a separate trait and may require a different evaluation method.
Furthermore, no reliable evaluation procedure is known that can effectively
and efficiently be used to identify drought-tolerant plants at different
developmental stages. These and other complexities have led to a limited
success in developing drought-tolerant plants or improving crop yields in
dry environments. Most commercial cultivars of tomato are sensitive to DS during different stages of plant development, yet genotypic variation for DT exist within the cultivated tomato (Wudiri and Henderson 1985) and related wild species, including L. cheesmanii, L. chilense, L. pennellii, L. pimpinellifolium, and L. esculentum var. cerasiforme (Yu 1972, Richards and Phillips 1979, Martin et al. 1989, Pillay and Beyl 1990, Rick 1973, 1978, 1979, 1982). The latter species, being native of coastal deserts of western South America (including Galapagos), witness rainless long periods but for the occasional El Niño episodes when warm ocean water generates heavy rains. The species grow at habitats where condensation of dew at night and fog drip are the main source of moisture. They are also remarkably capable of overcoming brief wilting. Rana and Kalloo (1990) evaluated 150 lines of cultivated and wild species of tomato under water-deficit conditions and identified a few selections within L. esculentum, and a few accessions of L. pimpinellifolium and L. chilense, with various DT attributes. However, only limited effort has been devoted to the characterization of the physiology or genetics of this variation (Kahn et al. 1993,
Martin et al. 1999, Pillay and Beyl 1990) to warrant its use in breeding programs to develop drought-tolerant tomato cultivars. This is unlike the extensive research that has been conducted on DT in many other crop species, including rice, Oryza sativa L. (Nguyen et al. 1997, Zhang et al. 2001), maize, Zea mays L. (Ribaut et al. 1997), sorghum, Sorghum bicolor L. Moench (Subudhi et al. 2000) and lettuce, Lactuca sativa L. (Johnson et al. 2000).

Comparatively, also less research has been done on tomato DT than its tolerance to other abiotic stresses such as salinity and extreme temperatures. The available information on genetics of DT in tomato and the prospect for developing drought-tolerant tomatoes is provided in the following sections.

Drought Tolerance during Seed Germination and Seedling Emergence

The ability of the tomato seed to germinate rapidly and uniformly under DS is a desirable trait for direct seeding in the field. Large areas of land are established for tomato production by sowing seed directly into the field instead of using transplants (Liptay and Schopfer 1983). Successful
establishment of direct-seeded crops depends on successful seed
germination and seedling emergence. Most commercial
cultivars of tomato
are sensitive to DS during SG, however, sources of
tolerance have been
identified within the related wild species of tomato,
including L. pennellii
and L. pimpinellifolium (M.R. Foolad et al, unpublished
data). Recently,
using one accession of L. pimpinellifolium in crosses with
L. esculentum, the genetic basis of DT during SG in tomato
was determined
in our investigations.

Inheritance
A BC 1 population (N = 1000) from a cross between a
drought-tolerant L. pimpinellifolium accession (LA722) and a drought-sensitive
tomato breeding
line (NC04173; maternal and recurrent parent) was evaluated
for SG
under DS (14% PEG, \( \psi \approx -680 \) kPa), and the most rapidly germinating
seeds (first 3% germinated) were selected. The 30 selected
BC 1
individuals were grown to maturity and self-pollinated to produce BC 1 S 1
progeny seeds. Twenty of the 30 “selected BC 1 S 1 ”
progeny families were
evaluated for germination under DS and their average performance was
compared with that of a “nonselected” BC 1 S 1 population of the same
cross. Results indicated that selection for rapid SG under DS was effective and significantly improved progeny SG rate under DS (selection gain = 19.6%). A realized $h^2$ of 0.41 was obtained for DT during SG in this population. The results indicated that DT during SG in tomato was genetically controlled and could be improved by directional phenotypic selection.

QTL Mapping

Two independent studies were recently conducted to identify QTLs for DT during SG in tomato. In one study, a trait-based marker analysis, using BC 1 individuals of a cross between drought-sensitive tomato breeding line NC84173 and drought-tolerant L. pimpinellifolium accession LA722, detected four QTLs on chromosomes 1, 4, 8, 9, and 12 for DT (M.R. Foolad et al., unpublished data). The results indicated that DT during SG in tomato was a quantitative trait, controlled by more than one gene. A few BC 1 S 1 families were identified with most or all of the QTLs and with a DT comparable to that of LA722. These families should be useful for developing germination drought-tolerant tomato lines using MAS. In a second study, 145 F 9 RILs of
the same cross were evaluated for germination rate under DS and, by using composite interval mapping analysis, several QTLs for DT during SG were identified on tomato chromosomes 1, 2, 3, 4, 8, 9, and 12 (M.R. Foolad et al., unpublished data). The results of this study were highly consistent with those of the previous study (M.R. Foolad et al., unpublished data). These results indicated the presence of stable QTLs for DT during SG across tomato populations derived from the NC84173 × LA722 cross, suggesting the usefulness of these QTLs for improving tomato SG under DS by MAS.

Drought Tolerance during Vegetative Growth and Reproduction

Potential sources of genes for DT during vegetative growth and later stages in tomato have been identified within the related wild species L. chilense and L. pennellii, mostly among accessions native to dry habitats. (Rick 1973, 1978, 1979, 1982). Different tolerance indices (TIs) have been employed to characterize the physiological and/or genetic basis of DT in tomato, including dry weight (DW) of shoot and root, root length, root morphology, leaf rolling, flower and fruit set, fruit weight, yield, water-use efficiency (WUE), recovery after re-watering, stomatal resistance, plant survival, leaf
water potential, leaf osmotic potential, osmoregulation, oxidative damages,

transpiration rate, photosynthetic rate, enzymatic (e.g. superoxide dismutase

and Rubisco) activities, and pollen viability (Richards and Phillips 1979,
Blum 1988, Martin and Thorstenson 1988, Rana and Kalloo 1989, Pillay

and Beyl 1990, Cohen et al. 1991, Kalloo 1991,
Lutfor-Rahman 1998). For

example, in a germplasm evaluation study, tomato cultivar Saladette

sustained severe water stress as determined by a smaller reduction in fruit

set compared to other cultivars, which was attributed to its ability to rolling

up leaves under a high evaporative demand and thereby maintaining a

high leaf water potential (Wudiri and Henderson 1985). Rick (1978)
suggested that the physiological basis for DT in L. chilense might be related
to its deep vigorous root system. Cultivar Red Rock performed better than

other cultivars under drought conditions and its DR was similarly

attributed to its deep and more vigorous root system (Stoner 1972). DT in

the L. pimpinellifolium was also attributed to root length
(Rana and Kalloo 1989). Conversely, the drought-tolerant L. pennellii
accession LA716 has a

limited root system, and the basis for its DT is most likely due to its ability
to conserve moisture during periods of limited rainfall. LA716 has been
characterized as having a greater WUE under DS than L. esculentum, as
measured by g DW produced per Kg of water consumed (Martin and
Thorstenson 1988). A higher WUE in this accession than in L. esculentum
was attributed to different characteristics of this accession, including
smaller leaf conductance due to fewer and smaller stomata, longer
trichomes, lower chlorophyll content and Rubisco activity per unit leaf
area, and larger mesophyll cell surface exposed to intercellular air space
(Martin et al. 1999). Although WUE may be a good indicator of DT in
tomato, its measurement under field condition is extremely difficult. Thus,

attempts have been made to determine the relationship between WUE and
stable carbon isotope discrimination ($\delta$), a measure for proportion of 13 C
relative to 12 C in plant organic matter, easier to measure when dealing
with large number of plants (Martin et al. 1999). A recent study suggested
that WUE in progeny of crosses between L. esculentum and L. pennellii LA716
could be increased by selecting for low $\delta$, however, this could lead to the
selection of small plants, an agriculturally undesirable
characteristic

(Martin et al. 1999). The authors suggest that the small plant size could be corrected by conventional breeding following selection for DT, but no such effort has been reported.

Inheritance and QTL Mapping

Limited research has been conducted to characterize the genetic control of or to develop tomatoes with improved DT. To facilitate selection for low \( \mu \), three QTLs associated with this trait were previously identified using F3 and BC1S1 progeny of a cross between L. esculentum UC82B and L. pennellii LA716 (Martin et al. 1989). The results were inconclusive in determining whether selection for these QTLs could increase WUE in tomato. Other related research studies on genetics of tomato DT include the identification of several genes or mRNAs whose expressions are elevated in response to DS. For example, four tomato drought induced genes, le4, le16, le25 and le20, were identified and characterized in L. esculentum (Cohen et al. 1991, Plant et al. 1991, Kahn et al. 1993). It was further determined that the increase in the expression of these genes occurred after a longer period of water deficit in L. pennellii than in L.
esculentum, although these genes did not appear to be responsible for DT in L. pennellii (Kahn et al. 1993).

Future Prospects for Developing Tomato Cultivars with Enhanced Drought Tolerance

From the preceding discussion it is evident that currently there is limited physiological and/or genetic information on DT in tomato to warrant development of drought-tolerant cultivars through plant breeding. In essence, research must be extended to identify additional genetic resources for DT in tomato. Collections from torrid areas should be tested for DT at different developmental stages, such as SG, VS, flowering and fruit set, to identify useful resources for basic physiological and genetic studies as well as for breeding purposes. Considering the normal climatic conditions for growing tomatoes, where short periods of drought may occur intermittently throughout the growing season, it seems that the ability of the plant to survive transient periods of water stress and to recover rapidly upon re-availability of water is far more important than the ability to survive long-term water stress. Thus, during germplasm evaluation and breeding process, the focus should be on
dehydration avoidance. From a practical point of view, the most reliable criteria for breeding tomatoes for DT are agronomic characteristics such as yield, and absolute and relative plant growth under stress and nonstress environments. Such criteria, however, may not be efficient or feasible to apply because in most initial germplasm evaluation and/or breeding projects often a large number of individuals, families or populations are used. Alternative criteria based on physiological characteristics such as photosynthetic rates, stomatal resistance and leaf water potential might be more efficient. These characteristics are easier to measure, compared to yield, and generally show rather strong correlations with agronomic characteristics. Other alternatives are the identification of biochemical characteristics such as enzyme activities and protein contents. These methods, however, often lack a strong correlation with agronomic characteristics and are expensive. Like other abiotic stresses, the identification and utilization of molecular markers associated with different tolerance-related physiological, morphological, or agronomic criteria might be an efficient way to improve DT in tomato.
Furthermore, transgenic approaches have been employed in other plant species to increase DT (Grover et al. 1999, Serrano et al. 1999), and an increasing number of DT-related genes or proteins are being discovered. Transgenic approaches should be successful in developing tomatoes with improved DT.

TOLERANCE TO COLD STRESS

Background Information

Although the cultivated tomato originated and was domesticated in the tropical and subtropical regions of South and Central America (Rick 1975), this crop is also commercially grown in many temperate regions of the world where it often experiences low temperatures (LTs) during at least part of the growing season. Temperatures in the range of 0 to 15 °C (chilling temperatures) injure many crops of tropical origin, including tomatoes. Most commercial cultivars of tomato are sensitive to LTs equally as to other abiotic stresses during all stages of plant development, SG, VS and reproduction (Patterson et al. 1978, Lyons et al. 1979, Graham and Patterson 1982, Patterson et al. 1987). This sensitivity limits the geographic distribution of tomato and puts constraints on cultivation time for annual
planting. For example, the spring soil temperatures in temperate regions, which are often below 15 °C, may restrict direct seeding of tomato crops (Liptay et al. 1982). LT sensitivity also affects greenhouse production of tomato, since cold-sensitive cultivars would require expensive greenhouse heating throughout the life of the plant. One approach to minimizing deleterious effects of cold stress (CS) is to develop cold-tolerant tomato cultivars. When grown under sub-optimal temperatures, cold-tolerant tomato genotypes could exhibit improved earliness, adaptability, water use, and yield of high-quality fruits. Cold-tolerant genotypes also could be planted earlier in the season and harvested earlier when the crop may have higher economic values. Furthermore, the crop could be grown in the field for longer periods of time (i.e., extended growing season), thus the final yield may be higher compared to cold-sensitive genotypes. Likewise, the production may improve because early plantings avoid high temperatures typically observed during the mid-summer in many climates which can reduce fruit set. Moreover, cold-tolerant genotypes may have lower demand for water in areas with a Mediterranean climate,
because early plant
growth would make better use of early-season rains and
available water in
the root zone. The search for genetic diversity for
temperature adaptation logically
leads one to examine cultivars or ecotypes adapted to
extreme environments.
The potential for this geographic approach in identifying
tomato genotypes
tolerant to LTs was examined by Thompson (1970) and
Patterson
et al. (1978), later reviewed by Vallejos (1979). Genetic
resources for cold
tolerance (CT) have been identified both in cultivated and
related wild
species of tomato (Patterson et al. 1978, Vallejos 1979,
Scott and Jones
1982, Patterson and Payne 1983, Wolf et al. 1986, Patterson
1988, Foolad
and Lin 2000, Henk-Venema et al. 2000). These resources
have been
employed to investigate the physiology and genetics of LT
tolerance
in tomato. The physiological aspects of CT in tomato and
other plant
species have been extensively studied and reviewed (Lyons
1973, Lyons
et al. 1979, Graham and Patterson 1982, Vallejos et al.
1983, Parkin
et al. 1994,
1995, Bohnert et al. 1995, Bradford 1995, Keller and
Steffen 1995).
Comparatively, however, less research has been done on genetics of CT in tomato. Recent findings regarding genetic characterization of tomato for CT during different developmental stages are presented below and the prospects assessed for developing cold-tolerant cultivars through various genetic approaches.

Cold Tolerance during Seed Germination and Seedling Emergence

Germination rate of tomato seed decreases progressively as the temperature in the germination medium is reduced from 25 to 10 °C, and is inhibited below 10 °C (Scott and Jones 1982). Low temperatures (10-15 °C) delay the onset, reduce the rate, and increase the dispersion of germination events. As a result, under such conditions, many seeds either do not germinate or germinate so sporadically that plants grow differentially, thereby delaying plant establishment and leading to variability in crop maturation. Most commercial cultivars of tomato are sensitive to LTs during SG, with considerable genetic variation within the cultivated and related wild species. Genetic variability for CT during SG in Lycopersicon was first reported by Smith and Millet (1964) and has since been the subject
of numerous studies. For example, Scott and Jones (1982) evaluated a total of 37 accessions of the cultivated and wild species of tomato and identified one accession of *L. chilense* that germinated better at 10 °C than PI120256, the fastest germinating *L. esculentum* accession known at that time.

Additional accessions exhibiting rapid SG at 10 °C were also identified within *L. peruvianum* and *L. hirsutum*. In a later study, Scott and Jones (1985b) evaluated SG of three fast germinating *L. esculentum* accessions, PI120256, PI174263 and PI341988, and a slow germinating tomato breeding line, T3, at temperatures 6-20 °C. They concluded that rapid germination of these PIs at LTs might not be due to CT, but to seed characteristics that promoted rapid SG. Furthermore, early seedling emergence of the same three PIs under LTs was attributed to their rapid SG rather than rapid hypocotyl development. More recently, Foolad and Lin (2000) evaluated germination CT of 31 tomato accessions (cultivars, breeding lines and plant introductions) representing six *Lycopersicon* species and identified significant phenotypic variation in the ability of the seed to germinate rapidly under LT. Several accessions were identified within
L. pimpinellifolium and L. hirsutum with high CT during SG. Potential genetic resources for CT during SG have also been identified in S. lycopersicoides (Rick 1988, Wolf et al. 1986). Generally, accessions germinating rapidly at LTs also exhibit fast seedling growth under such conditions (Scott and Jones 1985b). A few accessions within L. chilense (LA460) and L. peruvianum (PI126435 and PI127832) were identified with the ability of fast hypocotyl growth under LTs (Scott and Jones 1986). The identified phenotypic variation in these and other studies should be useful for improving LT germination and hypocotyl growth of commercial cultivars of tomato. To facilitate breeding for CT during SG in tomato, several researchers have examined the genetic basis of this seed-related characteristic using a number of LT-tolerant accessions of tomato, which is summarised below. The physiological basis of LT seed germination in tomato has been described elsewhere (Simon 1979, Maluf and Tigchelaar 1982, Dahal et al. 1990, Leviatov et al. 1994, 1995).

Inheritance

Studies on CT during SG in tomato attributed the genetic control of this
trait to either one recessive gene (Cannon et al. 1973), additive genes (Whittington and Fierlinger 1972), or three to five genes (Ng and Tigchelaar 1973, DeVos et al. 1981) using different germplasms. Broad-sense $h^2$ was estimated at 0.97 and narrow-sense $h^2$ at 0.67-0.69 (Ng and Tigchelaar 1973). These estimates were independently confirmed by DeVos et al. (1981).

In a recent study, an LT fast germinating L. esculentum accession (PI120256) and an LT slow germinating tomato cultivar (UCT5) and their reciprocal $F_2$, $F_3$ and $BC_1$ progeny (total of 10 populations) were evaluated for germination at a low (11 ± 0.5 °C) and normal (20 ± 0.5 °C) temperature regimes (Foolad and Lin 1998). Weighted least square regression analysis indicated that under CS most of the variation in germination time was due to additive genetic effects; dominance and epistatic interactions were not significant. Partitioning of the total genetic variance into those attributable to the effects of embryo, endosperm, testa, and the cytoplasm indicated that additive effects of endosperm could account for 80% of the total genetic variance for germination under CS. These results supported the previous physiological studies suggesting the significant role of
the endosperm in determining the rate of germination in tomato seed (Groot and Karssen 1987, Haigh and Barlow 1987). While examining the response to selection for improved CT during SG in tomato, an F2 population of a cross between UCT5 and PI120256 was evaluated under a LT (11 ± 0.5 °C) regime and the fastest germinating seeds (the first 5% that germinated) were selected (Foolad and Lin 1998).

The selected F2 progeny were grown to maturity and self-pollinated to produce F3 seed. The selected F3 progeny were evaluated for SG under the LT and their mean performance was compared to that of a nonselected F3 population of the same cross. The results indicated that selection was effective with significant improvement in germination performance; a realized h2 of 0.74 was obtained for CT during SG (Foolad and Lin 1998). This study demonstrated that the rate of tomato SG under CS was genetically controlled, with additive effect, and that it could be improved by directional phenotypic selection. A recent similar study, using interspecific BC1 and BC1 S1 progeny of a cross between a LT fast-germinating L. pimpinellifolium accession (LA722) and a LT slow-germinating tomato cultivar (NC04173), suggested a more moderate
realized heritability

(h2 = 0.43) for CT during SG in tomato (Foolad et al. 2002). To improve LT seed germination of processing tomatoes, breeders have

used L. esculentum lines screened at 10 °C, including PI120256, PI174263,

and derived lines PI341905 and PI341908 (Cannon et al. 1973, Ng and

Tigchelaar 1973, DeVos et al. 1981, Scott and Jones 1985a, b) or wild

accessions from high altitudes screened for high

germination at 6-7 °C,

including L. hirsutum accessions LA1363 and LA1777 and L. chilense

accession LA460 (Patterson et al. 1978, Patterson and Payne 1983). Several

processing breeding lines were released in Europe, and

field tests conducted

with early direct seeding confirmed an advantage of 3 to 5
days in terms of

emergence with lower variability compared to standard
cultivars

(Damidaux and Martinez 1992). In conclusion, it seems that,
due to its

moderate-to-high heritability, LT tolerance during SG in
tomato can be

relatively easily transferred to commercial cultivars by

hybridization and

phenotypic selection. Molecular mapping techniques have

also been

employed to examine its genetic basis and elucidate the

prospect for

improving this trait through MAS.
QTL mapping

The number, chromosomal locations and genetic effects of QTLs contributing to CT during SG in tomato have been determined in different interspecific populations of tomato. A BC 1 S 1 population of a cross between breeding line NC84173 (cold sensitive) and L. pimpinellifolium accession LA722 (cold tolerant during SG) was evaluated for the rate of SG under a LT regime. Interval mapping analysis detected QTLs on tomato chromosomes 1 and 4 with significant effects on CT during SG (Foolad et al. 1998b). The L. pimpinellifolium accession had favorable QTLs on chromosomes 1, and NC84173 had favorable QTLs on chromosome 4.

The percentage of phenotypic variation explained (PVE) by individual QTLs ranged from 11.9% to 33.4%. Multilocus analysis indicated that the cumulative action of all significant QTLs accounted for 43.8% of the total phenotypic variance. Digenic epistatic interactions were evident only between two QTL-linked markers and two QTL-unlinked markers.

Transgressive phenotypes were observed in the direction of cold sensitivity. The validity of the identified QTLs was examined in two different populations of the same interspecific cross. First, 1000 seeds
of BC 1 progeny were evaluated for rate of germination under CS, and the most rapidly germinating seeds (the first 3% that germinated) were selected as the cold tolerant individuals. These selected BC 1 plants were subjected to molecular marker analysis, using 119 RFLP markers spanned over 12 tomato chromosomes with an average distance of 9.7 cM between markers. A distributional extreme analysis resulted in the identification of QTLs for CT on chromosomes 1, 4, 8, 9 and 12 (Foolad et al. 2003). In a different study, seeds of 145 F9 RILs of the NC84173 × LA722 cross were evaluated for germination under CS and the time to 50% germination (T50) for each RIL was determined. The RILs were also subjected to marker analysis using 129 RFLP and 62 RGAs and a molecular linkage map was constructed. Interval mapping analysis detected QTLs for CT on tomato chromosomes 1, 2, 3, 7, 8, 9, and 12 (M.R. Foolad et al., unpublished data). The latter two studies validated all of the QTLs identified in the first study (Foolad et al. 1998b) and further detected a few additional QTLs. The combined results supported the suggestion that CT during SG in tomato was a quantitative...
trait controlled by more than one gene. Comparison of QTLs in different populations (e.g. BC 1, BC 1 S 1 and RILs) of the same L. esculentum × L. pimpinellifolium cross indicated that most QTLs were stable across populations whereas a few QTLs were population-specific. Although no attempt has yet been made to improve CT during SG in tomato via MAS, the consistency of QTLs across populations suggests that such an attempt might be fruitful.

Cold Tolerance during Vegetative Growth and Reproduction

Chilling tolerance during VS in tomato has been defined as the ability of the plant to resist damage below −10 °C but above the freezing temperature (Lyons 1973). Went (1957) determined that the cultivated tomato preferred a growth temperature Ca. 7 °C higher than the cultivated potato, (Solanum tuberosum L.), which, although closely related to tomato, is much less liable to chilling injury. Although both species originated from the Andean region of South America (Jenkins 1948, Correll 1962), the cultivated tomato originated from the lower altitudes whereas the cultivated potato came from the cooler higher elevations. Plants that regularly experience temperatures below 10 °C in their native habitat would be
expected to be comparatively chilling resistant. Near the Equator, night temperatures regularly are below 10 °C only at altitudes of 2000 m or more. High-altitude wild species of tomato have not contributed to the ancestry of the cultivated tomato, which may explain why the cultivated tomato is less tolerant to chilling than the potato. A green-fruited related wild species of tomato, L. hirsutum, grows naturally over a wide range of altitudes, from sea level in Ecuador to 3300 m in Peru (Rick 1973). Patterson et al. (1978) and Patterson and Payne (1983) evaluated several accessions of this wild species and reported that chilling tolerance was greatest in those which originated in the higher altitudes (e.g. LA1363 and LA1777) and reduced in accessions from low altitudes (e.g. LA407). In these studies, the authors used either a survival test (survival of seedlings after chilling at 0 °C for 4-7 days) or a night chill test (growing seedlings at a d/n temperatures of 20/0 °C for several days) to evaluate chilling tolerance of tomatoes. In addition, Wolf et al. (1986) evaluated CT of several high-altitude accessions of wild tomato species including L. hirsutum (LA1363, LA1777), L. chilense Dun. (LA1969, LA1971)
and Solanum lycopersicoides Dun. (LA1964) during vegetative growth and compared that with the CT of a L. esculentum breeding line, UC82B. These researchers used chlorophyll fluorescence (Walker et al. 1990, Bruggemann and Linger 1994), electrolyte leakage (Van-De-Dijk et al. 1985), and plastochron index (Coleman and Greyson 1976) as evaluation criteria and reported that high-altitude wild accessions were more tolerant to LT than UC82B.

Recently, by producing a cybrid between L. hirsutum LA1777 (source of the cytoplasm) and L. esculentum cultivar ‘Large Red Cherry’ (source of nucleus), Henk-Venema et al. (2000) demonstrated that the LT tolerance of LA1777 was not due to genes in the cytoplasm. The cybrid was as cold sensitive as the L. esculentum parent as determined by various growth- and photosynthesis-related characteristics under suboptimal (d/n 16/14 °C) temperatures. Vallejos et al. (1983) reported a high-altitude accession of L. hirsutum having less growth reduction under a low-temperature (d/n 12/5 °C), whereas at high-temperature regime (25/18 °C) the growth was comparable to a low-altitude L. hirsutum or a L. esculentum breeding line. The high-altitude L. hirsutum accession was capable of
growing faster and acquiring greater biomass than the low-altitude
L. esculentum and the low-altitude L. hirsutum accessions. In contrast,

Raison and Brown (1989) evaluated CT of a high (LA1777, 3200 m),
mid (LA1625, 1500 m) and low (LA1361, 50 m) altitudinal
cotypes of

L. hirsutum based on inhibition of photosynthesis at LTs
(d/n 15/5 °C)

and observed that the three ecotypes had similarity for chilling-induced

photoinhibition (at critical temperature between 10 and 15 °C) and

the rate of their response to chilling stress. Three ecotypes thus were

similarly sensitive to chilling. Photosynthesis, however, is only one

component of response to CS. Miltan et al. (1986) reported that dry weight

(DW) accumulation and leaf area increase in the wild species L. hirsutum

and L. chilense were less adversely affected by LTs as compared to

L. esculentum. Root growth is an important attribute for selecting genotypes adapted to

LTs. Scott and Jones (1986) evaluated hypocotyl and root growth elongation

of four wild accessions of L. chilense and L. peruvianum and two control

genotypes of L. esculentum under low (10 °C) and control (20 °C)

temperatures. Hypocotyl growth rates of the wild accessions
were less inhibited at the 10 °C relative to 20 °C than were either of the cultivated accessions, suggesting that these wild accessions had greater CT than the cultivated genotypes for early seedling growth. In a more recent study, 31 accessions of cultivated and related wild species of tomato were evaluated for CT during VS to identify genetic resources that could be potentially useful for improving chilling tolerance of modern tomato cultivars (Foolad and Lin 2000). In this study, plant vegetative growth was evaluated under two d/n temperature regimes of 12/5 °C (cold stress) and 25/18 °C (control) with 12 h photoperiod and photon flux of 350 μmol.m\(^{-2}\).s\(^{-1}\) in growth chambers. CT during VS was defined as the ratio of shoot DW under CS to shoot DW under control conditions, and referred to as vegetative stage TI. Across accessions, vegetative stage TI ranged from 0.12 to 0.39 indicating the presence of genotypic variation for CT. CT during VS was independent of plant vigor, as judged by the absence of a significant correlation ($r = 0.14, P > 0.05$) between vegetative stage TI and DW under control. Several accessions were identified that exhibited considerable CT,
including L. hirsutum PI127826, LA1777 and LA386, and L. esculentum PI174263 and PI120256. Several of the identified accessions have been used to examine the genetic basis of CT during VS in tomato, as briefly reviewed in the next section. In addition to Lycopersicon wild species, potential sources of LT tolerance for tomato breeding have also been identified in Solanum species, in particular S. lycopersicoides (Wolf et al. 1986, Rick 1988, Walker et al. 1990) The cultivated tomato sets fruit poorly at or below 10 °C (Charles and Harris 1972) and this has been attributed mainly to: 1) failure of pollen production, 2) reduced pollen viability, 3) limited anther dehiscence, and 4) failure of pollination under LTs (Maisonneuve 1982, Picken 1984). Pollen tube growth also may be very slow to affect fertilization below 10 °C (Dempsey 1970). Other important processes involved in fruit set of tomato, such as the position of stigma in the anther cone, the formation of fertile ovules, or the early development of the embryo, are not adversely affected by chilling temperatures (Fernandez-Munoz and Cuartero 1991, Fernandez-Munoz et al. 1995b). Exposure of immature flowers of L. esculentum to repeated night temperatures below 10 °C resulted in sterile
pollen grains (Patterson et al. 1987). However, high-altitude L. hirsutum accessions LA1363, LA1393 and LA1777 produced functional pollen under such conditions (Maisonneuve 1983, Patterson et al. 1987). Pollen of L. hirsutum accession LA1366 was able to germinate at 6 °C (Patterson et al. 1987). Similarly, pollen germination of L. hirsutum accession LA1777 was less inhibited at 5 °C than pollen from a cultivated tomato (Zamir et al. 1981). Furthermore, the frequency of L. hirsutum gametes contributing to hybrid zygote formation was doubled when controlled fertilizations with pollen mixtures of L. esculentum and L. hirsutum occurred at d/n temperatures of 12/6 °C as compared to crosses with the same mixtures at 24/19 °C (Zamir et al. 1981). The results of the latter study suggested competitive ability of high-altitude L. hirsutum pollens at LTs. Furthermore, this ability was shown to be under genetic control and heritable. In another study, evaluation of pollen fertility and anther desiscence of 170 accessions from 8 different Lycopersicon species indicated that, unlike for L. esculentum, accessions from L. hirsutum, L. peruvianum and L. pennellii generally produced fertile pollen below 10 °C and released pollen
satisfactorily (Fernandez-Munoz et al. 1995a). Lyakh (1992) demonstrated that pollen of some accessions of L. pennellii and L. hirsutum fertilized more efficiently than that of L. esculentum at 10 °C. In other germplasm evaluation studies, several L. esculentum lines were identified with the ability to produce flowers and fruit set at LTs, including Early North, PI205040, PI280597, Cold Set, Precocé, Apedice, Montfavet and Supermarmande (Kemp 1968, Philouze and Maisonneuve 1979, Gautam et al. 1981). The aforementioned studies indicate the presence of potential genetic resources within the cultivated and related wild species of tomato for improving CT of commercial cultivars.

The utility of such genetic resources for improving tomato CT has been examined in a few studies, as briefly described below.

Inheritance and QTL Mapping

A high-altitude ecotype of L. hirsutum, which was able to develop the first true leaf when grown at a night temperature of 0 °C, was hybridized with a cold-sensitive L. esculentum cultivar (pistillate and recurrent parent) and F 1 and BC 1 progeny were produced (Patterson and Payne 1983). Subsequent selections for survival under a night temperature of 0 °C in the BC 1 and its
selfed progeny indicated that the CT attributes of the L. hirsutum accession could be transferred to L. esculentum through hybridization and selection.

Using BC 1 population of an interspecific cross between a cold-sensitive L. esculentum line and a cold-tolerant L. hirsutum accession, Vallejos and Tanksley (1983) identified three QTLs responsible for growth at LTs. In a more recent study, parental and reciprocal F 1 , F 2 , F 3 and BC 1 progeny (total of 12 populations) of a cross between a cold-sensitive tomato breeding line (UCT5) and a cold-tolerant primitive cultivar (PI120256) were grown under two d/n temperature regimes of 15/10 °C (cold stress) and 25/15 °C (control) (Foolad and Lin 2001a). Plants were evaluated for shoot DW under CS and for tolerance index (TI), measured as the ratio of DW under CS to DW under control conditions. Shoot DW was reduced in all genotypes in response to CS, however, PI120256 exhibited the highest tolerance (TI = 90.5%) and UCT5 the lowest (TI = 38.9%). The TIs of the filial and backcross progeny were intermediate to the parents, suggesting that CT of PI120256 genetically transmitted in the progeny. Across generations, there was a positive correlation ($r = 0.76, P < 0.01$) between DW under
CS and DW

under control conditions, suggesting that growth under CS was influenced by plant vigor. However, the absence of a significant correlation between DW under control conditions and the TI ($r = 0.47$, $P > 0.05$) and, in contrast, the presence of a significant positive correlation ($r = 0.92$, $P < 0.01$) between DW under CS and the TI suggested that plant vigor was not a determining factor in the expression of CT in PI120256 and its progeny.

Generation means analyses of DW under CS and TI indicated that the variation among generations was genetically controlled, with additive effects accounting for most of the variation ($\approx 90\%$ for TI). There were no significant dominance effects, and epistatic effects were minor and involved only additive additive interactions. The results suggested that the inherent CT of PI120256 would be useful for improving LT tolerance of commercial cultivars of tomato. The inheritance of CT during reproduction has been characterized to vary in different tomato germplasms. Using parental and F 3 progeny of a cross between L. esculentum and a high-altitude cold-tolerant accession of L. hirsutum, Patterson et al. (1987) demonstrated that some F 3 plants
were able to produce pollen of normal appearance at LT, similar to the L. hirsutum parent. Fernandez-Munoz et al. (1995b) investigated LT pollen viability of parental, F1, F2 and BC progeny of a L. esculentum ⨀ L. pennellii cross and concluded that this trait was polygenic and heritable. In segregating progeny derived from crosses between L. esculentum and L. pimpinellifolium, the ability to set fruit under LTs was determined to be controlled by recessive factors (Kalloo and Banerjee 1990). In contrast, the CT of hybrid between the sensitive L. esculentum cv Sub Arctic Maxi and tolerant S. lycopersicoides was suggested to be due to dominant nuclear genes (Kamps et al. 1987). However, the available information in the literature suggests that sources of genetic variation for CT exist within the cultivated and related wild species of tomato and there is potential for improving tomato CT by hybridization and selection. Further research is needed to determine the number and genomic locations of QTLs or major genes in order to facilitate the development of cold-tolerant tomatoes via MAS or genetic transformation.

Comparison of Cold Tolerance during
Different Stages of Plant Development

Low temperature tolerance is required at all stages of plant development,

including SG, VS, and reproduction, and during post-harvest storage of

fruits. When searching for genetic resources for CT, it is desirable to identify

genotypes that exhibit CT throughout the life cycle of the plant. However,

CT at one stage of plant development may not be correlated with tolerance

at other development stages, and there might not be accessions bearing CT

at all critical stages (Herner and Kemps 1983). Patterson et al. (1978) studied

altitudinal ecotypes of L. hirsutum and reported variable tolerance to LTs at

the whole plant level. However, equivalent germination rates of L. hirsutum

(native to 3100 m altitude in Peru) occurred at 3 °C lower temperature than

L. esculentum cultivar Rutgers. This marginal performance suggests that CT

during VS is not necessarily a valid indication of CT during other stages,

including SG. In other studies, comparison of different accessions of L.
hirsutum indicated that the ability of the seed to germinate quickly at LTs

was not related with the ability of the pollen to germinate at LTs (Patterson

et al. 1979, Patterson 1988). Similarly, evaluation of L. hirsutum accessions
for CT indicated that tolerance at the seedling stage was not necessarily correlated with tolerance during reproduction (pollen development and germination) (Patterson et al. 1987), and pollen selection at LTs was not effective for improving tomato CT during plant development (Maisonneuve et al. 1986). Lack of positive relationships between CT during SG and reproduction and between seedling growth and reproduction were also reported in other studies (Patterson et al. 1978, Maisonneuve and Den-Jijs 1984, Maisonneuve et al. 1986, Patterson et al. 1987). Although most studies indicate the absence of relationships among CT during different stages of plant development, a few studies suggest otherwise. For example, Zamir et al. (1981) reported that L. hirsutum accessions that were cold tolerant during seedling stage also exhibited greater CT during pollen germination and pollen tube growth. Furthermore, the inhibition of root elongation at LTs was less in plants of the crosses in which pollen was used from LTs adapted L. hirsutum plants than from plants of normal temperatures (Zamir and Gadish 1987). Two recent studies examined in more detail the phenotypic and genetic relationships between CT during SG and VS in tomato. In one study, the
phenotypic relationship was investigated by evaluating 31
tomato
accessions (cultivars, breeding lines, and plant
introductions), representing
six Lycopersicon sp., for CT during both stages (Foolad and
Lin 2000). SG
was evaluated under two temperature regimes of 11 ± 0.5 °C
(cold stress)
and 20 ± 0.5 °C (control) in darkness. CT during SG was
defined as the
inverse of the ratio of germination time under CS to
germination time under
control conditions, and it was called germination TI. Across accessions,
germination TI ranged from 0.15 to 0.48, indicating the
presence of
substantial genotypic variation for CT during SG.
Vegetative growth was
evaluated in growth chambers with d/n temperatures of 12/5
°C (cold
stress) and 25/18 °C (control) and a 12-h photoperiod of
350 μmol.m⁻².s⁻¹
(pressynthetic photon flux). CT during VS was defined as the
ratio of
shoot DW under CS to shoot DW under control conditions and
referred to
as vegetative stage TI. Across accessions, vegetative TI
ranged from 0.12 to
0.39 indicating the presence of notable genotypic variation
for CT during
VS. CT during VS was independent of plant vigor, as judged by the absence
of a significant correlation (r = 0.14, P > 0.05) between
vegetative stage TI

and DW under control. Furthermore, CT during VS was independent of CT during SG, as suggested by the absence of a significant rank correlation 

($r_R = 0.14, P > 0.05$) between vegetative stage TI and germination TI.

However, a few accessions were identified with CT during both SG and VS (Foolad and Lin 2000). The results indicate that for CT breeding in tomato,

each stage of plant development would have to be evaluated and selected independently. To determine the genetic relationship between CT during SG and VS, an F2 population of a cross between L. esculentum PI120256 (cold tolerant during both SG and VS) and UCTS (cold sensitive during both stages) was evaluated for germination under CS and the most cold-tolerant individuals (the first 5% that germinated) were selected (Foolad and Lin 2001b). Selected F2 individuals were grown to maturity and self-pollinated to produce F3 families (referred to as the selected F3 population). The selected F3 population was evaluated for CT separately during SG and VS and its performance was compared with that of a nonselected F3 population of the same cross.

The results indicated that selection for CT during SG significantly improved
CT of the progeny during SG, and a realized $h^2$ of 0.75 was obtained for this trait. However, selection for CT during SG did not affect plant CT during the VS. There was no significant difference between the selected and nonselected F3 populations in either absolute CT (defined as shoot FW under cold stress) or relative CT (defined as shoot FW under cold as a percentage of control). The results indicated that, in PI120256, CT during SG was genetically independent of CT during VS (Foolad and Lin 2001b). The overall evidence indicates that there is very little or no relationships among CT in different development stages in tomato, including SG, VS and reproduction. Thus, to develop tomato cultivars with improved CT throughout the ontogeny of the plant, selection protocols that include all relevant developmental stages are necessary. In this regard, the use of molecular markers and MAS may be desirable for achieving this goal.

Future Prospects for Developing Tomato Cultivars with Enhanced Cold Tolerance

Genetic resources for CT during different stages of plant development, including SG, VS and RS, have been identified within the cultivated and related wild species of tomato, in particular L.
hirsutum. Significant progress has been made in characterizing the physiological mechanisms and metabolic aspects of CT in tomato. We have some knowledge of the genetic controls of CT during different stages of plant development in tomato. However, progress in developing tomatoes with improved CT has been very limited, in part due to an insufficient understanding of the genetic basis of CT and the limited breeding effort that has been devoted to this goal. Significant progress has been made in the molecular genetic basis of CT in many other plant species, in particular A. thaliana.

Several genes, proteins, enzymes and other compounds have been identified with direct or indirect effects on LT tolerance. Utilization of such genes or compounds in tomato using the available transgenic approaches may become a useful method for improving tomato CT. This will require a better understanding of the genetic basis of CT in tomato at the cellular and molecular levels and transferring of tolerance components from CT accessions of L. hirsutum into the cultivated tomato. Research to identify QTLs with major effects on CT may also be useful for improving tomato LT.
TOLERANCE TO HEAT STRESS

Background Information

Although tomato plants can grow in a wide range of climatic conditions, their vegetative and reproductive growth are severely impaired at high temperatures, resulting in reduced yield and fruit quality (Abdul-Baki 1991, Dane et al. 1991, Wessel-Beaver and Scott 1992, Scott 1993). Generally, when the ambient temperature exceeds 35 °C, tomato seedling and vegetative growth, flowering and fruit set, and fruit ripening are adversely affected (Kalloo 1991). This high-temperature sensitivity is particularly important in areas with tropical or subtropical climates. In such environments, heat stress (HS) may become a major limiting factor for field production of tomatoes. Although tomato plants are sensitive to high temperatures during all stages of plant development, flowering and fruit set are the most sensitive stages. Fruit set is somewhat affected at d/n temperatures above 26/20 °C and is severely affected above 35/26 °C (Rudich et al. 1977, George et al. 1984, Stevens and Rudich 1987, Berry and Rafique-Uddin 1988). For example, a 4-h exposure to a day temperature of
40 °C during the reproductive stage prevents fruit set in most cultivars of tomato (Charles and Harris 1972, Rudich et al. 1977). Villareal (1978) defined heat tolerance (HT) in tomato as the ability to set fruits under night temperatures not lower than 21 °C. Other researchers, however, have argued that day and night temperatures may not affect tomato fruit set independently, and that diurnal mean temperature is a better predictor of plant response to high temperature, with day temperature having a secondary role (Peet and Bartholemew 1996, Peet et al. 1997, Peet and Willits 1998, Peet et al. 1998). Accordingly, the sensitivity to high temperature should be determined as the diurnal mean temperature above which tomato fruit set is reduced. By examining different d/n temperature regimes, Peet et al. (1997, 1998) demonstrated that percent fruit set in tomato decreased as mean diurnal temperature rose above 25 °C and was severely impaired at 29 °C. Reproductive processes adversely affected by high temperature in tomato include meiosis in the microspore and megaspore mother cells (Kinet and Peet 1997), amount of pollen produced (El-Ahmadi and Stevens 1979b, Stevens and Rudich 1987), anther dehiscence and pollen
release (El-Ahmadi and Stevens 1979b), pollination (Charles and Harris 1972, Shelby et al. 1978), pollen germination and pollen tube growth (Weaver and Timm 1989), ovule viability (Kinet and Peet 1997), stigmatic and stylar position (Charles and Harris 1972, El-Ahmadi and Stevens 1979b), number of pollen grains retained by the stigma, fertilization as well as post-fertilization processes, growth of the endosperm, proembryo and fertilized embryo (Kinet and Peet 1997, Peet et al. 1998). However, the most noticeable morphological effect of high temperature is the production of an exserted style (when stigma is elongated beyond the anther cone), which may prevent self-pollination (Rick and Dempsey 1969). Poor fruit set at high temperature has also been associated with low levels of carbohydrates and growth regulators released in plant sink tissues (Kinet and Peet 1997). Furthermore, high-temperature effects on fruit set depend on the stage of floral development (Iwahori 1965) and the genotype (Rudich et al. 1977, Shelby et al. 1978, El-Ahmadi and Stevens 1979a, b, Shen and Li 1982). Growth chamber and greenhouse studies suggest that high temperature is most deleterious when flowers are
first visible and sensitivity continues for 10-15 days (Calvert 1969).

Reproductive phases most sensitive to high temperature are gametogenesis (8-9 days before anthesis) and fertilization (1-3 days after anthesis) (Iwahori 1966). Both male and female gametophytes are sensitive to high temperature and response varies with genotype (Shelby et al. 1978, El-Ahmadi and Stevens 1979a, b). Ovules are generally less heat sensitive than pollen (Charles and Harris 1972, Rudich et al. 1977, Peet et al. 1998). A strong positive correlation has been observed between fruit set and yield under high temperature (El-Ahmadi and Stevens 1979a, Abdul Baki 1991, Wessel-Beaver and Scott 1992). Therefore, evaluation of tomato germplasm to identify sources of HT has regularly been accomplished by screening for fruit set under high temperature (El-Ahmadi and Stevens 1979a, Berry and Rafique-Uddin 1988, Abdul-Baki 1991). Since poor fruit set at high temperature can not be attributed to a single factor, decreases in pollen germination and/or pollen tube growth are among the most commonly reported factors. Thus, pollen viability has been suggested as an indirect selection criterion for HT (Weaver and Timm 1989). Levi et al. (1978) reported that heat-tolerant cultivars often
exhibited higher pollen viability than heat-sensitive cultivars under high temperature. Production of viable seed also is often reduced under high temperature and thus, high seed set has been arguably reported as an indication of HT (Charles and Harris 1972, El-Ahmadi and Stevens 1979a, Berry and Rafique-Uddin 1988, Abdul-Baki 1991). High-temperature conditions also induce the incidence of fruit disorders, e.g., cracks, blossom-end rot, deterioration of fruit color, watery tissue, and small immature fruit. All these cause reductions in marketable yield (Charles and Harris 1972, Scott et al. 1986, Abdul-Baki 1991, Johjima 1995). The non-reproductive processes in tomato which are affected by HS include photosynthetic efficiency (Bar-Tsur et al. 1985), assimilate translocation (Tanaka et al. 1974), mesophyll resistance (Stevens and Rudich 1987), and disorganization of cellular membranes (Chen et al. 1982). Overall the criteria most often used for evaluating tomatoes for HT are yield, fruit set, fruit quality, and seed production. Genotypic variation has been observed in the cultivated and related wild species of tomato for the effect of high temperature on pollen and
ovule production and viability, anther dehiscence, pollination effectiveness

(Shelby et al. 1978), style elongation (Rudich et al. 1977, El-Ahmadi and Stevens 1979a), splitting of the antheridial cone, stigma exsertion (Levi et al. 1978), and the ability to set fruit (Rudich et al. 1977, Stoner and Otto 1975, Berry and Rafique-Uddin 1988, Abdul-Baki 1991, Dane et al. 1991, Abdul-Baki and Stommel 1995). However, a causal relationship between each of these characteristics and HT has not always been demonstrated (Charles and Harris 1972, El-Ahmadi and Stevens 1979a, Lohar and Peat 1998). Rudich et al. (1977) reported that in heat-tolerant lines generally stigma position was near the staminal cone whereas in heat-sensitive lines the style was elongated and exserted. However, Lohar and Peat (1998) suggested that the use of stigma exsertion as a criterion for selecting against fruit set at high temperature might be misleading and should be avoided.

El-Ahmadi and Stevens (1979a) reported that for an optimal HT response, a cultivar must exhibit a combination of essential characteristics under high temperature, including high number of flowers per plant, absence of stigma exsertion, high pollen production, ovule viability, and substantial
fruit and seed set. However, none of the heat-tolerant cultivars studied by these researchers possessed all of the model traits. Two common and undesirable characteristics generally observed in heat-tolerant tomato genotypes are production of small fruit and restricted foliar canopy (Rudich et al. 1977, Shelby et al. 1978, Villareal 1978, El-Ahmadi and Stevens 1979a, Hanna and Hernandez 1982, Scott et al. 1986, Dane et al. 1991, Wessel-Beaver and Scott 1992, Scott 1993, Scott et al. 1997). The production of small fruit is most likely due to adverse effects of high temperature on the production of auxins in the fruit, and the poor canopy is due to the highly reproductive nature of the heat-tolerant genotypes.

Dane et al. (1991) evaluated fruit set ability of 47 tomato genotypes under high temperatures in the field and/or in the greenhouse and concluded that small-fruited genotypes were generally less affected by heat than large fruited cultivars, consistent with many other reports on the negative correlations between HT and fruit size. However, despite all the complexities of HT and the difficulties encountered during the transfer of tolerance, heat-tolerant inbred lines and hybrid cultivars with commercial
acceptability have been developed and released (see Section: Inheritance and Breeding). Seed germination in tomato is greatly affected by high temperature.

Tomato seed germinate best between 20 °C and 25 °C. The germination is reduced at 30 °C, highly inhibited at 35 °C (Jaworski and Valli 1965, Thompson 1974, Lorenz and Maynard 1980, Coons et al. 1989), and almost nil at 39-39.5 °C (Thompson et al. 1977). There is significant genotypic variation in response to germination under high temperature, total germination ranging from 0% to 95% at or above 35 °C in some genotypes (Berry 1969, Thompson 1974, El-Hassan 1978, Taylor et al. 1982, Coons et al. 1989). The ability to germinate under high-temperature conditions is important for the fall production in tropical and subtropical environments where seeding is required during late summer and soil temperatures often exceed 35 °C (Coons et al. 1989).

Inheritance and Breeding

Development of tomato cultivars for improved fruit set and yield under high temperature is desirable for tomato production in regions where temperatures during the growing season reach 35 °C (Scott et al. 1986, Stevens and Rudich 1987, Scott 1993). However, breeding for
HT in tomato

is a difficult task, in part due to the complexity of the trait and its low to moderate h2 (Charles and Harris 1972, Rudich et al. 1977, Levi et al. 1978, Aung 1979, Villareal and Lai 1979, Kuo et al. 1979, El-Ahmadi and Stevens 1979b, Scott et al. 1986, Dane et al. 1991). Other drawbacks being that heat tolerant lines tend to produce smaller fruit than is commercially acceptable (Rudich et al. 1977, Shelby et al. 1978, Villareal 1978, Villareal and Lai 1979, El-Ahmadi and Stevens 1979a, Hanna and Hernandez 1982, Dane et al. 1991, Wessel-Beaver and Scott 1992, Scott 1993, Scott et al. 1997) and poor foliar canopy (Scott 1993, Scott et al. 1997). To overcome these impediments and for developing widely adapted lines with acceptable horticultural characteristics, it is recommended to utilize in a breeding program genotypes capable of fruit set at high temperatures in combinations with genotypes with large fruit and vigorous vine growth in different environmental conditions (Berry and Rafique-Uddin 1988, Wessel-Beaver and Scott 1992, Scott et al. 1997). To estimate h2 for HT and produce heat-tolerant tomato lines, Wessel-Beaver and Scott (1992) developed a synthetic population of tomato from
polycrosses among seven selected genotypes, and evaluated the population for HT in two different locations (Florida and Puerto Rico) during one summer. Single-location h2s were high for percent fruit set (0.74-0.77), yield (0.65-0.81), and fruit weight (0.89-0.97). Across-location h2 was low for yield (0.14), intermediate for fruit set (0.60), and high for fruit weight (0.92).

Genotype x environment interaction was most important for yield and least important for fruit weight. Large genetic correlations (r = 0.71-0.74) were observed between yield and fruit set under high temperatures at both locations (Wessel-Beaver and Scott 1992). El-Ahmadi and Stevens (1979b) reported that inheritance of fruit set under high temperature of heat-tolerant genotypes was due to additive and dominant gene actions with moderate h2. Hanna et al. (1982) concluded that additive gene action was more important than nonadditive gene action for percent fruit set, percent flower drop, and percent undeveloped ovaries under high field temperatures. In a diallel analysis using several heat-tolerant and heat-sensitive tomato genotypes, Dane et al. (1991) determined that pollen fertility and fruit set under high field temperatures were primarily under additive genetic
controls. However, evaluating parental and hybrid progeny of crosses between several genotypes with various levels of fruit-set ability under high temperature, Scott et al. (1986) reported the presence of dominance for high fruit set under high temperature and recommended the development and use of hybrid cultivars with improved HT. A more recent study, including parental and hybrid progeny of crosses among several heat tolerant and heat-sensitive genotypes, confirmed the presence of dominance for fruit-set ability under high temperature (Scott et al. 1997). The advantage of the dominant nature of high temperature fruit-setting ability is that commercial heat-tolerant fresh-market tomato cultivars can be feasibly developed by producing F1 hybrids between heat-tolerant and heat-sensitive parents. In this approach, defects of the heat-tolerant parent, such as disease susceptibility, small fruit and poor vine coverage, could be at least partially ameliorated, if the proper heat-sensitive parent is used for the hybrid production. A review of the literature indicates that, despite various difficulties in transferring HT characteristics in tomato, several breeding lines and hybrid cultivars with improved HT and rather acceptable
horticultural characteristics (medium to large size fruit, vigorous vine and good leaf coverage) have been developed and released (Scott et al. 1989, Hanna et al. 1992, Scott et al. 1995, Scott et al. 1997, Scott 2000). The problem of small fruit size has largely been overcome by crossing small-fruited heat-tolerant lines with extremely large-fruited heat-sensitive lines and producing heat-tolerant F1 hybrids. The use of such hybrid cultivars has already resulted in dramatic increases in tomato yield in areas with high day or night temperatures during the growing season, such as that in Florida (Scott et al. 1997, Scott 2000). Most of the heat-tolerant varieties developed to date have jointed pedicel, however, Scott and his colleagues were able to develop heat-tolerant, large fruited, jointless inbred lines. Such genotypes, however, do not perform as good as the equivalent genotypes with jointed pedicel (Scott et al. 1997, Scott 2000). The available heat-tolerant genotypes are currently used extensively to develop commercially acceptable tomato cultivars with enhanced HT.

Future Prospects for Developing Tomato Cultivars with
Enhanced Heat Tolerance

Germplasm resources for HT have been widely identified within Lycopersicon species. The physiological and morphological components of heat tolerance/sensitivity have been resolved and the genetic basis of HT at the whole plant level determined. During the past several years, many breeding lines and cultivars have been developed with improved fruit setting ability under high temperature. Many of these lines exhibit some undesirable horticultural characteristics, in particular small fruit size, low yield, poor vine coverage, and jointed pedicel. However, many years of tomato research and breeding efforts at the University of Florida have resulted in the development of new high-yielding breeding lines and hybrid cultivars with medium to large size fruit, high fruit setting ability, jointless pedicel, and other HT attributes. Currently, further efforts are being devoted to the development of heat-tolerant tomato cultivars with larger fruit size, higher yield, and more vigorous vines for production in tropical and subtropical environments. Judging from the recent advances in this area, we expect to witness improved cultivars, in particular F1 hybrids, with
excellent fruit setting ability under high temperature and acceptable horticultural characteristics in the near future. However, limited research has been conducted on molecular genetic basis of HT in tomato. This line of research as well as research on the relationship between tolerances to high and low temperatures during different stages of plant development should be strengthened.

RELATIONSHIPS OF TOLERANCES TO DIFFERENT ABIOTIC STRESSES

Tolerance to Different Stresses during Seed Germination

It has been hypothesized that similar or identical genes and physiological mechanisms might control the rate of tomato SG under different stresses, (SS, DS and CS). Excessive salt depresses the water potential of the germination medium, making water less available to the seed, and thus lowers the rate of, or completely inhibits, germination. Most studies suggest that under SS low water potential of the external medium, rather than ion toxicity effects, is the major limiting factor to germination in different crop species, including tomato (Kaufman 1969, Ungar 1978, Bliss et al. 1986, Haigh and Barlow 1987, Bradford 1995). Drought Stress also causes low
rate of SG due to reduced water potential of the germination medium

(Bradford 1995). Therefore, it is expected that seeds that germinate rapidly under SS would also germinate rapidly under DS, and vice versa. Low temperatures (cold stress) also affects the water status of the cell and thus, it could delay SG by causing water stress (Liptay and Schopfer 1983). The genetic and physiological processes that impart rapid SG under different stress conditions are exactly unknown. It is important, both for scientific and practical purposes, to determine whether the same genes contribute to rapid SG under different stress conditions. It is also equally important to determine whether genotypes with the ability to germinate rapidly under various environmental conditions can be identified. Such information may contribute to the development of cultivars with superior germination performance under a wide range of environmental conditions. Recently, three different undermentioned approaches were taken to determine the relationship in the rate of tomato SG to different stress conditions, including SS, CS and DS.

(i) Germplasm Evaluation
Three independent investigations were followed to evaluate germplasm by finding phenotypic relationships in the rate of tomato 
SG under different 
abiotic stresses. In the first study, germination responses 
of 30 tomato 
accessions representing six Lycopersicon species were 
examined under CS 
and SS (Foolad and Lin 1999). In the second study, 
germination responses 
of approximately 70 L. pimpinellifolium accessions were 
examined under 
CS, SS and DS (M.R. Foolad et al., unpublished data). In 
the third study, 
germination responses of 145 RILs, derived from a cross 
between a slow 
germinating L. esculentum breeding line (NC84173) and a 
fast germinating 
L. pimpinellifolium accession (LA722) were examined under 
CS, SS and DS. 

In these studies, the pair-wise phenotypic correlation 
coefficients between 
germination responses under the three stress conditions 
ranged between 
0.62 and 0.80, and all were statistically significant at 
the 0.01 probability 
level. The overall results indicated the presence of 
significant phenotypic 
relationship between germination ability under three stress 
conditions (CS, 
SS and DS). However, these results must be supported on the 
genetic basis 
of these relationships.
(ii) Analysis of Response, and Correlated Response, to Selection

Two independent studies were conducted to examine genetic relationships among tomato SG responses under different stress conditions. In the first study, seeds of F2 progeny of a cross between a slow germinating (UCT5) and a fast germinating tomato line (PI120256) were evaluated separately for germination under CS and SS, and in each treatment the most rapidly (first 5%) germinating seeds were selected. Selected seedling were grown to maturity and self-pollinated to produce F3 progeny. The F3 progeny from each selection experiment were evaluated separately for germination under CS and SS, and their performances compared with germination rate of non-selected F3 progeny of the same cross. The results indicated that selection under CS or SS significantly improved progeny germination rate under each of cold- or salt-stress conditions (Foolad, et al. 1999b).

The genetic correlation between the rate of SG under CS and SS was estimated as $r_G = 1.00$, as determined by $r_G = (CR_xCRY/RxRY)^{1/2}$, where, $CR_x$ is the correlated response under treatment $X$ due to selection under treatment $Y$, and $Rx$ is the direct response under treatment $X$. 
due to selection under the same treatment, X. The results supported the

suggestion that same genes might contribute to rapid SG under both CS

and SS. In the second study, seeds of BC 1 progeny of an

interspecific hybrid

between a slow germinating tomato breeding line (NC84173; maternal and

recurrent parent) and a fast germinating L. pimpinellifolium accession

(LA722) were evaluated separately for germination rate under CS, SS and

DS, and in each treatment the most rapidly (first 2%) germinating seeds

were selected. Selected individuals were grown to maturity and self

pollinated to produce BC 1 S 1 progeny. The selected BC 1 S 1 progeny from

each experiment were evaluated for germination rate under each of CS, SS

and DS, and their performances under each stress were compared with

those of a non-selected BC 1 S 1 population of the same hybrid. Results

indicated that selection for rapid SG in each of the three stress treatments

was effective and significantly improved progeny germination rate under

all three stress conditions (Foolad et al. 2002). The observation of large

genetic correlations (rG) between CS and SS (0.94), CS and DS (0.81), and

SS and DS (1.00) suggested that the same genes or
physiological mechanisms might control the rate of SG under these three stress conditions.

Furthermore, these studies suggest the presence of some common stress related genes that facilitate rapid SG under different stress conditions. In practice, therefore, selection for rapid SG under a single stress environment might result in progeny with improved SG under a wide range of stress conditions.

(iii) QTL Mapping

To further examine the genetic relationships of germination under different stress conditions, three QTL comparison investigations were conducted. In all three studies, different populations of the same interspecific hybrid between a slow germinating breeding line, NC84173, and a fast germinating L. pimpinellifolium accession, LA722, were used to compare QTLs contributing to rapid SG under CS, SS and DS. In the first experiment, QTLs for CT and ST during SG were identified in BC 1 S 1 populations using an interval mapping approach (Foolad et al. 1999a). In the second experiment, a selective genotyping approach was employed to identify QTLs for CS, SS and DS using different BC 1 populations of the same cross.
In the third experiment, a RIL population was evaluated for SG under CS, SS and DS and QTLs were identified for rapid SG under the three stress conditions (M.R. Foolad et al., unpublished data). The combined results indicated the presence of two types of QTLs. Some QTLs were identified that contributed to rapid SG under two or three stress conditions. These QTLs were referred to as germination-related, stress-nonspecific QTLs. Some QTLs that contributed to rapid SG only under one stress condition. Such QTLs were referred to as germination-related, stress-specific QTLs. The general conclusions from the above investigations suggest the presence of genes or physiological mechanisms in some accessions of tomato that facilitate rapid SG under different stress conditions, including CS, SS and DS. Apparently such genetic or physiological mechanisms can function under different stress conditions and stimulate rapid SG. The results also suggest the presence of other genes that might be expressed only under specific stress conditions and which may stimulate rapid SG under such conditions. However, isolation, characterization and comparison of
functional genes which facilitate rapid SG under different stress conditions would be necessary to validate these suggestions. The results of these studies, however, indicate that to develop tomato cultivars with rapid SG under diverse environmental conditions, it may be sufficient to conduct selection and breeding only under a single stress treatment.

Germination Responses under Stress and Nonstress Conditions

The ability of the seed to germinate rapidly and uniformly under different stress and nonstress (NS) conditions is a desirable trait for many crop species, including tomato. Germplasm evaluation studies have indicated that tomato seeds that germinate rapidly under NS conditions may also tend to germinate rapidly under stress conditions for some germplasm. For example, there was a significant phenotypic correlation ($r_P = 0.75, P < 0.01$) between the rate of SG in NS and CS treatments among 36 accessions of the cultivated and wild species of tomato (Scott and Jones 1982). Similarly, a significant phenotypic correlation ($r_P = 0.89, P < 0.01$) between the rate of SG under NS and CS was reported among 30 tomato accessions from six different Lycopersicon species (Foolad and Lin 1999). A smaller, phenotypic correlation ($r_P = 0.54, P < 0.01$)
between the rate of SG

under NS and CS was also observed among 145 F 9 RILs of a cross between

a slow germinating tomato breeding line (NC84173) and a fast germinating

L. pimpinellifolium accession (LA722) (M.R. Foolad et al., unpublished data).

Furthermore, significant phenotypic correlations between the rate of SG

under NS and SS were reported in different studies, including among 45

cultivated and wild accessions of tomato (rP = 0.62, P < 0.01) (Foolad and

Lin 1997b), among 30 accessions from different Lycopersicon species (rP =

0.63, P < 0.01) (Foolad and Lin 1999), and among 145 F 9 RILs of the cross

between NC84173 and LA722 (rP = 0.58, P < 0.01) (M.R. Foolad et al.,

unpublished data). Moreover, a significant phenotypic correlation (rP =

0.57, P < 0.01) between the rate of SG under NS and DS was observed

among 145 F 9 RILs of the hybrid between NC84173 and LA722 (M.R. Foolad

et al., unpublished data). These correlations are consistent with the

suggestion that similar physiological mechanisms might contribute to rapid

SG under both NS and stress conditions (Bradford 1995). Similarly, they

may indicate that genetic factors facilitating rapid SG under stress
conditions have no undesirable effects on performance in the absence of stress. Notably, however, in most of these studies, the magnitudes of the correlation coefficients for germination under NS and stress conditions were moderate or small. A common observation in these studies was that some accessions or lines that germinated rapidly under NS exhibited poor germination under stress conditions, and vice versa. This observation supports the presence of genes which might be stress-specific and contribute to rapid SG only under specific stress conditions. Several investigations were conducted to determine the genetic relationship between the rate of tomato SG under stress and NS conditions. In one study, an F2 population of a cross between a slow germinating (UCTS) and a fast germinating (PI120256) tomato lines was evaluated separately for germination under stress (SS and CS) and NS conditions, and in each treatment, selection was made for rapid SG. Evaluation of response and correlated response to selection in the F3 progeny indicated that selection for rapid SG under either SS or CS resulted in progeny with improved germination under both stress and NS conditions (Foolad et al. 1999b).

However, selection for rapid SG under NS conditions did not
significantly improve progeny SG under any of the three conditions. This lack of response to selection could be due to a limited expression of genetic variation in the F₂ population under NS conditions. Large genetic correlations were revealed between germination under NS and either CS (rₓ = 0.81) or SS (rₓ = 0.66). In a different study, selection for rapid SG under SS, CS or DS in a BC₁ population of a hybrid between NC84173 and L. pimpinellifolium accession LA722 resulted in moderate improvements (significant only at P < 0.05) in progeny SG under NS conditions (Foolad et al. 2002). No selection was made under NS condition because there was little variation in germination rate in the NS treatment. Furthermore, in this study, moderate phenotypic correlations were observed between SG under NS and either CS (rₚ = 0.53, P < 0.05), SS (rₚ = 0.50, P < 0.05), or DS (rₚ = 0.46, P < 0.05). The results suggested the presence of some genetic relationships between germination under NS and stress (salt, cold or drought) conditions. However, these studies indicated that, for practical purposes of improving tomato SG under NS conditions, it is better to conduct selections under a stress treatment. This is
because of the higher

variance for germination and higher selection efficiency
under stress

conditions. Higher genetic variance in stress environments
is one

of the more favorable situations for plant breeders
(Rosielle and Hamblin

1981) although it does not appear to be a common occurrence
(Daday et al.

1973). To further examine the genetic relationship between
rate of tomato SG

under stress and NS conditions, QTLs contributing to these
traits were

compared in three different studies M. R. Foolad et al.,
unpublished data).

First, QTLs were identified for germination under NS, CS
and SS in BC 1 S 1

populations of a cross between NC84173 and L.
pimpinellifolium accession

LA722 and compared for co-localization (Foolad et al.
1999a). In the second

study, QTLs for the rate of tomato SG under NS, CS, SS and
DS were
determined in different BC 1 populations of a NC84173 ×
LA722 hybrid

using selective genotyping. In the third study, QTLs for
the same four traits

were identified and compared using a RIL population of a
cross between

NC84173 and LA722. Results of the these investigations were
comparable

and indicated the presence of a genetic relationship
between the ability of
tomato seed to germinate rapidly under NS and stress conditions. A few QTLs were detected which contributed to rapid SG under both NS and stress conditions; these QTLs were referred to as germination-related, stress nonspecific QTLs. Several other QTLs were identified which affected germination under one or more of the stress conditions but not under NS conditions; these QTLs were referred to as germination-related, stress specific QTLs. The detection of stress-nonspecific QTLs indicated the presence of a genetic relationship between the ability of the tomato seed to germinate rapidly under NS and stress conditions. However, whether such a genetic relationship was due to pleiotropic effects of the same genes, physical linkage of different genes, or a combination of the two, could not be determined in these studies. Isolation and characterization of functional genes affecting germination rate under different stress and NS conditions may be necessary to determine the nature of the genetic relationship. The detection of stress-nonspecific QTLs, however, is consistent with the significant phenotypic and genetic correlations observed between germination rate under NS, CS, SS and DS conditions. The results are in
agreement with the suggestion that genetic parameters or physiological mechanisms that facilitate rapid tomato SG under NS conditions also contribute to improved germination rate under stress conditions (Jones 1986a, Foolad and Jones 1991, Bradford 1995). In this regard, the stress nonspecific QTLs could affect germination rate by controlling the vigor of the germinating embryo, the variation in the thickness of the endosperm, the physical and permeability properties of the endosperm cell walls, the time of onset or rate of activity of enzymes that modify the properties of the endosperm cell wall, the release of gibberellins by the embryo (which is necessary for endosperm weakening), or other unknown factors essential for the initiation of germination. Theoretically, such effects should contribute to rapid SG under both NS and stress conditions. Conversely, the detection of stress-specific QTLs indicates the presence of genes that facilitate rapid germination only under specific stress conditions. These QTLs may affect germination-related physiological processes that are triggered by a specific stress, and thus contribute to rapid germination under such conditions. For example, the QTLs for rapid
germination under CS might affect the rate of germination-related metabolic activities in the embryo or endosperm under LTs, the thermal time requirements for germination (Bradford 1995) or the rate of embryo growth and its ability to overcome the mechanical restraint imposed by the surrounding endosperm. Similarly, the QTLs for rapid germination under SS or DS might affect the base water potential required for SG, the hydrotome constant (Bradford 1995) or the rate of metabolic activities in the embryo or endosperm under osmotic stress. Moreover, such QTLs may contribute to a better osmoregulation in rapidly germinating seeds.

Finally, the finding that some QTLs affect germination only under specific stress conditions may explain the less-than-perfect correlations observed between germination under NS and stress conditions or between germination under different stress conditions in different studies (Foolad 1999a, Foolad, et al. 1999b). The QTL results are consistent with the previous report that selection for rapid SG under NS was less effective than selection under stress conditions (Foolad, et al. 1999a). For practical breeding purposes, however, the stress-nonspecific QTLs should be more useful as
they relate to rapid germination under a wide range of environmental conditions. The identification of germination-related, stress-specific and stress-non specific QTLs indicates that marker-assisted selection for such QTLs may result in the development of germplasm with improved germination under both NS and stress conditions. Between 5 and 6 QTLs have been identified in the wild accession LA722 for germination under each germination condition. Introgression of this rather small number of QTLs by MAS is feasible, providing opportunities to rapidly develop cultivars with enhanced germination under different conditions. However, further genetic and physiological investigations are needed to examine the nature of the common and/or stress-specific genes (or physiological mechanisms) which affect germination under different conditions.

Tolerance to Different Stresses during Vegetative Growth and Reproduction

Different abiotic stresses, salinity, drought and extreme temperatures, often adversely affect the same growth-related physiological, biochemical, and metabolic aspects of the plant and same protective mechanisms are often activated to these stresses, albeit sometimes through
different signaling pathways (Holmberg and Bulow 1998, Shinozaki and Winicov 1998, Zhu 2001, Yamaguchi 2005). Research in other plant species, including A. thaliana, has demonstrated that the expression of some common genes is increased in response to different stresses (Kasuga et al. 1999). In fact, single-gene transgenic A. thaliana plants have been developed with enhanced salt, cold and freezing tolerance (Holmberg and Bulow 1998, Kasuga et al. 1999). However, in tomato very little research has been done to determine relationships among tolerance to different stresses during vegetative growth or reproduction although some studies have been made in our laboratory.

The overall results from our investigations indicated the absence of a relationship between ST and CT across the accessions studied. Sometimes tomato genotypes that are capable of fruit setting at low temperatures also have the ability for high temperature fruit setting (Charles and Harris 1972).

Further research is therefore needed to determine tolerance to different stresses during vegetative growth and reproduction in tomato.

CURRENT STATUS AND FUTURE PERSPECTIVES

Abiotic stresses are major constraints to crop production...
worldwide. Most commercial cultivars of tomato are sensitive to abiotic stresses during all stages of plant development, and, thus, tomato production is limited in stressful environments. The cultivated species of tomato has a very narrow germplasm base due to several genetic bottlenecks during its domestication and evolution. Consequently, genetic resources having desirable agricultural characteristics, such as abiotic stress tolerance, are not found in the cultivated species. Fortunately, however, the eight related wild species within Lycopersicon are a rich source of desirable genes for tomato crop improvement. Although thus far only a superficial assessment of the extent of the genetic variation for abiotic stress tolerance within Lycopersicon has been made, some germplasms with significant tolerance to different abiotic stresses, including salinity, drought and extreme temperatures, have been identified. Such resources are extensively used to characterize the physiological mechanisms and cellular bases of stress tolerance in tomato. Comparatively, little progress has been made on genetics and breeding for stress tolerance in tomato. With the availability of advanced
tools of plant molecular biology the focus has largely been shifted to

the genetic basis of stress tolerance in tomato. Some notable progress

has been achieved. Tolerance components have been defined and their

 genetic controls characterized, and many controlling genes or QTLs

 with major effects have been identified and/or cloned. Several breeding

 lines, cultivars, or germplasms with improved tolerance to different abiotic

 stresses, in particular salt stress, cold stress and heat stress, have been

 developed. The new biotechnological approaches of gene transfer have

 provided opportunities to engineer tomatoes with enhanced stress

 tolerance. Although the transgenic plants have only been subjected to

 artificial laboratory tests of stress tolerance, the prospect for engineering

 tomato plants with field stress tolerance is improving. The recent

 achievements, however, should be considered only a beginning and

 it is predicted that the importance of breeding for stress tolerance in

 tomato will increase substantially in the future. To accelerate the

 development of tomato cultivars with improved tolerance to different

 abiotic stresses, various research activities deemed necessary are listed in
Appendix 16.1.

SUMMARY

Abiotic stresses are among the most important challenges to agricultural production worldwide. The cultivated tomato, L. esculentum Mill., is sensitive to most abiotic stresses, including salinity, drought, and extreme temperatures. Considerable genotypic variation for abiotic-stress tolerance, however, exists within the related wild species of tomato. During the past few decades, significant progress has been made in characterizing the physiological mechanisms and whole plant response to abiotic stress in tomato. Progress also has been made in discerning the genetic basis of tolerance to different stresses. Most physiological and genetic investigations indicate that tolerance to abiotic stress is a complex trait, controlled by more than one gene, and highly influenced by environmental variation. Furthermore, abiotic stress tolerance in tomato is a developmentally regulated, stage-specific phenomenon; tolerance at one stage of plant development is often not correlated with tolerance at other developmental stages. Thus, specific ontogenic stages have often been evaluated separately.
for the assessment of tolerance and for the identification, characterization
and genetic manipulation of tolerance components. Partitioning of the
tolerance into its developmental and physiological components has
provided a better understanding of the plant’s response to abiotic stress.
The use of traditional plant genetics and breeding protocols as well as
contemporary molecular biological techniques, such as molecular marker
maps, quantitative trait locus (QTL) mapping, marker-assisted selection
(MAS), and genetic transformation have resulted in genetic characterization
and/or development of tomato genotypes with improved stress tolerance.
The application of QTL mapping in particular has contributed to a better
understanding of the genetic relationship among tolerances to different
stresses. Furthermore, some tomato germplasms, comprising breeding lines
and cultivars, have been developed with improved stress tolerance, such
as heat tolerance, cold tolerance, and salt tolerance. With the recent
advancements in molecular genetic techniques, and the isolation, cloning,
and characterization of new stress-related genes and proteins, the prospect
for developing commercial cultivars of tomato with enhanced stress
tolerance is improving.

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APPENDIX 16.1

FUTURE PROSPECTS OF PRODUCING TOMATO TOLERANT TO

ABIOTIC STRESSES

1. Large screening experiments must be conducted in search of additional genetic resources for tolerance to different abiotic stresses, in particular drought tolerance. An almost inexhaustible supply of unexplored diversity exists within the wild species of Lycopersicon. A few species within Solanum have also been shown to be rich sources of genes for abiotic stress tolerance and which could be used for tomato breeding (Rick 1988). 2. For each abiotic stress, the major components of tolerance have to be identified and characterized. Often it is not only one physiological mechanism or genetic factor that contributes to plant stress tolerance. Furthermore, different physiological and genetic mechanisms may be involved in stress tolerance in different genotypes. The identification and characterization of major tolerance components in different germplasms would facilitate the transfer and possibly pyramiding of the tolerance contributing factors in desirable genetic backgrounds. 3. Research is needed to extend our knowledge of the genetic controls of tolerance related components in different germplasms and during different developmental stages. This includes studies on the inheritance of tolerance traits, the identification and characterization of controlling genes (or QTLs), and assessing the methods for transferring genes individually or together across genotypes. 4. The search for potential tolerance components, including genes and proteins, must go beyond the limits of species within Lycopersicon, and it should include other genera such as model plants and microbial organisms. 5. A close collaboration is highly needed among physiologists, geneticists, breeders and molecular biologists interested in plant stress tolerance. Successful development of commercial cultivars with proven field stress tolerance is beyond the capabilities of one individual scientist. This page intentionally left blank

Fig. 3.2 Examples of the use of genomic in situ hybridization (GISH) for genome analysis of tomato
interspecific/intergeneric hybrids, and monosomic addition (MA), substitution (SL) and introgression (IL) lines. (A) disrupted pairing at metaphase I of F1 L. esculentum x S. lycopersicoides; (B) partial differentiation at mitotic metaphase of homologous chromosomes in F1 S. lycopersicoides x S. sitiens; (C) S. lycopersicoides SL-8 at diakinesis with pairing between homeologous chromosomes (bivalent; arrow); (D) S. sitiens MA-8 at diakinesis showing pairing between homeologues (bivalent; arrow) and an unpaired L. esculentum chromosome (univalent; arrowhead); (E) S. lycopersicoides SL-7 at pachytene showing the unpaired S. lycopersicoides chromosome (arrow); (F) heterozygous introgression line containing a segment from S. lycopersicoides chromosome 7 of ~42 cM (TG499 - TG128) in length (arrow). (A, C, E-F) Red = S. lycopersicoides, Blue = L. esculentum; (B) Blue = S. lycopersicoides, Red = S. sitiens; (D) Blue = L. esculentum, Red = S. sitiens. Bars represent 5 µm. Color Plate Section

Chapter 3

Fig. 4.1 Sporogenous (ms 10) sterility in tomato. Flowers with exserted and non-exserted stigma Fig. 4.2 Structural (sl) sterility in tomato. Fig. 4.3 Functional (ps) sterility in tomato.

Chapter 4

Fig. 4.4 Functional (ps 2) sterility in tomato.
Longitudinal section of tomato anther cones; on the left: ps 2-indehiscent anthers; on the right: fertile anthers

Fig. 4.5 Functional (ex) sterility in tomato. On the left: fertile flower (P 2 ); on the right: exserted stigma sterile flower (P 1 ); in the middle: flower with exserted stigma (F 1 ) Fig. 4.6 Longitudinal section of tomato flowers with normal and short style.

Fig. 4.7 Emasculation in tomato ps 2- line possessing relatively low level stigma without using forceps

Chapter 5

Fig. 5.3 Genetic map of the tomato genome showing the regions where QTLs were detected in at least two different progeny for fruit weight (right of the chromosome) or soluble solid content (left). The map is based on data involving L. pimpinellifolium P I (Grandillo and Tanksley 1996a, Tanksley et al. 1996, Chen et al. 1999) L.
Fig. 10.1 Hormonal regulation of tomato fruit development; Upper panel: Developmental phases of tomato fruit. Middle panel: Changes in hormonal levels observed during fruit development (redrawn with approval from Gillaspy et al. (1993), copyright American Society for Plant Biologists). Lower panel: Cell division as mitotic index is redrawn with approval from Cong et al. (2002). Copyright (2002) National Academy of Sciences, U.S.A.; fruit weight gain and growth rate (weight gained/day) are redrawn and calculated from Abdel-Rahman (1977) with approval. All data are normalized as percent maximum observed during fruit development.

Chapter 10 Anthesis and fruit set Phase I Phase II Phase III Phase IV

Floral development Cell division Ripening/

Senescence Auxs GAs CKs ABA CH 2 4 Mitotic Index Growth rate Fruit weight Percent Maximum 100 75 50 25 0 10 20 30 40 50 Days after anthesis Cell expansion Fig. 10.2 Hormonal cross-talk during tomato fruit development. Ethylene Auxins Fruit set Cell division Cell expansion PAP Pollination / Fertilization Parthenocarpy? GAs CKs BRs JA Sources in krelations ABA Floral Senescence Missed signals? Ripening Genetic Improvement of Solanaceous Crops

Editors Maharaj K. Razdan Autar K. Mattoo Volume 2: Tomato

Genetic improvement of the tomato crop has been an on-going process with the objective of gaining high fruit yield, enhanced fruit nutritive value,
controlled fruit maturation and ripening, and developing resistance to phytophagous insects, microbial pathogens, and various abiotic stresses. This book presents a critical appraisal of the state-of-the-art findings on this Solanaceous crop in the form of overviews, emphasizing various approaches and strategies used for its improvement through research conducted at various research institutes, organizations and universities the world over. The book will be useful not only to breeders, or other specialists, but will equally benefit teachers and students seeking information on aspects of tomato biology, genetics and biotechnology.

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