# Tomato Breeders Roundtable

## October 17-20, 2004

## Annapolis, Maryland

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# Tomato Breeders Roundtable
# October 17–20, 2004
# Annapolis, Maryland

## Sunday, October 17

6:30 - 8:30 a.m. **Registration**

7:00 – 9:00 p.m. **Welcome Reception**
**Historic Inns, Governor Calvert House**

## Monday, October 18

7:00 – 8:30 a.m. **Registration and Continental Breakfast**
**Historic Inns, Governor Calvert House**

8:30 – 8:40 a.m. **John R. Stommel** (USDA, ARS, Beltsville, MD)
***"Welcome"***

### Session 1. Molecular Marker Technology

**Moderator, Chuck Rivara** (California Tomato Research Institute, Escalon, CA)

8:40 – 9:30 a.m. **Majid R. Foolad** (Department of Horticulture and the Intercollege Graduate Program in Genetics, The Pennsylvania State University, University Park, PA)
***"Molecular Markers as Selection Tools in Tomato Breeding"***

9:30 – 10:00 a.m. **Angela M. Baldo[1], Derek Huntley[2], Larry D. Robertson[1], and Joanne A. Labate[1]** ([1]USDA, ARS Plant Genetic Resources Unit, Geneva, NY, [2]Centre for Bioinformatics, Department of Biological Sciences, Imperial College, London, UK)
***"High-throughput SNP Prediction in Tomatoes Based on ESTs"***

10:00 – 10:30 a.m. **Wing Cheung, Martin Laforest, Benoit Landry, Louise O'Donoughue, Charles Pick** (DNA Landmarks, Quebec, Canada)
***"Markers in Lycopersicon esculentum"***

10:30 – 11:00 a.m. **Break**

## Session 1. continued

**Moderator, Joanne Labate** (USDA, ARS, Geneva, NY)

11:00 – 11:50 a.m. **Mikel R. Stevens** and **JoLynn J. Stevens** (Department of Plant and Animal Sciences, Brigham Young University, Provo, UT)
***"What are the Possibilities of Using Microarray Technology in Marker Assisted Selection Using Diversity Array Technology (DArT)?"***

11:50 – 12:30 p.m. **Ivan Simko** (Vegetable Laboratory, USDA, ARS, Beltsville, MD)
***"Association Mapping – A Lesson From Potato Genomics"***

12:30 – 1:00 p.m. **Depart for the Beltsville Agricultural Research Center and U.S. National Arboretum, *lunch enroute***

1:45 – 3:45 p.m. **Tour of the Beltsville Agricultural Research Center, Beltsville, MD**

3:45 – 4:00 p.m. **Depart for the U.S. National Arboretum**

4:30 – 6:30 p.m. **Tour and reception at the U.S. National Arboretum, Washington, D.C.**

6:30 p.m. **Depart for the Historic Inns, Annapolis**

## Tuesday, October 19

7:00 – 8:30 a.m. **Continental Breakfast**
**Historic Inns, Governor Calvert House**

### Session 2. Tomato Disease Resistance

**Moderator, James Brusca** (Harris Moran, Davis, CA)

8:30 – 9:00 a.m. **W. Yang[1], John W. Scott[2], Sally A. Miller[3], Jeffrey B. Jones[4], and David M. Francis[1]** ([1]Horticulture and Crop Science, [3]Plant Pathology, The Ohio State University, OARDC, Wooster, OH, [2]Gulf Coast Research and Education Center, University of Florida, Bradenton, FL, [4]Plant Pathology, University of Florida, Gainesville, FL)
***"The Genetic Basis of Resistance to Multiple Races of Bacterial Spot in PI 114490"***

9:00 – 9:30 a.m. **John W. Scott[1], Jeffrey B. Jones[2], and David M. Francis[3]** ([1]Gulf Coast Research and Education Center, University of Florida, Bradenton, FL, [2]Plant Pathology Department, University of Florida, Gainesville, FL, and [3]Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, Wooster, OH)
***"Resistance to Bacterial Spot Race T4 in Tomato"***

9:30 -9:50 a.m. **Liliana Stamova** (California Tomato Research Institute, Davis, CA)
***"Indication of Dominant Resistance to Corky Root"***

9:50 – 10:20 a.m. **Break**

### Session 2. continued

**Moderator, Lisa Piccinino** (Syngenta, Naples, FL)

10:20 – 10:50 a.m. **Majid R. Foolad, Arun Sharma and Guoyang Lin** (Department of Horticulture, The Pennsylvania State University, University Park, PA)
***"Genetics and Breeding of Early Blight Resistance in Tomato"***

10:50 – 11:20 a.m. **Randy G. Gardner** (Dept. of Horticultural Science, NC State University, MHCREC, Fletcher, NC)
***"Progress in Breeding for Combined Resistance to Early Blight and Late Blight of Tomato"***

11:20 – 11:50 a.m. **Martha A. Mutschler[1], Min Jea Kim[1] and Tom Zitter[2]** ([1]Dept. of Plant Breeding and Genetics, [2]Dept. of Plant Pathology, Cornell University, Ithaca, NY)
***"Completing Late Blight Resistance Characterization and Transfer to Tomato"***

11:50 – 12:50 p.m. **Lunch**

## Session 2. continued

**Moderator, Richard Ozminkowski** (Heinz Seed, Stockton, CA)

12:50 – 1:15 p.m. **Raquel Salati[1], Raquel Mello[2], Maria Shorey[1], Anna Frampton[3], Jaap Hoogstraten[4], and Phyllis Himmel[3]** ([1]Seminis Vegetable Seeds, Inc., San Juan Bautista, CA, [2]Paulinia, SP, Brazil, [3]Woodland, CA, [4]Wageningen, The Netherlands)
***"Evaluation of Commercial Tomato Variety Resistance to New World Begomoviruses"***

1:15 -1:40 p.m. **Yuanfu Ji and Jay W. Scott** (GCREC, University of Florida, Bradenton, FL)
***"Finding RAPD Markers Linked to Lycopersicon chilense Derived Geminivirus Resistance Genes on Chromosome 6 of Tomato"***

1:40 – 2:05 p.m. **Luis F. Gordillo,** and **Mikel R. Stevens** (Department of Plant and Animal Sciences, Brigham Young University, Provo, UT)
***"Screening of the USDA Lycopersicon peruvianum Germplasm Collection for Resistance to TSWV Isolates Overcoming Sw-5 and an Update on the Breeding for Resistance to TSWV-6"***

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|---|---|
| 2:05 – 2:30 p.m. | **Clarissa J. Maroon-Lango**[1], **Mary Ann Guaragna**[1], **Ramon L. Jordan**[1], **John Hammond**[1], **Murali Bandla**[2], **Steve K. Marquardt**[2,4] **and John Stommel**[3] ([1]Floral and Nursery Products Research Unit, [3]Vegetable Laboratory, USDA, ARS, Beltsville, MD; [2] Agdia, Inc., Elkhart, IN; [4] Current Address: North Dakota State Seed Department, Fargo, ND)<br>***"Pepino Mosaic Virus: Variability in U.S. isolates"*** |
| 3:00 – 5:00 p.m. | **Schooner Woodwind sailing excursion – departure from Annapolis Marriott dock**<br>***or*** **Annapolis sightseeing** |
| 7:30 – 9:00 p.m. | ***Tomato Crop Germplasm Committee Business Meeting*** **at Historic Inns, Governor Calvert House**<br>**Moderator, Martha Mutschler** (Cornell University, Ithaca, NY) |

## Wednesday, October 20

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| 7:00 – 8:30 a.m. | **Continental Breakfast**<br>**Historic Inns, Governor Calvert House** |
| 8:30 – 8:40 a.m. | **Martha Mutschler** (Cornell University, Ithaca, NY)<br>***Tomato Crop Germplasm Committee Update*** |

### Session 3. Tomato Fruit Quality

**Moderator, Halley Vick** (Campbell Soup Co., Davis, CA)

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| 8:40 – 9:05 a.m. | **Peter J. Mes and James R. Myers** (Department of Horticulture, Oregon State University, Corvallis, OR)<br>***"Breeding a True Purple Tomato for Increased Antioxidant Activity"*** |
| 9:05 – 9:30 a.m. | **David Francis, Audrey Darrigues, Wencai Yang, and Alba McIntyre** (Department of Horticulture and Crop Science, The Ohio State University, OARDC, Wooster, OH)<br>***"A Strategy for Improving the Color and Nutritional Value of Tomatoes by Breeding for a Reduction in Color Disorders"*** |

9:30 – 9:55 a.m. **Beverley Clevidence** (Diet and Human Performance Laboratory, USDA, ARS, Beltsville Human Nutrition Research Center, Beltsville, MD)
***"Lycopene: Factors Affecting Bioavailability"***

9:55 – 10:20 a.m. **Break**

## Session 3. continued

**Moderator, Pablo Salgado** (BHN Research, Huron, CA)

10:20 – 10:50 a.m. **Arthur A. Schaffer[1], Ilan Levin[1], David Granot[1], Marina Petreikov[1], Lena Yeselson[1], Shmuel Shen[1], Ran Hovav[1], Michal Moi[1], Nehama Gilboa[1], Orit Amir[1], Moshe Bar[2], Golan Abend[2], Rachel Platin[2]** ([1]Department of Vegetable Crops and Genetics, Volcani Center, Bet Dagan, Israel, [2] Gedera Seed Co., Gedera, Israel)
***"Improving Sugar Accumulation in Tomato Fruit via Introgressions from Wild Species"***

10:50 – 11:15 a.m. **David Smith and Kenneth Gross** (Produce Quality and Safety Laboratory, USDA, ARS, Beltsville, MD)
***"Role of β-Galactosidase/Exo-Galactanases in Tomato Fruit Development"***

11:15 – 11:35 a.m. **John R. Stommel[1], Judith Abbott[2], Austin Campbell[3], and David Francis[4]** ([1]Vegetable Laboratory, [2]Produce Quality and Safety Laboratory, [3]Soybean Genomics Improvement Laboratory, USDA, ARS, Beltsville, MD, [4]Ohio State University, OARDC, Wooster, OH)
***"Inheritance of Tomato Firmness Components in Genotypes Derived from Crosses Between Lycopersicon esculentum and L. hirsutum"***

11:35 – 12:00 p.m. **Yaguang Luo** (Produce Quality and Safety Laboratory, USDA, ARS, Beltsville, MD)
***"Fresh-Cut Tomatoes – Challenges and Opportunities"***

12:00 – 1:00 p.m. **Lunch**

## Session 4. Tomato Germplasm Updates and Area Reports

**Moderator, John R. Stommel** (USDA, ARS, Beltsville, MD)

1:00 – 1:20 p.m. **John R. Stommel** (USDA, ARS, Beltsville, MD)
***"Tomato Breeders Roundtable Business Meeting – Where and When for Next Meeting"***

1:20 – 1:30 p.m. **Larry Robertson** (USDA, ARS, Geneva, NY)
***Curator Report***

1:30 – 1:40 p.m. **Jay Scott** (University of Florida, Bradenton, FL)
***Tomato Genetics Cooperative Report***

1:40 – 2:30 p.m. **Area Reports**

***United States***

***Northeast/Midwest***
**Dorothy Eyberg** (Seminis Vegetable Seeds, Naples, FL)

***Southeast***
**James Frantz** (Seminis Vegetable Seeds, Felda, FL)

***Florida***
**Rogelio Hernandez** (Harris Moran, Immokalee, FL)

***California Fresh Market***
**Felix Serquen** (Syngenta, Woodland, CA)

***California Processing***
**Nankui Tong** (Heinz Seed, Stockton, CA)

***Canada***
**James Dick** (Tomato Solutions, Chatham, Ontario, Canada)

***Northern Europe***
**Ruud Verhoef** (Rijk Zwaan Breeding B.V., Utrecht, The Netherlands)

***Southern Europe***
**Jan Barten** (DeRuiter Seeds, Almeria, Spain)

***Far East***
**Peter Hanson** (AVRDC, Tainan, Taiwan)

***Israel***
**Matti Sarfati** (Hazera Genetics, M.P. Lanchish Darom, Israel)

2:30 p.m. **Adjourn!**

# Tomato Breeders Roundtable

## October 17-20, 2004

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## Molecular Markers as Selection Tools in Tomato Breeding

**Majid R. Foolad**
Department of Horticulture and the Intercollege Graduate Program in Genetics
The Pennsylvania State University, University Park, PA 16802
(Email: mrf5@psu.edu)

One of the applications of markers and maps is to identify and locate genes and QTLs of interest and subsequently transfer them via marker-assisted selection (MAS) and breeding. In tomato, currently there are more >500 morphological/physiological markers, 35 isozymes, >1000 RFLPs, and >160,000 ESTs. Markers have been used to tag genes conferring vertical resistance to many diseases, including bacterial spot (*rx-1, rx-2, rx-3*), bacterial speck (*Pto*), root knot nematode (*Mi* and *Mi-3*), potato cyst nematode (*Hero*), potyvirus (*pot-1*), tobacco mosaic virus (*Tm-1* and *Tm-2*$^a$), tomato spotted wilt virus (*Sw-5*), tomato yellow leaf curl virus (*Ty-1* and *Ty-2*), alternaria stem canker (*AscI*), cladosporium leaf mould (*Cf* series), fusarium wilt (*I-1, I-2, I-3*), fusarium crown and root rot (*Frl*), verticillium wilt (*Ve*), powdery mildew (*Lv* and *OI-1*), late blight (*Ph* series), corky root rot (*Py-1*) and *Stemphilium* (*Sm*). Markers have also been identified exhibiting associations with QTLs for horizontal resistance to tomato diseases such as blackmold, early blight, late blight, bacterial wilt, bacterial canker and powdery mildew. However, in only a few cases have markers been employed for gene (e.g. growth habit and self-compatibility) or QTL transfer (e.g. blackmold and late blight). This is similar to the situation in other plant species, although there are several examples of successful use of markers for MAS in crops such as maize (earliness, grain yield and quality), rice (disease and insect resistance, grain quality, drought tolerance), beans (different traits), cotton (fiber quality), barley (disease resistance, malting quality, grain yield), wheat (disease resistance) and pear millet (disease resistance). The potential benefits of marker deployment to plant breeding are undisputed, in particular for pyramiding disease resistance genes with different gene actions or pathogen specificity. Also, considering the development of newer marker technology, such as SNPs, there seems to be no limitation in marker discovery even in genetically less diverse species such as tomato. The high cost of marker assays, however, may still be a limiting factor for their routine application in plant breeding programs. An overview of the prospects for affordable use of marker technology in plant breeding will be presented.

## High-Throughput SNP Prediction in Tomatoes Based on ESTs

**Angela M. Baldo**[1], **Derek Huntley**[2], **Larry D. Robertson**[1], **and Joanne A. Labate**[1]
[1]USDA-ARS, Plant Genetic Resources Unit, Geneva, NY, USA http://www.ars-grin.gov/gen; [2]Centre for Bioinformatics, Department of Biological Sciences, Imperial College, London, UK http://www.bioinformatics.ic.ac.uk
(Email: abaldo@pgru.ars.usda.gov)

Cultivated tomato is extremely low in genetic diversity. Only one nucleotide polymorphism was observed in more than 7 kb in four modern cultivars (Nesbitt and Tanksley, 2002). This monomorphism is a result of microevolutionary processes such as founder events, genetic bottlenecks, and intense selection, and creates a challenge for characterizing germplasm collections. A computational approach to predicting single nucleotide polymorphisms (SNPs) is a powerful way to discover rare polymorphic regions. We have developed a method to screen an entire NCBI Unigene set for potential SNPs using the SEAN SNP Prediction Program (Huntley, 2003). Predictions are based on established criteria (Picoult-Newberg et. al. 1999). Polymorphisms were further examined in the context of the cultivars and clones in which they were identified. Using this method we discovered 2,527 potential SNPs among 764 clusters from the unigene set. We have verified 62 SNPs within 21 unigenes by DNA sequencing two or three cultivars, Based on our results, the verified SNPs have been found 21 times more frequently (approximately three SNPs per kb in two to three cultivars) than we'd expect with random sequencing. We have virtually mapped some of the markers by BLASTing the unigenes against published DNA sequences of mapped markers.

# Markers in *Lycopersicon esculentum*

**Wing Cheung, Martin Laforest, Benoit Landry, Louise O'Donoughue, and Charles Pick**
DNA Landmarks, 84 rue Richelieu, St. Jean Suv Richelieu, Quebec, Canada
(Email: pickc@dnalandmarks.ca)

Within cultivated tomato, *Lycopersicon esculentum* Mill., the level of polymorphism is very low and hence hampers the use of marker-assisted selection and association mapping of traits in tomato breeding. We propose here two different approaches to increase the number of available markers for cultivated tomato: (1) Development of high-throughput single nucleotide polymorphism (SNP) discovery and validation and (2) Inter-mite polymorphism (IMP) markers.

**1) High-throughput single nucleotide polymorphism (SNP) discovery and validation**

Single nucleotide polymorphism (SNP) has, compared to other marker systems, the most frequent variations among DNA of different germplasms within the same species. The availability of Expressed Sequence Tags (EST) allows *in silico* data mining for SNP discovery. Using sequence clustering and SNP discovery tools, 10,389 clusters of sequences coming from five different genotypes have been analyzed and over 1,000 putative SNPs identified. Validation of these SNPs will be carried out and they will be used to characterize a number of elite cultivars.

The second SNP discovery approach is complementary to the development of IMP markers described below. In this targeted approach, IMP polymorphisms linked to one or more characteristics of interest will be identified in a segregating population. The linked polymorphic region identified through the IMP technology will serve as a basis for the development of locus specific SNP markers in the same area. In order to do so, the IMP band together with flanking sequences will be sub-cloned and sequenced. This sequence will be used to amplify and sequence the targeted genomic regions of lines differing for the presence of the IMP marker band. Multiple alignments of high quality sequences will lead to the identification SNPs and these will be converted into validated SNP assays.

The goals of the present project are as follows:

- to develop 1,000 working assays,
- to characterize a panel of genetically diverse cultivars and,
- to map SNPs that show polymorphism in segregating populations.

**2) Development of IMP markers for cultivated tomato**

Inter MITE Polymorphism or IMP is a DNA LandMarks proprietary DNA marker system. This system takes advantage of the abundance of small class II transposable elements called Miniature Inverted Repeats Transposable elements (MITEs) in plant genomes. Primers are designed to amplify genomic DNA between MITE insertions. A single PCR reaction results in the detection of 25 to 75 loci making this technology a very cost effective marker system. IMP markers are useful for various marker-assisted breeding functions however they are also valuable for use in targeted SNP discovery as described above.

Thirty six IMP primers generating a total of 913 loci have been identified as being useful for Lycopersicon species confirming that this technology would be applicable to tomato. To further increase the number of loci with the highest polymorphism level is best to use MITE elements found in the target or very closely related species. There are currently over 300 entries of Solanaceae genomic sequences of over 5Kb in length deposited in NCBI GenBank. It is proposed to develop Solanaceae IMP primers by mining these genomic sequences for the identification of Solanaceae MITE families and to design primers based on such families. The new markers along with the markers identified previously in cross-applicability will be evaluated for polymorphism on a panel of tomato cultivars and the distribution of the IMP marker bands determined by mapping in a segregating population.

# What are the Possibilities of Using Microarray Technology in Marker Assisted Selection Using Diversity Array Technology (DArT)?

**Mikel R. Stevens and JoLynn J. Stevens**
Department of Plant and Animal Sciences, Brigham Young University, Provo, UT 84602
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Since the earliest molecular markers were developed in plants hypotheses were proposed for using those markers in plant breeding (Tanksley, 1983). By the end of the 1980's theories of using molecular markers for studying quantitative inheritance, in crop, were discussed (Lander and Botstein, 1989). In 1996 the ground breaking paper on quantitatively inherited characteristics and molecular markers was published using tomato (Tanksley et al.). However, there are several aspects of such research that make it impractical for most tomato breeding programs. Specifically, time, cost, polymorphism identification within the germplasm of interest, high throughput capabilities, facilities, technical expertise and so-on are just a few issues that make such goals impractical to most breeding programs. Nevertheless, scientists from around the world have continued to "chip away" at these challenges. Recently a technology known as DArT (diversity array technology) has been advanced as a method to overcome several of the difficulties listed above (Jaccoud, 2001).

DArT utilizes microarray technology as a method to compare 1000's of markers, in one test, to one DNA sample from a single specimen. Thus, an experiment could take place where100's of segregating plants from an $F_2$ population (including parents and the $F_1$) were tested and an entire genome be monitored with hundreds of genome wide polymorphic markers then be compared to the phenotypic data in a week or two of a single technician's laboratory time. Plus, of course, the time required for taking the appropriate phenotypic data in the field or greenhouse. Although this kind of data throughput seems improbable it is now a working methodology in barley (Wenzl et al., 2004). Recently, we initiated two studies using DArT in tomato. The first study involves two interspecific *L. esculentum* x *L. pennellii* segregation population, which included all possible combinations of the BC, $F_2$, and $F_1$'s. The *L. pennellii* used were two homozygous inbreed accessions (LA 716 and LA 2963). The *L. esculentum* parent was the inbred Fla 7613. The second study utilized the 20 NIL (near isogenic lines) lines developed by H. Laterrot (1996). Our preliminary data provide clear evidence that this technology has potential and clearly suggests resolutions to the weaknesses of high throughput, and reduced time issues as well as the possibilities of lower cost per data point. However, questions still remain in the areas of technical competence, facilities required, and other issues that still need further examination. In the final analysis DArT clearly holds promise; then again, significant technical issues exist as barriers. Nevertheless, the group working to develop DArT to be applicable for marker assisted selection is making impressive headway on many of the formidable technical challenges remaining in this technology.

Jaccoud, D., K. Peng, D. Feinstein, A. Kilian. 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. Nuclei Acids Res. 29:4e25.

Laterrot, H. 1996. Stock list. Rep. Tomato Genet. Coop. 46:34.

Lander, E.S., and D. Botstein. 1989. Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185-199.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Molecular Biology Reporter 1:3-8.

Tanksley, S.D., S. Grandillo, T.M. Fulton, D. Zamir, Y. Eshed, V. Petiard, J. Lopez, and T. Beck-Bunn. 1996. Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor. Appl. Genet. 92:213-224.

Wenzl P, J. Carling, D. Kudrna, D. Jaccoud, E. Huttner, A. Kleinhofs, and A. Kilian. 2004. Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proc. Natl. Acad. Sci. USA 101: 9915-9920.

# Association Mapping – A Lesson From Potato Genomics

**Ivan Simko**
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(Email: simkoi@ba.ars.usda.gov)

The GENETIC LINKAGE ANALYSIS is currently the most common method for mapping plant genes. The method involves generating mapping populations derived from F1 and estimating recombination frequencies between marker loci and the genes of interest. Such mapping populations, however, sample only a small proportion of all possible alleles in elite cultivars. Detecting variation in economically important traits within genetic backgrounds that are relevant to plant breeders requires additional mapping techniques, such as those based on LINKAGE DISEQUILIBRIUM. The ASSOCIATION-MAPPING METHOD uses linkage disequilibrium to detect segregating genes after several meiotic generations and without developing new mapping populations. A significant difference between association mapping in a general population and genetic linkage mapping in a defined segregating population is that the association mapping generally identifies the association of common alleles (rare alleles do not reach statistical significance), whereas a population originating from a biparental cross enables the identification of alleles rare in the population at large. The association mapping techniques that provide means for detecting genes underlying the variation of a trait among elite cultivars are thus complementary to linkage mapping methods that effectively locate genes segregating in a population originating from two individuals. Results from mapping VERTICILLIUM resistance genes in cultivated potato (through a candidate gene approach) will be presented and discussed.

# The Genetic Basis of Resistance to Multiple Races of Bacterial Spot in PI 114490

**W. Yang[1], J. W. Scott[2], S. A. Miller[3], J. Jones[4], and D. M. Francis[1]**
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Bacterial spot (caused by *Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas vesicatoria)* is an economically important disease of tomato (*Lycopersicon esculentum*) grown for processing and fresh-market. The development of varieties with resistance has been difficult due to the emergence of new races, the lack of a correlation between a hypersensitive response and resistance in the field, and the quantitative inheritance of resistance. In addition, the best-characterized sources of resistance to bacterial spot have been *L. esculentum* breeding lines or plant introductions, and the use of molecular marker assisted breeding has been limited due to low levels of genetic polymorphism. To discover polymorphisms appropriate for the genetic characterization of bacterial spot resistance we have compared DNA sequences in public databases to develop genetic markers based on single nucleotide polymorphisms (SNP). We have extended our analysis to the estimation of intron position, *de novo* sequencing, and experimental verification. The frequency of polymorphism is low within coding regions, but once a SNP is discovered it has a high probability of being polymorphic in multiple elite populations. The frequency of SNPs in introns is 5.3 fold higher than SNPs in coding regions. We have used SNP markers to select for recombinants that bring resistance to bacterial speck and race T1 of bacterial spot into coupling phase. We have further used markers to characterize an Inbred Backcross population derived from PI 114490. A putative QTL explained 14.6% of total variation for T2 resistance, 63.8% of total variation for T3 resistance, and 44.3% of total variation for T4 resistance.

# Resistance to Bacterial Spot Race T4 in Tomato

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Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) race T4 is a mutation from race T3 that is virulent on the hypersensitive T3 resistant inbred Hawaii 7981 (Jones, unpublished data). It has been found in tomato (*Lycopersicon esculentum* Mill.) fields in south and west Florida but its prevalence is not known because no race T3 resistant varieties are being grown that would allow for easy detection of T4. Race T4 was first isolated in 1999 from south Florida breeding plots when three of four lines with resistance to races T1 and T3 were badly infected with bacterial spot. Fla. 7835 was the T1 and T3 resistant line that was not badly infected. In 2002, T4 was isolated from a bacterial spot epidemic in plots of T3 resistant breeding lines in west Florida. Fla. 8233, an inbred with PI 114490 and Hawaiian ancestry, was the only inbred with resistance to that mixed T3 and T4 infection. In summer 2003 and spring 2004, the resistance of Fla. 8233 and PI 114490 was confirmed. An inbred backcross population based on PI 114490 was screened for T4 and a major resistance locus on chromosome 11 accounted for 74% of the variation. A population is presently being tested to confirm the resistance at this locus. In spring 2004, Fla. 8326 had good T4 resistance. The pedigrees of Fla. 8326 and Fla. 7835 both have PI 126932 as the source of T3 resistance. Thus, we surmise that PI 126932 is resistant to race T4 as well as race T3. In summer 2004 we are testing PI 126932 and 18 other accessions for T4 resistance. We are also testing heterozygous hybrids from Fla. 8326 for T4 resistance. Results from these experiments will be discussed if available at meeting time.

# Indication of Dominant Resistance to Corky Root

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Corky root on tomatoes is an important disease of open field and greenhouse crops (Campbell & Moon, 1979). The disease has become a serious concern especially to early planting of processing and fresh market tomatoes in California. *Pyrenohaeta lycopersici* (Gerl. & Sch.), the causal agent of corky root, is a soil-born fungus. Jones et al. (1989) reported that CA isolates might differ in their pathogenicity from certain European strains. Several green-fruited species - *L. hirsutum*, *L. peruvianum* and *L. glandulosum*, have been reported to carry factors for resistance to corky root (Szteyn, 1962; Hogenboom, 1970; Volin & McMillan, 1978; Laterrot, 1983). Gene *py-1*, a single recessive gene from *L. glandulosum* controls resistance to corky root (Laterrot, 1983). Although sources of resistance have been known for a long time, until now a few processing tomato hybrids are available to tomato growers. The recessive nature of the gene *py-1* is partially responsible for this situation. For expressing the resistance to corky root, commercial hybrids should be homozygous for the gene *py-1*.

Author's resistant line Pirelly - 38, having in its pedigree an wild species from Subgenus *Eriopersicon*, showed high level of resistance, when grown in heavily infected field, near Davis, CA (Stamova, 2003). In previous investigation this line was found to assure a satisfactory level of resistance in heterozygous condition. The objective of this work was to confirm the mode of inheritance to corky root in Pirelly -38 when inoculated with CA population of *P. lycopersici*.

To study the inheritance of the resistance, the response of the following lines and populations were evaluated: Pirelly - 38 (P1), CTRI 4863 VFFNPto (P2), F1, F2, BC1P1, BC1P2 and line 41-6 (susceptible control). The work was performed in the CTRI greenhouse, near Davis, CA.

The seed were sown and the plants were grown in 10 cm pots until the time of inoculation. One month old plants were transplanted into big pots, filled with soil taken from naturally heavily infected field, and mixed with perlite to improve the aeration. All plants were grown with regular pruning and tying to one meter long stakes. Three month later, after fruit harvest, the plants were extracted, roots washed in water and rated for disease severity. They were ranked on a scale 1 - 5, where 1 = no symptoms and 5 = the whole surface of the main root and some fine feeder roots covered with corky lesions. Ratings 1 and 2 were considered resistant. The disease severity index (DSI) was calculated by averaging plants with each line and population.

The susceptible reaction of the susceptible control line 41-6 was well documented (DSI = 4.40) (Table 1; Fig. 1). The main roots were heavily damaged - dark brown corky lesions covering the whole surface of the taproot and fine feeder roots were badly decayed on plants with many corky lesions. Plants of Pirelly - 38 line showed again, a high level of

resistance in this greenhouse test (DSI =1.26) (Fig. 1). The root system was strong, very well developed, with clean taproots and fine feeder roots. Most of the plants were without corky lesions on their taproots. Some plants showed a few small (0.5 cm) light brown lesions. The level of resistance of F1 population was comparable to that of the resistant line (DSI = 1.28) (Fig. 1). The segregation ratio in F2 was roughly 3 resistant plants : 1 susceptible plant. The resistant plants had DSI = 1.26 and the susceptible plants had DSI = 4.0. Plants from the first backcross to Pirelly - 38 (BC1P1) had a DSI = 1.27. BC1 to CTRI 4863 - still to be evaluated

The response of the above-mentioned lines and populations suggest the conclusion, that the gene controlling resistance to CA population of *P. lycopersici* in Pirelli - 38 is dominantly inherited. More investigations are needed to determine completely the genetics of resistance to corky root. It will be useful to challenge the line Pirelly-38 with different isolates of *P. lycopersici* and to check the performance of F1 population in the field.

The availability of a dominant source of resistance to *P. lycopersici* will help speed up the development of tomato hybrids resistant to corky root.

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Table 1. Reactions of lines and populations to inoculation with *P. lycopersici* population in CA

| Lines and populations | Disease Severity Index (DSI) | Ratio R : S |
|---|---|---|
| Pirelly - 38 (P1) | 1.26 | 1 : 0 |
| 4863 | 3.80 | 0 : 1 |
| $F_1$ (Pirelly-38 x 4863) | 1.28 | 1 : 0 |
| $F_2$ (Pirelly-38 x 4863) | 1.27 and 4.00 | 3 : 1 |
| $BC_1P_1$ | 1.27 | 1 : 0 |
| $BC_1P_2$ | to be evaluated | |
| 41-6 - susceptible control | 4.40 | 0 : 1 |

Rating scale 1 – 5: 1 = no symptoms, 5 = the whole surface of the main root covered with brown corky lesions. Plants with ratings 1 and 2 considered resistant.

Fig. 1. Roots of the lines 41-6 (susceptible control), Pirelly-38 and F1 (Pirelly-38 x 4863)

## Genetics and Breeding of Early Blight Resistance in Tomato

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Early blight (EB), caused by fungus *Alternaria solani* Sorauer, is a destructive disease of tomato (*Lycopersicon esculentum* Mill.) in the U.S. and elsewhere. Sources of genetic resistance have been identified within tomato related wild species, including green-fruited *L. hirsutum* Humb. and Bonpl. and *L. peruvianum* (L.) Mill. and red-fruited *L. pimpinellifolium* (Jusl.) Mill.. Resistance was previously reported to be horizontal and quantitative, and transmitted as dominant, additive, or a recessive trait, depending on the cross used. Furthermore, EB resistance does not follow the gene-for-gene model of host-pathogen interaction. We have used traditional protocols of plant genetics and breeding and contemporary molecular markers technology to better understand the genetic basis of and to develop tomato germplasm with improved EB resistance. Traditional backcross breeding using resistant donors within *L. pimpinellifolium* has resulted in the development of fresh-market and processing tomato germplasm with improved resistance and other desirable horticultural characteristics, including high yield, excellent fruit quality (e.g. high lycopene), and adaptation to Pennsylvania conditions. Several interspecific populations derived from crosses between *L. esculentum* and either *L. hirsutum* or *L. pimpinellifolium* were developed and used for QTL mapping. In each population, an average of seven QTLs were identified for EB resistance. While similar QTLs were detected in different populations of the same cross, generally different QTLs were identified in populations derived from different crosses. This suggests stability of the QTLs across environments and populations derived from the same cross but their variability across populations derived from different crosses. Marker-assisted pyramiding of resistance QTLs from different sources may result in development of germplasm with strong and durable resistance. Further inspection of the results led to the identification and selection of six QTLs with stable and independent effects for use in marker–assisted selection (MAS) and breeding. To facilitate 'clean' transfer and pyramiding of these QTLs, however, we are developing six near-isogenic lines (NILs), each containing one QTL in a *L. esculentum* background. The NILs will be used to develop series of overlapping sub-NILs for fine-mapping of the QTLs within ~5 cM intervals for marker-assisted introgression and possible cloning and characterization of underlying genes. Furthermore, recent studies have resulted in the identification of several resistance-gene-analogs (RGAs) as well as candidate disease-resistance/defense-response genes co-localizing with QTLs for EB resistance. These findings may lead to the identification genes underlying EB resistance in tomato.

## Progress in Breeding for Combined Resistance to Early Blight and Late Blight of Tomato

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Because of moderate temperatures, frequent rainfall, and heavy morning dew, early blight and late blight are important diseases of tomato in the mountains of western North Carolina. Breeding for resistance to early blight has been ongoing since 1976 and has resulted in the release of cultivars and breeding lines with moderate resistance to early blight. 'Mountain Supreme', a large fruited tomato, and the breeding lines NC EBR-2, -3, and -4, all derived from ‘Campbell 1943’, have been released and distributed world-wide. ‘Campbell 1943’ has moderate foliar resistance to early blight, a high level of resistance to the stem lesion (collar rot) phase of early blight and is also resistant to fruit lesions around the stem end of the fruit. Two fresh market plum (Roma type) $F_1$ hybrids, 'Plum Dandy' and 'Plum Crimson,' along with the parental lines NC EBR-5, -6, -7, and -8 have been released. These carry moderate foliar resistance derived from 71B2, a processing tomato line released by USDA. An additional breeding line, NC EBR-1, with moderate foliar resistance derived from *Lycopersicon hirsutum* PI 126445 has been released. Since 1991, when aggressive strains of late blight which overcame the chemical Ridomil first appeared in North Carolina, late blight has been a problem during most growing seasons. Breeding for combined resistance to early blight and late blight has focused on backcrossing useful sources of late blight resistance into the ‘Campbell 1943’ derived early blight resistant line NC 215E. The *Ph-3* gene derived from the *L. pimpinellifolium* line L 3708, obtained from the Asian Vegetable Resource and Development Center in Taiwan, has been backcrossed into NC 215E. The *Ph-2* gene, derived from 'Richter's Wild Tomato,' has also been backcrossed into NC 215E. In 2002 and 2003, $F_1$ hybrids heterozygous for both *Ph-2* and *Ph-3* were tested in the field and found to be superior in resistance to breeding lines carrying only one of the major genes. In both years severe late blight developed on lines with either *Ph-2* or *Ph-3* alone, but the disease was very limited on hybrids combining both genes. We have selected isolates which specifically overcome the *Ph-2* and *Ph-3* resistance genes and are maintaining their pathogenicity through weekly transfers onto live tomato leaves. We recently tested an $F_2$ population segregating for both *Ph-2* and *Ph-3* using a detached leaf inoculation technique and identified segregates which were resistant when inoculated with a combination of two isolates, one which overcomes *Ph-2* and the other which overcomes *Ph-3*. Progeny testing of $F_3$ populations is underway in field plots in the 2004 summer season to identify lines homozygous for both *Ph-2* and *Ph-3* and to determine whether having both genes in homozygous condition provides superior resistance to use of the genes in heterozygous condition. Additional late blight resistance derived from *L. hirsutum* LA 1033 has been backcrossed into NC 215E. This resistance appears to be controlled by two or more genes and has been much more difficult to advance into lines with good horticultural type compared to using *Ph-2* and *Ph-3*. Breeding is underway to incorporate the LA 1033 resistance along with *Ph-2* and *Ph-3* in an effort to develop more durable resistance.

Screening is also underway to identify other potential sources of useful resistance to both early blight and late blight.

# Completing Late Blight Resistance Characterization and Transfer to Tomato

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This reports updates work finalizing late blight transfer and characterization, and tests of late blight and early blight resistance in tomato

In prior work, the breeding program succeeded in producing a first generation of late blight resistant processor tomato lines, transferring resistance derived from *L. pimpinellifolium* accession L3708. These first generation lines were strongly resistant in inoculated fields in Ithaca NY and under heavy natural infection in Mexico. Field trials in California indicated that some lines approach the horticultural and fruit quality requirements for processing tomato varieties. A series of the resulting late blight resistant processing tomato lines were released. One set of these late blight resistant processing tomato lines were early maturity, possessed Ve, I2 resistance genes, were a bit small in fruit and plant size, and jointed. The second set of these late blight resistant processing tomato were late maturity, possessed Ve, I2 resistance genes, were a bit large in fruit and plant size, and jointless. Some lines in both sets had the Pto and/or Mi resistance genes as well. In the last few years a new generation of late blight resistant processing tomato lines have been produced combining the jointless, the desired fruit and plant size, and main season maturity. These lines are in production trials this summer in California.

Prior work generated indirect evidence that the late blight resistance in these lines was not due to one resistance gene. More direct evidence for more than one gene was desired. A genetic test for this hypothesis was performed. The results indicate that more than the one major gene on chromosome 9 is involved in this resistance, but does not clearly indicate if one or two additional epistatic genes is involved in the resistance. The results also confirm that use of full resistance system in homozygous condition provides the strongest resistance.

Hybrids combining late blight and early blight resistance indicate that both resistances can be used. There are no negative or positive interactions between the genes controlling these two types of resistance.

# Evaluation of Commercial Tomato Variety Resistance to New World Begomoviruses

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Diseases caused by begomoviruses can be production limiting wherever commercial tomatoes are grown worldwide. Of this group, *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf curl sardinia virus* (TYLCSV) are well characterized and their distribution documented in Europe, the Middle East, the Caribbean basin and Southeastern US. Resistant varieties are available commercially. Additional members of this group of viruses are still being identified in South and Central America, Mexico, and Asia. The increasing incidence of these new begomoviruses in tomato production areas makes it important to assess the efficacy of existing resistance in commercial hybrids.

Nineteen commercial tomato hybrid varieties resistant to TYLCV were tested in field trials in Italy (Sicily), Spain (Murcia), and Brazil (Sao Paulo, Goias, Pernambuco, and Ceara states) in 2002. In 2003, 10 varieties from the 2002 test were again planted in field trials in Brazil (Sao Paulo, Minas Gerais, Goias, and Ceara states). At each location, 10 plants per hybrid were sewn in 5 replications for a total of 50 plants per hybrid. Evaluations were visual using a 1 (resistant) to 5 (susceptible) scale. Leaf tissue samples were collected from TYLCV resistant and susceptible varieties to determine begomovirus presence using nucleic acid extractions. PCR was run using degenerate primers, the amplified product was cloned and sequenced. Obtained DNA sequences were compared to the sequences of known geminiviruses for identification.

In Italy, while TYLCV alone was detected in most samples, a mixed infection of TYLCV and TYLCSV was detected sporadically. In Spain, TYLCV was again detected in the majority of the collected samples, however TYLCV-Mild, and TYLCSV were also detected. In Brazil, *Tomato yellow vein streak virus* (TYVSV), *Tomato rugose mosaic virus* (ToRMV), *Tomato severe rugose virus* (TSRV), and *Tomato mottle leaf curl virus* (ToMLCV) were detected in all collected samples.

Commercial hybrid varieties, Hilario, Anastasia, Densus, Marcela, Eldiez, Stylus, Boludo, Elamane, Quadro, Silver, Poket, Tiway, Trinity, Birloque, Noelia, Ulises, Gardel, Ty-Fanny, and Scala, are know to be resistant to TYLCV. These same varieties also displayed a usable level of resistance to TYLCSV, TYVSV, ToRMV, TSRV, and ToMLCV compared to susceptible commercial varieties.

## Finding RAPD Markers Linked to *Lycopersicon chilense* Derived Geminivirus Resistance Genes on Chromosome 6 of Tomato

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Tomato yellow leaf curl virus (TYLCV) and tomato mottle virus (ToMoV) are two of the most destructive whitefly-transmitted geminiviruses of tomato (*Lycopersicon esculentum*). Resistance to these viruses has been found in several accessions of the wild species *L. chilense*. Three different regions in chromosome 6 of *L. chilense* have been shown to associate with the resistance, indicating that at least three genetic loci contribute to the resistance. In the present study, we aim to identify molecular markers tightly linked to the resistance loci, by using *L. chilense* (accessions LA2779, LA1932, and LA1938) derived breeding lines that have been phenotypically selected for TYLCV and ToMoV resistance. Three plants from each line displaying resistance to both viruses were selected to screen against previously mapped RAPD (randomly amplified polymorphic DNA) markers. We also employed *L. chilense* derived $F_3$ and segregating lines in our study. Markers displaying high reproducibility of the polymorphism between the susceptible control 'Horizon' and the *L. chilense* derived lines were identified. These include UBC697 and UBC621 from region 1 (*Ty-1* gene in this region); UBC264, UBC169, UBC197, and UBC365 from region 2; and UBC131, UBC137, UBC236, and UBC389 from region 3. Among these markers, UBC697 of region 1 and UBC264 of region 2 showed tight linkage to the resistance genes, while markers from region 3 showed varied degrees of segregation from the resistance gene. We have cloned the polymorphic bands from these markers. The conversion of these linked RAPD markers to co-dominant sequence characterized amplified region (SCAR) markers is in progress.

# Screening of the USDA *Lycopersicon peruvianum* Germplasm Collection for Resistance to TSWV Isolates Overcoming *Sw-5* and an Update on the Breeding for Resistance to TSWV-6

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*Lycopersicon peruvianum* has demonstrated resistance to TSWV in several studies. *Sw-5* derived from this species, has been introgressed into a number of tomato cultivars. Due to the discovery of new TSWV isolates that have overcome *Sw-5*, we screened the entire *L. peruvianum* germplasm collection. TSWV isolates were maintained on *Nicotiana rustica* plants from which virus infected leaves were used to prepare inoculum for two mechanical inoculations applied to *L. peruvianum* accessions one week apart. Visual infection symptoms were used to eliminate obviously infected plants, followed by ELISA tests of the apparently healthy plants. It is important to note that some of these apparently healthy plants were infected according to ELISA. In this comprehensive study, we screened two TSWV isolates; the TSWV-6 isolate found in Hawaii that totally overcame *Sw*-5, and the Anemone isolate ($An_{wa}^{-1}$) found in Banjup, Western Australia that occasionally overcame *Sw-5* in our trials. The Hawaiian isolate showed total infection on our control plants 89S (*Sw-5*$^{+}$/*Sw-5*$^{+}$) and 89R (*Sw-5*/*Sw-5*), of which 89R was more severely infected than 89S. The Australian isolate was not as virulent as the Hawaiian, since 163 89R control plants were free of the virus out of 566 (≈29%). For the Hawaiian (TSWV-6), 11,014 *L. peruvianum* plants were tested from 285 accessions of which 1383 showed resistance, with all control (640) 89S and (630) 89R plants infected. For the Australian isolate (Anemone) 5679 *L. peruvianum* were tested of which 1688 showed resistance to this isolate; there were a total of 171 accessions tested out of which 112 showed resistance, with control (456) 89S and (404) 89R plants infected out of 485 and 566 respectively.

The breeding project of introgressing and identifying resistance to the TSWV-6 isolate is in the process of gathering data. This project consists of four populations of , $BC_1$, $BC_2$, and $BC_3$ derived from separate $F_1$ interspecific hybrids of *L. esculentum* x *L. peruvianum* (resistant to TSWV-6). Preliminary data suggests that the resistance is heritable with dominance characteristics although it has weak penetrance. AFLP markers are being used to identify the possible markers linked to the resistance.. These two studies (screening the germplasm and introgression) provide important data for breeding programs interested in utilizing new resistant genes to TSWV derived from *L. peruvianum*.

# *Pepino Mosaic Virus*: Variability in U.S. Isolates

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*Pepino mosaic virus* (PepMV) was first found in pepino (*Solanum muricatum*) growing in coastal Peru in 1974 and described in 1980. The economic importance of the virus was realized when PepMV reappeared in protected tomato (*Lycopersicon esculentum*) in the Netherlands early in 1999. Since then, it has been reported to occur in tomato in several countries including the United States. Within the United States, PepMV has been reported to occur in Arizona, California, Colorado, Florida, Maryland, New York, Nevada, Ohio, Oklahoma, Texas, and Virginia.

The occurrence of PepMV in the US was first observed in 2000 at Agdia, Inc. (Elkhart, IN) in symptomatic tomato leaves from Arizona. This led to the molecular characterization of the three US strains of PepMV, two of which were from limited amounts (< 0.2 g) of the original co-infected tomato leaf samples from Arizona (i.e., PepMV-US1 and PepMV-US2), and the third from infected tomato fruits obtained from stores in Maryland (i.e., PepMV-US3). The full-length genomic sequences of PepMV-US1 and US2, and the 3'-end sequence of US3, were all obtained by RT-PCR using total RNA from infected tissue as template, and degenerate potexvirus- and PepMV species- and strain-specific primers. The genome organizations of PepMV-US1 and US2 were typical of the genus *Potexvirus*, with the following reading frame order: ORF1 encoding a 163 kDa-replicase; ORF2, triple gene block protein (TGBp) 1 (26 kDa); ORF3, TGBp2 (14 kDa); ORF4, TGBp3 (9 kDa); and ORF5, coat protein (CP) (25 kDa). Gene-for-gene comparison between PepMV-US1 and US2 revealed the following amino acid identities: 91% in replicase, 89% in TGBp1, 92% in TGBp2, 85% in TGBp3, and 93% in the CP. While unique, PepMV-US1 is more closely related to the previously reported European strains than is PepMV-US2. The CP of PepMV-US3 is nearly identical to the European isolates at the amino acid level.

To further enhance our ability to diagnose PepMV-infected tomato plants, expressible His-tagged CP clones of PepMV-US1 and PepMV-US2 strains were generated. Bacterially expressed CPs of both PepMV-US1 and PepMV-US2 reacted to commercially available PepMV antisera as did the original source sample for PepMV-US3. These expressed proteins can be used to develop strain-specific monoclonal antibodies.

To understand the epidemiology of PepMV, potential hosts, particularly those grown in greenhouses, were evaluated for susceptibility to the virus. PepMV-US3 failed to infect twenty-three herbaceous plant species, including twenty one ornamental plant species and

*N. tabacum*, when mechanically inoculated with PepMV-infected sap of *N. benthamiana*. In contrast, *Hyoscyamus niger*, *N. edwardsonii* and *N. benthamiana* exhibited symptomatic infection upon inoculation. None of the eighteen tomato germplasm accessions or ten tomato cultivars were resistant to mechanical inoculation with PepMV-US3; in contrast, no infection was detected in nine pepper cultivars or four pepper germplasm accessions. Tomato fruit symptoms were most prominent when plants were inoculated at or after initial fruit set, and least visible or not apparent when plants were inoculated prior to flowering. It may be possible that symptom expression in fruits can be avoided by early inoculation of plants, and that a mild strain of PepMV could be selected for cross protection against infection by more severe isolates.

## Breeding a True Purple Tomato for Increased Antioxidant Activity

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Tomatoes contain a variety of antioxidants relevant to human nutrition. These include the carotenoids, tocopherols, ascorbic acid, flavonoids and other phenolics, and ubiquinone. Anthocyanins, antioxidant not found in commercial tomatoes, are expressed in tomato lines carrying the genes *Aft*, *atv*, *Abg*, *hp-1*, and an as yet undetermined gene from the wild species accession LA2099. We have selected lines for elevated anthocyanin content through combining these genes, resulting in increased pigment intensity. Anthocyanins are expressed primarily in the subepidermal tissue, while no anthocyanins have been detected in the inner wall and radial pericarp tissues. Anthocyanin expression is light induced, such that portions of the fruit that are shaded do not express pigment. The anthocyanins petunidin-, malvidin-, and delphinidin-3-p-coumaryl glucoside 5-rutinoside have been identified through HPLC-PDA/MS detection. Antioxidants characterized in purple, red and yellow tomatoes included total anthocyanins, measured by pH differencial, total phenolics, measured by Folin-Ciocaltea, and the carotenoids, tocopherols, and vitamin C, identified and quantified by HPLC. The antioxidant activity of juice made from anthocyanin-expressing tomatoes was compared to juices made from yellow and red tomatoes. The antioxidant activity of the anthocyanin and phenolic-containing fractions of purple tomatoes was determined by oxygen radical absorbance capacity, and was significantly higher in anthocyanin-expressing tomatoes. This is believed to be relevant to human nutrition as both the anthocyanins and phenolics are detected in the bloodstream after consumption. The potential for breeding a high-antioxidant tomato will be discussed.

Figure 1. Tomato with Aft and atv genes expressing anthocyanin in the greenhouse

Figure 2. Tomato with Aft and atv genes expressing anthocyanin in the field

# A Strategy for Improving the Color and Nutritional Value of Tomatoes by Breeding for a Reduction in Color Disorders

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Yellow shoulder disorder (YSD) affects the color uniformity and nutritional quality of tomatoes. Fruit affected by YSD have a 15% lower content of the nutritional carotenoids lycopene and pro-vitamin A than unaffected fruit. Quality standards dictate that affected fruit cannot be used for high value peeled products. Both environment and genetics contribute to YSD expression. A major component of genotype by environment interactions that affect YSD severity relates to potassium nutrition. Reciprocal grafting experiments indicate that the genotypic differences may be due to utilization or transport, but not uptake of potassium, as scion effects dominate. Classical genetic analysis and directional selection experiments suggest a moderate heritability for resistance to YSD. Genetic improvement can therefore reduce the severity of the disorder. Exploiting existing genetic variation to breed YSD resistant varieties would be facilitated by a more complete understanding of the genetic basis of resistance and susceptibility. Achieving this goal is complicated by the lack of genetic markers that detect differences between elite breeding lines. Tomato has extensive genetic resources, but the focus on wide crosses and introgression has left a void in our knowledge of and ability to manipulate many traits of agricultural importance within elite germplasm. We have begun to systematically identify polymorphisms within elite germplasm through data mining, de novo sequencing, and hybridization to oligo-nucleotide arrays. We are applying these resources to the problem of breeding within elite germplasm pools. Although we estimate that as many as 5,000 Single Nucleotide Polymorphisms (SNPs) exist within *L. esculentum* germplasm, these polymorphisms are not evenly distributed within market classes or within elite populations. Two independent *L. esculentum* quantitative trait loci (QTL) associated with improved color were identified based on linkage to markers mapping to chromosome 4 and chromosome 11. Epistatic interactions were identified between the two *L. esculentum* loci. Alternating cycles of Marker assisted selection and field selection based on replicated trials has the potential to develop varieties with improved color uniformity on an accelerated time frame.

## Lycopene: Factors Affecting Bioavailability

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Consumption of tomato products, the dominant source of lycopene in the American diet, has been linked with protection against cancer, cardiovascular disease, and diabetes. Although lycopene has been the component most closely associated with the health protections that tomato provides, lesser tomato carotenoids may act synergistically with lycopene, thus the bioavailability of these carotenoids is also of interest. A number of food processing and dietary factors are known to affect the bioavailability of lycopene. Food processing techniques that use oil and disrupt the cellular matrix are among the well known factors promoting lycopene absorption. Dietary factors that increase lycopene absorption include co-consumption of dietary fat; those that decrease lycopene bioavailability include consumption of sucrose polyesters, which are used as fat replacers, and plant sterols, which are used as lipid lowering agents. Dose-response studies have demonstrated that plasma lycopene response is blunted with increasing levels of lycopene consumption, thus unlike other major carotenoids, lycopene absorption may be saturated at modest levels of intake. As determinants of lycopene bioavailability are elucidated, strategies can be developed for enhancing lycopene absorption and thereby accruing expected health benefits.

# Improving Sugar Accumulation in Tomato Fruit via Introgressions From Wild Species

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The sugar content of the tomato fruit is a major determinant of the quality and value of the crop, whether for the fresh produce market or for processing. Soluble sugar levels contribute strongly to the total soluble solids content and tomato flavor, and have therefore been the subject of many research efforts.

Within the *Lycopersicon* germplasm there is a broad range of genetic variability for fruit carbohydrate metabolism. The cultivated *L. esculentum* accumulates little, if any, sucrose and its soluble sugars are comprised of approximately equimolar quantities of the hexoses, glucose and fructose. Among the wild species of *Lycopersicon* there are genetic traits of sucrose accumulation and modified fructose to glucose ratios in the mature fruit, as well as for modulated transient starch accumulation in the immature fruit, which can serve as a reservoir for soluble sugar in the mature fruit. This genetic variability is being utilized for the development of tomato genotypes with altered carbohydrate metabolism and accumulation patterns in the fruit.

## Role of β-Galactosidase/Exo-Galactanases in Tomato Fruit Development

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At least seven tomato β-galactosidase (TBG) genes are expressed during tomato fruit development. Six are known to be expressed during ripening, and of these six, the products of five are predicted to be localized in the wall. β-galactosidases are enzymes that can hydrolyze a β-galactosyl residue linked to a variety of aglycones (e.g., lactose, PNP-gal, etc.); while exo-galactanases are enzymes specific for the non-reducing end of galactans (cell wall). In order to determine the function of these enzymes during tomato fruit development, transgenic plants have been created to suppress endogenous mRNA levels. The results of suppressing four of the genes (TBG1, TBG3, TBG4 and TBG6) have been published and will be reviewed. Antisense fruit were examined for morphological, textural, physiochemical, biochemical and molecular changes in comparison to controls. In summary: TBG1 suppression had no detectable effect on fruit quality. TBG3 suppression led to processing fruit with reduced exo-galactanase activity, increased wall galactosyl content, improved long-term storage, pastes with an increased proportion of insoluble solids and slightly increased viscosity. TBG4 suppression led to fresh-pick fruit with reduced exo-galactanase activity, reduced free galactose content before ripening, increased cell wall galactosyl content in lines with lowest TBG4 mRNA levels, up to 40% increase in whole fruit firmness. TBG6 suppression led to a high percentage of cracked fruit and fruit with compact locular spaces, increased cuticle thickness, reduced level of exo-galactanse activity but no changes in cell wall galactosyl content, soluble galactose or fruit firmness.

# Inheritance of Tomato Firmness Components in Genotypes Derived from Crosses Between *Lycopersicon esculentum* and *L. hirsutum*

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Tomato fruit firmness is a key quality component of tomatoes produced for processing applications. Fruit firmness is generally considered a quantitatively inherited trait. Pericarp firmness of modern tomato cultivars is derived from a fairly narrow genetic background and is the result of the cumulative effort of numerous breeders over many years. Despite inferior phenotypes, wild species contain loci that can substantially increase tomato fruit quality. In the current study, inheritance of fruit firmness in firm and ultra-firm processing tomato germplasm developed from transgressive segregants of interspecific *Lycopersicon esculentum* x *L. hirsutum* and intraspecific *L. esculentum* crosses was characterized. Large-fruited breeding lines that varied in fruit firmness from soft to firm were identified for genetic analyses. A six-parent diallel of these advanced breeding lines was developed for field trials over multiple locations. Fruit firmness in the resulting 36 lines was determined by measuring fruit elastic properties during fruit puncture and compression. Following loading for compression, stress relaxation was recorded for 15 seconds. A three-parameter model was used to fit the relaxation curves. There was little correlation between firmness (maximum force) and the three relaxation parameters, i.e., firmness measured the elastic component and the relaxation parameters measured the viscous portions of the texture. General and specific combining ability for firmness derived from the respective genetic backgrounds was determined. Environmental effects were small and crosses performed quite uniformly across environments. Since general combining ability was the principal source of genetic variation, breeding methods that exploit additive variation would be most appropriate for improving fruit firmness.

# Fresh-cut Tomatoes – Challenges and Opportunities

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Fresh-cut produce industry has been growing with a double digit growth rate over the past decade. Fresh-cut tomatoes are on high demands from both food service and retail sectors. However, many technical challenges exist in maintaining the quality and microbial food safety of fresh-cut tomatoes have impeded the growth and expansion of fresh-cut tomato industry. This presentation will discuss the market trend in fresh-cut tomato industry, food quality and safety issues, including chilling injury, tissue damage, ethylene response, tissues softening, drip loss, microbial spoilage and pathogen growth and survival, as well as the important quality traits desired for fresh-cut processing. Technology advancement in modified atmosphere packaging system, sanitization, storage and transportation, and fresh-cut processing techniques that are important to fresh-cut tomatoes will also be discussed.