Report of the Tomato Genetics Cooperative



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14625 County Road 672

Wimauma, FL 33598 USA

Foreword

The Tomato Genetics Cooperative, initiated in 1951, is a group of researchers who share and interest in tomato genetics, and who have organized informally for the purpose of exchanging information, germplasm, and genetic stocks. The Report of the Tomato Genetics Cooperative is published annually and contains reports of work in progress by members, announcements and updates on linkage maps and materials available. The research reports include work on diverse topics such as new traits or mutants isolated, new cultivars or germplasm developed, interspecific transfer of traits, studies of gene function or control or tissue culture. Relevant work on other Solanaceous species is encouraged as well.

Paid memberships currently stand at approximately 39 from 14 countries. Requests for membership (per year) at US\$10 for the online edition should be sent to Dr. J.W. Scott, jwsc@ufl.edu. Please send only checks or money orders. Make checks payable to the **University of Florida**. We are sorry but we are **NOT** able to accept cash, wire transfers or credit cards.

Cover: The feature article of this volume deals with fruit shapes used in the breeding of plum or Saladette tomatoes and is written by Dr. Bob Heisey of United Genetics Seed. The cover photo was provided by Bob and shows the shapes of tomatoes that are referenced in the article. There is virtually nothing in the literature on this topic be sure to check it out if you are interested in breeding plum tomatoes or in tomato fruit shape in general. The key to the shapes follows:

- A: Manzana or apple-shape parent
- B: Typical large round parent
- C: Saladette to Roma
- D: Typical chontos
- E: Typical pear with "waist"
- F: Fat San Marzano, chonto parent

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From the editor:

Happy 2015 to all TGC members and others who have found us on the web. No matter that it is February 2016 and the TGC has been posted late yet again. I retired as of August 31, 2015 and now am Professor Emeritus, but have carried on the "heavy burden" of being Tomato Genetics Cooperative Managing Editor until now. Several TGC Volumes have been late before so I can't blame the tardiness of this Volume on my retirement. Somehow everyone seems to be able to handle the delays without major disruption to their professional and personal lives. If you do have any complaints perhaps you would like to become Managing Editor.....maybe threats like this are the reason why I never get any TGC complaints! As has been indicated in the past we typically have a shortage of reports for the TGC in this age of Impact Factors and other demands on the time of tomato and other researchers. Thus, each year I ponder if the TGC should continue with new Volumes. I will solicit reports for 2016 and then, based on the response, decide if there will be a Volume 66 or not. Regardless, we will maintain the website so that the existing TGC reports will not be lost. Up to now the most recent volume has been password protected for a year to stimulate the number of dues paying members. However, Volume 65 will be available right away to members and non-members alike.

This year's feature article by Bob Heisey of United Genetics provides insights on the breeding of plum tomatoes providing information that has not been available in print until now. It should be useful for both breeders and for those interested in identifying genes controlling fruit shape in tomato. So thanks Bob for sharing some of your vast experience in this area. Thank you also to the other people who wrote reports for this issue.

Over the years a few of our predecessors who have made major contributions to tomato genetics and breeding have been recognized upon their passing. See the In Memorium section for a piece largely written by Haim Rabinowitz on Professor Nachum Kedar; a great tomato breeder/geneticist. He was an even greater human being and he is sorely missed.

Thank you to our members for your support over the years. Thanks also to Dolly Cummings for her expert help in putting this volume together. She actually does most the work. Dolly works for Sam Hutton so thank you Sam for allowing her to do the TGC work amidst all of her other responsibilities in your breeding program. Christine Cooley helps maintain the website <u>http://tgc.ifas.ufl.edu/</u> and kudos to her as well.

My contact information:

Jay W. Scott, Ph.D. Gulf Coast Research & Education Center 14625 CR 672 Wimauma, FL 33598 USA Fax: 813-634-0001 Email: jwsc@ufl.edu

Jay W. Scott, Managing Editor

Upcoming Meetings

Tomato Breeders Roundtable, March 13-16, Santo Domingo, Dominican Republic (see TGC website-above)

V International Symposium on Tomato Diseases: Perspectives and Future Directions in Tomato Protection, Malaga, Spain <u>http://www.ishs.org/symposium/546</u>

The 13th Solanaceae Conference SolGenomics: from Advances to Applications, Sept. 12-16, Davis, CA, USA <u>http://solgenomics2016.ucdavis.edu/</u>

31st Tomato Disease Workshop, November 1-2, Kanuga Conference Center, near Hendersonville, NC. <u>www.ncsu.edu/mckimmon/cpe/opd/TDW/</u>

FEATURE ARTICLE

Inheritance of Fruit Length in Tomato; Implications for a Saladette Breeding Program

Bob Heisey^z, United Genetics Seed, 8000 Fairview Rd., Hollister, CA 95023. email: heisey1@att.net

Since the beginning of classical tomato genetics studies, many researchers have examined the inheritance of fruit length of tomatoes. Most of these researchers have come to the conclusion that there are probably two genes that result in elongated, pear or San Marzano fruit, one major recessive gene (*o* gene) and another minor additive gene. What does this mean in a practical sense, in a modern saladette breeding program?

Interest in the saladette tomato fruit type has expanded dramatically over the last 50 years, and is now a large segment of the market in many countries around the world. To define the "saladette" type, we are generally talking about a tomato fruit with a length-to-width (L/W) ratio of around two. From the first major variety of this type, Rio Grande, we have moved to many varieties around the world with this type of fruit. The plant type been expanded to include both has determinate and indeterminate plant habits. The reasons for the increased interest in the saladette types is not totally clear, but I



suspect that this type of fruit combines quality characteristics of both round fresh market and long or square-round processing tomatoes. Saladettes have the internal structure and wall thickness of processing types, which allow for longer shelf life and the ability to tolerate handling during transport and marketing, yet the fruit retain some of the gel and juice which allows for acceptable quality and flavor for fresh consumption.

How do we as breeders use the available genetic variability to come up with a saladette tomato type? Rio Grande is an open- pollinated, fixed variety, with L/W ratio of around two, but we have many varieties with much longer fruit. For example, the San Marzano types can have L/W ratios of more than four. It is clear that the length of the fruit is not the result of a single gene. Probably there is a major gene, possibly with many alleles, but more importantly there are probably tens of major and minor modifying genes. This situation would probably be best demonstrated by selfing down an F_2 of a cross between a round-fruited and a San Marzano type, and looking at hundreds of inbred lines from this cross. I suspect you would end up with a continuum of fruit types from round to San Marzano, but probably the round and San Marzano classes would be over-represented.

The existence of many modifying genes is also supported by the existence of significant transgressive segregation for length in many segregating populations. This happens with

crosses between many types of tomato; round by long, round by saladette, saladette by saladette, and not just between crosses of long types. Many of these modifying genes for length appear to be "hiding" in round or square-round types. Of interest also is the appearance of long-fruited types in F₂ populations of inter-specific crosses between round types such as *S.lycopersicum* X *S.pimpinellifolium and S.lycopersicum* X *S. galapagense*.

Breeding saladettes

How do we deal with the inheritance of fruit length in a real-world saladette breeding program? First, we will define classes based on fruit type;

Round - typical garden or fresh market tomato. They can be oblate, flat oblate, deep oblate, flattened globe, globe, deep globe. L/W is generally one or less.

Square-round - generally reserved for processing types, L/W is generally about one.

Chonto or **Santa Clara** - large square-round fresh market type (mainly grown in Colombia and Brazil). L/W is generally 1 to 1.25. Blocky shoulder.

Saladette and Roma- covers a range of lengths, generally from 1.25 to 2.0 L/W, with a generally rounded shoulder (Romas tend to have a "waist" or indentation around the neck region).

Pear, San Marzano - both types are long, generally more than 2.5 L/W. San Marzanos are more cylindrical, usually with a blocky shoulder, and little to no "waist". Note that pear as used here is different than pear tomatoes that are plum types that are highly constricted stem end.

If we are trying to develop varieties with the chonto to saladette shape, we have several options from a fruit length point of view, limited of course by the genetics of fruit length.

1) **Saladette by saladette** crosses would seem to be our most obvious choice. However, when we add another important trait, fruit size, we have problems. Many of our markets in Central and South America require large sizes, 150 gm and more. We have found it difficult to obtain these sizes by making "saladette by saladette" crosses. We find that making crosses with fresh-market rounds or "manzana" (apple-shaped) types is the easiest way to bring size into the equation (manzana types can be selected out of some segregating saladette populations. The origin of these manzana types is not clear; it is possible that this phenotype is related to the "deep globe" phenotype that appears in fresh-market germplasm). The length of these hybrids will be determined by the depth of the round parent, the length of the long parent, and by the presence of modifier genes.

2) Large round by San Marzano crosses sometimes result in very nice, large saladettes. These crosses can result in some defects, including tapering, pointed fruit, and blocky shoulders. It is possible that these crosses between parents of very different phenotypes lead to hybrids with unstable phenotypes.

Large round by pear crosses generally do not make useful hybrids; most of the pear phenotype, especially the "waist" ends up being negative in these hybrids. The San Marzano types seem to make better parents.

3) Chontos in particular seem to require a large round parent crossed with a shorter San Marzano type. Large size is a requirement in most of the chonto market area, so a large round is useful. Using these large rounds is also a good way to bring extra disease resistance into chonto types.

What we have learned, or not learned, from years of breeding saladettes

1) Many saladette hybrids have a relatively unstable phenotype, sometimes too long, sometimes too short. There are probably many causes of this instability. Crosses between extreme types seem to be more unstable. Environmental conditions are probably responsible for a large part of the problem, but defining the conditions causing this instability is difficult. In one area, warm temperatures seem to cause fruit to elongate, in another area cool temperatures seem to be responsible. And the effects are probably different depending on the stage of flowering and fruit set. Day length may also be a factor in this instability. Here in Hollister, CA, we have warm to hot days and cool nights, so we can expect chaos, which we generally have ,, in determining fruit length. Of course stability of fruit length is an important characteristic of saladette hybrids; there are hybrids which show good stability.

2) When selecting within segregating populations for new saladette parents, a breeder must select into the F_5 or F_6 to make sure the selection is going to breed true for phenotype. Other types such as square-round and large round seem to stabilize at F_4 , but this is not true of saladettes. This situation gives the possibility of improving phenotype of a breeding line by selection into the F_5 or F_6 generations.

3) The saladette phenotype has been changing over the decades. Larger size is valued and in some markets required, fruit with tapering toward the blossom-end is now accepted, and blocky shoulders are no longer considered a defect.

4) The gene(s) for fruit length appears to be partially dominant, with many modifying genes distributed throughout cultivated tomato germplasm. This rather plastic genetic reality allows for breeding of hybrids with almost any L/W ratio, from 0.5 for a flat-oblate round to 4+ for a San Marzano, with any ratio in between.

Because of the benefits to consumers of the saladette fruit type, this category will probably increase in importance worldwide. With the wealth of germplasm available, and the large pool of genetic variability available, breeders should be able to continue to satisfy market requirements of the saladette type.

^zThanks to Dr. Cathy Thome for suggestions and help with editing

RESEARCH REPORTS

A new multiplex PCR reaction for the screening of the nematode resistance gene *Mi*, and the tomato spotted wilt virus resistance gene *Sw-5* in tomato

Pablo D. Asprelli, Guillermo S. Gallardo. EEA La Consulta INTA. ExRuta 40 Km 96. CC 8 (5567) La Consulta, Mendoza, Argentina. Telefax: +54-02622-470304/470753/ 470501 <u>asprelli.pablo@inta.gob.ar</u>

Abstract

Root-knot nematodes and tomato spotted wilt virus (TSWV) are two of the most important pests in the cultivated tomato (*Solanum lycopersicum* L). The introduction of resistance to TSWV and nematodes in breeding lines is mainly prompted by the serious damage that these pests can cause to tomatoes, and with the purpose of reducing the use of pesticides in agriculture. *Sw-5* and *Mi* are single dominant genes introgressed into cultivated tomato from wild tomato species that confer resistance to TSWV and root-knot nematodes respectively. Markers linked to the root-knot nematode resistance gene *Mi* and to the tomato spotted wilt virus (TSWV) resistance gene *Sw-5* were developed. In order to accelerate the process of screening for resistances, we developed a new multiplex PCR reaction for the screening of both genes. PCR reactions and the digestion of the products yielded the expected bands for susceptible or resistant alleles. The new multiplex reaction is a very useful tool in marker-assisted selection systems in a large range of tomato accessions, and it allowed us to identify tomato plants homozygous for both *Sw-5* and *Mi* resistance genes in a straightforward manner.

Key words: *Solanum lycopersicum, Meloidogyne* spp., Tospovirus, genetic resistance, marker-assisted selection.

Introduction

Root-knot nematodes (Meloidogyne spp.) are important endoparasitic pests of numerous crop species, including tomato (Solanum lycopersicum L). They have a worldwide distribution in warm temperate to tropical regions, and are also prevalent in greenhouse and other controlled environment production systems (Roberts and May 1986, Williamson and Colwell 1991, Williamson and Hussey 1996, Johnson 1998). Gene Mi-1, introgressed from S. peruvianum L., confers resistance to three species of root-knot nematodes, Meloidogyne arenaria, M. incognita and M. javanica (Dropkin 1969a), as well as to the potato aphid (Macrosiphum euphorbiae) (Rossi et al. 1998). Genetic and physical mapping localized Mi-1 in the introgressed region on the short arm of chromosome 6. Mi-1 was cloned and shown to belong to the class of resistance genes that contains a leucine zipper, a nucleotide binding site and leucine rich repeats (Milligan et al. 1998). This gene has been exploited extensively for modern tomato cultivar development, and is the only commercially available source of resistance to root-knot nematodes in tomato. Williamson et al. (1994) developed a SCAR marker, REX-1, tightly linked to the gene Mi. The amplified band of 750 bp from susceptible and resistant tomato lines was distinguishable after cleavage with the restriction enzyme TagI. The amplified DNA from resistant plants is cleaved into two bands of 570 and 180 bp, whereas the DNA from susceptible plants did not contain a *Taq*I cleavage site.

Although *Mi-1* is a very effective source of root-knot nematode resistance in the field, Mi-1mediated resistance is inactive above 28 °C soil temperature (Holtzmann 1965, Dropkin 1969b). The break-down of Mi-1-mediated resistance due to high temperature has been reported in both greenhouse and field conditions (Philis and Vakis 1977, Tzortzakakis and Gowen 1996, Noling 2000). Temperature sensitivity appears to be a characteristic of several Meloidogyne resistance genes, as this trait has also been described in other crop species such as alfalfa (Griffin 1969), sweet potato (Jatala and Russell 1972) and cotton (Carter 1982). Several S. peruvianum accessions have been identified to possess heat-stable resistance (Ammati et al. 1986, Veremis and Roberts 1996a). Genetic analysis using an interspecific population between LA2157 and cultivated tomato, generated by embryo rescue (Ammati et al. 1986, Poysa 1990, Veremis and Roberts 1996b) revealed that the heat-stable resistance in LA2157 is governed by a single dominant gene (Veremis et al. 1999, Veremis and Roberts 2000). Using molecular markers, this gene was mapped to chromosome 6 (Veremis et al. 1999), very tightly linked to Mi-1 (Dixon et al. 1995, Kaloshian et al. 1998, Ammiraju et al. 2003), and formally designated as Mi-9, based on recombination and molecular data and phenotype specificity (Veremis and Roberts 2000).

On the other hand, a number of related Tospovirus species (family *Bunyaviridae*) are responsible for the disease known as "spotted wilt" (Silberschmidt 1937, Williams *et al.* 2001), that causes severe annual yield losses of fresh-market and processing tomatoes around the world (Gordillo *et al.* 2008). In tropical and sub-tropical areas of South America, Tomato spotted wilt virus (TSWV), Tomato chlorotic spot virus (TCSV), Groundnut ringspot virus (GRSV) and Chrysanthemum stem necrosis virus (CSNV) are the four prevalent Tospovirus species infecting tomatoes (de Ávila *et al.* 1993, Giordano *et al.* 2000, Williams *et al.* 2001). These viruses are transmitted by thrips (*Thysanoptera: Thripidae*) (Wijkamp *et al.* 1995). Susceptible plants show a wide range of symptoms, and early infection of highly susceptible cultivars often leads to complete decay and death. The symptom expression of these viruses is highly influenced by environmental conditions (Nagata *et al.* 2000). This resistance can be broken by some Tospovirus species and some TSWV isolates, by high inoculum pressure, or by drastic temperature variation (Lathan and Jones 1998, Roselló *et al.* 1998, McMichael *et al.* 2002, Aramburu and Marti 2003, Ciuffo *et al.* 2005).

The complex nature of the Tospovirus species might lead to the occurrence of escapes (false resistant plants) in screening systems of breeding programs. An almost complete correlation was observed between the *Sw-5*-derived marker result and the resistance to tospoviruses under field conditions. The presence of a small fraction of symptomatic plants is commonly observed even in highly inbred lines carrying the *Sw-5* locus under field conditions (Boiteux and Giordano 1993). This result has been explained by the incomplete penetrance of the *Sw-5* gene (Stevens *et al.* 1992) rather than by the occurrence of distinct viral strains and/or environmental effects on gene expression.

Due to the economic losses caused by tospoviruses in many areas of the world, genetic resistance became the major research focus related to the disease management (Soler *et al.* 2003, Gordillo *et al.* 2008). Sources of genetic resistance to tospoviruses have been found in domesticated and wild *Solanum (Lycopersicon)* accessions (Soler *et al.* 2003). So far, the best levels of broad-spectrum resistance to tospoviruses are conferred by the *Sw-5* gene from *S. peruvianum* (Van Zijl *et al.* 1986, Stevens *et al.* 1992). The *Sw-5* gene is located on the telomeric region of the long arm of chromosome 9 and it was isolated via positional cloning

(Brommonschenkel *et al.* 2000, Spassova *et al.* 2001). The *Sw-5* belongs to the same class of resistance genes as the *Mi*, which confers resistance to *Meloidogyne* species; RPM1 (resistance gene to *Pseudomonas syringae* pv. *maculicola*) and many other R-genes (Brommonschenkel *et al.* 2000) that contain leucine-rich repeats and a highly conserved nucleotide binding site (Martin *et al.* 2003). The *Sw-5* gene resides within a complex cluster comprising five linked gene copies, named *Sw-5a* through *Sw-5e* (Spassova *et al.* 2001). The efficiency of each copy in conferring resistance to tospovirus species is not yet completely clear, but separate analysis of the copies in transgenic plants indicated that the *Sw-5b* is the single gene for the expression of the resistance phenotype (Spassova *et al.* 2001).

The dominant nature of the *Sw-5* gene allowed a broad use of this locus in the development of cultivars. The tomato 'Stevens' (GenBank AY007366) is the source of the *Sw-5* locus introgressed from *S. peruvianum*. However, the *S. peruvianum* accession used for the introgression of the *Sw-5* locus into the cultivar 'Stevens' has not been disclosed (Dianese *et al.* 2010). Plants carrying the *Sw-5* gene are able to restrict the systemic spread of the virus, showing only localized symptoms represented as tiny local lesions caused by a hypersensitive reaction. It was found that cultivars carrying the *Sw-5* locus had broad-spectrum resistance with high stability to distinct TSWV isolates (Stevens *et al.* 1992, Roselló *et al.* 1998) and also to the related Tospovirus species GRSV and TCSV (Boiteux and Giordano 1993, Boiteux *et al.* 1993).

Although sequences of the *Sw-5* locus are available at public databases, there are few reports of molecular markers derived from them (Stevens *et al.* 1995, Chagué *et al.* 1996, Smiech *et al.* 2000, Langella *et al.* 2004, Garland *et al.* 2005). Markers derived from the resistant gene itself, capable of differentiating among susceptible and resistant plants, would be the ideal ones (Folkertsma *et al.* 1999, Garland *et al.* 2005). Stevens *et al.* (1996) found a RAPD (Random Amplified Polymorphic DNA) primer tightly linked (1 cM) to *Sw-5*. The SCAR-421 (Sequence Characterized Amplified Region) developed from that RAPD is a co-dominant marker showing two bands at 940 bp for the susceptible and at 900 bp for the resistant. However, this *Sw-5* locus-specific and co-dominant PCR marker is not publicly available (Masuelli *et al.* 2000).

Dianese *et al.* (2010) report that the 'Sw-5-2' co-dominant, within-locus, SCAR marker system is more advantageous than the other closely linked markers reported. The analyses indicated a first DNA pattern displaying an amplicon of 574 bp, exhibited by three Tospovirus resistant accessions carrying the *Sw-5* locus; a second group displayed an amplicon of ca. 510 bp and encompassed susceptible accessions; and a third group displayed an amplicon of 464 bp and was composed by other Tospovirus-susceptible accessions. The polymorphic amplicon encompass a conserved sequence of the promoter region from the functional *Sw-5b* gene near its open reading frame. The differences in the amplicon sizes were due to a number of insertions/deletions in this genomic region, and a number of point mutations were also observed (Dianese *et al.* 2010).

For both diseases, an almost complete correlation was found between resistance under greenhouse or field conditions and the presence of the markers (Stevens *et al.* 1992, Boiteux and Giordano 1993, Boiteux *et al.* 1993, Roselló *et al.* 1998, Veremis *et al.* 1999, Ammiraju *et al.* 2003). For Marker Assisted Selection (MAS), pyramiding the two resistance genes into one breeding line requires performing two PCR reactions per plant and a digestion with the enzyme *Taq*I. Multiplex polymerase chain reaction (PCR) is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction (Henegariu 1997). In order to advance the MAS, we developed a new protocol for a multiplex PCR

reaction with free primer sequences for the selection of *Mi* and *Sw-5* genes in tomato that could be easily used in simple PCR assays.

Materials and Methods

The adjustment of this new molecular system was carried out with four tomato materials with a known allelic composition (ILC913: double homozygous resistant, Zorzal: double heterozygous resistant, SakataHP: homozygous resistant to spot wilt, UC82: double susceptible). Genomic DNA from one leaf of individual tomato seedlings was isolated with the minipreparation procedure of DNAzol[®]ES, following the manufacturer's protocol, but with some modifications.

Plant tissue was pulverized in a precooled (-80°C) mortar and transferred to a centrifuge tube, filling half of the cone. To each sample, 400 μ l of DNAzol[®]ES plus 3 μ l of β mercaptoethanol were added, mixed vigorously, and incubated at room temperature for 15 min. Samples were then supplemented with 400 µl of chloroform and shook until an emulsion was formed. Following centrifugation at 14,000 rpm for 10 min, the upper aqueous phase was transferred to a clean tube and DNA was precipitated by mixing with 500 μ l of ethanol 96% and storing it at room temperature for 5 min. The samples were centrifugated at 14,000 rpm for 5 min, discarding the resulting supernatant. The pellets were resuspended in 200 μ l water with 2 μ l of RNase (1 mg/ml) and incubated for 15 minutes at room temperature. Samples were treated with 500 µl of DNAzol®ES:ethanol 96% (1:0.75) during 10 minutes by inverting the tubes, and centrifuged at 14,000 rpm for 5 min. DNA was washed with 700 µl of ethanol 70% for 10 min, and then centrifuged at 14,000 rpm for 5 min, discarding the supernatant. A second wash was performed with 700 μl of ethanol 96% for 10 min, and then the samples were centrifuged at 14,000 rpm for 5 min, discarding the supernatant. The tubes were inverted for a few minutes for alcohol drying. The pellet was solubilized in 200 µl of sterile distilled water and stored at 4°C.

The PCR reactions were carried out in a 20 μ l volume in the presence of 0.25 μ M of each primer, 0.1 mM dNTPs, 1 U of Taq polymerase and the buffer provided by the manufacturer. The first set of multiplex reaction, an adaptation of the protocol developed by Masuelli et al. (2000), was carried out with the primers for markers REX-1 (Williamson et al. 1994), REXF (5'-TCGGAGCCTTGGTCTGAATT-3'), and REXR (5'-GCCAGAGATGATTCGTGAGA-3'), and the primers 421-1 and 421-2 for the amplification of the SCAR-421 marker (Stevens et al. 1996). In a second set of multiplex PCR reactions, we replaced the SCAR-421 marker by the Sw-5-2 al. marker primers developed by Dianese et (2010), Sw-5-2F (5'-AATTAGGTTCTTGAAGCCCATCT-3') and Sw-5-2R (5'-TTCCGCATCAGCCAATAGTGT-3'), maintaining the remaining reaction conditions. The amplification routine consists of an initial denaturation step of 94 °C for 3 min; 35 cycles of 94 °C for 45 sec, 52 °C for 45 sec and 72 °C for 1 min 45 sec; and a final extension step of 72 °C for 5 min.

Reviewing the 574 bp amplicon sequence for the resistance allele of the Sw-5-2 marker (Dianese *et al.* 2010), we found a restriction site for the *Taq*I enzyme, also present in the REX-1 amplicon, tightly linked to the gene *Mi* (Williamson *et al.* 1994), and in the SCAR-421 amplicon (Masuelli *et al.* 2000). The resistant Sw-5-2 allele produced after digestion bands of 400 bp and 174 bp, and the susceptibility alleles (464 bp and 510 bp) remained uncut because the restriction site belongs to an insertion/deletion fragment. All the PCR products were subjected to restriction digestion with the *Taq*I enzyme. The reaction mixture

consisted of 5 μ l of PCR products, 0.42 μ l restriction buffer 10x, 0.125 μ l of (2000 U/ μ l) and distilled sterile water for a final volume of 10 μ l. The reaction mixture was incubated for 1 h at 65 °C. The DNA fragments were resolved by electrophoresis in a 2% agarose gel with 1x TBE buffer, stained with Gelgreen[®] 0.03%, and visualized under a Dark Reader[®].

A total of 371 individual plants were analyzed with this protocol, and the results of both marker combinations in the multiplex PCR reactions (REX-1/SCAR-421 and REX-1/Sw-5-2) were compared. Plants arose from different crosses, generations, and breeding strategies of the tomato breeding program of the Agricultural Station La Consulta INTA, Mendoza, Argentina.

Results

Multiplex reaction products show the expected allele size arrangements. For the gene *Mi*, susceptible and resistant tomato lines are distinguishable after cleavage of the amplified band of 750 bp with the restriction enzyme *Taq*I (Williamson *et al.* 1994). The SCAR-421 marker for the TSWV resistance amplified both alleles at 900 bp and 940 bp for the susceptible and resistant variants respectively. The PCR profile obtained with the primer pair for Sw-5-2 was a single amplicon of 574 bp for the resistance allele, while susceptibility displayed only the allele of 464 bp.

The digestion with *Taq*I of the PCR products resulted in a double co-dominant band pattern. The *Mi* gene produces bands of 750 bp for the susceptible and 570 bp + 180 bp for the resistant alleles. For the SCAR-421 marker, the digestion resulted in a band of 500 bp and in another band of 440 bp for the resistant variant, or 400 bp for the susceptible allele, as reported by Masuelli *et al.* (2000). For the Sw-5-2 marker (Dianese *et al.* 2010), bands of 464 bp for the susceptibility allele, and bands of 400 bp and 174 bp for the resistant variant were obtained (Figure 1A, 1B).

The 371 analyzed plants from the tomato breeding program of the EEA La Consulta INTA show different combinations of resistance and susceptibility alleles for Mi and Sw-5 genes (Table 1). Taking into account both genes, 88 plants were double-homozygous for the resistant alleles (23.7%), and 111 plants were double-susceptible (29.9%). Also, we notice the lack of heterozygous plants for the *Mi* gene and a very low frequency of heterozygous plants for the *Sw-5* gene (Figure 2).

Discussion

The 371 analyzed plants from the tomato breeding program of the EEA La Consulta INTA show different combinations of resistance and susceptibility alleles for *Mi* and *Sw-5* genes (Table 1). Taking into account both genes, 88 plants were double-homozygous for the resistant alleles (23.7%), and 111 plants were double-susceptible (29.9%). Also, we notice the lack of heterozygous plants for the *Mi* gene and a very low frequency of heterozygous plants for the *Sw-5* gene (Figure 2). This is a direct effect of the applied selection tending to fix the dominant alleles for the resistance to nematodes and tospoviruses in tomato breeding lines. In addition, the alternative susceptible allele of 510 bp for the Sw-5-2 marker was observed in 17 plants (0.8%) making evident at least two different lineages in the parental set (Figure 1C). The presence of more than one gel pattern for the susceptible accessions demands either the comparison with PCR samples from DNA template extracted from sources of the *Sw-5* locus or a careful estimation of the amplicon size.

Most disease resistance genes are members of multigene families and seem to be clustered in the plant genome. Duplication and subsequent divergence seem to be a common process in plant gene evolution. For example, the Cf2 locus isolated from tomato contained two functional sequences encoding the same specificity in recognizing the same avirulence factor from the fungus *Cladosporium fulvum* (Dixon *et al.* 1996). Such is the case of the *Mi*-1 locus where two highly homologous genes were identified, but only one conferred resistance to root-knot nematodes (Milligan et al. 1998). On the other hand, the Mi-9 gene has the same phenotypic expression as the Mi-1 in terms of Meloidogyne species and biotype specificity, and it differs phenotypically only in the stability of the resistance at high temperature (Ammiraju et al. 2003). It is possible that the Mi-9 is a member of the Mi-1 family and that this member has evolved to confer heat-stable resistance (Veremis and Roberts 2000). Mapping analysis has revealed that *Mi-9* maps on the short arm of chromosome 6. There was no evidence of recombination between heat-stable and heatsensitive resistance genes since no recombination was found between REX-1 and Mi-9, indicating that these markers are in close proximity to the gene (Ammiraju et al. 2003). On the other hand, the sequences of the Sw-5-resistant lines analyzed by Dianese et al (2010) were 100% identical to that of Stevens et al. (1996), and they were highly conserved across two S. peruvianum loci.

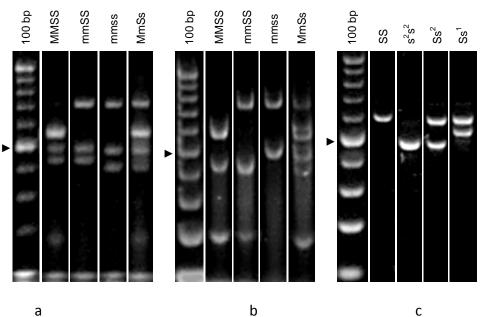
In order to screen the genomic region encoding a given phenotype, the so-called genederived (Varshney *et al.* 2005) and/or locus-specific markers represent robust tools since they minimize the risk of accidental separation through genetic recombination (crossingover) events as reported for closely linked DNA markers. From the marker-assisted selection point of view, a reliable, fast, and simple PCR assay to screen for the presence of the interest loci is highly desirable (Stevens *et al.* 1995, Chagué *et al.* 1996, Smiech *et al.* 2000, Langella *et al.* 2004). A practical application of MAS requires that markers could be identified with a high level of accuracy and efficiency, be cost effective and be easy to use. The results obtained with the new primer pair combination suggests this protocol as a robust selection tool to monitor the gene introgression in tomato breeding lines. It is important to mention that, in all situations, it was not necessary to assay special PCR reaction conditions for the multiplex PCR; both loci were amplified and the results were reproducible in several repetitions. Also, heterozygous plants were able to produce amplicons with quite similar intensity allowing, therefore, a reliable co-dominant marker system.

The new multiplex reaction described here allowed us to pyramid tomato lines with *Sw-5* and *Mi* genes in a straightforward manner, and the homozygous lines were readily selected from F2 generations without the need for progeny testing. Markers like the REX-1 can be used to select the Mi-9 trait and will be valuable for incorporating the *Mi-9* into cultivated tomato using conventional breeding approaches (Veremis *et al.* 1999). On the other hand, the advantages of the Sw-5-2 marker over the SCAR-421 marker are that the first one is publicly available, is located inside the gene, shows a better gel resolution due to a greater band size difference, and allows the possibility to differentiate at least two genetic pools in the parental sets of these 371 analyzed plants from the tomato breeding program of the EEA La Consulta INTA.

Table 1. Number of different genotypes observed for each molecular marker over 371 tomato plants procedent from the tomato breeding program of the EEA La Consulta INTA.

Marker	Bands (bp)	Genotype	Phenotype	Plants
	570+180	MM	Nematode resistant	153
REX-1	570+180+750	Mm	Nematode resistant	20
	750	mm	Nematode	
	750		suceptible	198
	500+440	SS	TSWV resistant	167
SCAR-421	500+440+400	Ss	TSWV resistant	32
	500+400	SS	TSWV suceptible	172
	400+174	SS	TSWV resistant	167
	510+400+174	Ss ¹	TSWV resistant	1
Sw-5-2	464+400+174	Ss ²	TSWV resistant	31
	510	s ¹ s ¹	TSWV suceptible	2
	464	s ² s ²	TSWV suceptible	170

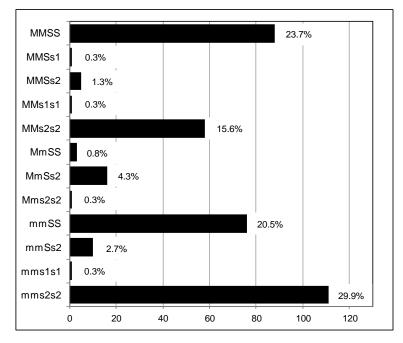
Fig 1 PCR amplification profile (in 2% agarose gel in TBE buffer) obtained with the primer pair combination Mi/SCAR-421 after cutting with the endonuclease Tagl (a) and Mi/Sw-5-2 after cutting with the endonuclease *Taq*I (b), and with the primer pair Sw-5-2 (c) (**b** 500 bp). PCR assays in (a) and (b) were carried out using as template genomic DNA extracted from four tomato cultivars with a known allelic composition (ILC913: MMSS, double homozygous resistant; Sakata: mmSS, homozygous resistant to spot wilt, UC82: mmss, double susceptible, Zorzal: MmSs, double heterozygous resistant). PCR assays in (c) were carried out using as template genomic DNA extracted from four tomato plants procedent from the tomato breeding program of the EEA La Consulta INTA



а

С

Fig 2 Number of plants and proportions of different genotypes observed for resistance to root-knot nematodes and to tomato spotted wilt virus in 371 tomato plants procedent from the tomato breeding program of the EEA La Consulta INTA



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References

- Ammati M, Thomason IJ, McKinney HE (1986) Retention of resistance to Meloidogyne incognita in Lycopersicon genotypes at high soil temperature. J Nematol 18:491–495.
- Ammiraju JSS, Veremis JC, Huang X, Roberts PA, Kaloshian I (2003) The heat-stable root-knot nematode resistance gene Mi-9 from Lycopersicon peruvianum is localized on the short arm of chromosome 6. Theor Appl Genet 106:478–484.
- Aramburu J, Marti M (2003) The occurrence in north-east Spain of a variant of Tomato spotted wilt virus (TSWV) that breaks resistance in tomato (Lycopersicon esculentum) containing the Sw-5 gene. Plant Pathol 52:407.
- Boiteux LS, Giordano LB (1993) Genetic basis of resistance against two Tospovirus species in tomato (Lycopersicon esculentum). Euphytica 71:151–154.
- Boiteux LS, Giordano LB, de Ávila AC, Santos JRM (1993) 'TSW 10': uma linhagem de tomate com resistência a três espécies de Tospovirus. Hort Bras 11:163–164.
- Brommonschenkel SH, Frary A, Tanksley SD (2000) The broad-spectrum Tospovirus resistance gene Sw-5 of tomato is a homolog of the root-knot nematode resistance gene Mi. Mol Plant Microbe Interact 13:1130–1138.
- Carter WW (1982) Influence of soil temperature on Meloidogyne incognita resistant and susceptible cotton. J Nematol 14:343–346.
- Chagué V, Mercier JC, Gue´nard M, de Courcel A, Vedel F (1996) Identification and mapping on chromossome 9 of RAPD markers linked to Sw-5 in tomato by bulked segregant analysis. Theor Appl Gen 92:1045–1051.
- Ciuffo M, Finetti-Sialer MM, Gallitelli D, Turina M (2005) First report in Italy of a resistancebreaking strain of Tomato spotted wilt virus infecting tomato cultivars carrying the Sw5 resistance gene. Plant Pathol 54:564.
- De Ávila AC, de Haan P, Kormelink R, Resende RO, Golbach RW, Peters D (1993) Classification of Tospovirus based on phylogeny of nucleoprotein gene sequences. J Gen Virol 74:153–159.
- Dianese EC, de Fonseca MEN, Goldbach R, Kormelink R, Inoue-Nagata AK, Resende RO, Boiteux LS. (2010) Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the Sw-5 (Tospovirus resistance) gene cluster in a wide range of tomato accessionsMol Breeding 25:133-142.
- Dixon MS, Jones DA, Hatzixanthis K, Ganal MW, Tanksley SD, Jones JDG (1995) Highresolution mapping of the physical location of the tomato Cf-2 gene. Mol Plant-Microbe Interact 8:200–206.
- Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrisom K, Jones JDG (1996) The tomato Cf-2 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451–459.
- Dropkin VH (1969a) Cellular responses of plants to nematode infections. Annu Rev Phytopathol 7:101–122.
- Dropkin VH (1969b) The necrotic reaction of tomatoes and other hosts resistant to Meloidogyne: reversal by temperature. Phytopathology 59:1632–1637.

- Folkertsma RT, Spassova MI, Prins M, Stevens MR, Hille J, Goldbach RW (1999) Construction of a bacterial artificial chromosome (BAC) library of Lycopersicon esculentum cv. Stevens and its application to physically map the Sw-5 locus. Mol Breed 5:197–207.
- Garland S, Sharman M, Persley D, Mcgrath D (2005) The development of an improved PCRbased marker system for Sw-5, an important TSWV resistance gene of tomato. Aust J Agric Res 56:285–289.
- Giordano LB, de Ávila AC, Charchar JM, Boiteux LS, Ferraz E (2000) 'Viradoro': A Tospovirusresistant processing tomato cultivar adapted to tropical environments. Hort-Science 35:1368–1370.
- Gordillo LF, Stevens MR, Millard MA, Geary B (2008) Screening two Lycopersicon peruvianum collections for resistance to Tomato spotted wilt virus. Plant Dis 92:694–704.
- Griffin GD (1969) Effects of temperature of Meloidogyne hapla in alfalfa. Phytopathology 59:599–609.
- Henegariu O, Heerema NA, Dlouhy SR, Vance GH, Vogt PH. (1997) Multiplex PCR: Critical parameters and step-by-step protocol. Biotechniques 23:504-511.
- Holtzmann OV (1965) Effects of soil temperature on resistance of tomato to root-knot nematode (Meloidogyne incognita). Phytpathology 55:990–992.
- Jatala P, Russell CC (1972) Nature of sweet potato resistance to Meloidogyne incognita and the effects of temperature on parasitism. J Nematol 4:1–7.
- Johnson AW (1998) Vegetable crops. In: Pederson GA, Windham GL, Barker KR (eds) Plantnematode interactions. Agronomy Society of America, Madison Wisconsin, pp 595– 635.
- Kaloshian I, Yaghoobi J, Liharska T, Hontelez J, Hanson D, Hogan P, Jesse T, Wijbrandi J, Simons G, Vos P, Zabel P, Williamson VM (1998) Genetic and physical localization of the root-knot nematode resistance locus Mi in tomato. Mol Gen Genet 257:376–385.
- Langella R, Ercolano MR, Monti LM, Frusciante L, Barone A (2004) Molecular marker assisted transfer of resistance to TSWV in tomato elite lines. J Hort Sci Biotech 79:806–810.
- Lathan LJ, Jones RAC (1998) Selection of resistance breaking strains of Tomato spottedwilt tospovirus. AnnAppl Biol 133:385–402.
- Martin GB, Bogdanove AJ, Sessa G (2003) Understanding the functions of plant disease resistance proteins. Ann Rev Plant Biol 54:23–61.
- Masuelli RW, Cuesta C, Piccolo R. (2000) A multiplex PCR reaction for the screening of the nematode resistance gene Mi, and the tomato spotted wilt virus resistant gene Sw-5 in tomato. Journal of Genetics and Breeding 54:233-235.
- McMichael LA, Persley DM, Thomas JE (2002) A new Tospovirus serogroup IV species infecting capsicum and tomato in Queensland, Australia. Australasian Plant Pathol 31:231–239.
- Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root-knot nematode resistance gene Mi from tomato is a member of leucine zipper nucleotide binding leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319.

- Nagata T, Inoue-Nagata AK, Prins M, Goldbach R, Peters D (2000) Impeded thrips transmission of defective Tomato spotted wilt virus isolates. Virology 90:454–459.
- Noling JW (2000) Effects of continuous culture of resistant tomato cultivars on Meloidogyne incognita soil population density and pathogenicity. J Nematol 32:452.
- Philis J, Vakis N (1977) Resistance of tomato varieties to the rootknot nematodes Meloidogyne javanica in Cyprus. Nematol Medit 5:39–44.
- Poysa V (1990) The development of bridge lines for interspecific gene transfer between Lycopersicon esculentum and L. peruvianum. Theor Appl Genet 79:187–192.
- Roberts PA, May DM (1986) Meloidogyne incognita resistance characteristics in tomato genotypes developed for processing. J Nematol 18:353–359.
- Roselló S, Diez MJ, Nuez F (1998) Genetics of tomato spotted wilt virus resistance coming from Lycopersicon peruvianum. Eur J Plant Pathol 104:499–509.
- Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene Mi of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95:9750–9754.
- Silberschmidt KM (1937) A doença de vira-cabeça do fumo. O Biológico 3:183–184.
- Smiech M, Rusinowski Z, Malepszy S, Niemirowicz-Szczytt K (2000) New RAPD markers of tomato spotted wilt virus (TSWV) resistance in Lycopersicon esculentum Mill. Acta Phys Plantarum 22:299–303.
- Soler S, Cebolla-Cornejo J, Nuez F (2003) Control of diseases induced by tospoviruses in tomato: an update of the genetic approach. Phytopathol Mediterr 42:207–219.
- Spassova MI, Prins TW, Folkertsma RT, Klein-Lankhorst RM, Hille J, Goldbach RW, Prins M (2001) The tomato gene SW5 is a member of the coiled coil, nucleotide binding, leucine-rich repeat class of plant resistance gene and confers resistance to TSWV in tobacco. Mol Breed 7:151–161.
- Stevens MR, Heiny DK, Rhoads DD, Griffiths PD, Scott JW. (1996) A linkage map of the tomato spotted wilt virus resistance gene Sw-5 using near isogenic lines and an interspecific cross. Acta Horticulturae 431:385-392.
- Stevens MR, Lamb EM, Rhoads DD (1995) Mapping the Sw-5 locus for Tomato spotted wilt virus resistance in tomatoes using RAPD and RFLP analyses. Theor Appl Gen 90:451– 456.
- Stevens MR, Scott SJ, Gergerich RC. (1992) Inheritance of a gene for resistance to tomato spotted wilt virus (TSWV) from Lycopersicum peruvianum Mill. Euphytica 59:9-17.
- Tzortzakakis EG, Gowen SR (1996) Occurrence of resistance breaking pathotypes of Meloidogyne javanica on tomatoes in Crete, Greece. Fund Appl Nematol 19:283–288.
- Van Zijl JJB, Bosh SE, Coetzee CPJ (1986) Breeding tomatoes for processing in South Africa. Acta Hort 194:67–75.
- Varshney RK, Graner A, Sorrells ME (2005) Genomics-assisted breeding for crop improvement. Trends Plant Sci 10:621–630.

- Veremis JC, Roberts PA (1996a) Relationships between Meloidogyne incognita resistance genes in Lycopersicon peruvianum differentiated by heat sensitivity and nematode virulence. Theor Appl Genet 93:950–959.
- Veremis JC, Roberts PA (1996b) Differentiation of Meloidogyne incognita and M. arenaria novel resistance phenotypes in Lycopersicon peruvianum and derived bridge lines. Theor Appl Genet 93:960–967.
- Veremis JC, Roberts PA (2000) Diversity of heat-stable genotype specific resistance to Meloidogyne in Maranon races of Lycopersicon peruvianum complex. Euphytica 111:9–16.
- Veremis JC, van Heusden AW, Roberts PA (1999) Mapping heat-stable resistance to Meloidogyne in Lycopersicon peruvianum. Theor Appl Genet 98:274–280.
- Wijkamp I, Almarza N, Goldbach R, Peters D (1995) Distinct levels of specificity in thrips transmission of tospoviruses. Phytopathology 85:1069–1074.
- Williams LV, López-Lambertini PM, Shohara K, Biderbost EB (2001) Occurrence and geographical distribution of Tospovirus species infecting tomato crops in Argentina. Plant Dis 85:1227–1229.
- Williamson VM, Colwell G (1991) Acid phosphatase-1 from nematode resistant tomato isolation and characterization of its gene. Plant Physiol 97:139–146.
- Williamson VM, Ho JY, Wu FF, Miller N, Kaloshian I (1994) A PCR based marker tightly linked to the nematode resistance gene, Mi, in tomato. Theor. Appl. Genet. 87:757-763.
- Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. Plant Cell 8:1735–1745.

RESEARCH REPORTS

Underutilized Germplasm - S. galapagense

Bob Heisey, United Genetics Seed, 8000 Fairview Rd., Hollister, CA 95023. email: <u>heisey1@att.net</u>



The above picture shows the F₂ progeny of a cross between a large oxheart-type *S. lycopersicum*, weighing about 300 gms (bottom left and right in the picture) and *S.galapagense*, weighing about 1 gm, (top, middle). My original idea was to investigate the high-solids characteristics of this species. However, it has become increasingly clear that this species has many other interesting traits to investigate. Besides the high brix of many of these individuals, up to 11 % (non-replicated), we noticed probable resistance to Powdery Mildew (*Leveillula taurica*), *Oidium* powdery mildew, Cladosporium leaf mold, and tolerance to greenhouse whiteflies. Based on the environmental conditions of the native habitat of these accessions, we expect them to be tolerant to drought and salinity. The flavor is also interesting, with many individuals having complex flavor with "fruity and floral" notes (however, watch out for the tomatine).

Important in this discussion is that fact that *S.galapagense* crosses easily with cultivated tomato, and segregation is normal.

Designing new DNA markers and determining the effective size of *Ph-2* and *Ph-3* introgressions for late blight resistance stacking purposes in tomato.

Reza Shekasteband*, Samuel F. Hutton, and Jay W. Scott, University of Florida, Institute of Food and Agricultural Sciences, Gulf Coast Research & Education Center, 14625 CR 672, Wimauma, FL 33598 rezash@ufl.edu 1-813-633-7485

Introduction

Late blight caused by the fungal pathogen Phytophthora infestans (Mont.) de Bary is one of the most destructive diseases of potato (Solanum tuberosum) and tomato (S. lycopersicum) crops under moist, cool, rainy, and humid environments (Birch and Whisson, 2001; Kamoun and Smart, 2005; Foolad et al., 2008). The late blight of potatoes and tomatoes resurfaced as an important disease in the United States and Canada during the late 1980s and early 1990s (Fry and Goodwin, 1997). An aggressive application of systemic fungicides is necessary to prevent late blight under favorable weather conditions for the pathogen. However, frequent applications of fungicides to prevent a sudden and highly distractive epidemic of late blight is expensive and environmentally undesirable and may lead to the emergence of new exotic strains of P. infestans with higher pathogenic fitness (Ko, 1994). These more aggressive strains of the pathogen have been introduced into United States and Canada causing more problems for growers of potato and tomato in many production regions. Late blight prevention and management in some vulnerable regions can add an extra \$200/acre or more than 10% to the production cost which can be higher than the profit margin in many cases (Smart and Fry, 2001). Breeding for resistance to P. infestans in cultivated tomato has led to identification of three resistant genes; Ph-1, Ph-2 and Ph-3, located on chromosome 7, 10 and 9, respectively derived from S. pimpinellifolium (Peirce, 1971; Moreau et al., 1998; AVDRC, 1994). The Ph-1 gene is no longer effective against present pathogen strains. Furthermore, due to incomplete resistance achieved by Ph-2 or Ph-3 alone and emerging new exotic strains of the pathogen, the single resistant gene approach is not effective enough to prevent the damage caused by late blight (Goodwin et al., 1995; Black et al., 1996). Tomato breeders are using combinations of resistance genes to provide varieties with improved and more durable resistance (Pedersen and Leath, 1988; Yang and Francis, 2005; Vidavski, 2008). Hybrids heterozygous for Ph-2 and Ph-3 genes have held up well to the pathogen in several tomato production regions. However, to be able to effectively pyramid multiple genes using marker assisted selection (MAS) and avoid the linkage drag effects, the size of the introgression carrying the resistant genes should be minimized (Ji et al., 2009; Robbins et al., 2010). In this study we used several approaches to design new DNA markers linked to the Ph-2 and Ph-3 genes. These markers were then used to minimize the introgressions as we developed late blight resistant breeding lines from NC2-CELBR, a LB resistant line from North Carolina State University (Gardner and Panthee, 2010).

Materials and Methods

Plant Material

NC2 CELBR was used as a donor of the *Ph-2* and *Ph-3* genes for MAS backcrossing to 32 University of Florida recurrent parents. Nearly-isogenic lines were developed for each recurrent parent after five or six backcrosses. CAPS markers kindly provided by Martha Mutschler at Cornell University were used to select the plants with the resistance genes for most of the early backcrossing. These markers were dTG63 and dTG422 for *Ph-2* and TG328 and TG591, for *Ph-3*. These lines along with more than 200 Recombinant Inbred Lines (RILs), carrying *Ph-2* or *Ph-3* only, have provided enough plant material to develop new markers and delimit the introgression sizes. Some of the new markers were utilized for the later part of the backcrossing.

Late Blight Bioassay

An isolate of *P. infestans*, US-23, from Florida was freshly maintained on plants of the dwarf cultivar Fla. Lanai year-round by sub-culturing every other week. Inoculated plants were kept in a semitransparent plastic box, sprayed daily with water to keep them moist and the boxes were kept in a growth room under controlled cycles of 16 h light at 21°C and 8 h dark at 19°C. The same growth room and conditions were used to maintain inoculated seedlings to evaluate their resistance. To obtain spores to inoculate tomato seedlings, 2-3 well infected Fla. Lanai leaves were dipped and shaken gently in 500 ml distilled water to release sporangia. The sporangia were then filtered through fine cheesecloth to remove plant tissues and incubated in 4°C for an hour to facilitate spore release from the sporangia. For inoculations 6 weeks old, well hardened and fertilized, tomato seedlings were transferred into a semitransparent plastic box, 26.5 x 16.0 x 12.25 (L x W x H), in a 128 cell Speedling[®] (Speedling, Inc., Sun City, FL) planter trays. The seedlings were sprayed uniformly with 200 ml of the inoculum in each box and the boxes were kept in the growth room for 6 days under the above mentioned conditions (Fig 1).



Fig 1: Late blight bioassay A) Inoculation box B) Fla.7781 homozygous susceptible for *Ph-2* and *Ph-3* on the left and Fla.7781 heterozygous for *Ph-2* and *Ph-3* on the right, 6 days after inoculation

Marker development

To study the introgression size and develop new markers we used three molecular approaches and designed the DNA markers, covering upstream and downstream of the original *Ph-2* and *Ph-3* markers.

SolCAP SNPs genotyping. A collection of 30 $F_1BC_4S_1$ seedlings along with the recurrent resistant and susceptible parents were genotyped with the 7,720 SNP tomato Infinium chip. The resistant recurrent parents were NC1-CELBER (*Ph-2* and *Ph-3*), NC-25p (*Ph-3* only), Richter's Wild (*Ph-2* source), L 3707 (*Ph-3* source), and 11 susceptible recurrent parents; Fla.7770, Fla.7776, Fla.7781, Fla.7804, Fla.8000, Fla.8059, Fla.8083, Fla.8111B, Fla.8124C, Fla.8249 and Fla.8820.

Genome comparison. A simple comparative sequence alignment of inbred tomato cultivar 'Heinz 1706 against *S. pimpinellifolium* LA1589 was used to identify possible polymorphic SNPs and Indels for the regions of interest on Chromosome 9 and 10. Several scar markers were designed using polymorphic Indels in those regions and used to screen different *Ph-2* and *Ph-3* segregating populations to investigate possible similar polymorphic patterns.

Gene Resequencing. The gene Solyc10g085460 (Cc-nbs-Irr resistance protein) closely linked to the original *Ph-2* marker, was re-sequenced in three LB resistant backgrounds including: NC1-CELBER, NC-25p, and Richter's Wild plus susceptible line Fla.7776. The comparative sequence alignment of resistant lines against the susceptible line revealed several polymorphic SNPs from which a new CAPS marker was designed.

PCR conditions

We used Primer 3.0 software (http://www.ncbi.nlm.nih.gov/tools/primer-blast) with modified settings to design all the primers. All PCR amplifications were performed using a thermal cycler with 0.2-ml tubes and primers given in Table 3. One μ l of DNA, extracted according to a standard CTAB protocol (Fulton et al., 1995) from 3-week-old seedlings, was used as template in a 10- μ l PCR reaction according to the guidelines provided by Phire DNA polymerase (Finnzymes) product manual (Table 1). Following PCR amplification, the products were separated and visualized on 3% Agarose gel containing 0.05 μ l/ml ethidium bromide.

	Temperature	Time	Cycle
Initial denaturation	98°C	30 s	1
Denaturation	98°C	5 s	
Annealing	Primer Specific (56°C)	5 s	42
Extension	72°C	20 s	
Final Extension	72°C	1 min	1

Table 1: Cycling instructions

Results and Discussion

As a part of our parallel backcrossing scheme to transfer *Ph-2* and *Ph-3* genes into 32 University of Florida recurrent parents more than 3,000 $F_1BC_4S_1$ seedlings were genotyped. From this 212 RILs were homozygous for one gene; 152 carrying *Ph-2* and 60 carrying *Ph-3*. All these RILs were confirmed to be resistant to *P. infestans* by the seedling bioassay with resistance levels expected from their genotype (Fig 2). A random collection of 30 RILs from these LB resistant RILs were selected to be genotyped using SolCAP SNPs array (Fig. 2). This collection of resistant RILs; 8 lines homozygous for *Ph-2* and *Ph-3* markers, 11 lines homozygous for *Ph-2* only, 10 lines homozygous for *Ph-3* only and 1 line heterozygous for *Ph-2* only were genotyped using the 7,720 SNPs array. The recurrent resistant parents (*Ph-2* and *Ph-3* homozygous) and the susceptible parents were also compared with these SNPs. Ten and 15 polymorphic SNPs in the regions of interest on chromosome 10 and 9, respectively were identified. According to these data the source *Ph-2* introgression starts from 64.63 and extends beyond 64.77 Mb on chromosome 10, while the original *Ph-3* introgression spans from 70.9 to 71.8 Mb on chromosome 9 (Table 2). Short introgressions were found for RIL-01, RIL-02, RIL-29 and RIL-30 on chromosome 10 and for RIL-26 and RIL-27 on chromosome 9. It seemed that NC 25p has a short introgression on chromosome 9 as well. To confirm this finding the remaining 212 RILs were genotyped using new *Ph-3* SCAR markers and more recombinants with similar short introgressions on chromosomes 10 or 9 but as resistant as Richter's Wild (Ph-2 source) and LA 3707 (Ph-3 source) were identified containing *Ph-2* or *Ph-3*, respectively (data not shown).



Table 2: Polymorphic SolCap SNPs, *Ph-2* and *Ph-3* markers on tomato chromosome 9 and 10 of 30 LB resistant RILs and their susceptible recurrent parents, along with NC1-CELBER, NC-25p, L3707 and Richter's Wild, used to identify short introgressions on the corresponding chromosomes.

		Chromosome	SL2.5	Rich	RIL-18	Fla.7776	RIL-	RIL-	RIL-04	RIL-	RIL-06	Ē	RIL-	RIL-10	RIL-	RIL-12	Fla.7	NC2	L3707	Fla.8000	Fla 8021	RIL-14	RIL-	RIL-	RIL-17			RIL-21	RIL-	Fla 8059	RIL-	RIL-	RIL		RIL	RIL-	RIL-	Fla.8	NC1	Fla.8249	5 9 1	
		BO	30 ()	ter	18	10	202	03	04	Ş,	8	38	3	10	Ė	12 /8	804	SP	5	<u> </u>	35	14	5	16	17	8	120	21	22	202	23	,-24	25	2	3 28	29	3	8111R	Œ	249	22	
Marker ^z	Description ^w	some	SL2.50 (Mbp ^v)	Richter's Wild																																		.8124C	I CELBR			ene
solcap_snp_sl_22329	-	9	70.84	+	-			-	-	-		· -	-	-	-		-	-	-		- -	·	-	-		- -		-	-		-	-	•		-	-	•	• •	+		-	
solcap_snp_sl_69787	-	9	70.94	-	-	- -	· -	-	-	-	- 1	· -	-	-	-		-	+	+	- 1	- L-	· +	-	+	+ -	+ +	+ +	+	+	- -	/	+	+ +	+ +	Ŀ.,	+	+	- -	+		-	
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^z Marker genotypes represented by (+) are homozygous resistant, (/) heterozygous, (-) homozygous susceptible, and (.) are missing data for LB *Ph-2* and *Ph-3* markers. Lines with (RP-No Ph) are recurrent parents susceptible to late blight.

^y Newly designed scar markers.

^x Markers that are linked to the resistance genes and no recombination has been identified.

^w CAPS = Cleaved Amplified Polymorphic Sequence, dCAPS = derived Cleaved Amplified Polymorphic Sequences SCAR = Sequence Characterized Amplified Region, SNP = Single-Nucleotide Polymorphism.

^v Mbp = Mega base pair.

^u R = LB resistance and S = LB susceptible.

In this study 8 additional indels in the *Ph-3* region were found by using the draft genome sequence of the *S. pimpinellifolium* LA1589 accession, and 8 new scar markers were designed by targeting these regions (Table 3). Based on our observations, there was a strong similarity between *S. pimpinellifolium* LA1589 and the *Ph-3* introgression from LA 3707 on Chromosome 9. These new markers extend from 71.22 to 71.91 Mb which is 0.21 to 0.48 Mb upstream and downstream, respectively of the newly mapped *Ph-3* gene located at 71.43 Mbp on Chr. 9 (Zhang et al., 2014). The original cleaved amplified polymorphic sequences (CAPS) *Ph-3* markers, TG328 and TG591, are located at 71.36 and 71.44-71.45 Mb, respectively. These newly designed SCAR markers were useful in screening the larger population of RILs for the shortest introgression on chromosome 9. Furthermore, one sequence characterized amplified region (SCAR) DNA marker, UF-Ph3-5, was only 0.04 Mbp away from the recently mapped *Ph-3* gene (Fig 3).

Table 5. De	.301	iption of one Fir-2 marker and	Seveni		useu in this stut	у
Marker Name		Sequence (5'->3')	Tm	S Product Size	R Product Size	Description
UF-Ph2-1 ^z	F	TTGGGGCAGTGTTGTATTCGT	60	480 & 27 bp	355, 125 & 27 bp	CAPS /Hinf I
	R	TCGACATCTTGAGCTGGTAGG	59			
UF-Ph3-1	F	TGATAAAGAAAAAGGGAAAAATGA	53	105 bp	114 bp	SCAR
	R	CTCTAGTTTTTGAACGGCAG	54			
UF-Ph3-2	F	TCATGCATTGTTTAGCCTGACA	58	131 bp	149 bp	SCAR
	R	ACTGCAAAGAGAATAGGGTTTCCT	59			
UF-Ph3-3	F	ATGTCCCAGTTCCTCCAGGT	60	130 bp	120 bp	SCAR
	R	AGCAATTCCTAAACGTATTGAAGG	57			
UF-Ph3-4	F	TCATCATGCCTTGAGATAAGT	54	149 bp	165 bp	SCAR
	R	AATTAAAGCTGCAAATTCTGA	52			
UF-Ph3-5 ^z	F	TGGAACAATTTTCACCACCCA	58	153 bp	172 bp	SCAR
	R	TGACAAAGGACAAATGCATGG	57			
UF-Ph3-6	F	CCGAACTTGCCAACAAAAAGC	59	1100 bp	380 bp	SCAR
	R	CCTCCCAATTCCCCAACCTG	60			
UF-Ph3-7	F	TTGGACTAGATCATTGGCATCC	57	120 bp	138 bp	SCAR
	R	CACGAGAAGGCATTGGAGGA	59			

Table 3: Descrip	ntion of one Ph	-2 marker and	seven Ph-3 m	arkers used in t	his study
I able J. Deschi	puon or one rn		SEVEN FIFS III	ai kei s useu iii t	ins study

^z These markers are highly associated with the corresponding genes on chromosome 9 and 10.

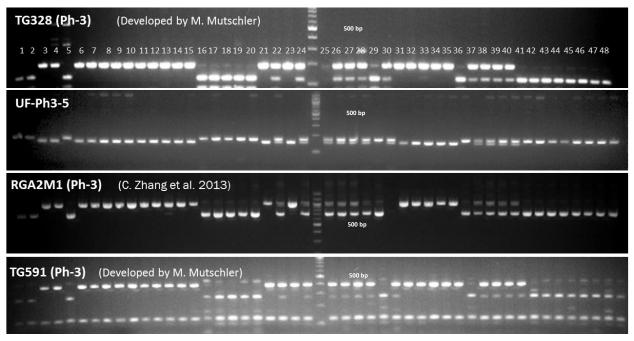


Fig 3: Newly designed molecular marker, UF-Ph3-5, is flanked by TG328 and TG591 markers and highly associated with the *Ph-3* gene on chromosome 9. The germplasm by lane are: 1: NC1 CELBER, 2: NC 25P, 3: Fla.7776, 4: Richter's Wild, 5: LA3707 and 6-15, 21, 23 and 31-35: *Ph-3* homozygous susceptible, 22, 24-28, 30 and 37-40 *Ph-3* heterozygous, 16-20, 29, 36 and 41-48 *Ph-3* homozygous resistant samples.

The *Ph-2* introgression is located at the distal end of chromosome 10 (Moreau et al., 1998) and SolCAP SNPs did not yield any informative polymorphic data. Furthermore, the absence of sequence similarity between the *S. pimpinellifolium*, LA1589 accession (Tomato Genome Consortium, 2012), and the *Ph-2* introgression on Chromosome 10 made marker development more challenging. Instead, a Cc-nbs-Irr resistance gene, Solyc10g085460, located upstream of the original *Ph-2* marker, in three different LB resistant backgrounds including: NC1 CELBER, NC25p, Richter's Wild and one susceptible line, Fla.7776, were resequenced and comparative sequence alignment revealed several polymorphic SNPs on which a new CAPS marker was designed, UF-Ph2-1 (Fig. 4).

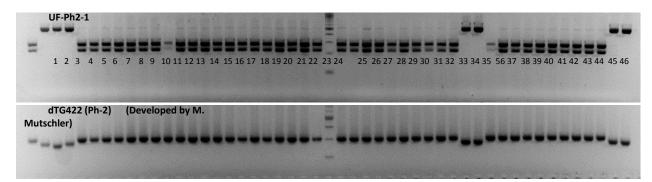


Fig 4: Newly designed molecular marker, UF-Ph2-1, is highly associated with dTG422 (shown) and dTG63 (not shown) and the *Ph-2* gene on tomato chromosome 10. The germplasm by lane are: 1: NC1 CELBER, 2: NC 25P, 3: Fla.7776, 4: LA3707, 5-34 and 37-46 *Ph-2* homozygous resistant e and 35, 36, 47 and 48 are homozygous susceptible.

These new *Ph-2* and *Ph-3* markers have been successfully used in our large scale MAS program; more than 6,000 seedlings have been genotyped and around 1,000 seedlings, carrying different combinations of *Ph-2* and *Ph-3* genes heterozygously, have been phenotyped using the seedling bioassay and no recombination between UF-Ph2-1 and UF-Ph3-5 markers and their respective resistance genes was identified. These new *Ph-2* and *Ph-3* markers may provide more affordable alternatives for small-scale genotyping operations (Table 3). Those with interest should contact Sam Hutton.

References

- AVDRC, 1994. AVDRC Progress Report 1993, Asian Vegetable Research and Development Center, Shanhua, Tainan, Taiwan. pp.201–203.
- Birch P. R. J., Whisson S., 2001. Phytophthora infestans enters the genomics era. Mol Plant Pathol 2, pp.257–263
- Black, L.L., T.C. Wang, P.M. Hanson and J.T. Chen, 1996. Late blight resistance in four wild tomato accessions: Effectiveness in diverse locations and inheritance of resistance. Phytopathology 86(11):S24 (abstr.).
- Foolad, M.R., Merk, H.L. and Ashrafi, H., 2008. Genetics, genomics and breeding of late blight and early blight resistance in tomato. Critical Reviews in Plant Sciences, 27(2), pp.75-107.Fry W. E., Goodwin S. B., 1997. Re-emergence of potato and tomato late blight in the United States Plant Dis 81, pp.1349–1357
- Fulton, T.M., Chunwongse, J. and Tanksley, S.D., 1995. Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Molecular Biology Reporter, 13, pp.207-207.
- Gardner, R.G. and Panthee, D.R., 2010. NC 1 CELBR and NC 2 CELBR: Early blight and late blight-resistant fresh market tomato breeding lines. HortScience, 45(6), pp.975-976.
- Goodwin, S.B., Sujkowski L. S., Fry W. E., 1995. Rapid Evolution of Pathogenicity within Clonal Lineages of the Potato Late Blight Disease Fungus. Phytopathol. 85, pp.669– 676.
- Ji Y., Scott J.W., Schuster D.J., Maxwell D.P., 2009. Molecular mapping of Ty-4, a new Tomato yellow leaf curl virus resistance locus on chromosome 3 of Tomato. Journal of the American Society for Horticultural Science 134: 281–288. doi: 10.1007/s11032-007-9102-1Kamoun S., Smart C. D., 2005. Late blight of potato and tomato in the genomics era. Plant Dis 89, pp.692–699
- Ko W. H., 1994. An alternative possible origin of the A2 mating type of Phytophthora infestans outside Mexico. Phytopathology 84, pp.1224-1227.
- Moreau, P., Thoquet, P., Olivier, J., Laterrot, H. and Grimsley, N., 1998. Genetic mapping of Ph-2, a single locus controlling partial resistance to Phytophthora infestans in tomato. Molecular Plant-Microbe Interactions, 11(4), pp.259-269.Pedersen, W.L. and Leath, S., 1988. Pyramiding major genes for resistance to maintain residual effects. Annual Review of Phytopathology, 26(1), pp.369-378.
- Peirce, L. C. 1971. Linkage tests with Ph conditioning resistance to race 0, Phytophthora infestans. Tomato Genet. Co-op. Rep. 21, pp.30.
- Robbins, M.D., Masud, M.A., Panthee, D.R., Gardner, R.G., Francis, D.M. and Stevens, M.R., 2010. Marker-assisted selection for coupling phase resistance to tomato spotted wilt virus and Phytophthora infestans (late blight) in tomato. HortScience, 45(10), pp.1424-1428.Smart C. D., Fry W. E., 2001. Invasions by the late blight pathogen: renewed sex and enhanced fitness. Biol Invasions 3, pp.235–243
- Tomato Genome Consortium, 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature, 485(7400), pp.635-641.

- Vidavski, F., Czosnek, H., Gazit, S., Levy, D. and Lapidot, M., 2008. Pyramiding of genes conferring resistance to Tomato yellow leaf curl virus from different wild tomato species. Plant Breeding, 127(6), pp.625-631.
- Yang, W., and D. M. Francis. 2005. Marker-assisted selection for combining resistance to bacterial spot and bacterial speck in tomato. Journal of the American Society for Horticultural Science 130, pp.716–721.
- Zhang, C., Liu, L., Wang, X., Vossen, J., Li, G., Li, T., Zheng, Z., Gao, J., Guo, Y., Visser, R.G. and Li, J., 2014. The Ph-3 gene from Solanum pimpinellifolium encodes CC-NBS-LRR protein conferring resistance to Phytophthora infestans. Theoretical and Applied Genetics, 127(6), pp.1353-1364.

Breeding Tomato (*Solanum lycopersicum* L.) Varieties for Tolerance to Tomato Yellow Leaf Curl Virus (TYLCV) Disease

Mohamed Suliman Mustafa Faculty of Agriculture, Nile Valley University, Sudan, Tel.: +249919431494 E-mail: <u>bd1966mar8@gmail.com</u>

Abstract

Fifteen tomato genotypes were screened for tolerance to tomato yellow leaf curl virus (TYLCV) disease during season 2001/2002 at EL Hudeiba Research Station Experimental Farm, 290 Km north of Khartoum, Sudan. Two breeding lines FLA505 (BL1172) and F3 DRD8071 were selected as tolerant to TYLCV disease. FLA505 (BL1172) was artificially crossed pollinated with the cultivar Peto 86 PS. Pedigree method of selection was adopted. Five elite breeding lines tolerant to TYLCV disease were selected, named (BL03, BL04, BL05, BL06 and BL07). F3 DRD8071 was let for normal self pollination. The pedigree method of selection also was adopted. One elite breeding line tolerant to TYLCV disease named (BL012) was selected. The six lines were tested in variety trials conducted at three locations (El Hudeiba at northern Sudan, Khashm El Girba at eastern Sudan and Um Dom at central Sudan) for two seasons (2007/2008 and 2008/2009). BL03 and BL012 showed tolerance to TYLCV disease and high fruit yield compared to the check standard cultivars Strain-B and Baladiya.

ملخص ملخص خمسة عشر صنف وسلالة من الطماطم تمت غربلتهم لمقاومة مرض تجعد أور اق الطماطم الأصفر الفيروسي خلال موسم 2001/ 2002م بالمزرعة التجريبية لمحطة بحوث الحديبة بالدامر, 290 كيلومتر شمال الخرطوم . تم انتخاب السلالتين (2012م المزرعة التجريبية لمحطة بحوث الحديبة بالدامر, 290 كيلومتر شمال الخرطوم . تم انتخاب السلالتين (2012 BL 505 (BL 1172) و DRD7071 كسلالتين مقاومتين للمرض المذكور أعلاه. السلالة باستعمال طريقة انتخاب النسب تم انتخاب خمسة سلالات تربية مقاومة للمرض أعلاه وهي محمل الانعز الية الماستعمال طريقة انتخاب النسب تم انتخاب خمسة سلالات تربية مقاومة للمرض أعلاه وهي BL03, BL04, BL05, BL05 باستعمال طريقة انتخاب النسب تم انتخاب خمسة سلالات تربية مقاومة المرض أعلاه وهي BL03, BL04, BL05, BL05 باستعمال طريقة انتخاب النسب تم انتخاب خمسة سلالات تربية مقاومة للمرض أعلاه وهي BL03, BL04, BL05, BL05 باستعمال طريقة انتخاب النسب تم انتخاب خمسة سلالات تربية مقاومة للمرض أعلاه وهي BL03, BL04, BL05, BL05, BL05, BL05 باريقة انتخاب النسب ايضا. تم انتخاب معالة واحدة مقاومة للمرض أعلاه وهي BL05, BL05, BL05, BL05 و يعن ثم تمت متابعة الأجيال الانعز الية ودر استها باستعمال طريقة انتخاب النسب ايضا. تم انتخاب سلالة واحدة مقاومة للمرض أعلاه وهي BL05 . السلالات الست تم اختبار ها في عرارب تقويم أصناف لموسمين هما (2007/2007 و 2009/2008) بكل من الحديبة ' خشم القربة وأم دوم. السلالتان عارية بالصنفين المزروعين BL03 والبلدية. و BL03 و BL03 أعطنا أعلى إنتاجية للمحصول وأظهرتا مقاومة جدة لمرض تجعد أور اق الطماطم الأصفر الفيروسي مقارنة بالصنفين المزروعين Brain B والبلدية.

Introduction

The commercial tomato belongs to a species referred to as *Solanum lycopersicum* L. (David *et al.*, 2005). It is one of the most popular and widely consumed vegetable crops throughout the world, both for the fresh fruit market and the processed food industry. Several fungal, bacterial and viral diseases have directly hampered the cultivation of tomato in eastern and southern Africa (AVRDC reports 1995, 1996 and 1997). The most serious disease of tomato throughout the Mediterranean region, the Middle-East and tropical region of Africa and Asia is tomato yellow leaf curl virus (TYLCV) which is caused by a geminivirus transmitted by the whitefly, *Bemisia tabaci* (Kasrawi, 1991). TYLCV was endemic throughout the Sudan with epidemic often reaching 100% and disease incidence was greater during the summer and autumn seasons than during winter Yassin (1975).

Considerable progress has been made to identify resistance sources to TYLCV by introgressing genes from the wild species of tomato especially *S. chilense, S. peruvianum* and *S. habrochaites* (previously known as *L. hirsutum*) (Vidavski *et al.*, 2008). Five genes conferring resistance to TYLCV have been reported, which included *Ty-1* from *S. chilense* LA 1969 (Zamir *et al.*, 1994). *Ty-2* from *S. habrochaites* (Hanson *et al.*, 2006), *Ty-3* from *S. chilense* LA 2779 (Ji and Scott, 2006). *Ty-4* from *S. chilense* LA1932, LA2779 and LA1938 and *Ty-5* from *S. peruvianum* (Ji *et al.*, 2008).

In the Sudan, the first breeding program for resistance to TYLCV was started at Sennar Station, Agricultural Research Corporation, Sudan (ARC). The program released two open pollinated varieties tolerant to TYLCV under the names Sennar-1 and Sennar-2 (Geneif, 1984).

The present breeding program aimed at breeding tomato variety/s tolerant to TYLCV and has good fruit yield and quality under open field condition in the Sudan.

Materials and Methods

Screening

Germplasm used in this study were received from Asian Vegetable Research and Development Centre (AVRDC) at Taiwan, National Institute for Promotion of Horticultural Exports (NIPHE) at University of Gezira, Sudan and seed shop (table 1). Screening for tolerance to TYLCV disease was conducted under open field condition during the period from October 2001 to March 2002 at EL Hudeiba Research Station Experimental Farm, 290 Km north of Khartoum, Sudan. Plants were grown in infected field naturally infested by *B. tabaci* and occurrence of the disease symptoms was recorded. Ample supply of viruliferous whiteflies was insured by growing two rows of the tested genotypes in between two rows of susceptible checks that were directly seeded four weeks earlier.

The disease severity was scored on a 1-3 scale as described by Kasrawi (1989) as follows:

- 1 = no observed symptoms (resistant).
- 2 = mild symptoms not affecting or slightly affecting the plant growth and vigour (tolerant).
- 3 = severe symptoms include leaf size reduction, leaf curling, between veins yellowing, and plant stunting (susceptible).

Breeding program

Selected line, from the screening experiment, which was tolerant to TYLCV, was artificially crossed pollinated with the cultivar Peto 86 PS and the segregating generations were followed by pedigree method of selection. Many plants of other selected line were let for normal self pollination and the segregating generations were also followed by pedigree method of selection (table 2).

Selected breeding lines were evaluated in replicated multi-locations trials. The variety trials were conducted at EL Hudeiba at northern Sudan, Khashm El Girba at eastern Sudan and Um Dom at central Sudan. At each location a trial was carried out in the winter season of the years 2007/2008 and 2008/2009. The six selected breeding lines with two check cultivars (Strain B and Baladiya) were grown in randomized complete block design (RCB) with three replications. Plot size was 8 meters x 3.6 meters with spacing of 0.5 meter

between plants. All technical packages recommended by Agricultural Research Corporation, Sudan (ARC), for tomato cultivation were done. Statistical analyses were performed using the software packages SAS version 9 and MSTATC.

Results and Discussion

Accessions FLA505 (BL1172) and F3 DRD8071 were selected as tolerant to TYLCV disease. Artificial cross pollination between FLA505 (BL1172) and Peto 86 PS followed by pedigree method of selection resulted in five breeding lines tolerant to TYLCV disease, named BL03, BL04, BL05, BL06 and BL07. Self pollination of F3 DRD8071 followed by pedigree method of selection was resulted in one breeding line tolerant to TYLCV disease named BL012. Results obtained from the trials conducted at the three locations during the two years are shown in (table 3). Highly significant ($P \le 0.01$) differences among the tested genotypes were detected at the three locations in the two years. The breeding line BL03 showed the highest fruit yield in the three locations in the two seasons except in the year 2007/2008 at Um Dom. The breeding line BL012 showed significant differences from the check cultivars Strain B and Baladiya in terms of fruit yield per unit area. Tolerance to TYLCV in BL03 is due to the presence of TYLCV resistance genes inherited from S. chilense through FLA505 (BL1172). DRD8071 is already recommended by INRA, France as tolerant to TYLCV (Prof. Ali Elamin Eljack, Faculty of Agricultural Sciences University of Gezira, Sudan, Personal communication, 2001). The program suggested following the segregating population of F3 DRD8071 through pedigree method of selection without any artificial crossing to any other variety because of the good fruit quality and high yield of many of the plants in the segregating generations.

From the combined analysis of variance (table 4), significant differences ($P \le 0.05$) were detected for fruit yield (ton/hectare). Least significant difference (LSD) for the genotypes mean yield showed that line BL03 had the highest mean value, averaged over the three locations (table 5). Line BL012 showed significant difference ($P \le 0.05$) from the two check varieties Strain B and Baladiya.

Because the variance due to genotype x environmental interaction was found significant stability of genotypes yield was assessed. Considering the values of linear regression of mean genotype yield on environmental means and coefficient of determination, BL012 and BL03 appear to be stable (table 6).

Conclusion

On the basis of tolerance to TYLCV disease and fruit yield, I recommend that the two breeding lines BLO3 and BLO12 be released for tomato production in central and northern Sudan.

No.	Entry	Resistance source	Source
1	TY52 (990106)	Ty-1 allele from LA1969 (S. chilense)	AVRDC
2	FLA 456-4 (990109)	Tyking, LA2779 (S. chilense)	AVRDC
3	FLA505 (BL1172)	LA1969 (S. chilense), Tyking, Fiona	AVRDC
4	FLA478-6-3-1-11(23404-0)	LA1938 (S. chilense), Tyking)	AVRDC
5	FLA653-3-1-0 (16611-1)	LA2779 (S. chilense); Tyking	AVRDC
6	FLA496-11-6-1-0(23422-0)	LA1932	AVRDC
7	99S-C-39-20-11-24-17-0(23430)	Unknown	AVRDC
8	H24	Unknown S.hirsutum accession	AVRDC
9	TLB111	H24	AVRDC
10	CLN2026D	Check	AVRDC
11	F3 Destina10	Unknown	NIPHE
12	F3 DRD8071	Unknown	NIPHE
13	Strain B	check	Seed shop
14	Peto 86 P.S.	check	Seed shop
15	Baladia	check	Seed shop

Table 1: Tomato germplasm screened for tolerance to TYLCV disease during season 2001/2002

AVRDC=Asian Vegetable Research and Development Centre, Taiwan

NIPHE = National Institute for Promotion of Horticultural Exports, University of Gezira, Sudan

Generation	Season	Procedure
Parental	2001/2002	Crossing between the selected parents
F1	2002/2003	Growing of F1 plants as bulk
F2	2003/2004	Growing of more than 1000 F2 plants separately
F3	2004/2005	Growing of 100 families resulted from the selected F2 plants
F4	2005/2006	Growing of the families resulted from the selected F3 plants from the selected families
F5	2006/2007	Growing of new families resulted from the selected F4 plants.
F6	2007/2008	First variety trail
F7	2008/2009	Second variety trail

Table (2): Summary of the pedigree method of selection adopted during this breeding program

Table 3: Fruit yield (ton/ hectare) of eight tomato genotypes over three locations and two seasons

										1	-		
	Year		Genotype							F.	S.E	C.V	LSD
L											(±)	(%)	0.05
		В	В	BL	BL	BL	BLO	Stria	Bala				
		L03	L04	05	06	07	12	n B	diya				
Н	2007/	12.39	9.11	6.17	17.7	20.3	26.8	23.5	15.0	**	1.56	12.8	4.74
	2008												
	2008/	14.74	14.15	9.11	30.8	32.4	34.9	34.6	27.7	**	0.60	3.3	1.82
	2009												
U	2007/	6.93	6.80	6.38	17.9	16.8	13.3	11.9	11.4	**	1.37	16.1	4.19
	2008												
	2008/	6.132	4.24	5.83	14.1	10.1	10.6	11.8	9.5	**	1.47	21.6	4.46
	2009												
К	2007/	7.35	4.11	2.68	8.3	6.4	13.7	12.7	8.3	**	1.28	20.8	3.10
	2008												
	2008/	4.28	3.65	1.59	7.4	7.7	9.2	9.2	6.1	**	0.61	13.4	3.1
	2009												

Key: L= Location, H= Hudeiba, U= Um Dom, K= Khashm el Girba,

F= Fisher test value, **= significant at the 1% level of probability

Table 4: Combined analysis of variance for the genotypes mean yield (ton/ hectare) combined over three locations and two seasons of eight tomato genotypes

source	Degr	Sum of	Mean of	F.	F. test
	ee of	squares	squares	value	
	freedom				
Year (Y)	1	181.214	181.214	40.72261	**
Location (L)	2	3176.047	1588.023	356.8621	**
YL	2	427.804	213.9022	48.06824	**
R(LY)	12	146.7421	12.2283	2.748018	**
Variety (V)	7	253.1647	36.1662	8.127336	**
YV	7	43.22976	6.17568	1.387806	**
LV	14	303.4756	21.67704	4.871244	**
YLV	14	82.29648	5.87832	1.320984	**
Error	84	156.9947	1.869	40.72261	* *
Total	143	4770.969			

Table 5: Least significant difference (LSD) for the genotypes mean yield (ton/ hectare) combined over three locations and two seasons of eight tomato genotypes.

Genotype	Mean yield (ton / hectare)
BLO3	8.643
BLO7	6.993
BLO6	6.955
BL012	6.942
BLO4	6.728
Strain B	6.640
BL05	6.598
Baladiya	5.262

S.E (±) = 0.208 LSD_{0.05}= 0.587 Table 6: Stability Parameters for Yield of eight tomato genotypes combined over three locations and two seasons.

Breeding Line	Regression	Coefficient of
Dieeding Lille	Negi ession	coefficient of
	coefficient (b)	determination (R ²)
BL03	0.921	0.968
BL04	1.174	0.943
BL05	1.066	0.926
BL06	1.256	0.988
BL07	0.667	0.829
BL012	1.057	0.923
Strain B	1.277	0.975
Baladiya	0.582	0.984

References

- Abu-Gharbieh, W.I.; Makkouk, K.M. and Saghir, A.R. (1978) Plant diseases reports, 62:263-266.The American phytopathological society, ISSN 2330-3123.
- AVRDC (Asian Vegetable Research and Development Center) (1997), Progress report 1997, Shanhua, Taiwan.
- AVRDC (Asian Vegetable Research and Development Center) (1996), Progress report 1996, Shanhua, Taiwan.
- AVRDC (Asian Vegetable Research and Development Center) (1995), Progress report 1995, Shanhua, Taiwan.
- David, M.S.; Iris, E.P. and Sandra, K. (2005) Comparison of AFLPs with other markers for phylogenetic inference in wild tomatoes (*Solanum* L. section *Lycopersicon* (Mill.) Wettst.), Taxon-Journal of international association for plant taxonomy (IAPT), 54(1): 43-61.
- Geneif, A.A. (1984) Breeding for resistance to tomato yellow leaf curl virus in tomatoes in the Sudan, Acta Horticulturae, 143:469-484.
- Hanson, P.M., Green, S.K. and Kuo, G. (2006) *Ty-2,* gene on chromosome 11 conditioning geminivirus resistance in tomato, Report. Tomato Genetic Cooperative, volume 56.
- Ji, Y., Scott, J.W., Maxwell, D.P. and Schuster, D.J. (2008) *Ty-4*, a tomato yellow leaf curl virus resistance gene on chromosome 3 of tomato, Tomato Genetic Cooperative Reports, 58:29-31.
- Ji, Y. and Scott, J.W. (2006b) Ty-3, a begomovirus resistance locus linked to Ty-1 on chromosome 6, Report of Tomato Genetic Cooperative, volume 56.
- Kasrawi, M.A. (1991) Tomato production and yellow leaf curl viruses in London, In: Resistance of the tomato to TYLCV, Proceeding of Seminar of EEC Contract DGX-TS2-055F (CD) Partners. 14-16.
- Vidavsky, F., Czosnek, H., Gazit, S., Levy, D. and Lapidot, M. (2008) Pyramiding of genes conferring resistance to Tomato yellow leaf curl virus from 5 different wild tomato species, Plant Breeding Journal, 1-7, Blackwell Verlag, Berlin.
- Yassin, A.M. (1975) Epidemic and chemical control of leaf curl disease of tomato in the Sudan. Agricultural Experiments reports, ARC,Sudan.11: 161-165.
- Zamir, D., Ekstein-Michelson, I., zakay, Y., Navot, N., Zeidan, M., Sarfatti, M., Eshed, Y., Harel, E., Pleban, T., Van Oss, H., Keder, N., Rabi-nowitch, H.D. and Czosnek, H. (1994) Mapping and introgression of tomato yellow leaf curl virus tolerance gene, TY-1, Theoretical and Applied Genetics, ISSN 0040-5752, 88:141-146.

Revised List of Miscellaneous Stocks

Roger T Chetelat

C.M. Rick Tomato Genetics Resource Center, Dept. of Plant Sciences, University of California, Davis, CA 95616

This list of approximately 1,790 miscellaneous genetic stocks is a revision of the previous one issued in TGC 62 (2012). Extinct, obsolete, or faulty accessions have been dropped and recently acquired accessions have been added. The new stocks include a set of 148 recombinant inbred lines from the cross *S. lycopersicum* NC EBR-1 x *S. pimpinellifolium* LA2093, synthesized by Prof. Majid Foolad at Penn State University. A group of trichome chemistry mutants induced by EMS treatment of M-82 were isolated by Prof. Rob Last at Michigan State Univ. Other new lines include stocks of *Abg, B,* and *Lac* from *S. lycopersicoides* bred into *S. lycopersicum*, and the cultivars NC EBR-1 and Reinmenn Phillippe (a parthenocarpic variety).

We attempt to maintain all listed accessions in adequate seed supply for distribution. However, some stocks, including some multiple marker combinations, aneuploids, and prebreds, are weak and require special cultural care; consequently, seed supplies may at times be too low to permit distribution. Other accessions may be temporarily unavailable during seed regeneration or for other reasons.

Names and phenotypic classes of individual mutations are given in our most recent Monogenic Stocks List (see TGC 64). Additional information is available through our website (<u>http://tgrc.ucdavis.edu</u>).

Types of Miscellaneous Genetic Stocks

- 1. Modern and Vintage Cultivars
- 2. Latin American Cultivars
- 3. Introgression Lines
- 4. Recombinant Inbred Lines
- 5. Alien Substitution Lines
- 6. Monosomic Alien Addition Lines
- 7. Other Prebred Lines
- 8. Interspecific Hybrids
- 9. Stress Tolerant Stocks

- 10. Translocations
- 11. Trisomics
- 12. Autotetraploids
- 13. Cytoplasmic Variants
- 14. Chromosome Marker Stocks
- 15. Linkage Screening Testers
- 16. Miscellaneous Marker Combinations
- 17. Provisional mutants

1. Modern and Vintage Cultivars (210 accessions)

We maintain the following set of cultivars, inbreds, and breeding lines for various purposes, mainly as isogenic or nearly isogenic stocks for specific mutants, standards for genetic comparison, sources of disease resistances, or other purposes. Marglobe is considered the standard for tomato gene (mutant) nomenclature. Most lines have been maintained by selfing for many generations.

LA	Cultivar	LA	Cultivar	LA	Cultivar
LA0818	A-1	LA3244	Antimold-B	LA4451	Black Cherry
LA0516	Ace	LA3527	Apex 1000	LA4449	Black Plum
LA2838A	Ailsa Craig	LA0657	Beaverlodge	LA1499	Break O'Day
LA2463	Allround	LA2973	Big Rainbow	LA4346	Bryan Self-Topper
LA0655	Anahu	LA2972	Big Yellow Red Ctr.	LA3341	C5
LA1995	Angela	LA4347	B-L-35	LA0198	Cal 255

LA	Cultivar	LA	Cultivar	LA	Cultivar
LA2414	Cal Ace	LA1089	John Baer	LA3321	Ohio 7663
LA0337	Calif. Red Cherry		Kallio's Alaskan	LA1088	Ohio Globe A
LA1439	Calmart	LA1131	Dwarf	LA2447	Ontario 7717
LA3316	Campbell 24	LA0025	King Humbert #1	LA2449	Ontario 7517
LA3317	Campbell 28	LA3240	Kokomo	LA2396	Ontario 7710
LA3228	Canary Export	LA3526	L04012	LA2448	Ontario 7818
LA2374	Caro Red	LA0505	Laketa	LA2970	Orange, Red Ctr.
LA2400	Castlemart	LA3203	Large Plum	LA4450	Paul Robeson
LA3121	Chico Grande	LA3118	Laurica	LA0012	Pearson
LA4285	CLN2264F	LA0791	Long John	LA0020	Pennheart
LA4286	CLN2264G	LA0534	Lukullus	LA3528	Peto 95-43
LA3213	Columbian	LA3475	M-82	LA3243	Platense
LA0533	Condine Red	LA3120	Malintka 101	LA3312	Platense
LA0817	CP-2	LA3007	Manapal		Pomodorini
LA3247	Craigella	LA0502	Marglobe	LA3125	Napolitan
LA1219	Dwarf San Marzano	LA1504	Marmande	LA2715	Porphyre
LA0313	Dwarf Stone	LA0278	Marzano Grande	LA3820	Potentate
LA3245	E.S.1	LA3151	Mecline	LA3903	Primabel
LA4024	E-6203		Michigan State	LA0089	Prince Borghese
LA3238	Earliana	LA0011	Forcing	LA3233	Pritchard
LA2006	Earlinorth	LA3911	Micro-Tom	LA3229	Prospero
LA3010	Earlipak	LA2825	Mobaci	LA2446	Purdue 135
LA0266	Earlipak	LA2824	Moboglan	LA2377	Purple Calabash
LA0517	Early Santa Clara	LA3152	Moboline	LA2378	Purple Smudge
LA2711	Edkawi	LA2821	Mobox	LA4350	Red River
LA3800	Fargo Self-pruning	LA2830	Mocimor	LA0276	Red Top VF
LA3801	Farthest North	LA3471	Mogeor	LA3129	Rehovot 13
LA3024	Fireball	LA2828	Momor	LA4443	Reinmenn Phillippe
LA3242	Flora-Dade	LA2829	Momor Verte		Rey de Los
LA3840	Florida 7060	LA2818	Monalbo	LA2356	Tempranos
LA4026	Florida 7481	LA2706	Moneymaker	LA0535	Rheinlands Ruhm
LA4025	Florida 7547	LA2819	Monita	LA3343	Rio Grande
LA4442	Florida 8516	LA2713	Montfavet 167	LA3145	Rockingham
LA3030	Gardener	LA2714	Montfavet 168	LA0503	Roumanian Sweet
LA2969	Georgia Streak	LA2827	Moperou	LA3214	Rowpac
LA2802	Globonnie	LA2822	Mossol	LA2088	Royal Red Cherry
LA4355	Gold Nugget	LA2820	Motabo	LA3215	Roza
LA4011	GT	LA2826	Motaci	LA1090	Rutgers
LA3231	Gulf State Market	LA2823	Motelle	LA2662	Saladette
LA0314	Hardin Miniature	LA3472	Movione	LA3216	Saladmaster
LA4441	Hawaii 7981	LA2661	Nagcarlang	2-297	San Marzano
LA3202	Hawaii 7997	LA4354	NC 84173	LA3008	San Marzano
LA3856	Hawaii 7998	LA4504	NC EBR-1	LA0180	San Marzano
LA4345	Heinz 1706-BG	LA3845	NC EBR-5	LA2375	San Marzano
LA0806	High Crimson	LA3846	NC EBR-6	LA1021	Santa Cruz B
LA3237	Homestead 24	LA3847	NC HS-1	LA2413	Severianin
LA3320	Hotset	LA3625	NC265-1 (93)-3-3	LA2912	Short Red Cherry
LA3144	Hunt 100		New Hampshire	LA3234	Sioux
LA2805	Indehiscent Currant	LA3802	Victor	LA4444	Stack's Cherry
LA3201	IRB 301	LA2009	New Yorker	LA3632	Start 24
-		LA4452	Nyagous		

LA	Cultivar	LA	Cultivar	LA	Cultivar
	Stemless	LA2939	UC-T338	LA1222	VF145 78-79
LA0030	Pennorange	LA2940	UC-TR44	LA0742	VF-34
LA2443	Stirling Castle	LA2941	UC-TR51	LA0490	VF-36
LA1091	Stokesdale	LA0021	Uniform Globe	LA0743	VF-6
LA1506	Stone	LA2445	V-121	LA2086	VFN Hi Sugar
LA4432	Sunseeds 1642	LA0745	V-9 Red Top	LA0815	VFN-14
LA0164	Sutton's Best of All	LA3246	Vagabond	LA1022	VFN-8
LA2399	T-5	LA3905	Vantage	LA1221	VFNT Cherry
LA2590	T-9	LA3122	Vendor	LA3630	Vrbikanske nizke
LA0154	Tiny Tim	LA2968	Vendor (Tm-2a)	LA3465	Walter
LA1714	UC-134	LA2971	Verna Orange	LA0279	Webb Special
LA4437	UC-204B	LA2444	Vetomold	LA2464A	White Beauty
LA3130	UC-204C	LA0744	VF-11	LA2804	Yellow Currant
LA1706	UC-82	LA1023	VF-13L	LA2357	Yellow Peach
LA2937	UC-MR20	LA1507	VF-145 21-4	LA3148	Zemer Kau
LA2938	UC-N28	LA0816	VF-145 22-8		

2. Latin American Cultivars (252)

This collection of Latin-American cultivars has been assembled from various sources but principally from our collecting trips, often at local markets. With a few exceptions they are indigenous in the sense that they are not recently introduced lines. Many of them are extinct in the source region, having been replaced by modern cultivars.

Country	Collection Site	LA	Country	Collection Site	LA
Bolivia	Santa Cruz	LA0172			LA0408-
Bolivia	Coroica	LA2699	Ecuador	Guayaquil	LA0410
Bolivia	Chamaca	LA2871	Ecuador	Daular	LA0415
Bolivia	Lote Pablo Luna	LA2873	Ecuador	Puna	LA0416
Bolivia	Playa Ancha	LA2874	Ecuador	Puna Polvora	LA0417
Brazil	Coop. Agric. Cotia	LA1021	Ecuador	San Cristobal Island	LA0423
Brazil	Florianopolis	LA2402	Ecuador	Puyo	LA1224
Chile	Hacienda Rosario	LA0466	Ecuador	Viche	LA1238
Chile	Lluta Valley	LA0467			LA1239-
Chile	Iquique	LA0468	Ecuador	Esmeraldas	LA1241
		LA0356-	Ecuador	Boca de los Sapos	LA1244
Colombia	Buenaventura	LA0358	Ecuador	Loja	LA1249
Colombia	Villa Hermosa	LA1425	Ecuador	Loja – La Toma	LA1250
Colombia	Cali	LA1426	Ecuador	Loja	LA1251
Colombia	Cali to Popayan	LA1539	Ecuador	Lago Agrio	LA1420
Colombia	El Paramillo	LA2696	Ecuador	Santa Cecilia	LA1421
	Vereda Mata de		Ecuador	El Naranjo	LA2094
Colombia	Cana	LA2697	Ecuador	Chuchumbetza	LA2132
	Vereda La				LA2381-
Colombia	Esperanza Belgica	LA2698	Ecuador	Malacatos	LA2384
Costa Rica		LA1215	Ecuador	Malacatos	LA3126
		LA3453A	Ecuador	Santa Rosa	LA3624
Costa Rica	Turrialba	-3453D	El Salvador	Comasagua	LA0763
Cuba		LA1162	El Salvador	Cojutepeque	LA0765
Ecuador	Quito	LA0126	El Salvador	San Salvador	LA1210
Ecuador	Santa Cruz Island	LA0292	El Salvador	San Salvador	LA1211

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Country	Collection Site	LA
Guatemala	Quetzaltenango	LA0767
Guatemala		LA0767
	Antigua	
Honduras	Tegucigalpa	LA0147
Honduras	Tegucigalpa	LA0148
Mexico	Mexico City	LA0146
Mexico	Vera Cruz	LA1218
Mexico	Huachinango	LA1459
Mexico	Merida	LA1462
Mexico	Xol Laguna	LA1544
Mexico	Papantla	LA1546
Mexico	Culiacan	LA1564
Mexico	Val. nacionale	LA1565
Mexico	Val. nacionale	LA1566
Mexico	Sinaloa	LA1567
Mexico	Yucatan	LA1568
Mexico	Sinaloa	LA1702
Mexico	Rio Tamesi	LA1703
Mexico	Rio Tamesi	LA1704
Mexico	Tamaulipas	LA1994
Mexico	Guaco, Culiacan	LA2083
Mexico	Comala, Culiacan	LA2084
Nicaragua		LA1212
Nicaragua		LA1213
Panama		LA1216
Panama		LA1217
Panama	Cerro Azul	LA1570
Peru	Hacienda Calera	LA0113
Peru	Chiclayo	LA0116
Peru	Piura	LA0117
Peru	Trujillo	LA0125D
Peru	Arequipa	LA0131H
Peru	Ayacucho	LA0134C
	Луасасно	LA0393-
Peru	Chiclayo	LA0395
	omolayo	LA0401-
Peru	Piura	LA0405
Peru	Tacna	LA0457
Peru	Tacna	LA0472
Peru	Calana	LA0472
Peru	Chincha	LA0477
Peru	Chincha	LA0477
	Convento de Sivia,	LA0478 LA1313-
Peru	Pichari	1313-6
Peru	Ayna, San Francisco	LA1315
Peru	La Molina	LA1390
Peru	Iquitos	LA1390
		LA1397 LA1398
Peru	Iquitos Puerto Maldonado	
Peru		LA1632
Peru	Fundo Bogotalla	LA1650
Peru	Tarapoto	LA1655
Peru	Jahuay	LA1669

Country	Collection Site	LA
Peru	Kradolfer Chacra	LA1698
Peru	Trujillo	LA1701
		LA1976A
Peru	Calana	-1976C
Peru	Iquitos	LA1988
		LA2207-
Peru	Bajo Naranjillo	LA2212
		LA2213-
Peru	Nueva Cajamarca	LA2220
		LA2221-
Peru	Moyobamba	LA2235
		LA2237-
Peru	La Habana	LA2244
		LA2245-
Peru	Soritor	LA2253
		LA2254-
Peru	Puerto Moyobamba	LA2256
	Hotel Abricias,	
Peru	Moyobamba	LA2257
Peru	Yantalo	LA2258
		LA2259A
Peru	Moyobamba	-2259D
		LA2260-
Peru	Lahuarpia	LA2264
	Casaria de	LA2265-
Peru	Pacaisapa	LA2268
	Km 57 from	LA2269-
Peru	Tarapoto	LA2276
_		LA2278-
Peru	Tabalosas	LA2282
_	-	LA2283-
Peru	Tarapoto	LA2307
_		LA2309-
Peru	Puerto Santa Cruz	LA2311
Peru	Sargento	LA2316
Peru	Mangual Pucallpa	LA2622
Peru	Pucalepillo Pucallpa	LA2623
D		LA2665,
Peru	San Juan del Oro	LA2666
Peru	San Juan del Oro	LA2676
Peru	Chinuna	LA2841
Peru	Santa Rita	LA2842
Peru	Moyobamba	LA2843
Peru	Shanhao	LA2844
Peru	Moyobamba	LA2845
		LA3221-
Peru	San Isidro	LA3326
Peru	Puente Tincoj	LA3646
Sri Lanka	Kandy	LA2703

3. Introgression Lines

3.1. S. pennellii Introgression Lines (84)

The following group of introgression lines (ILs) was developed by Y. Eshed and D. Zamir (*Euphytica* 79:175; TGC 49:26). Each IL is homozygous for a single introgression from *S. pennellii* (LA0716) in the background of cv. M-82 (LA3475). (IL 8-1 is heterozygous for a short and a long introgression.) The entire *pennellii* genome is thereby represented by 50 lines with overlapping introgressions. Recombinant sublines provide increased mapping resolution in some regions. (The IL 5-4 sublines are described in *Amer. J. Bot.* 94: 935 and *Theor. Appl. Genet.* 117: 221.)

LA	Line	LA	Line	LA	Line	LA	Line
LA4028	IL 1-1	LA4049	IL 4-1-1	LA4064	IL 7-1	LA4086	IL 9-3-2
LA4029	IL 1-1-2	LA4050	IL 4-2	LA4065	IL 7-2	LA4087	IL 10-1
LA4030	IL 1-1-3	LA4051	IL 4-3	LA4066	IL 7-3	LA4088	IL 10-1-1
LA4031	IL 1-2	LA4052	IL 4-3-2	LA4067	IL 7-4	LA4089	IL 10-2
LA4032	IL 1-3	LA4053	IL 4-4	LA4068	IL 7-4-1	LA4090	IL 10-2-2
LA4033	IL 1-4	LA4054	IL 5-1	LA4069	IL 7-5	LA4091	IL 10-3
LA4034	IL 1-4-18	LA4055	IL 5-2	LA4070	IL 7-5-5	LA4092	IL 11-1
LA4035	IL 2-1	LA4056	IL 5-3	LA4071	IL 8-1	LA4093	IL 11-2
LA3480	IL 2-1	LA4057	IL 5-4	LA4072	IL 8-1-1	LA4094	IL 11-3
LA4036	IL 2-1-1	LA4434	IL 5-4-1	LA4073	IL 8-1-3	LA4095	IL 11-4
LA4037	IL 2-2	LA4435	IL 5-4-2	LA4074	IL 8-2	LA4096	IL 11-4-1
LA4038	IL 2-3	LA4436	IL 5-4-4	LA4075	IL 8-2-1	LA4097	IL 12-1
LA4039	IL 2-4	LA4439	IL 5-4-5-137	LA4076	IL 8-3	LA4098	IL 12-1-1
LA4040	IL 2-5	LA4429	IL 5-4-5-44	LA4077	IL 8-3-1	LA4099	IL 12-2
LA4041	IL 2-6	LA4430	IL 5-4-5-49	LA4078	IL 9-1	LA4100	IL 12-3
LA4042	IL 2-6-5	LA4438	IL 5-4-8	LA4079	IL 9-1-2	LA4101	IL 12-3-1
LA4043	IL 3-1	LA4058	IL 5-5	LA4080	IL 9-1-3	LA4102	IL 12-4
LA4044	IL 3-2	LA3500	IL 6-1	LA4081	IL 9-2	LA4103	IL 12-4-1
LA3488	IL 3-3	LA4060	IL 6-2	LA4082	IL 9-2-5		
LA4046	IL 3-4	LA4061	IL 6-2-2	LA4083	IL 9-2-6		
LA4047	IL 3-5	LA4062	IL 6-3	LA4084	IL 9-3		
LA4048	IL 4-1	LA4063	IL 6-4	LA4085	IL 9-3-1		

3.2. S. habrochaites ILs (93)

The following group of introgression lines represent the genome of *S. habrochaites* LA1777 in the background of cv. E-6203 (LA4024) via homozygous chromosome segments (*Genome* 43:803). The first 57 lines (LA3913 - LA3969) represent approximately 85% of the donor genome, while the remaining lines (LA3970 - LA4010) contain different introgressions, mostly derivatives of the first group. Unlike the *pennellii* ILs above, each *habrochaites* IL may contain more than one introgression, representing one to several chromosomes, as indicated below.

LA	Line	Chrom.	LA	Line	Chrom.	LA	Line	Chrom.
LA3913	TA1258	1	LA3919	TA1128	1	LA3925	TA1111	3
LA3914	TA523	1	LA3920	TA1536	1	LA3926	TA1276	3
LA3915	TA1229	1	LA3921	TA1105	2	LA3927	TA1277	3
LA3916	TA1223	1	LA3922	TA1266	2	LA3928	TA1540	3
LA3917	TA1535	1	LA3923	TA1537	2	LA3929	TA1541	3
LA3918	TA1127	1	LA3924	TA1538	2	LA3930	TA1133	4

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LA	Line	Chrom.		LA	Line	Chrom.		LA
LA3931	TA1280	4	1	LA3957	TA1325	9	1	LA39
LA3932	TA1562	4	1	LA3958	TA1330	9		LA39
LA3933	TA1542	4	1	LA3959	TA1331	9	1	LA39
LA3934	TA1459	4	1	LA3960	TA1550	10	1	LA39
LA3935	TA517	4	1	LA3961	TA1551	10	1	LA39
LA3936	TA1475	4	1	LA3962	TA1552	10	1	LA39
LA3937	TA1473	4	1	LA3963	TA1337	10	1	LA39
LA3938	TA1287	5	1	LA3964	TA1339	10	1	LA39
LA3939	TA1293	5	1	LA3965	TA1555	11	1	LA39
LA3940	TA1112	5	1	LA3966	TA1554	11	1	LA39
LA3941	TA1543	5	1	LA3967	TA1342	11	1	LA39
LA3942	TA1117	5	1	LA3968	TA1350	12	1	LA39
LA3943	TA1544	5	1	LA3969	TA1121	12		LA40
LA3944	TA1539	6	1	LA3970	TA1219	1		LA40
LA3945	TA1545	6	1	LA3971	TA1218	2	1	LA40
LA3946	TA1546	6		LA3972	TA1173	2		LA40
LA3947	TA1559	6	1	LA3975	TA1629	3		LA40
LA3948	TA1303	7	1	LA3976	TA1138	4		LA40
LA3949	TA1304	7	1	LA3977	TA1467	4		LA40
LA3950	TA1547	7		LA3978	TA1468	4		LA40
LA3951	TA1312	7	1	LA3979	TA1630	4		LA40
LA3952	TA1315	8	1	LA3980	TA1290	5		LA40
LA3953	TA1316	8	1	LA3981	TA1116	5		LA40
LA3954	TA1548	8]	LA3983	TA1631	5		
LA3955	TA1320	8]	LA3984	TA1632	5		
LA3956	TA1324	9		LA3985	TA1306	7		

LA	Line	Chrom.
LA3986	TA1309	7
LA3988	TA1318	8
LA3989	TA1319	8
LA3990	TA1560	8
LA3991	TA1326	9
LA3993	TA1549	10
LA3994	TA1635	10
LA3995	TA1553	11
LA3996	TA1120	11
LA3997	TA1563	1-10
LA3998	TA1637	1-11-12
LA3999	TA1638	1-12
LA4000	TA1557	1-4
LA4001	TA1644	1-7-12
LA4002	TA1645	1-8-12
LA4003	TA1648	2-11
LA4004	TA1649	2-3-6
LA4005	TA1652	3, 5
LA4006	TA1654	4-10-11
LA4007	TA1655	4-12
LA4008	TA1656	5-6-9
LA4009	TA1564	5-7-10
LA4010	TA1561	8-2

3.3. S. lycopersicoides ILs (101)

The following group of ILs have been bred from *S. lycopersicoides* into the background of cv. VF36. These lines represent ~96% of the donor genome and are described in *Genome* 48:685, and *Theor. Appl. Genet.* 76:647. While some lines are available in the homozygous condition, others are partially or completely sterile as homozygotes, thus are maintained via heterozygotes. In this case, marker analysis is required to identify the desired genotypes in segregating progenies. Seed of some lines may be limited or temporarily unavailable.

LA	Line	Chr.	- 1	LA	Line	Chr.	LA	Line	Chr.
LA3866	LS1-1	1		LA3869	LS42-4	2	LA4243	LS1-3	3
LA3867	LS11-9	1		LA3870	LS38-10	2	LA4244	LS10-9	4
LA4230	LS15-2H	1		LA3871	LS41-3	2	LA4245	LS10-11A	4
LA4231	LS15-2B	1		LA4236	LS49-8A	2	LA4246	LS49-8B	4
LA4232	LS11-11A	1		LA4237	LS40-8	2	LA4247	LS12-9	4
LA4233	LS20-9	1		LA4238	LS5-1	2	LA4314	LS12-9B	4-10
LA4234	LS21-2	1		LA4239	LS41-20	2	LA3875	LS24-14	4-12
LA4235	LS10-2	1		LA4420	C2S	2	LA3878	LS24-6	5
LA4293	LS5-8	1		LA3882	LS43-14	2-6	LA4248	LS11-6	5
LA4294	LS15-2AD	1		LA3344	Mdh-1	3	LA4249	LS9-1	5
LA4295	LS15-2A	1		LA3874	LS20-9	3	LA4250	LS49-8C	5
LA4296	LS15-2AA	1		LA4240	LS1-13	3	LA4251	LS49-3	5
LA4297	LS15-2AAA	1		LA4241	LS40-2	3	LA4252	LS32-11	5
LA4298	LS15-2BA	1		LA4242	LS14-8	3	LA4299	LS4-9	5

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LA	Line	Chr.	LA	Line	Chr.	LA	Line	Chr.
LA4426	ILX	5	LA4305	LS9-26C	7-8	LA4273	LS12-8	10
LA3879	LS1-5	5-11	LA3876	LS29-1	8	LA4274	LS4-14	10
LA3893	LS16-6	5-12	LA3889	LS41-13	8	LA4275	SL-10	10
LA4300	LS9-7B	5-6	LA3906	Wa, DI	8	LA4276	LS12-12	10
LA4253	LS11-11B	6	LA4262	LS20-16	8	LA3892	LS48-2	11
LA4254	LS32-14	6	LA4263	LS46-6A	8	LA4277	LS24-11	11
LA4255	LS38-5	6	LA4264	LS9-26A	8	LA4278	LS3-2	11
LA4256	LS9-22	6	LA4265	LS9-26B	8	LA4279	LS19-11	11
LA3886	LS48-5	7	LA4266	SL-8A	8	LA4310	LS19-10A	11
LA4257	LS46-3	7	LA4267	LS16-10	8	LA4422	PROS	11
LA4258	LS19-7	7	LA4306	LS46-6	8	LA4280	LS1-5	11-5
LA4259	LS32-4	7	LA4307	SL-8	8	LA4281	LS13-13	12
LA4260	SL-7F	7	LA3345	Dia-3	9	LA4282	LS45-7	12
LA4261	LS8-11	7	LA4268	LS14-7	9	LA4283	LS8-9	12
LA4301	SL-7A	7	LA4269	LS12-2	9	LA4284	LS9-13	12
LA4302	SL-7C	7	LA4270	LS10-6	9	LA4311	LS14-2	12
LA4303	SL-7D	7	LA4271	LS49-5	9	LA4312	LS45-7C	12
LA4304	LS8-11A	7	LA4272	LS41-11	9	LA4313	LS8-12A	12
LA4315	SL-7	7	LA4308	LS32-10	9	LA4427	C12S	12
LA3883	LS48-6	7-11	LA4309	LS10-6D	9			

4. Recombinant Inbred Lines

4.1. Backcross Recombinant Inbred Lines (90)

The following group of backcross recombinant inbred lines originated from the cross *S. lycopersicum* E-6203 × *S. pimpinellifolium* LA1589 (*Genome* 45:1189). The result of 2 BC's and at least 6 generations of inbreeding via single seed descent, the lines are highly homozygous (residual heterozygosity ~3%). The population has been genotyped at 127 marker loci, and the corresponding maps, map files, and QTL data are available from the Solanaceae Genome Network (www.sgn.cornell.edu). This set of 90 lines has been selected for optimum mapping resolution using the MapPop software, and provide a permanent, high resolution mapping population.

LA	ТА	LA	Т	4		LA	ТА		LA	ТА
LA4139	TA2874	LA4	155 T/	42891		LA4172	TA2908		LA4189	TA2927
LA4140	TA2875	LA4	156 T/	42892		LA4173	TA2909		LA4190	TA2928
LA4141	TA2876	LA4	157 T/	42893		LA4174	TA2910		LA4191	TA2929
	TA2877,	LA4	158 T/	42894		LA4175	TA2911		LA4192	TA2930
LA4142	TA2149	LA4	159 T/	42895		LA4176	TA2912		LA4193	TA2931
LA4143	TA2878	LA4	160 T/	42896		LA4177	TA2914		LA4194	TA2932
LA4144	TA2879	LA4	161 T/	42897		LA4178	TA2915		LA4195	TA2933
LA4145	TA2880	LA4	162 T/	42898		LA4179	TA2916		LA4196	TA2934
LA4146	TA2881	LA4	163 T/	42899		LA4180	TA2917		LA4197	TA2935
LA4147	TA2882	LA4	164 T/	42900		LA4181	TA2918		LA4198	TA2936
LA4148	TA2883	LA4	165 T/	42901		LA4182	TA2919		LA4199	TA2937
LA4149	TA2884	LA4	166 T/	42902		LA4183	TA2920		LA4200	TA2938
LA4150	TA2885	LA4	167 T/	42903	1	LA4184	TA2922	1	LA4201	TA2939
LA4151	TA2886	LA4	168 T/	42904		LA4185	TA2923		LA4202	TA2940
LA4152	TA2887	LA4	169 T/	42905		LA4186	TA2924		LA4203	TA2941
LA4153	TA2888	LA4	170 T/	42906		LA4187	TA2925		LA4204	TA2942
LA4154	TA2890	LA4	171 T/	42907		LA4188	TA2926		LA4205	TA2943

LA	ТА	LA	ТА
LA4206	TA2944	LA4213	TA2951
LA4207	TA2945	LA4214	TA2952
LA4208	TA2946	LA4215	TA2953
LA4210	TA2948	LA4216	TA2954
LA4211	TA2949	LA4217	TA2955
LA4212	TA2950	LA4218	TA2956

4.2 Recombinant Inbreds (148)

The following set of 148 recombinant inbred lines (RILs) were developed from a cross between *S. lycopersicum* NC EBR-1 x *S. pimpinellifolium* LA2093, followed by multiple generations of single seed descent from the F2 (Ashrafi et al. 2009 *Genome* 52: 935). NC EBR-1 is an early blight resistant breeding line developed by Randy Gardner at North Carolina State University. LA2093 is an accession of *S. pimpinellifolium* collected by Charley Rick and colleagues at La Union, Ecuador. The RILs have been used to generate a high density molecular marker map (Ashrafi et al. 2009 *Genome* 52: 935) and to map QTLs for horticultural and fruit quality traits (Ashrafi et al. 2012 *Mol. Breeding* 30: 549). The RILs are currently at the F9 generation.

LA	RIL	LA	RIL	LA	RIL	LA	RIL
LA4504	NC EBR-1	LA4535	RIL-37	LA4566	RIL-75	LA4598	RIL-109
LA4505	RIL-1	LA4536	RIL-38	LA4567	RIL-76	LA4599	RIL-111
LA4506	RIL-2	LA4537	RIL-39	LA4568	RIL-77	LA4600	RIL-112
LA4507	RIL-3	LA4538	RIL-40	LA4569	RIL-78	LA4601	RIL-113
LA4508	RIL-4	LA4539	RIL-41	LA4570	RIL-79	LA4602	RIL-114
LA4509	RIL-5	LA4540	RIL-43	LA4571	RIL-80	LA4603	RIL-115
LA4510	RIL-6	LA4541	RIL-45	LA4572	RIL-81	LA4604	RIL-116
LA4511	RIL-7	LA4542	RIL-46	LA4573	RIL-82	LA4605	RIL-117
LA4512	RIL-10	LA4543	RIL-47	LA4574	RIL-83	LA4606	RIL-118
LA4513	RIL-11	LA4544	RIL-48	LA4575	RIL-84	LA4607	RIL-120
LA4514	RIL-12	LA4545	RIL-49	LA4576	RIL-85	LA4608	RIL-121
LA4515	RIL-13	LA4546	RIL-50	LA4577	RIL-86	LA4609	RIL-122
LA4516	RIL-14	LA4547	RIL-52	LA4578	RIL-88	LA4610	RIL-123
LA4517	RIL-16	LA4548	RIL-53	LA4579	RIL-89	LA4611	RIL-124
LA4518	RIL-18	LA4549	RIL-54	LA4580	RIL-90	LA4612	RIL-125
LA4519	RIL-19	LA4550	RIL-55	LA4581	RIL-91	LA4613	RIL-126
LA4520	RIL-20	LA4551	RIL-56	LA4582	RIL-92	LA4614	RIL-127
LA4521	RIL-21	LA4552	RIL-57	LA4584	RIL-94	LA4615	RIL-128
LA4522	RIL-22	LA4553	RIL-58	LA4585	RIL-95	LA4616	RIL-129
LA4523	RIL-23	LA4554	RIL-59	LA4586	RIL-96	LA4617	RIL-130
LA4524	RIL-24	LA4555	RIL-62	LA4587	RIL-97	LA4618	RIL-131
LA4525	RIL-25	LA4556	RIL-63	LA4588	RIL-98	LA4619	RIL-132
LA4526	RIL-26	LA4557	RIL-65	LA4589	RIL-99	LA4620	RIL-133
LA4527	RIL-27	LA4558	RIL-66	LA4590	RIL-100	LA4621	RIL-134
LA4528	RIL-28	LA4559	RIL-67	LA4591	RIL-101	LA4622	RIL-135
LA4529	RIL-30	LA4560	RIL-68	LA4592	RIL-102	LA4623	RIL-137
LA4530	RIL-31	LA4561	RIL-69	LA4593	RIL-103	LA4624	RIL-139
LA4531	RIL-32	LA4562	RIL-71	LA4594	RIL-105	LA4625	RIL-140
LA4532	RIL-33	LA4563	RIL-72	LA4595	RIL-106	LA4626	RIL-142
LA4533	RIL-34	LA4564	RIL-73	LA4596	RIL-107	LA4627	RIL-143
LA4534	RIL-35	LA4565	RIL-74	LA4597	RIL-108	LA4628	RIL-144

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LA	RIL	L	Α	RIL	LA	RIL		LA	F
LA4629	RIL-145	L	A4637	RIL-153	LA4645	RIL-162		LA4653	F
LA4630	RIL-146	L	A4638	RIL-154	LA4646	RIL-164	1		
LA4631	RIL-147	L	A4639	RIL-155	LA4647	RIL-165	1		
LA4632	RIL-148	L	A4640	RIL-156	LA4648	RIL-167	1		
LA4633	RIL-149	L	A4641	RIL-158	LA4649	RIL-168	1		
LA4634	RIL-150	L	A4642	RIL-159	LA4650	RIL-169	1		
LA4635	RIL-151	L	A4643	RIL-160	LA4651	RIL-170	1		
LA4636	RIL-152	L	A4644	RIL-161	LA4652	RIL-171]		

5. Alien Substitution Lines (7)

In the course of his study of segregation and recombination in *S. lycopersicum* x *S. pennellii* hybrids, Rick (*Genetics* 26:753; *Biol. Zbl.* 91:209) backcrossed certain chromosomes of *S. pennellii* LA0716 into the background of several chromosome marker stocks in cultivated tomato. Selected heterozygotes of later generations were selfed and subsequent progenies containing the wild type alleles at the marker loci were selected. The chromosome 6 substitution (LA3142) was further selected with RFLP markers to eliminate residual heterozygosity (*Genetics* 135:1175). The mutant loci used to select each substitution are indicated. In addition, three *S. lycopersicoides* chromosome substitutions (SL-7, -8 and -10) are listed above under introgression lines.

LA	Chrom.	Marker Loci
2091	1	au, dgt, inv, scf
1639	2	Me, aw, m, d
1640	3	sy, bls, sf
3469	4	clau, ful, ra, e, su³

LA	Chrom.	Marker Loci
3142	6	yv, ndw, m-2, c
1642	8	l, bu, dl, al
1643	11	j, hl, a

6. Monosomic Alien Addition Lines (10)

Each of the following group of monosomic additions (MAs) contains a single extra chromosome from *S. lycopersicoides* LA1964 introgressed into the genome of cultivated tomato (Chetelat et al. 1998 *Genome* 41:40). The integrity of the *S. lycopersicoides* chromosomes in these stocks has been verified with relatively few markers, and some may be recombinant. (Our stock of MA-8 lacks *S. lycopersicoides* markers distal to TG330 on the long arm, for example.) Like other types of trisomics, progeny of the monosomic additions include 2n as well as 2n+1 plants; the rate of transmission of the alien chromosomes varies, ranging from ca. 5% to 25%. Identification of 2n+1 plants in each generation is facilitated by their phenotypic resemblance to the corresponding primary trisomics in *S. lycopersicum*. Therefore, the guidelines of Rick (TGC 37:60) for identifying trisomics in the seedling stage are useful for selecting monosomic additions as well. Some of the monosomic alien addition lines carry dominant morphological markers, listed below, that can be used to distinguish them from diploid progeny. Phenotypes of each gene or trait are described on the TGRC website and Rick et al. (1988, Theor. Appl. Genet. 76: 647)

LA	Chrom.	2n+1	2n
3454	MA-2	+	+
3455	MA-3	+	+
3456	MA-4	+	+
3457	MA-5	obv+	obv
3459	MA-7	Bco	+

LA	Chrom.	2n+1	2n
3460	MA-8	Wa, DI ^s	+
3461	MA-9	Fmb, Bif	+, +
3462	MA-10	Abg, u+	+, U
3463	MA-11	+	+
3464	MA-12	+	+

7. Other Prebreds (26). This group of prebreds contain selected morphological traits bred into cultivated tomato from related wild species. Some traits may be simply inherited, others likely involve multiple genetic loci.

LA	Trait
LA0214	Dark anthers from S. peruvianum
LA1015	Compressed fruits from S. cheesmaniae
LA1016	Yellow green from S. cheesmaniae
LA1017	'Pachypericarp' fruit from S. cheesmaniae
LA1018	Odorless from S. cheesmaniae
LA1019	'Pachypericarp' fruit from S. cheesmaniae
LA1500-LA1503, LA1563	High fruit solids, intense pigment from S. chmielewskii
LA1996	Anthocyanin fruit (Aft) from S. chilense
LA2380	Exserted stigmas from S. pimpinellifolium
LA3855	High leaf 2-tridecanone levels from S. habrochaites
LA3897-LA3899	High fruit beta-carotene levels from S. galapagense
LA4104, LA4453, LA4454	High fruit sucrose levels from S. chmielewskii
LA4136	Tissue culture regeneration ability from S. peruvianum
LA4421	B, Lac from S. lycopersicoides
LA4424	'Poodle' syndrome from S. lycopersicoides
LA4425, LA3668	Aubergine (Abg) fruit from S. lycopersicoides
LA4428	Virescent leaves from S. lycopersicoides

8. Interspecific hybrids (2).

- LA4135 F_1 *S. lycopersicum* VF36 × *S. pennellii* LA0716. This hybrid is useful as a rootstock. We use it for maintenance of *S. sitiens*, and sometimes *S. juglandifolium*, and *S. ochranthum*.
- LA4488 F₁ S. *lycopersicum* NC 84173 × S. *pennellii* LA0716. A rootstock hybrid with ToMV resistance.

9. Stress Tolerant Stocks (60+)

We receive many requests for stocks with tolerances to environmental stresses (abiotic or biotic). This group of mostly wild species accessions have been chosen based on observations of plants in their native habitats and/or reports in the literature.

Stress	Species	Accessions
Drought	S. pimpinellifolium	LA1578, LA1595, LA1600, LA1607, LA2718
Drought	S. pennellii (general feature)	LA0716, and others
Drought	S. chilense (general feature)	LA1958, LA1959, LA1972, and others
Drought	S. sitiens (general feature)	LA1974, LA2876, and others
Flooding	S. lycopersicum 'cerasiforme'	LA1421, and others
Flooding	S. juglandifolium, S. ochranthum	LA2120, LA2682
	(general feature)	
High temperatures	S. lycopersicum	LA2661, LA2662, LA3120, LA3320
Low temperatures	S. habrochaites	LA1363, LA1393, LA1777, LA1778
Low temperatures	S. chilense	LA1969, LA1971, LA2883, LA2773, LA2949,
		LA3113, LA4117A
Low temperatures	S. lycopersicoides	LA1964, LA2408, LA2781
Low temperatures	S. sitiens	LA4331 and others

Stress	Species	Accessions
Aluminum toxicity	S. lycopersicum 'cerasiforme'	LA2710 (suspected)
Salinity	S. chilense	LA1930, LA1932, LA1958, LA2747, LA2748, LA2880, LA2931
Salinity	S. galapagense	LA1401, LA1508, LA3909
Salinity	S. cheesmaniae	LA0749, LA3124
Salinity	S. lycopersicum	LA2711
Salinity	S. lycopersicum 'cerasiforme'	LA2081, LA1310, LA2079, LA4133
Salinity	S. pennellii	LA0716, LA1809, LA1926, LA1940, LA2656
Salinity	S. peruvianum	LA0462, LA1278, LA2744
Salinity	S. pimpinellifolium	LA1579 and others
Salinity	S. sitiens (general feature)	LA4113 and others
Arthropods	S. habrochaites	LA0407 and others
Arthropods	S. pennellii	LA0716 and others

10. Translocations (38)

The following group of translocation stocks have been assembled from the collections of their originators - D.W. Barton, C.D. Clayberg, B.S. Gill, G.R. Stringham, B. Snoad, and G. Khush. As far as we know, they are all homozygous for the indicated structural changes. They are described by Gill *et al.* (TGC 23: 17-18; TGC 24:10-12). Accessions with an asterisk comprise the tester set.

LA	Chrom.s	LA	Chrom.s	LA	Chrom.s
*LA1115	T9-12	LA1121	T4-9	LA1882	T12-3 or -8
*LA1119	T3-8	LA1122	T2-9	LA1883	T3-7
*LA1120	T6-12	LA1123	T2-9	LA1884	2 IV T3-8,9-12
*LA1876	T1-2	LA1124	T3-9	LA1886	T12-3 or 8
*LA1885	T5-7	LA1125	T5-7	LA1892	2 IV T9-12, ?-?
*LA1898	T2-10a	LA1126	T7-9	LA1894	T2-9a
*LA1899	T6-11	LA1127	T3-5	LA1895	T2-9b
*LA1903	T4-7	LA1129	T3-9	LA1896	T1-12
		LA1877	T2-4	LA1897	T7-11?
LA1049	T1-9	LA1878	T2-7	LA1902	T2- ?
LA1116	T1-11	LA1879	T2-9	LA1904	T2-9d
LA1117	T5-7	LA1880	T2-11	LA1905	T1-3 or 8
LA1118	T7-11	LA1881	T2-12	LA1906	T2-10b

11. Trisomics (34)

The following series of trisomics contain various kinds of extra chromosomes. Since the extras are transmitted irregularly, each stock necessarily produce a majority of diploid progeny, the remainder aneuploid. Primary trisomics yield mostly 2n and 2n+1, and rarely tetrasomics (2n+2). Telotrisomics yield telos and an occasional rare tetratelosomic. Secondary, tertiary, and compensating trisomics transmit other trisomic types as expected. Because transmission is irregular and reproduction of stocks requires much labor, our stocks are limited. In requesting our aneuploids, researchers are asked to keep these points in mind. To assist in the identification of primary trisomics at the seedling stage, the key features of each have been summarized by Rick (TGC 37:60). Additional 2n+1 stocks are listed under Monosomic Alien Addition Lines above.

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Accession	Genotype	Accession	Genotype
			ary trisomics
Primary trisomics		Second	
delta-10	Triplo-1	delta-44	2n + 2S-2S
delta-06	Triplo-2	delta-43	2n + 5L•5L
delta-08	Triplo-3	delta-36	2n + 7S•7S
delta-02	Triplo-4	delta-26	2n + 9S•9S
delta-04	Triplo-5	delta-31	2n + 9L•9L
delta-12	Triplo-6	delta-28	2n + 10L·10L
delta-07	Triplo-7	delta-41	2n + 11L·11L
delta-03	Triplo-8	delta-29	2n + 12L·12L
delta-05	Triplo-9	Tertia	ry trisomics
delta-01	Triplo-10	delta-18	2n + 2L·10L
delta-40	Triplo-11	delta-16	2n + 4L•10L
delta-09	Triplo-12	delta-39	2n + 5L•7S
Telo	trisomics	delta-15	2n + 7S·11L
delta-14	2n + 3S	delta-25	2n + 9L·12L
delta-17	2n + 3L	delta-23	2n + 1L•11L
delta-21	2n + 4L	Compens	ating trisomics
delta-20	2n + 7L	delta-32	2n - 3S·3L + 3S + 3L·3L
delta-19	2n + 8L	delta-33	2n - 3S·3L + 3S·3S + 3L·3L
delta-35	2n + 10S	delta-34	2n - 7S·7L + 7S·7S + 7L·7L

12. Autotetraploids (17)

We are currently maintaining the following group of tetraploids. Whereas we formerly stocked many more lines, their rapid deterioration, low seed yields, and lack of demand required that we prune them to a smaller group of more frequently used genotypes. Most are stocks of *S. lycopersicum*, unless otherwise noted, and arose from either induced or spontaneous chromosome doubling.

LA	Genotype
2-095	cv. San Marzano
2-483	cv. Red Cherry
LA0794	ag, t ^v
LA1917	S. chilense
LA2335	S. pimpinellifolium
LA2337	cv. Stokesdale
LA2338	cv. Break O'Day
LA2339	cv. Pearson
LA2340	S. pimpinellifolium

LA	Genotype
LA2342	cv. Danmark
LA2343	cv. Waltham Fog
LA2581	S. peruvianum
LA2582	S. arcanum
LA2583	S. arcanum
LA2585	S. pimpinellifolium
LA2587	S. lycopersicum 'cerasiforme'
LA3255	cv. Ailsa Craig

13. Cytoplasmic Variants (3)

The following three lines are cytoplasmically-inherited chlorotic variants maintained by the TGRC and included in the miscellaneous group for want of better classification. They were induced by mutagens and are inherited in strictly maternal fashion. They are not transmitted by pollen but in reciprocal crosses -- no matter what male parents we have used -- the progeny are 100% variant.

LA1092 Uniform yellow, induced by fast neutrons in hybrid background (G.S. Khush)

LA1438 Light green, induced by X-rays in cv. Moneymaker (K. Kerkerk)

LA2979 Cyto-variegated, in cv. Glamour (R.W. Robinson)

14. Chromosome Marker Stocks (178)

This group consists of stocks in each of which has been assembled a series of marker genes for a single chromosome. In a few cases markers on other chromosomes are also present (listed in parentheses). Some of the more useful stocks have been combined with male steriles in order to facilitate large scale test crossing. These stocks are listed below according to chromosome, and within each chromosome group by accession number. Asterisks indicate the preferred marker combination for each chromosome (i.e. that which provides the best map coverage).

LA	Genotype	LA	Genotype	LA	Genotype
Chr	omosome 1	LA0650	aw, d	LA0992	clau, ra, su ³ (com)
LA0910	per, inv	LA0715	Wo ^m , Me, aw, d	LA0993	ra, si
LA0984	scf, inv	LA0732	suf, d	LA0994	cm, ver
LA0985	inv, per	LA0733	Wo ^m , d, ms-10	LA1073	clau, afl
LA1003	scf, inv, per	LA0754	aw, p, d, m, o	LA1074	clau, ver
LA1082	era, um	LA0777	dil, d	LA1075	ver, e, su ³
LA1107	inv, co	LA0789	Me, aw, d, m	LA1536	clau, su³, ra; icn
LA1108	inv, dgt	LA0790	wv, Me, aw, d	Chror	nosome 5
LA1169	scf, dgt	LA0986	s, bk, Wo ^m , o, aw,	LA0512	mc, tf, wt, obv
LA1173	gas, co		p, d	LA1188	frg, tf
LA1184	au ^{tl} , dgt	LA1525	aa, d	LA3850*	af, tf, obv
LA1185	au ^{tl} , scf, inv	LA1526	are, wv, d		mosome 6
LA1186	au ^{tl} , scf, inv, dgt	LA1699	Wo ^m , bip	LA0336	с, sp (а, у)
LA1431	au ^{tl} , dgt	LA1700*	wv, aa, d	LA0640	yv, c
LA1490	au ^{tl} , co, inv, dgt	LA3132	Prx-2 ¹ , ms-10, aa	LA0651	<i>m-</i> 2, <i>c</i>
LA1492	ms-32, bs		nosome 3	LA0773	yv, m-2, c
LA1529*	au ^{tl} , co, scf, inv, dgt	LA0644	r, wf	LA0802	yv, m-2, c (ms-2)
LA2354	br, y (p, l)	LA0782	sy, sf	LA0879	tl, yv
LA3209	imb, irr, y	LA0880	sf, div	LA1178	yv, coa, c
LA3301	fla, com ⁱⁿ	LA0987	pli, con	LA1189*	pds, c
LA3302	imb, com ⁱⁿ	LA0988	ru, sf	LA1190	pds, yv
LA3303	imb, inv	LA1070	ru, sf, cur	LA1489	yv, ves-2, c
LA3305	imb, Lpg	LA1071	sy, bls, sf	LA1527	d-2, c
LA3306	com ⁱⁿ , inv	LA1101	cn, sy, sf	LA3805	m-2, gib-1
LA3307	com ⁱⁿ , Lpg	LA1175	bls, aut	LA3806	yv, Mi, B ^{og} , sp, c
LA3346	au, bs	LA1430*	sy, Ln, bls, sf	LA3807	tl, yv, c
LA3347	au, ms-32	Chro	omosome 4		omosome 7
LA3348	au, com	LA0774	ful, e	LA0788	La/+, deb
LA3349	au, imb	LA0885	ful, e, su³	LA0882	La/+, deb, adp
LA3350	au, br	LA0886	ful, ra, e	LA0923	ig, La/+
LA3351	imb, Lpg/+	LA0888	ful, ven, e	LA0924	La/+, not
LA3352	imb, au, Lpg/+	LA0889	ra, su³	LA1083	ig, flc
Chro	omosome 2	LA0890	ra, ven	LA1103*	var, not
LA0271	aw, O	LA0902	ful, ra², e (ms-31)	LA1104	deb, not
LA0286	d, m	LA0915	clau, ful	LA1172	La/+, lg-5
LA0310	Wo ^m , d	LA0916	clau, ra, su³		omosome 8
LA0330	bk, o, p, d, s (r, y)	LA0917*	clau, ful, ra, e, su³	LA0513	I, bu, dl
LA0342	Wo ^m , d (ms-17)	LA0920	ful, ra, e, su³	LA0712	l, bu, dl; ms-2
LA0514	aw, Wo ^m , d	LA0989	afl, ful	LA0776	I, va ^{virg}
LA0639	Me, aw, d	LA0990	cm, ful, e, su ³	LA0897	l, bu, dl, al

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LA	Genotype	LA	Genotype	LA	Genotype
LA0922	bu, dl, spa		lg)	LA4341	h, hy, u
LA0998	l, bu, dl, Pn/+	LA1085	h, res	Chro	mosome 11
LA0999	tp, dl	LA1086	h, ten	LA0259	hl, a
LA1012	dl, l	LA1110	icn, ag	LA0291	hl, a (ms-2)
LA1191	spa, ae	LA1192	hy, ag	LA0729	neg, a
LA1442	dl, glg, marm	LA1487	icn, t ^v	LA0761	a, hl, j
LA1666*	l, bu, dl, ae	LA2493	Xa-2, hy, h, ag	LA0803	hl, a, pro (ms-2)
Chr	omosome 9	LA2495	Xa-2, h, ten, ag, al	LA0881	neg, hl, a
LA0883	pum, ah	LA2496	Xa-2, h, l-2, t	LA0925*	j, hl, a, f
LA0884	wd, marm	LA2497	hy, u, icn, h, ag	LA1102	a, hl, tab
LA1000	nv, ah	LA2498	u, Xa-3, h	LA1109	j, hl, mnt
LA1001	pum, ah, marm	LA2499	u, nor, t	LA1488	neg, ini
LA1100	ah, pla, marm	LA2500	u, icn, h	LA1786	j, f, a, bi (c)
LA1112	marm, lut	LA2501	u, icn, h, ag	LA2352	j, f (p, c)
LA1176	Crk, ah, marm	LA2502	u, h, auv, I-2, t ^v	LA2364	j, a, f (y, wt, c, l, u)
LA3297	Tm-2ª, nv (Tm)	LA2503	u, h, I-2, t ^v , ag	LA2489	neg ^{ne-2} , a
LA3353*	ah, marm, pct	LA2504*	u, h, t, nd, ag	LA4290	a, bks
Chro	omosome 10	LA2505	u, I-2, t, ag, Xa	LA4291	a, bks²
LA0158	Xa/+, u, t (y)	LA2506	ag, h, l-2, oli, t ^v	LA4292	j-2, up, wv-3
LA0339	ag, u	LA2507	h, t, nd, ag	LA4344	a, mon
LA0341	h, ag (ms-2)	LA2508	h, t, ag, Xa	Chro	mosome 12
LA0643	u, l-2	LA2509	oli, I-2, t ^v , ag (wf)	LA1111	fd, alb
LA0649	t ^v , ag	LA2591	Xa-2, h, ag	LA1171	yg-2 ^{aud} , fd
LA0711	t ^v , ag (ms-2)	LA2592	u, h, t, nd, ag	LA1177*	alb, mua
LA1002	h, u, l-2, t, ag (pe,	LA2593	u, auv, ag		

15. Linkage Screening Testers (15)

The following set of linkage testers each combines two pairs of strategically situated markers on two different chromosomes (see TGC 22: 24). They are intended primarily for assigning new, unmapped markers to a chromosome. The more complete chromosome marker combinations (list 6.1 above) should be used for subsequent testing to delimit loci more accurately. Whereas six of these stocks should pretty well cover the tomato genome, we list below the entire series of the current available testers because alternative stocks differ in their usefulness, depending upon the phenotype of the new mutant to be located. The chromosomal location of each pair of markers is indicated in parentheses.

LA	Genotype
LA0780	<i>yv, c</i> (chr 6) <i>; h, ag</i> (chr 10)
LA0781	<i>ful, e</i> (chr 4) <i>; neg, a</i> (chr 11)
LA0784	<i>ful, e</i> (chr 4) <i>; hl, a</i> (chr 11)
LA0982	<i>clau, e</i> (chr 4) <i>; hl, a</i> (chr 11)
LA0983	<i>l, dl</i> (chr 8) <i>; ah, marm</i> (chr 9)
LA1163	<i>d, wv</i> (chr 2); <i>obv, tf</i> (chr. 5)
LA1164	var, not (chr 7); ah, marm (chr 9)
LA1166	<i>clau, su</i> ³ (chr 4) <i>; icn, ag</i> (chr 10)

LA	Genotype
LA1182	sy, sf (chr 3); alb, mua (chr 12)
LA1441	<i>coa, c</i> (chr 6) <i>; hl, a</i> (chr 11)
LA1443	<i>scf, dgt</i> (chr 1) <i>; l, al</i> (chr 8)
LA1444	<i>wv, d</i> (chr 2) <i>; af, tf</i> (chr 5)
LA1445	<i>clau, su</i> ³ (chr 4); <i>h, icn, ag</i> (chr 10)
LA1491	scf, dgt (chr 1); spa, ae (chr 8)
LA1665	scf, dgt (chr 1); l, ae (chr 8)

16. Miscellaneous Marker Combinations (299)

The following list groups stocks in which various mutant genes have been combined for various purposes. A few of these items include linked genes, but are classified here because other linkage testers provide the same combinations or because they are more useful as markers of several chromosomes. Some multiple marker combinations that are of limited

usefulness, difficult to maintain, and/or redundant with other genotypes, have been dropped from the current list.

LA	Genotype	LA	Genotype	LA	Genotype
LA0013	a, c, d, l, r, y	LA1219	d, u	LA3254	a, c, I, Ve
LA0014	al, d, dm, f, j, wt, h	LA1663	Ln, Wo ^m	LA3256	at, t
LA0052	j, wt, br	LA1664	hp, lp	LA3257	gf, gs, r
LA0085	Wo, d, h	LA1783	ad, sp	LA3258	u, Ve
LA0137	dl, wd, gq	LA1787	Bk-2, en	LA3261	Del, gs
LA0158	t, u, Xa, y	LA1789	sl ^{cs} , a	LA3262	Del, ug
LA0159	a, e, mc, t, u, y, wf	LA1796	Rs, d, h	LA3267	Cf-4, u
LA0169	ps, wf, wt	LA1804	sr, sp, u	LA3268	Tm-2, nv, u
LA0189	bl, cl-2	LA1805	sr, y	LA3269	Tm-1, u
LA0190	wf, br, bk	LA1806	ti, y, wf, al, j	LA3271	Cf-?, Tm-1, u
LA0215	at, y, u	LA2350	<i>y, ne, p, c, sp, a</i>	LA3273	Gp, Tm-2 ²
LA0281	e, t, u	LA2353	<i>y, wt, n</i>	LA3274	ah, Tm-2, nv, u
LA0296	br, bk, wf	LA2355	sp, ug	LA3275	ah, Gp, Tm-2 ²
LA0297	tf, ug, Nr	LA2360	e, wt, I, u	LA3276	Tm-1, u, Ve
LA0299	ag, rv	LA2363	y, Wo, wt, c, t, j	LA3279	at, Del
LA0345	ch, j-2	LA2369	p, Tm-1	LA3284	at, gf
LA0497	ch, j-2, sf	LA2370	wf, n, gs	LA3286	r, ug, y
LA0499	Od, sn, at, cm/+	LA2372	sp, fl	LA3287	hp, r, ug
LA0508	gf, d, c, a, r, y	LA2441	d, m-2, mc, rvt, t, u	LA3288	hp, ug, y
LA0638	ht, d, r	LA2452	B, f, gf, y	LA3289	gf, r, y
LA0648	rv, e, Wo, wf, j, h	LA2453	Gr, u	LA3209	gf, hp, y
LA0040	Jau, clau	LA2454	neg ^{ne-2} , u	LA3291	at, hp, t
LA0713	wv, d, c, r	LA2457	u, so	LA3291	Tm-2, u
LA0727	a, lut	LA2457	Pto, sp, u	LA3292	
LA0728		LA2458		LA3294	bl, d, u Tm-1, Tm-2, nv
LA0759	lg, vi, pe, t	LA2461	sp, stu, u	LA3297	
LA0700	lg, vi clau, pa	LA2464A	aer-2, r, upg, y	LA3299	ep, u
LA0775		LA2404A	r, u, upg, y	LA3315	og ^c , u sp, pst, u, j-2, up,
LA0775	tf, h, au, +/d atv, slx	LA2465	sp, u, v-2	LA3315	sp, psi, u, j-2, up, vo
LA0875		LA2400 LA2467	d, t, v-3	LA3362	
LA0875	hp, u, sp	LA2407 LA2473	pe, u, vi	LA3363	gs, t
LA0875	hp, sp	LA2473 LA2477	alb, c, gra, sft vo, cjf, wf, sp, l, u,	LA3364	at, gs
LA0895	tp, sp, u, Hr	LAZ4//	h	LA3365	gs, u
LA0907	lut, pr	LA2478	ae ^{afr} , r, gs, h	LA3366	gf, gs
LA0908	per, var	LA2476	inc, pds, sp, u, t	LA3367	t, y
LA0909 LA0912	con, sf ht, su ³	LA2480		LA3368	hp, t
		LA2490	pdw, mc, pst, dl	LA3369	hp, y
LA0913	ful, su ³ , ht	LA2492 LA2524	ti, wf, e, mc, u, a		at, y
LA0914	com, ful	LA2524	af, sd	LA3370	at, hp
LA0991	ful, e, com		dp, sp, u	LA3371	hp, u
LA0995	deb, um	LA2527 LA2595	l allele, sp, u	LA3372	gs, y
LA0996	um, ig	LAZ595	br, d, dm, wt, al, h, j, f	LA3373	at, u
LA1018	h, Od, ptb	1 4 2 5 0 7		LA3374	U, Y
LA1038	e, ht, su	LA2597	y, r, wf, mc, m-2,	LA3375	gs, r
LA1072	sy, sf, um	LA2797	c, gs, gf, marm, h	LA3376	Del, hp
LA1078	ria, ves-2		bu, j	LA3381	<i>Г</i> , У
LA1079	c, ves-2	LA3128	Ln, t, up	LA3382	r, u
LA1105	con, cur	LA3212	tmf, d, sp, u	LA3383	gs, hp
LA1106	fsc, ah	LA3217	glg, Pts	LA3384	gf, y
LA1170	cn, con	LA3252	Del, t	LA3385	gs, Nr

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LA	Genotype
LA3386	gf, t
LA3387	Nr, t
LA3389	Nr, y
LA3390	Nr, ug
LA3391	gf, hp
LA3393	r, t
LA3394	at, ug
LA3395	
LA3396	gs, hp, y
LA3397	at, u, y
LA3398	gs, t, y
LA3399	gs, hp ,t
LA3399	at, gs, hp
LA3400	at, hp, u
	at, gs, y
LA3403	gf, gs, u
LA3404	hp, u, y
LA3405	gs, hp, u
LA3406	at, hp, y
LA3407	gs, u, y
LA3408	<i>t, u, y</i>
LA3409	gs, t, u
LA3410	at, gs, u
LA3411	gs, r, u
LA3412	gf, gs, hp, u
LA3413	at, gf
LA3414	t, ug
LA3415	ug, y
LA3416	hp, ug
LA3417	r, ug
LA3418	gf, gs, ug
LA3419	at, gf, gs
LA3420	gf, ug
LA3421	Nr, u
LA3422	at, gs, ug
LA3423	gf, gs, hp, u, y
LA3424	gs, hp, u, y
LA3425	gf, gs, hp, t, u
LA3426	gs, hp, t, u
LA3427	gf, gs, t, u
LA3428	I, u, Ve
LA3429	Del, gs, hp
LA3432	Tm-1, Tm-2, nv, u
LA3433	ah, Tm-2, nv, u
LA3437	at, Nr
LA3442	de, dil, u
LA3443	cor, de, u
LA3444	cor, dil, u
LA3445	cor, pum, u
LA3446	cor, sp, u
LA3447	dil, sp, u
LA3448	in, u
LA3449	d, sp, u
	· · ·

LA	Genotype
LA3450	bls, sp, u
LA3451	bl, sp, u
LA3540	I, U
LA3541	gs, r, ug
LA3542	u, ug
LA3543	bls, o, u
LA3545	Del, u, y
LA3546	bls, Cf-?, u
LA3547	ah, u
LA3548	pum, u
LA3549	bls, Gp, Tm-2 ² , u
LA3557	
LA3557	Del, gf
	gf, Nr
LA3559	Del, gs, y
LA3561	gf, gs, hp, Nr, u
LA3562	gf, gs, u, y
LA3563	sp, u
LA3585	gf, u, ug
LA3587	r, u, ug
LA3589	u, ug, y
LA3590	Nr, gs, y
LA3591	Nr, u, y
LA3593	hp, u, ug
LA3594	gs, hp , ug
LA3595	gf, hp, ug
LA3596	hp, t, ug
LA3597	at, hp, ug
LA3598	r, t, ug
LA3599	at, t, ug
LA3600	t, ug, y
LA3601	gf, r, t
LA3603	at, gf, y
LA3604	hp, r, t
LA3605	at, ug, y
LA3606	r, t, y
LA3607	gs, hp, Nr
LA3608	hp, Nr, t
LA3609	hp, Nr, y
LA3615	d ^x , U
LA3675	hp, Nr, u
LA3676	gf, hp, t
LA3677	gf, hp, r
LA3678	Nr, u, ug
LA3679	gs, Nr, ug
LA3680	Nr, t, u
LA3682	
LA3683	gs, t, ug
	gs, ug, y
LA3684	Nr, t, y
LA3686	gs, Nr, t
LA3688	gf, gs, hp
LA3689	gs, hp, r
LA3691	r, u, y

LA	Genotype
LA3692	at, r, y
LA3693	g, t, u
LA3694	Del, gs, u
LA3695	Del, hp, t
LA3697	gs, r, t
LA3698	gs, r, y
LA3699	gt, u, y
LA3700	at, gf, u
LA3701	at, t, u
LA3702	gf, gs, y
LA3702	gf, gs, y gf, hp , u
LA3703	at, gf, hp
LA3704	at, gs, t
LA3706A	Del, t, y
LA3700A	Del, gf, gs, hp, u
LA3703	
LA3741 LA3742	pum, u de, u
LA3742 LA3743	cor, u
LA3743 LA3744	
LA3744 LA3745	sph, u
	bl, u
LA3771	hp, B ^c
LA3811	gf, r
LA3812	bls, Tm, Tm-2, nv
LA3815	Del, t, ug
LA3821	dil, pum, u
LA3826	mon, u
LA3827	dil, cor, sp, u
LA3830	ep, B ^c , u
LA4136	Rg-1, r
LA4342	oli, u, y
LA4343	gq, h
LA4348	yg-2, c ^{int}
LA4361	fri, tri
LA4362	fri, phyB2
LA4363	cry1, fri
LA4364	phyB2, tri
LA4365	cry1, tri
LA4366	fri, phyB2, tri
LA4367	cry1, tri, fri
LA4368	fri, hp-1, tri
LA4369	fri, hp-1, tri, phyB2
LA4455	ah, B ^c
LA4456	aw, B ^c
LA4457	B ^c , bls
LA4458	aw, c, ex, ps
LA4460	Cnr, y
LA4464	bl, uf
LA4465	bl, sp, uf
LA4466	j, uf
LA4467	j, sp, uf
LA4468	sp, uf
LA4469	sp, ui s, uf

17. Provisional mutants (122).

The following group of provisional mutants are listed here, rather than with the monogenic stocks because they have not been fully characterized. For some, a monogenic segregation has not been verified, for others complementation tests were either not performed or did not detect allelism with existing mutants of similar phenotype. Most of these lines resulted from mutagenesis experiments, the remainder occurring spontaneously. More information on these stocks is available at our website.

-	
Access.	Traits
2-293	Snout
2-305	Broad
2-473	Yellow fruit, pale corolla
2-493	Purple tipped leaves, puny
2-575	Poxed fruit
2-585	Balloon
2-621	Turbinate
2-625	Prolific leaves
2-629	<i>Me</i> -oid
2-633	Hooded flowers
2-643	Yellow green
3-003	<i>yv</i> -oid
3-055	Round cotyledons and leaves
3-073	Abnormal flowers, are
3-077	Dwarf
3-082	Dwarf
3-083	Yellow virescent
3-084	Yellow green
3-088	Light green, dark veins
3-097	Yellow green
3-098	Slow chlorotic
3-101	<i>tl</i> mimic
3-106	Strong anthocyanin
3-107	Bright yellow virescent
3-112	Crippled
3-115	<i>rv</i> -oid
3-118	Rugose recurved leaves
3-127	Bright yellow
3-241-1	Yellow, anthocyanin
3-243	Long narrow
3-303	Slow, narrow leaves
3-305	<i>La</i> -mimic
3-307	Broad, grey green
3-309	Bunchy growth, mitten leaves
3-311	Slow, rugose
3-313	Acute, olive green
3-315	Glossy dwarf
3-317	<i>ra</i> -oid
3-319	Striated, divided
3-321	Narrow, dissected
3-323	Spirally coiled
3-325	Short, yellow virescent
3-329	Bronzing
3-331	Serrated leaves
	1

Access.	Traits
3-335	Gold dust virescent
3-337	Glossy dwarf
3-341	Dwarf
3-403	Fimbriate leaves
3-404	Speckled white
3-405	Streaked virescent
3-406	Streaked variegated
3-408	bu mimic
3-411	Blue green; bushy roots
3-423	ra-oid
3-424	Extreme dwarf
3-424	d^cr like
3-434	Overall yellow
3-430	Singed hairs
3-601	<i>clau</i> mimic
3-612	wiry mimic
3-613	La mimic
3-614	<i>pds</i> -oid
3-617	Dwarf
3-618	mimic of a
3-619	wiry mimic
3-621	<i>d</i> mimic
3-622	<i>d</i> mimic
3-624B	Yellow virescent
LA0506	Triplo-8 mimic
LA0652	calycine poxed, ch
LA0739	ag mimic
LA0765	Acute leaves
LA0791	Long slender fruit
LA0801	Pseudopolyploid, atv, slx
LA0870	frizzled virescent
LA0871	Calico
LA1012	Mottled, chlorotic petiole, <i>dl</i> , <i>l</i>
LA1060	<i>spl</i> -oid
LA1065	Miniature
LA1066	Speckled
LA1095	fy-oid
LA1098	Multiple inflorescence
LA1144	<i>ful</i> mimic
LA1148	Light green
LA1149	Xanthoid
LA1154	pale virescent, twisted leaves
LA1160	Fused cotyledons
LA1193	Yellow-sectored
LA1201	<i>rv</i> -oid
LA1202	Dirty orange cherry
LA1436	Withered cotyledons
LA1494	Adventitious roots
LA1532	<i>rv</i> -oid
LA1533	Purple stem
LA1707	Short stature, <i>btl</i>

Access.	Traits
LA2018	Anthocyanin deficient
LA2019	t ^v mimic
LA2020	Dark green foliage
LA2021	Variegated yellow
LA2358	Marginal leaf chlorosis
LA2806	Incomplete anthocyanin mutant
LA2817	<i>lg</i> mimic
LA2897	Virescent gold top
LA2899	Wrinkled fruit
LA3851	Virescent
LA4370	'multiflor' mutant
LA4371	multiflorous mutant
LA4489-	Trichome chemistry mutants
LA4503	

MEMBERSHIP

- Aarden, HarrietteMonsanto Holland BV, Dept. Tomato Breeding, LAAN VAN BRAAT 21, DELFTTHE NETHERLANDS2627CLh.aarden@gmail.com
- Augustine, Jim BHN Research/ BHN Seed, PO Box 3267, Immokalee, FI USA 34143 mgonzalez@bhnseed.com
- Ballardini, Massimiliano Edasem Spa, Via G. Marconi 56, Casaleone, Verona ITALY 37052 mballardini@esasem.com
- Barker, Susan University of Western Australia, School of Plant Biology M084, 35 Stirling Hwy, Crawley WA AUSTRALIA 6009 susan.barker@uwa.edu.au
- Brown, Loretta Rijk Zwaan USA, 19040 Portola Dr Ste B, Salinas, CA USA 93908 L.brown@rijkzwaan.com
- Buonfiglioli, Carlo Della Rimembranze nr. 6A, San Lazzaro di Savena, Bologna ITALY 40068 red@prorainbow.com
- Chetelat, Roger University of California, Dept of Veg Crops (mail stop 3), One Shields Ave, Davis, CA USA 95616-8746 trchetelat@ucdavis.edu
- DeHoop, Simon Jan East West Seed Co. Ltd, Hortigenetics Research (SE Asia) Ltd., 7 Moo 9, Maefackmai subdistrict Sansai district, Chiangmai THAILAND 50290 simon.dehoop@eastwestseed.com
- Dick, Jim Tomato Solutions, 23264 Mull Rd, Chatham, Ontario CANADA N7M 5J4 seed@tomatosolutions.ca
- East-West Seed Hortigenetics Research LTD, S.E. Asia, No. 7 Moo 9, Chiang Mai-Praw Rd Tamboon Maefack Mai, Amphur Sansai Chiang Mai THAILAND 50290 www.eastwestseed.com
- Galvez, Hayde U Philippines Los Banos Genetics Lab, CSC-1PB, CA, UP Los Banos College, Laguna PHILIPPINES 4031 haydegalvez@gmail.coM
- Hanson, Peter AVRDC, PO Box 42, Shanhua, Tainan, TAIWAN REPUBLIC of CHINA 741 peter.hanson@worldveg.org
- Hoogstraten, Jaap Seminis Veg Seeds, Postbus 97, 6700 AB Wageningen THE NETHERLANDS jaap.hoogstraten@monsanto.com
- Hutton, Sam University of Florida, Gulf Coast Research and Education Center, 14625 County Rd 672, Wimuama, FL USA 33598 sfhutton@ufl.edu
- Inai, Shuji Nippon Del Monte Corp., Research and Development, 3748 Shimizu-Cho Numata-shi, Gunma-ken JAPAN 378-0016 sinai@delmonte.co.jp, tfujii@delmonte.co.jp
- Kaplan, Boaz Nirit Seeds LTD Ltd, P.O. Box 95, Moshav Hadar Am ISRAEL 4293500 boazk@niritseeds.com

MEMBERSHIP

Krishidhan Veg Seeds, Private LTD, Krishidhan Bhanwan, Additional MIDC Karwa, Anup Jalna, Maharashtra INDIA 431203 anupkarwa@gmail.com Kobori Romulo F. Sakata Seed Sudamerica Ltda, PO Box 427, Av Dr. Plinio Salgado N. 4320, Bairro Braganca Paulista, Sao Paulo BRAZIL 12-906-840 romulo.kobori@sakata.com.br Liedl, Barbara WVSU , 100 Toney House, Institute, WV USA 25112 liedlbe@wvstateu.edu Martin, Gregory Boyce Thompson Institute, 533 Tower Rd, Ithaca, NY USA 14853 gbm7@cornell.edu Massoudi, MarkAg Biotech Inc., P.O. Box 1325, San Juan Bautista, CA USA 95045 info@agbiotech.net Maxwell, Douglas P. USA Max- Tach Services, 7711 Midtown Rd, Verona, WI 53593 douglas.maxwell08@gmail.com Technisem, Zone d'activite Anjou Actiparc de Jumelles Longue Jumelles Moisson, Chloe 49160 chloe.moisson@technisem.com FRANCE Myers, Jim Oregon State University Dept. of Horticulture, rm 4017, Ag & Life Sci Bldg., Corvallis, OR USA 97331 myersja@hort.oregonstate.edu Nukal, Balaji Seedworks India, 155 Ocean Lane Dr., #515, Key Biscayne, FL USA 33149 bnukal@seedworks.com O'Brochta, William John Roanoke Valley Governor's School, 3226 Peakwood Dr, Roanoke, VA USA 24014 william@obrochta.net Ozminkowski, Richard Heinz N.A., 6755 CE Dixon St, Stockton, CA USA 95206 Rich.Ozminkowski@us.hjheinz.com Randhawa, Parm California Seed and Plant Lab, 7877 Pleasant Grove Rd, Elverta, CA USA 95626 randhawa@calspl.com Rascle, Christine CLAUSE Centre de Recherche, Domaine de Maninet, Route de Beaumont, Valence FRANCE 26000 christine.rascle@hmclause.com University of Florida, Tomato Breeders, 14625 County Rd 672, Wimauma, Fl Scott, Jay USA 33598 jwsc@ufl.edu Sharma, Sundrish Syngenta Seeds, Inc., 10290 Greenway Rd, Naples, FL USA 34114 sundrish.sharma@syngenta.com Shashikumar, KT Noble Agro Biotech Pvt. Ltd., Horticulture, 579 23rd Cross, 16th Main, Judicial Layout Yelahnka, Bangalore (Bengaluru)/Karnataka INDIA 560065 SKUMAR.HORT@gmail.com Shintaku, Yurie 2-10-2, Shimizu Suginami-ku, Tokyo JAPAN 167-0033 napoleon@jcom.home.ne.jp

MEMBERSHIP

- Stack, Stephen Colorado State U, Dept of Biology, 1878 Campus Delivery, Fort Collins, CO USA 80523-1878 sstack@lamar.colostate.edu
- Stevens, Mikel Brigham Young Univ., 275 Widtsoe Bldg, PO. Box 25183, Provo, UT USA 84602 mikel_stevens@byu.edu
- Thome, Catherine United Genetics Seeds Co., 764 Carr Ave., Aromas, CA USA 95004 cathy@unitedgenetics.com
- van der Knaap, Koen Axia Vegetable Seeds, info@axiaseeds.com, Delft The Netherlands 262gHH koen@axiaseeds.com
- Zhiqi, JiaHenan Agricultural University, room 313, no 2 building, No 95 Wenhua Rd, JinshuiDistrict, Zhengzhou City, Henan ProvinceCHINA 450002zhiqijia@yahoo.com.cn

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In Memoriam Adapted by Jay Scott from a Eulogy given and kindly provided to TGC by Haim Rabinowitch.

Nachum Kedar (1920-2015)

Nachum Kekar was an incredible plant breeder/ geneticist at The Hebrew University. He was an even better human being. Nachum was born in Vienna Austria in July 1920, where he grew up and joined the Jewish Zionist Youth movement (Maccabi* Tzair = Young Maccabees in Hebrew). In 1938, after the Nazi invasion of Austria, he was sent by the leaders of the movement to Denmark to practice farming/agriculture (and get shelter from the Nazis) in preparation for his planned immigration to what was then a British mandate, Palestine. There he was to join a Kibbutz and to help build the country to be. When Denmark became occupied by the Nazis in 1943, he and nine of his friends decided to find shelter in Sweden. This happened about half a year before the Danish endeavor to smuggle the Danish Jewry to Sweden in an effort to save them from the German atrocities. Nachum & friends offered the German guards some drinks and when these guards were hammered by the alcohol they stole a boat and headed to Sweden. Unusually, they were not deported by the Swedish authorities and were allowed to stay.

Nachum enrolled at Lund University where he obtained his Bachelor's degree and later at Uppsala University where he got his Master's degree in 1947. He also started working in the Swedish National Station for Plant Diseases, in Stockholm.

In 1950 he fulfilled his dreams and immigrated to Israel, became member of a kibbutz and a farmer. A year later he left and joined the Department of Plant Genetics at the Weizmann Institute of Science in Rehovot. He then assisted Dr Oved Shifriss in studying castor bean plants (*Ricinus communis*) including breeding, production, evaluation of hybrids and field technologies.

In 1954 he started his studies towards a Ph.D. degree at the Faculty of Agriculture of the Hebrew University of Jerusalem, in Rehovot, researching potato late blight (*Phytophthora infestans*) and the role of polyphenol oxidases in the response of plants to infection. While carrying out this research he was appointed as a Research Assistant. He submitted his dissertation in 1958. Later, when hired by the Faculty of Agriculture, he initiated research on Fusarium wilt of tomatoes, and applied his knowledge and results in developing the first Fusarium wilt resistant tomato OP variety (named Rehovot 13) for the Israeli market, thus enabling growers to keep on producing tomatoes on soils infested with this fungus.

Katy (Nachum's widow) told me that at the time he served as a Research Assistant and in his first years in position, his Hebrew was very poor, but teaching was (and mainly still is) done in Hebrew. Nachum prepared his lectures in English, Katy translated the texts into Hebrew, Nachum recited the texts and delivered his lectures in Hebrew. Mind you, in the early 1960s, I became his assistant and his student. I never guessed that this was the way he succeeded in delivering extremely interesting mind opening lectures. The only weird things were some of the professional terms he used (Katy did not know what these terms meant, checked the dictionary and selected some peculiar words that neither she nor Nachum knew the meaning of). From 1959 until his retirement in 1988, Nachum served as Senior Teacher (Full Professor) on vegetable physiology, genetics and breeding. In this capacity he educated and trained thousands of students and supervised Masters and PhD theses of 20-30 students.

*Maccabee named after the family of priests that led the victorious revolt of Judea against the Greek Empire, 2nd century BCE <u>https://en.wikipedia.org/wiki/Maccabees</u>

He also served as Head of the Department, as Head of Teaching and Curriculum in Field Crops, Vegetables and Genetics Committee. Knowing him well, I can vouch that these administrative tasks were the parts of academic life less favored by him.

Nachum served the Israeli parallel to the USAID, and contributed his knowledge and experience to improving horticulture in developing countries, mainly in SE Asia.

Due to the level of science in the neighborhood, Israeli scholars are scientifically isolated. Actually, the closest scientific neighbors work in Italy. Hence, spending sabbaticals abroad is quite a must for Israeli scientists. Nachum spent sabbaticals in Illinois, California, Florida, Holland and France where people learned to appreciate his intellect, original ideas and creative thinking.



Nachum used to think outside of the box, the most significant work he initiated was in the late 1960s early 1970s when reports on the discovery of random mutations, *rin* and later *nor* were published. Living in a hot climate country, despite the short transportation distances we experience rapid fruit softening (e.g, fruit picked on Thursday won't store well by Sunday and many fruit would have to be discarded in the production field; mind you, in Israel fruit are picked at the ripe stage), and export (to Europe) could be made only by air. He was intrigued by the potential of these mutations and was not discouraged by the recessive nature of these genes. Together we made in the early 1970s numerous crosses with a great variety of genotypic backgrounds in an attempt to identify the right genetic combinations that will produces red fruit with considerable longer shelf life than what was common.

In 1973 we got the first evidence that the wild idea is not that wild. The next obstacle was, how not surprising, financial support. No one in Israel or in Europe believed that this idea of using these recessive genes for extension the shelf-life of ripe tomatoes was possible. It took a couple of years or more before we were able to convince a group (today we refer to them as "angels") of non-professionals that had trust in us. The rest is history. It made a difference; in the local Israeli market and its exports, all over the Middle Eastern countries, in all Mediterranean basin countries, for Culiacan (Sinaloa) Mexico, and to the two small Israeli seed companies. Despite their initial skepticism and unwillingness to support the research, they were very happy to get the rights to produce these seeds. Very quickly, tomato varieties from Israel (our lab) occupied about 40% of the total indoor acreage in the Mediterranean basin and both companies are now recognized as leaders in the vegetable seed development and production. From here on, we invested much of the time in understanding the mode of action of ripening inhibitory genes and in developing better and different tomatoes for yearlong production. Nachum was fascinated by the potential of cherry tomatoes, then by hybridizing some cherry genotypes with sources for long shelf-life two goals were achieved; excellent flavor and long shelf-life thus making cherry tomatoes a successfully marketed commodity. Up to that time cherry tomatoes were picked individually, one by one which was labor intensive and costly. By developing tomatoes for picking in clusters (like grapes) we managed to save considerably on labor. We also initiated a program on high flavor salad size tomatoes and another one on improving the functional qualities of the fruit. Both were premature and failed, not because we did not reach the goals but because of marketing and companies' considerations. But now, new varieties bred for flavor are produced, manufactured and warmly accepted by consumers, and the demand for this product is continuously on the increase.

Nachum's main research themes revolved around tomatoes with some work on onions. The main thrusts of his research included: resistance to diseases, both pathological and physiological (e.g. catface; stem scar blossom-end rot, and more); heat-setting (actually out of season production); shelf-life and many other fruit qualities (firmness, color, flavor, size, shape, nutritional value and more).

Nachum published numerous refereed publications in scientific journals, chapters in books, and wrote a lot of papers in farmer journals to acquaint them with the developments in the lab. He received numerous awards including the Rothschild Prize, the Israel Prize, the Kaye Prize, the ASHS Award Best Vegetable Paper Award (for our publication on use of *rin* and *nor* genes to extend tomato shelf-life), and awards from a number of Israeli Grower Associations.