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Identification and Manipulation of Resistance to Tomato Spotted Wilt Virus Derived From Solanum peruvianum

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IDENTIFICATION AND MANIPULATION OF TSWV RESISTANCE IN *Solanum peruvianum*

by

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GRADUATE COMMITTEE APPROVAL

of a dissertation submitted by

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This dissertation has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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As chair of the candidate's graduate committee, I have read the dissertation of Luis Felipe Gordillo Morales in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACTS

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Chapter 1 Abstract

The domesticated tomato *Solanum lycopersicum* (L.), formerly known as *Lycopersicon esculentum* is a genetically well-studied crop species with high-density linkage and molecular maps based on crosses done between cultivated tomato and its distant related wild species. Wild tomato species harbor a wealth of resistance to many pathogens that have been introgressed into domesticated tomato for genetic control of diseases and pests and for improvement of many agronomic traits. The wild tomato *S. peruvianum* (L.) is the source of the *Sw-5* gene, characterized and mapped to chromosome 9 of the tomato genome and introgressed into elite tomato germplasm, providing resistance to the tospovirus *Tomato spotted wilt virus* (TSWV).

TSWV has been reported to be a major problem for tomato growers in many parts of the world, which in some cases, has resulted in tomato fields having been

abandoned for some time. Additionally, there are reports that new races of TSWV have evolved that overcome *Sw-5*.

TSWV replicates in both, plant cells and in the alimentary canal cells of thrips and then transmitted to plants by this insect acting as a vector. Both, TSWV and thrips have co-evolved to infest and infect more than 1090 plants species in over 100 families, thrips becoming resistant to pesticides and easily escaping by hiding deep in plant parts. World trade has disseminated thrips all over the world and environmental pressures have forced TSWV to recombine its RNA to overcome new resistance.

Chapter 2 Abstract

The United States Department of Agriculture Research Service (USDA) and the Tomato Genetics Resource Center (TGRC) *Lycopersicon peruvianum* germplasm collections (16,335 plants from 285 accessions) were screened with the TSWV isolates TSWV6 from Hawaii, and An_{wa}-1 from anemone in Western Australia. Using TSWV6 to screen for resistance, 10,634 *L. peruvianum* plants from 280 accessions were screened; resulting in, 168 (60%) accessions with 1437 (14%) plants indicating resistance, with all 1,404 89S (*Sw-5*$^{+}$/*Sw-5*$^{+}$) and 1,456 89R (*Sw-5*/*Sw-5*) controls infected. When using An_{wa}-1 for screening 864 (15%) of 5,701 *L. peruvianum's* uninfected from 106 of the 181 accessions tested, and 472 (95%) of the 495 89S and 421 (73%) of the 574 89R controls infected. Fifty-four of the 172 accessions tested with both isolates were resistant to one isolate but not the other. Additionally, more accessions from the USDA than from the TGRC collection indicating resistance. TSWV resistant accessions were somewhat equally distributed throughout the *L. peruvianum* geographic range with an observation

that northern Chile/southern Perú seemed to have an unusually high portion of accession indicating resistance. The value of *Sw-5* is discussed in relationship to potential additional sources of TSWV resistance.

Chapter 3 Abstract

Sw-5 derived from *Solanum peruvianum* (L.) confers resistance to *Tomato spotted wilt virus* (TSWV). A unique TSWV isolate from Hawaii (TSWV6) overcomes *Sw-5* in our trials when tested on near isogenic lines (NILs) controls 89R (*Sw-5/Sw-5*), and 89S (*Sw-5*$^{+}$*/Sw-5*$^{+}$). Both lines were 100% infected. Our TWSV6 resistance screening trials of *S. peruvianum* accessions indicate resistance in line PI 128660, although repeated testing of cuttings of putatively resistant plants suggests less than 100% penetrance. We have developed interspecific hybrids and some backcrosses between *S. lycopersicum* (L.) and TSWV6 resistant PI 128660 lines. To circumvent the postzygotic genetic barrier, we used embryo rescue to obtain the F_1 and the BC_1P_1 generations. We screened the F_1, BC_1P_1, BC_2P_1, and BC_3P_1 generations by mechanically inoculating them with TSWV6. Apparently uninfected plants were selected and tested for the presence/absence of TSWV6 by enzyme-linked immunosorbent assay (ELISA). Fewer plants indicating TSWV6 resistance in the BC_2P_1 and BC_3P_1 to the point where there were no significant differences between the TSWV susceptible controls and BC_3P_1 when tested in either the greenhouse or in the field with common isolates of TSWV or the TSWV6 isolate.

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CHAPTER 1

LITERATURE REVIEW

Abstract

The domesticated tomato *Solanum lycopersicum* (L.), formerly known as *Lycopersicon esculentum* is a genetically well-studied crop species with high-density linkage and molecular maps based on crosses done between cultivated tomato and its distant related wild species. Wild tomato species harbor a wealth of resistance to many pathogens that have been introgressed into domesticated tomato for genetic control of diseases and pests and for improvement of many agronomic traits. The wild tomato *S. peruvianum* (L.) is the source of the *Sw-5* gene, characterized and mapped to chromosome 9 of the tomato genome and introgressed into elite tomato germplasm, providing resistance to the tospovirus *Tomato spotted wilt virus* (TSWV).

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TSWV replicates in both, plant cells and in the alimentary canal cells of thrips and then transmitted to plants by this insect acting as a vector. Both, TSWV and thrips have co-evolved to infest and infect more than 1090 plants species in over 100 families, thrips becoming resistant to pesticides and easily escaping by hiding deep in plant parts. World trade has disseminated thrips all over the world and environmental pressures have forced TSWV to recombine its RNA to overcome new resistance.

Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is grown worldwide and its consumption provides vitamins A and C, carotene, minerals, anthocyanin, lycopene and other antioxidants (Causse et al. 2002; Foolad 2007; Rodrigues do Nascimento et al. 2009). Tomato cultivars vary widely in their resistance or susceptibility to many bacterial, fungal and viral diseases as well to other pests (Saidi and Warade 2008). Modern hybrids focus on improved disease resistance, such as resistance to *Tobacco mosaic virus* (TMV), *Curly top virus* (CTV), powdery mildew (*Oidium lycopersicum* Cooke and Mass); bacterial spot (*Xanthomonas campestris* pv. Vesicatoria, ex Doidge 1920), late blight (*Phytophthora infestans*, (Mont.) de Bary), early blight (*Alternaria solani*, Ell. and Mart.; Jones and Grout), among others (Bai and Lindhout 2008; Knott and Dvorák 1976).

125 million metric tons of tomatoes were produced in the world in 2005. China, the largest producer, accounted for about one quarter of the global output (31.6 metric tons), followed by United States (11.0 metric tons), Turkey (9.7 metric tons), Egypt (7.6 metric tons), and India (7.6 metric tons), among other countries. For processed tomato, in 2006 California produced 10.1 million metric tons (90% share in the USA); Florida produced 611,216 metric tons (5% share in the USA), and the other 5% share was from other states. Average grower price for U.S. fresh tomatoes in 2006 was about $900 per ton but prices fluctuate depending on the market. Processed tomato price is steadier at below $100 per ton (United States Foreign Agricultural Service, Horticultural & Tropical Products Division, 2007). The economic importance of tomato production plus the

source of vitamins, minerals, and antioxidants makes the tomato crop very important for human nutrition since it is used daily in diets in many different forms worldwide.

Prospects for genetic improvement in tomato

Due to the domestication of tomato and its self-pollinating nature, its genetic base is very narrow, providing only about 5% of the total genetic variability of the total tomato gene pool (including the wild relatives). Consequently it is vulnerable to many diseases, insects, physiological disorders and to biotic and abiotic stress (Johal et al. 2008; Sharma et al. 2008). This puts tomato production at risk worldwide, since pathogens have the ability to mutate, thereby creating the potential to wipe out entire tomato fields (Maluf et al. 1991). The survival of tomato cultivars is dependent upon the presence of resistance genes introgressed from wild tomato species that have co-evolved in nature with pathogens. Genetically variable wild tomato species are good sources of new resistance genes for many agriculturally and biologically important traits. These could be used to improve the genetic base of cultivated tomato to avoid possible losses to pathogens that may attack tomato crops (Barone and Frusciante 2007; Gordillo et al. 2008; Sharma et al. 2008). Human population is estimated to increase to 9 billion by the year 2050. Continued improvement of our crops to meet this future demand is needed through exploiting the genetic diversity of the wild relatives (Johal et al. 2008; Knott and Dvorák 1976).

Resistance to over 42 different diseases have been identified among the tomato wild relatives (Knott and Dvorák 1976; Kumar et al. 1993; Maluf et al. 1991), as well as the presence of volatiles (essential oils), waxes, and leaf trichomes that confer resistance

to many insects. One of these diseases is *Tomato spotted wilt virus* (TSWV), which has become a worldwide threat to tomato production due in part to the vectoring of the virus via the insect thrips (Groves et al. 2001; Kumar et al. 1993; Roselló et al. 2001; Ryley and Pappu 2004)

Success at breeding the cultivated tomato with distant related wild species has been limited due to severe genetic crossing barriers between these species. When crossing *S. lycopersicum* with *S. peruvianum*, pollen tubes germinate and successfully fertilize the ovaries; however, post-zygotic barriers prevent the seed from reaching maturity. Fruits develop normally but are generally seedless (Barbano and Topoleski 1984; Poysa 1990; Stevens and Rick 1986).

To circumvent this genetic barrier, tissue culture techniques such embryo culture, embryo rescue (Smith 1944), ovule culture (Imanishi 1988; Sacks 1996), and callus culture from ovules (LanZhuang and Adachi 1996; Thomas and Pratt 1981) have been used. These techniques require the use of expensive equipment, laboratories, and trained personnel to extract and culture the embryos.

Tomato spotted wilt virus (TSWV)-thrips interaction

TSWV is in the *Tospovirus* genus of the *Bunyaviridae* family of mainly arthropod-borne viruses (de Haan et al. 1991). The immense economic impacts of tospoviruses are mainly due to their extensive host range and worldwide distribution (Brommonschenkel and Tanksley 1997; Maluf et al. 1991; Rosello et al. 1999; Saidi and Warade 2008) occurring in over 1090 plant species in over 100 families reported (German et al. 1992). TSWV is transmitted by western flower thrips *Frankliniella*

occidentalis (Pergande), and the tobacco thrips *Frankliniella fusca* (Hinds) (Riley and Pappu 2004).

Thrips go through six stages in their lifecycle; beginning with the egg found inside plant tissue for 2-4 days followed by two larval (instars) stages in which they are wingless, small, pale yellow that resemble adults. The first instar larva stage lasts 1-2 days and second instar larval stage lasts 2-4 days. The non-feeding prepupal and pupal stages begin at the end of the second larval phase, the insect drops to the ground and pupate in the soil; in some species, the prepupal (1-2 days) and pupal stages (1-3 days) stay on the plant. The pupae stage is somewhat resistant to insecticides. From the pupae adults emerge returning to feed and breed on the plant for between 30-45 days and females lay between 150 to 300 eggs (Whitfield et al. 2005). The total life cycle of thrips may take between 10 to 12 days in the summer to a month or more in cool weather. Adult thrips are poor fliers with feathery wings that allow them to be carried long distances by air currents, invading field crops and weeds around banks and ditches, escaping from insecticides by hiding deep inside floral parts (Groves et al. 2001; Latham and Jones 1998; Riley and Pappu 2004; Saidi and Warade 2008).

Acquisition of TSWV by thrips is a pH dependent process, and low pH conditions favor immature (first and second instars larvae) stages to acquire TSWV from infected host plants (Whitfield et al. 2005). Virus replicates in the guts of these vector thrips, becoming viruliferous adults that quickly spread the virus. Filho et al. (2002) used immunolocalization of the non-structural proteins (NSs) encoded by the small RNA of TSWV and fluorescence microscopy analysis and Whitfield et al. (2005) used a protein A-gold tag immunostain procedure to study virus entry and fusion to the midgut of thrips

during development from larva to adults following virus uptake by first instar larva. Both found that thrips acquire TSVW by feeding on infected plants, and replication takes place in the alimentary canal. Virus was observed first in the epithelial cells of the midgut, then virus particles move to the muscle cells, to the rest of the midgut and finally it moves to the salivary glands via the ligaments that connect the salivary glands to the gut. Replication of virus was observed within 24 hours of the acquisition period and thrips were able to transmit the virus when feeding (Filho et al. 2002). Larval stages that do not acquire TSWV will develop into adult thrips that are incapable of transmitting the tospovirus even if they feed on infected plants due to a midgut barrier that prevents virus ingested by the adult from moving to the salivary gland (Whitfield et al. 2005).

There is not clear evidence if TSWV is a pathogen of thrips. Some reports indicate that there is a decreased thrips environmental fitness, having a shorter life span. Additionally, there is some evidence that the virus affects the larval stage in that it may take longer to develop into adults and adults may appear smaller than non-infected thrips. However, other studies have been unable to confirm these findings (Whitfield et al. 2005).

Nature of TSWV

TSWV virions are spherical and pleiomorphic particles composed of a lipid bi-layer envelope with a diameter of 80-120 nm, studded with the two viral glycoproteins G1 and G2 involved in recognition of receptors in the vector (German et al. 1992). Its tripartite negative strand genome consists of ambisense single-stranded linear segments of RNA containing a small RNA (2.9 kb), a medium RNA (5.4 kb), and a large RNA (8.9

kb) (de Haan et al. 1990, 1991). Each RNA segment is associated with nucleocapsid (N) proteins and a few copies of the large protein to form pseudo-circular nucleocapsid structures (Kormelink et al. 1992). The large RNA codes for the RNA-dependent-RNA polymerase (RdRp) associated with each segment of the genome and required for the TSWV transmission process supported by the L protein. The small RNA encodes the nucleocapsid proetin (N) that has been used as the source of pathogen-derived resistance (Pang et al. 1996) as well as contributing to viral replication cycle. It also encodes the non-structural protein associated with cell-to-cell movement and suppression of gene silencing during the plant-infection stage of the virus life cycle and found at high concentrations in infected plant cells (Bucher et al. 2003; Whitfield et al. 2005). The N protein encapsidates the RNA genome segments with possible roles in virus replication (ICTdB 2006).

The medium RNA encodes the non-structural protein (NSm) and the glycoproteins (GPs) Gn and Gc. The NSm is involved in the cell-to-cell movement forming tubules as a regular viral movement protein, and altering the size exclusion limit of plasmodesmata (Whitfield et al. 2005).

The GPs Gn and Gc are important in virulence of Bunyaviruses for attachment and entry into host cells of vertebrates or invertebrates. Both proteins play a role in virus entry, binding, acquisition, and fusion via GP-receptors found in guts of thrips. Furthermore, Gc is cleaved at low pH, suggesting a conformational change that helps it in the pH-dependent endocytosis of viral particles in the thrips guts (Whitfield et al. 2005).

The phospho-lipid bi-layer is made up of more than 50% proteins and about 20-30% lipids of the viral particle's weight. The composition of the lipid bi-layer is similar

to the viral host since it is derived from the host's cellular Golgi membrane and or plasma membranes, which includes glycolipids, fatty acids, sterols, and phospholipids (ICTdB 2006; Whitfield et al. 2005).

Sin et al. (2005), worked with 30 TSWV single lesion isolates (SLIs) derived from a single TSWV inefficiently vectored by thrips; three of these isolates were transmitted by thrips and 27 did not. They analyzed the M RNA, thrips transmissibility, the Gc protein, and by TEM studies, and found out that the mutation C1375A in the glycoproteins Gn/Gc in the open reading frame of the medium RNA resulted in thrips losing transmissibility without affecting virion assembly. An opposite result was obtained with SLIs with nonsense and frameshift mutations where the SLIs were defective in virion assembly. They detected Gc in the C1375A but not in the frameshift/nonsense mutants. They concluded that the glycoproteins may be required for transmissibility by thrips, but not required for TSWV infection of plant hosts. Gc may not be required to TSWV replication in its plant host, but it is required for transmissibility by its thrips vector. This demonstrates the importance of the TSWV M RNA in determining thrips transmissibility to plants (Sin et al. 2005)

TSWV particle morphogenesis in plant cells

The site for TSWV particle morphogenesis is the Golgi apparatus. Viral glycoproteins G1 and G2 accumulate in the Golgi, obtain a double membrane and later fuse to each other, forming single enveloped particles clustered in membranes in the endoplasmic reticulum (ICTdB 2006; Sin et al. 2005). TSWV particle maturation pathway in plants cells is characterized by typical structures associated with tospovirus

infection that become clustered within the endoplamic reticulum membranes. Viroplasms (VP), nucleocapsid aggregates (NCA), paired parallel membranes (PPM) (involved in budding processes), doubly enveloped particles (DEV), and single enveloped particles (SEV) are some of these typical structures observed under TEM studies (Kikkert et al. 1999). VP, NCA, PPM, and DEV are present in the early stages of infection; and clustered SEV are present in late or final stages of maturation of the viral particles. TSWV morphogenesis in thrips also shows some of these typical structures (G1/G2 proteins) derived from the Golgi apparatus in the cells of the midgut epithelium but the rest of the pathway is different in animals infected by tospoviruses (Kikkert et al. 1999; Sin et al. 2005; Whitfield et al. 2005).

TSWV disease expression in tomato

Symptoms caused by TSWV are diverse such as chlorotic, necrotic, ring-shape, and purple spots, mottling, wilting, stunting, and finally, death of the plant. Normally after infection, symptoms will appear within a period of three to ten days. On the fruit, they appear as concentric rings that are not visible at the early stage; as the fruit matures these concentric rings will appear rendering the fruit unmarketable (Aramburu et al. 2000; Maluf et al. 1991; Saidi and Warade 2008; Stevens et al. 1992).

The expression of symptoms depends on several factors such as the host plant, virus strains, age of the plant, density of thrips and environmental interactions (Maluf et al. 1991**;** Roselló et al. 1999). Sometimes the viral infection is latent in plant cells and is expressed within a short (two to three months) to a long time (three months to a year) (Maluf et al. 1991; Roselló et al. 1997).

Plant-pathogen interactions

Plants respond to pathogen attack (elicitation) by expressing a broad-spectrum disease resistance that is active against further pathogen attack. They have controlled-inducible defense mechanisms by activating signal transduction pathways, through the use of inducible promoters and cis-regulatory elements that correspond to resistance genes and pathogen-specific responses. The phytohormones salicilic acid (SA), Jasmonic acid (JA) and ethylene are plant defense signal transduction molecules used by these pathways mechanisms (Mazarei et al. 2008). Many aspects of these responses are expressed in infected and even in noninfected plant parts, producing the systemic acquired resistance (SAR) response, characterized by a local hypersensitive response (HR) that leads to programmed cell death around the infected area, isolating the pathogen to avoid more infection (Zehnder et al. 1999). Other expressed responses are local changes in cell-wall structure and composition, especially plasmodesmata to prevent further invasion of pathogens and local and systemic expression of pathogenesis-related proteins (PR) such as chitinases, peroxidases, and glucanases which are required for polymerization of cell wall components and have antifungal properties (Heil and Ploss 2006). Specific interactions between pathogen avirulence (*avr*) gene loci and alleles of corresponding plant resistance (R) locus in both, host and pathogen bring about resistance; disease results if either is absent or inactive (gene-for gene resistance) (Cook 1998; Gachomo et al. 2003). R products recognize *avr*-dependent signals and trigger the pathway of signal-transduction events, producing the defense signal molecules that in turn activates the defense mechanism and pathogen growth is arrested.

R genes encode five classes of proteins of which, the largest class encodes a highly evolved nucleotide-binding site plus leucine-rich repeat (NB-LRR) class of proteins that function exclusively as R genes. A subdivision of this class of proteins contains putative coiled-coil domains (CC-NB-LRR) that comprise multiple subfamilies varying in size and in the location of the coiled-coil domain. Sequencing analysis of these proteins show that R specificity resides mainly in the LRRs (Dangi and Jones 2001; Oldroyd and Staskawicz 1998).

Sources of resistance to TSWV

Stevens et al. (1995) screened 188 accessions of seven different *Lycopersicon* spp. for resistance to isolates of TSWV, finding that *L. peruvianum* (L.) Mill. and *L. chilense* Dun. provided the highest level of resistance to TSWV. All available *L. chilense* accessions and 12 *L. peruvianum* accessions were screened for resistance from the US germplasm collections (Stevens 1993; Stevens et al. 1994). In 1946, Norris reported in relationship to TSWV, that "*L. peruvianum* possesses true resistance amounting almost to immunity" (Norris 1946).

Gordillo et al. (2008) screened the United States Department of Agriculture Research Service (USDA) and the Tomato Genetics Resource Center (TGRC) *Lycopersicon peruvianum* germplasm collections (16,335 plants from 285 accessions) for resistance to the Hawaiian TSWV6 and the Australian anemone (An_{wa}-1) isolates. Using TSWV6 to screen for resistance, 10,634 *L. peruvianum* plants from 280 accessions were screened; resulting in, 168 (60%) accessions with 1437 (14%) plants indicating resistance, with all 1,404 89S (*Sw-5*$^{+}$/*Sw-5*$^{+}$) and 1,456 89R (*Sw-5*/*Sw-5*) controls

infected. When using An_{wa}-1 for screening 864 (15%) of 5,701 *L. peruvianum's* uninfected from 106 of the 181 accessions tested, and 472 (95%) of the 495 89S and 421 (73%) of the 574 89R controls infected. Fifty-four of the 172 accessions tested with both isolates were resistant to one isolate but not the other. Additionally, more accessions from the USDA than from the TGRC collection indicated resistance. The unilateral resistance response (either TSWV6 or An_{wa}-1) of 54 accessions suggests multiple alleles or genes for resistance to TSWV within the *L. peruvianum* germplasm collections. This theory is further supported by other studies of smaller populations showing unique *L. peruvianum* resistance responses to varying isolates of TSWV (Gordillo et al. 2008; Iizuka et al. 1993; Jordá et al. 1993; Norris 1946).

Transgenic resistance to plant viruses

Pathogen-derived resistance (PDR) is the integration of pathogen components that interfere with the normal life cycle of the virus that is inappropriately expressed in a host organism, disrupting the parasitic relationship that results in host resistance. During host-pathogen interaction, the pathogen brings with it essential components and functions required for its life cycle that are disrupted by the presence of a corresponding pathogen gene. This pathogen gene may be dysfunctional, over-expressed, or shows up at the wrong stage of the life cycle of the pathogen (Oldroyd and Staskawicz 1998). The viral nucleic acid is responsible for the infectivity, transmissibility, and symptomatology of viruses. The protein itself has not infectivity but its presence increases the infectivity of the virus (Freitas-Astúa et al. 2002).

Viral genes encode structural, non-structural (polymerase, helicase, movement

proteins, transmission protein, and proteases) and coat proteins. The movement protein allows the passage of viral particles through the plasmodesmata and the coat protein may help viruses move short distances. Virus enters the phloem and is quickly transported to many parts of the plant, mostly towards apical meristems (Abbas et al. 2008).

PDR techniques

Coat protein-mediated protection (CPMP)

Transformed tobacco plants expressing coat protein from tomato mosaic virus (TMV) were highly resistant when challenged with the virus. CPMP is expressed as a reduction of lesions on inoculated leaves of tobacco, a reduced rate of systemic infection, and lower accumulation of challenging virus when compared with non-transgenic control plants (Freitas-Astúa et al. 2002).

Movement protein-mediated resistance (MPMR)

This is a new strategy to block the viral infection process by the production of dysfunctional movement proteins that do not allow viruses to move via plasmodesmata. This strategy provides a broad spectrum of resistance in transformed plants by expressing a modified TMV movement protein, providing protection from several viruses (Abbas et al. 2008; Freitas-Astúa et al. 2002).

Protease-mediated resistance (PMR)

In the case of potyvirus, their genes express a polyprotein that is cleaved by virus-encoded proteases that inhibit the challenge viral proteinase activity, resulting in disruption of the viral infection cycle. This technique provides resistance to tobacco vein mottling virus (TVMV) and to potato virus Y (PVY) (Freitas-Astúa et al. 2002).

RNA mediated resistance

The production of an inappropriate RNA that pairs up with viral RNA prevents accessibility of the viral RNA for replication or gene expression.

Virus replication is prevented by using antisense RNA that has a polarity opposite to that of the viral messenger RNA as is the case within tobacco expressing complementary RNA to the coat protein untranslated regions of cucumber mosaic virus (CMV), tobacco mosaic virus (TMV), and potato virus X (PVX). A mechanism to degrade RNA after transcription occurs when extensive methylation of the transgene sequence is associated with induction of the specific cytoplasmic RNA degradation mechanism typical of gene silencing called post-transcriptional gene silencing (PTGS). No product is made and viral particles cannot reassemble to create more virus particles. This mechanism is activated by the presence of high levels of a specific transcript associated with viral RNA degradation (Freitas-Astúa et al. 2002).

The most important agricultural application of PDR is coat protein mediated protection (CPMP) against plant virus diseases. Numerous crop species have been genetically transformed with viral coat protein genes with the intent of producing virus-resistant varieties. Several species so transformed have been evaluated for disease resistance under field conditions that simulate commercial situations, and resistance has ranged from zero to immunity. The total body of work substantiates that PDR based on transformation with viral coat protein genes, is a valid and potentially effective method of controlling virus diseases in crops (Abbas et al. 2008; Freitas-Astúa et al. 2002; Oldroyd and Staskawicz 1998).

Transgenic resistance to plant viruses in tomato

Abbas et al. (2008) created transformed plants with resistance to *Fusarium oxysporum* using the biolistic plasmid DNA coated particles delivery system and transgenic tomato plants expressing the chitinase protein acquired antifungal activity against *Fusarium*. Huang et al. (2007) were able to transform tomato plants expressing the sweep pepper ferredoxin-I (PLFP) that showed resistance to the root-infecting pathogen *Ralstonia solanacearum* at different levels. Transformation was performed by using leaf discs with *Agrobacterium tumefasciens* containing a plasmid with a coding sequence of the *pflp* gene into a vector and kanamycin was used to select successful transformants.

Bucher et al. (2006) obtained boad tospovirus resistance at a high frequency levels by using the N gene sequence fragments of the four major tomato-infecting tospoviruses, *Tomato spotted wilt virus* (TSWV), *Groundnut ringspot virus* (GRSV), *Tomato chlorotic spot virus* (TCSV), and *Watermelon silver mottle virus* (WSMoV). They showed that efficient simultaneous targeting of the four different tospoviruses can be achieved by using a single small transgene based on the production of minimal size chimaeric cassettes. They found up to 82% resistance against the four viruses in the transformed plant lines. Brommonschenkel et al. (2002), Gal-On et al. (1998), and Oldroyd and Staskawics (1998), and Ultzen et al. (1995), also have reported success in transforming tomato plants showing diasease resistance to several pathogens affecting tomato.

The *Sw-5* gene

The use of resistant cultivars provides the most effective and durable way to minimize crop losses due to TSWV infection (Saidi and Warade 2008). Such resistance

is found in many wild tomato species such as *S. peruvianum*. This species is the source of the TSWV resistance gene *Sw-5* that has been introgressed into cultivated tomato (Aramburu et al. 2000; Roselló et al. 2001, 1999; Stevens et al. 1992; Saidi and Warade 2008). *Sw-5* provides resistance to common isolates of TSWV, *Tomato chlorotic spot virus* TCSV, and *Groundnut ring spot virus* GRSV (Boiteux and Giordano 1992; Gordillo et al. 2008; Maluf et al. 1991; Stevens et al. 1992). Stevens et al. (1995) mapped *Sw-5* near the sub-telomeric region of the long arm on chromosome 9 between the RFLP markers CT71 and CT220. This last marker is tightly linked to *Sw-5*. Brommonschenkel and Tanksley (1997) cloned the tomato genomic region that spans the *Sw-5* by means of the chromosome landing technique (Tanksley 1993) using yeast artificial chromosome YAC clones and the CT220 RFLP marker as a probe. Spassova et al. (2001) identified two-candidate resistance genes within the previously determined 40 kilo-base distance of CT220 obtained by Brommoschenkel et al. (1997). These two genes were named *Sw-5a* and *Sw-5b*, which encode proteins of 1245 and 1246 amino acids, respectively. These two candidate genes share 95% homology and are members of the coiled-coil nucleotide binding-leucine-rich repeat group of resistant gene candidates. Both candidate resistance genes resemble the tomato nematode and aphid resistance gene *Mi* and to a lesser extent the *Prf* gene which confers resistance to *Pseudomonas syringae* (Spassova et al. 2001).

The value of the *Sw-5* gene.

There is clear evidence that variant isolates of TSWV can overcome *Sw-5* (Aramburu and Martí 2003; Cho et al. 1996; Roselló et al. 1999; Thompson and van Zijl

1996). Furthermore, studies have demonstrated that tospoviruses can readily overcome both pathogen derived resistance and natural resistance through reassortment of the TSWV genome (Hoffmann et al. 2001; Qiu et al. 1998; Qiu and Moyer 1999). With these two lines of evidence in mind, the question arises as to why *Sw-5* has still been used worldwide providing a high level of resistance in most areas where utilized. In regions where a TSWV isolate has overcome *Sw-5*, two related conditions have been noted.

First, isolate TSWV6 came from a situation where virtually 100% of the *Sw-5*$^{+}$/*Sw-5*$^{+}$ genotypes were naturally infected; thus the disease pressure was extremely high. Second, where clear case histories can be identified; there have often been year-around cropping of *Sw-5* genotype tomatoes with consistently "high" tospovirus pressure. Curiously, TSWV6 like isolates have not been found in Hawaii since *Sw-5* was no longer used in the area. In fact, tomatoes, with *Sw-5*, were again planted several years later in the same region and *Sw-5* provided its normal level of resistance (John Cho, personal communication). This "*Sw-5* rest" allowed the indigenous tospovirus to revert to an "environmentally fit" tospovirus that did not include the ability to readily overcome *Sw-5*.

These anecdotal observations coupled with the data we obtained when using An_{wa}-1 opens the question to the environmental "fitness" of TSWV isolates that overcome *Sw-5*. If isolates overcoming *Sw-5* are generally less "environmentally fit" it would explain why growers who seasonally utilize *Sw-5* genotype cultivars where TSWV pressure is high expect as much as 20% of their crop infected with the virus. However, regions that have consistently high TSWV pressure and never allow a rest from *Sw-5* are purported to be areas having troubles with TSWV overcoming this resistance. In

considering the evidence that tospoviruses can quickly and easily "reshuffle" their genome, developing stable TSWV resistance may be a challenge **(**Hoffmann et al. 2001; Qiu and Moyer 1999; Qiu et al. 1998) However, the slow adaptation of the virus to overcome *Sw-5* coupled with information that this event is documented mostly in areas where TSWV is infecting nearly 100% of known susceptible lines are in complete harmony with Leach et al. (Leach et al 2001). Their results suggest that environmental fitness of this pathogen is directly related to the durability of resistance genes.

Conclusion

Cultivated tomato species often lack genes needed to confer disease resistance. Wild relatives of cultivated plants posses a wealth of resistance and agronomic traits that can be used to improve cultivated species (Foolad 2007; Poysa 1990). Screening for resistance in wild species and introgressing these genes into our cultivated species leads to pathogen adaptation, mutation, and change to overcome these new resistances (Knott and Dvorák 1976). Due to overcoming of existing resistance genes introgressed into tomato cultivars and varieties by new pathogen isolates, the need for resistance breeding, screening for new resistance sources, gene identification through marker assisted selection and the introgression of these resistances must continue. Durable resistance needs to be found in quantitative trait loci genes (Bai and Lindhout 2008; Tanksley 1993). However, while breeding for general or nonspecific resistance the possibility of loosing resistance in our cultivated species may occur since polygenic resistance creates an additive resistance effect towards pathogens and as we breed for resistance, some of these genes may get lost as we backcross to the recurrent parent, creating a dilution effect of the resistance (Tanksley 1993).

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CHAPTER TWO

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Screening Two *Lycopersicon peruvianum* Collections for Resistance to *Tomato spotted wilt virus*

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Abstract

The United States Department of Agriculture Research Service (USDA) and the Tomato Genetics Resource Center (TGRC) *Lycopersicon peruvianum* germplasm collections (16,335 plants from 285 accessions) were screened with the TSWV isolates TSWV6 from Hawaii, and An_{wa}-1 from anemone in Western Australia. Using TSWV6 to screen for resistance, 10,634 *L. peruvianum* plants from 280 accessions were screened; resulting in, 168 (60%) accessions with 1437 (14%) plants indicating resistance, with all 1,404 89S (*Sw-5*$^+$/*Sw-5*$^+$) and 1,456 89R (*Sw-5*/*Sw-5*) controls infected. When using An_{wa}-1 for screening 864 (15%) of 5,701 *L. peruvianum's* uninfected from 106 of the 181 accessions tested, and 472 (95%) of the 495 89S and 421 (73%) of the 574 89R controls infected. Fifty-four of the 172 accessions tested with both isolates were resistant to one isolate but not the other. Additionally, more accessions from the USDA than from the

TGRC collection indicating resistance. TSWV resistant accessions were somewhat equally distributed throughout the *L. peruvianum* geographic range with an observation that northern Chile/southern Perú seemed to have an unusually high portion of accession indicating resistance. The value of *Sw-5* is discussed in relationship to potential additional sources of TSWV resistance.

Introduction

Tomato spotted wilt virus (TSWV), classified as a *Tospovirus*, infects cultivated tomato (*Lycopersicon esculentum* Mill.), causing plant stunting, necrotic leaf spotting, mottling, epinasty, plant and fruit deformity, and, in severe cases, death (3,7). Thrips are the natural vectors of tospoviruses, with species of the genus *Frankliniella* and *Thrips* being the most common (8,9,32,43). Because of the globalization of trade these insects have spread worldwide along with their vectored disease (4,28,44,57,60-62). Tospoviruses infect at least 15 monocotyledonous and 69 dicotyledonous families, of which Asteraceae, Solanaceae, and Fabaceae are most commonly affected. More than 1,090 plant species have been reported to be infected by this virus, including many crops of economic importance, with vegetable yield losses worldwide estimated at one billion dollars annually (32). In the 2000 growing season, TSWV epidemics in tomato were estimated to cause $8.8 million in losses in Georgia (43). Cho et al. (7) reported that yield losses in Hawaii have been high enough to cease tomato production in some areas due to TSWV. In Brazil, Argentina, Spain, Portugal, and Italy, it is becoming the most limiting factor for tomato production (1,2,4,30,38,51,65).

Growers have tried to manage TSWV by controlling thrips through different approaches such as crop rotation, leaving the land fallowed, eliminating crop residue, insecticides, weed control, and reflective plastic mulch (29,42). However thrips are minute winged insects that easily migrate into fields, regardless of grower deterrents, then infect plants before insecticides have time to control them effectively (8). Of all the cultural practices tried, weed control and reflective mulches have had the most positive effect on increased marketable tomato yields (29,42,43).

A viable method to control TSWV is through natural resistance found in wild relatives of domesticated tomato plants, which provide a possible control to TSWV and subsequent reduction in fruit damage (8,43). Stevens et al. (58) screened 188 accessions of seven different *Lycopersicon* spp. for resistance to isolates of TSWV, finding that *L. peruvianum* (L.) Mill. and *L. chilense* Dun. provided the highest level of resistance to TSWV. All available *L. chilense* accessions and 12 *L. peruvianum* accessions were screened for resistance from the US germplasm collections (54,58). Additionally, a number of other reports (4,10-12,15,18,20,23,24,27,31,33,47,49,50,52,53,62,63) have identified a high level of TSWV resistance in *L. peruvianum* beginning as early as 1939 (64). In 1946, Norris reported in relationship to TSWV, that "*L. peruvianum* possesses true resistance amounting almost to immunity" (31). Despite this observation, no comprehensive study of the *L. peruvianum* U.S. germplasm collections have been conducted, even though the TSWV resistance gene "*Sw-5*" was introgressed into the cultivated tomato from this species (7,57,61). Additionally, new TSWV isolates overcoming *Sw-5* have been identified in several parts of the world (1,7,46,61). Latham et al. (25) deliberately developed two TSWV isolates, under controlled conditions, which

completely infected plants homozygous for *Sw-5*. Some of these TSWV isolates that infect *Sw-5*/*Sw-5* genotypes have been collected and used in this study.

In recognition of the paucity of comprehensive information on TSWV resistance in *L. peruvianum* and the identification of isolates that infect *Sw-5*/*Sw-5* plants, we initiated a screening of the *L. peruvianum* germplasm. In this study, 285 *L. peruvianum* accessions at the United States Department of Agriculture (USDA) Research Service, Cornell University Geneva, NY and the Tomato Genetics Resource Center (TGRC) at the University of California, Davis were screened for TSWV resistance to isolates completely overwhelming and partially overcoming *Sw-5*.

Materials and methods

Management of *Lycopersicon* germplasm

In all, 169 TGRC accessions were screened, 38 of which belong to the core collection (henceforth identified as "TGRC Core") and 116 from the USDA collection, totaling 285 *L. peruvianum* accessions (Tables 1 and 2). Seeds of *L. peruvianum*, and controls (near-isogenic tomato lines [NIL] 89R [*Sw-5/Sw-5*] and 89S [$Sw\text{-}5^{+}/Sw\text{-}5^{+}$]) were scarified to increase germination with household bleach (2.7% sodium hypochlorite) and distilled water at a 1:1 ratio for 30 min., as recommended by Rick and Hunt (41). They then were washed three to four times with tap water and spread on moist germination paper (Anchor Paper Company, St. Paul, MN) in Petri dishes. Germinated seedlings were transplanted to approximately 74 cm^3/plant cells filled with peat-based media in commercially available plastic flats. The objective was to test 50 plants per accession; however, actual numbers depended on germination and availability of seed.

Eight cells of each flat were dedicated to 89R and 89S (four cells each) as a TSWV inoculation check. The 40 remaining cells were filled with one or more accessions depending on available seedlings.

Maintenance and use of TSWV isolates

Isolate TSWV6 was identified in Hawaii infecting (*Sw-5*/*Sw-5*) tomato plants (7). The anemone isolate (An_{wa}-1), to which *Sw-5* provides some resistance, was identified on an *Anemone* sp. in Banjup, Western Australia in August 1998, and maintained in cv. Grosse Lisse (*Sw-5*$^+$/*Sw-5*$^+$) (25). These isolates were selected based on three criteria. First, the isolates infected controls differently; TSWV6 infected both *Sw-5* an *Sw-5*$^+$genotypes (7). However, *Sw-5* genotypes generally resisted An_{wa}-1 (25) but not *Sw-5*$^+$ genotypes. General symptoms of both TSWV6 and An_{wa}-1 were "tip blight" to whole-plant chlorosis and stunting in the controls, with An_{wa}-1 being the less aggressive of the two. Second, our experience suggested that both isolates produced reliable disease expression with our inoculation method. Finally, these two isolates were from different areas of the world.

Isolate maintenance and resistance screening trials were conducted in controlled access greenhouses at Brigham Young University, Provo, UT. Night temperatures ranged between approximately 18 and 25°C and day temperatures between approximately 21and 32°C through 10 months of screening.

Both isolates were maintained in *Nicotiana rustica* L. and were transferred by rub-inoculating Carborundum-dusted (600-mesh) leaves with sterile cheesecloth pads dipped in 1 to 2°C buffer (0.1 M phosphate buffer, pH 7.4, 0.01 M sodium sulfite), with

homogenized infected tissue from both previously inoculated and systematically infected leaves. Isolates were transferred to young, healthy *N. rustica* every ten days to maintain optimum virulence (57). Transfer an isolate within one to four days after the appearance of visual symptoms on the inoculated *N. rustica* second to fourth leaves produced the most reliable infection for mass inoculations (6,54-58; *unpublished data*).

Mass inoculation of accessions and controls were accomplished using techniques previously described (54,57,58). Between 20 and 60 flats were inoculated in one setting. TSWV rapidly deactivates once inoculum is prepared; as a result, a set of up to ten flats was inoculated within 12 to 15 min, after which fresh inoculum was prepared for the next flat set. 89R and 89S TSWV checks were arranged such that two check plants were inoculated before and after every ten *L. peruvianum* plants. All plants were inoculated between their second and fourth true leaves and re-inoculated with the same isolate six to eight days later.

To monitor uncontrolled TSWV spread, 10 to 20 uninoculated 89R and 89S plants were omnipresent in the greenhouse throughout the study. Utilizing mechanically transferred TSWV greatly reduces the possibility of unintended thrips transmission of this virus (19,21). Additionally, a rotation of a regular set of insecticides (Maveric [Welllmark, Schaumburg, IL], Azatin XL [Olympic Horticultural Products, Mainland, PA], Conserve SC [Dow Agro Sciences, Indianapolis, IN], and Orthene 75 [Ortho, Marysville, OH]) were used at labeled rates, approximately once a week, to minimize thrips and other arthropods. No instance of uncontrolled TSWV was identified throughout the study.

Evaluation of TSWV infection

Visual symptoms were used initially for eliminating obviously infected plants. Plants not showing characteristic TSWV symptoms were evaluated by peroxidase double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), supplied by Agdia Inc. (Elkhart, Indiana), to detect the presence or absence of the virus (57). Plants not responding in ELISA were maintained and visually reevaluated once a week for two additional months. We have observed (*unpublished data*) that plant stress often encourages phenotypic expression of symptomless TSWV carriers. Plants showing visual viral symptoms were eliminated and counted as infected. Consistently, those plants identified with optical density (OD) values near the threshold used by Stevens et al. (57) were found to become visually infected during the subsequent monitoring period.

Statistical analysis and ArcGIS program

SAS was used to develop the analysis of variance tables for statistical comparisons. The ArcGIS program (Environmental Systems Research Institute, Redlands, California was used to position the accessions to their geographical location according to accession passport information.

Results

Summary of accessions response to TSWV isolates

Either the TSWV6 or An_{wa}-1 isolates were screened on a total of 16,335 *L. peruvianum* plants, from 285 accessions, and 3,929 (1,899 89S and 2,030 89R) controls

(Table 1). Of the 285 accessions screened for TSWV resistance, 172 (about 60%) were tested with both isolates (6,683 and 5,516 *L. peruvianum* plants with TSWV6 and An_{wa}-1 respectively; Table 2). No resistance was detected in 39 (about 23%) of the 172 accessions to either TSWV isolate. Of the 172 accessions tested with both isolates, 23 (about 13%) indicated resistance to An_{wa}-1 but not to TSWV6 and 31 (about 18%) had plants with a resistance response to TSWV6 and not to An_{wa}-1. In all, 79 accessions had plants indicating resistance to both isolates (Table 2).

A distinct difference in the TSWV resistance response was identified between germplasm collections. The USDA and TGRC collections had a significantly ($P<0.0001$) higher level of resistance than the TGRC Core collection. Of the 172 accessions tested with both isolates, 35 of 42 (approximately 83%) USDA, 86 of 109 (approximately 79%) TGRC, and 12 of 21 (approximately 57%) TGRC Core indicated resistance to at least one of the two isolates (Table 2).

Germplasm responses to TSWV6 and An_{wa}-1

When inoculated with TSWV6, all 1,404 89S and 1,456 89R controls were infected (Table 1). Of the 10,634 *L. peruvianum* plants tested from 280 accessions, 14% of the plants were found to be virus free, which was significantly higher than the controls. The 116 USDA, 129 TGRC, and 35 TGRC Core accessions tested with TSWV6 were significantly different from each other (P-value <0.0001) with 21, 9, and 3% of the plants, respectively, virus (Table 1, Fig. 1A). This pattern was similar when comparing accessions with plants free of the virus (Table 1; Fig. 1B).

An_{wa}-1 was less virulent than TSWV6; 95% of the 495 89S and 73% of the 574 89R controls were infected (Table 1). Of the 5,705 *L. peruvianum* tested with An_{wa}-1, 15% indicated resistance, and these plants were found within 106 of the 177 (59%) accessions tested (Table 1). Using An_{wa}-1, the 42 USDA, 111 TGRC, and 24 TGRC Core accessions were found to be significantly different from each other (P-value <0.0001) with 33, 12, and 5% of the plants, respectively, virus free (Table 1; Fig. 1B).

Geographic distribution of *L. peruvianum* TSWV resistance

We were able to examine the geographical distribution of 260 *L. peruvianum* accessions with area specific passport data. Of those 260 accessions 154 were tested with both TSWV isolates, 256 were tested with TSWV6 (Fig. 2A; Table 2) and 158 with An_{wa}-1 (Fig. 2B; Table 2). Resistance to both TSWV isolates was somewhat equally distributed throughout Peru with perhaps more emphasis along the Peruvian Pacific coastal plane (Fig. 2). However, the Arica, Chile/southern Peru region indicated an unusually high ratio of accessions with resistant plants (Fig. 2, insert 2). Arica is a town in the Atacama Desert in northern Chile (Fig. 2). Specifically 23 of 26 (approximately 88%) and 16 of 17 (approximately 94%) of the accessions had >8% of their plants resistant to TSWV6 and An_{wa}-1 respectively (Fig. 2). This region can be compared with the insert 1 area of Figure 2A and 2B (surrounding Lima, Peru) in which 45 of 102 (about 44%) and 29 of 58 (about 50%) accessions had >8% of the plants, indicating a resistance response to TSWV6 and An_{wa}-1 respectively.

There is even a greater difference in the accessions from the Arica region (Fig. 2, insert 2), which had ≥33% of their plants indicate a resistant response. In all, 19 of 26

(about 73%) and 12 of 17 (about 71%) accessions had a resistance response to TSWV6 and An_{wa}-1 respectively (Fig. 2, insert 2) compared with the region around Lima (Fig. 2, insert 1) where 12 accessions tested with either TSWV6 (about 12%) or An_{wa}-1 (about 21%) had ≥33% of their plants free of TSWV infection.

Discussion

Mechanical inoculation

Several reports have utilized thrips for screening smaller germplasm populations for TSWV resistance (24,44,47). However, due to the impracticalities of managing or controlling thrips, we utilized mechanical inoculation to screen our large plant numbers with two TSWV isolates. Especially when some plants or accessions could be considered TSWV resistant when, in reality, thrips could be either deterred (preference) or possibly resisted by some *L. peruvianum* accessions (24).

A noted drawback to serial mechanical inoculation has been the generation of defective interfering RNAs (DI RNAs) causing attenuation of the symptoms by the TSWV isolate. Generation of these defective isolates can be aggravated during some environmental regimes (19,21,39,40). Nonetheless, there are usable stable TSWV isolates with DI RNAs. Ie (19) described TSWV isolates with DI RNAs which still produced disease after almost 20 years of mechanically transferring the isolates. Norris (31) first identified resistance in *L. peruvianum*, and Finlay (11,12) differentiated Sw_1^a, Sw_1^b, sw_2, sw_3, and sw_4 utilizing mechanical inoculations. Additionally, *Sw-5* was named (54,57), mapped (55,56), and subsequently cloned (13) based on TSWV mechanical inoculation methods. We have been working with a new source of TSWV resistance

derived from *L. chilense* (6,48) using mechanical inoculation methods. This resistance source has demonstrated its utility under naturally high TSWV pressure in repeated field trials (6,48; *unpublished data*). These studies demonstrate that mechanical serial inoculation methods have provided reliable data. In the study reported here, we were unable to identify discernible phenotypic differences in the TSWV symptoms of the controls over the duration of the screening with either TSWV6 or An_{wa}-1, which is in accordance with our previous studies (6,54-58).

TSWV evaluations using both ELISA and visual techniques

Both isolates (TSWV6 and An_{wa}-1) produced a range of systemic symptoms throughout the study and within a given inoculation, such as, tip blight, necrotic and chlorotic lesions, epinasty, and stunting in both the controls and *L. peruvianum* plants. However, both TSWV6 and An_{wa}-1 required more time before the typical symptoms appeared in either the controls or the accessions being tested, than in our experiments with either 85-9 or HR-1 (6,54,56-58; *unpublished data*). Using these isolates, we found that many of the inoculated plants indicated TSWV infection according to ELISA and did not always indicate visual symptoms for several weeks post inoculation.

Utilizing ELISA as a selection technique has been used for a number of TSWV resistance experiments (6,34,54-58). The conservative ELISA threshold suggested by Sutula et al. (59) has demonstrated a reasonable method of selecting infected plant material (6,34,54-58).

Differences in controls between the TSWV6 and An_{wa}-1 TSWV isolates

The reaction of 89R and 89S controls demonstrated that TSWV6 was significantly (P-value <0.0001) more virulent than An_{wa}-1. It was unexpected to have 73% of the 89R plants infected using An_{wa}-1 because *Sw-5* genotypes reportedly were resistant to An_{wa}-1 (25). Using typical TSWV isolates from various areas within the United States, we have found that the degree of resistance provided by *Sw-5* (*Sw-5*/*Sw-5* or *Sw-5*/*Sw-5*$^{+}$) results in 80 to 100% of plants being free of TSWV infection (6,57,58, *unpublished data*). This study shows that An_{wa}-1 overcomes 89R significantly (P-value<0.0001) less often than it infects 89S plants but at a much greater frequency than isolates we have used for past studies (6,57), suggesting that the TSWV isolates used in the Latham and Jones study (25) had a propensity to overcome *Sw-5* in their study.

Accession resistance responses to TSWV6 and An_{wa}-1

Several studies (20,22,31) have found, when testing two unique isolates, that a given accession may demonstrate resistance to one and not another. Fifty-four accessions tested with both isolates showed resistance to one isolate (Table 2). It is reasonable to attribute some of the purported resistance response in the *L. peruvianum* accessions when working with An_{wa}-1 to individual plants escaping infection because 5% of the 89S controls were not infected by this isolate (Table 1). However, there were 11 of the 23 accessions with a resistance response to An_{wa}-1 which had more than 10% of the plants not infected. Conversely, more than 10% of the plants (13 of the 31 accessions), showed resistance to TSWV6 but not to An_{wa}-1 (Table 2).

The unilateral resistance response (either TSWV6 or An_{wa}-1) of 54 accessions suggests multiple alleles or genes for resistance to TSWV within the *L. peruvianum* germplasm collections. This theory is further supported by other studies of smaller populations showing unique *L. peruvianum* resistance responses to varying isolates of TSWV (20,22,31).

Geographical distribution of resistant to TSWV

The unusually high percentage (≥81%) of the accessions with >8% plants free of infection to either isolate found in northern Chile and southern Peru is intriguing (Table 2; Fig. 2, inserts 2 and south to map edge, about 325 km). When examining the passport data of the accessions from this area we found that they were not all collected in the same year or by the same collection parties. Without additional in-depth studies, it is not possible to determine why apparent higher levels of TSWV resistance have identified in this area. Curiously, our data suggest that immediately north of this region, a general paucity of TSWV resistance occurs. However, in the northern part of Peru (near Lima and to the north), there are "pockets" of multiple accessions with higher levels of resistance to both isolates. For instance, the Culebras, Peru region (Fig. 2, northwest corner of inset 1) has a set of four accessions (PI 126944, PI126945, PI 126946, and PI 127839) with relatively higher levels of resistance to both isolates used in this study. Additionally, the Ecuadorian accessions (not found in Fig. 2) PI 128663, PI 129152, PI 129147, and PI 143679 tested only with TSWV6, indicated high percentages of virus-free plants ranging from 33 to 54%.

TSWV resistance distribution within the germplasm collections

The USDA and TGRC are collaborative centers each collection is somewhat representative of the wider indigenous geographical range of the species (Table 2). Hence, it is natural to expect that TSWV resistance would be somewhat equally distributed between these two germplasm collections. The TGRC Core collection was carefully selected using multiple criteria to represent as much *L. peruvianum* diversity as possible. Therefore it would be expected to have approximately the same percentages of TSWV resistance as found in the greater collections. However, we found that the USDA and the "non-core" TGRC collections had a similar percent of accessions with plants that had a resistant response to either TSWV6 or An_{wa}-1 (Fig. 1B), whereas the TGRC Core has significantly less ($P<0.0001$). Furthermore, the USDA collection had significantly more ($P<0.0001$) resistant plants per individual accession (Fig. 1A).

Previous studies of TSWV resistance within *L. peruvianum* accessions

Of the 285 accessions used in our study, 23 (Table 2) had been screened for TSWV resistance in other studies (4,7,15,20,22-24,34,47,49,50,52-54,57,58). Of those 23 accessions 13 were tested with both isolates, 9 with TSWV6, and 1 with An_{wa}-1 (Table 2). Of the 13 accessions previously reported (4,7,20,22,34,47,49,50,52,58) and tested with both isolates, 12 (PI 126929, PI 126930, PI 126441, PI 126444, PI 126928, PI 126935, PI 126944, PI 126945, PI 126946, PI 128652, LA 111, and LA 444) had no less than 15% of the plants, within an accession, resistant to at least one of the two isolates (Table 2).

Of the 13 accessions tested with both isolates, 3 indicated dramatic differences in the resistance responses between the isolates. Two of these three accessions (LA 111 and PI 126929) had little to no resistance to TSWV6 (1 and 0% respectively) and one (PI 126444) indicated 23% of the plants resistant to TSWV6 and 9% resistant to An_{wa}-1 (Table 2). PI 126444 and LA 111 had been tested previously with multiple isolates. Stevens et al. (58) tested PI 126444 with three isolates and found a similar level of resistance response between the isolates. In contrast to our results, Iizuka et al. (20) found that 100% of the LA 111 plants were resistant to two isolates of TSWV whereas 16% of the plants were susceptible to two other isolates.

Of the 23 accessions examined in previous TSWV resistance studies (7,15,20,24,27,34,47,49,50,52,57), 10 were tested with either TSWV6 or An_{wa}-1 (Table 2). Only three plants of accession LA 441 (tested with TSWV6) germinated; however, the remaining nine accessions (PI 128654, PI 128657, PI 128659, PI 128660, PI 129146, PI 143679, PI 251311, LA 372, and LA 385) had sufficient plants per accession to provide clear evidence of possible resistance (Table 2). Finding TSWV resistance in these accessions is in agreement with the previous reports (7,15,24,27,34,47,49,50,52,57). However, little or no resistance was found in LA 372 and LA 385 to TSWV6 and An_{wa}-1, respectively. Both of these accessions demonstrated resistance to three of four isolates used by Iizuka et al. (20).

Tomato lines purported to derive their resistance from *L. peruvianum*

Four tomato cultivars or lines (Anahu, UPV 1, UPV 32, and Stevens) attribute their purported TSWV resistance to *L. peruvianum*. Cv. Anahu (15) was reported to have

the TSWV resistance gene *Sw-1*, from PI 128657 (14). Previous research has found Anahu to be easily infected with various TSWV isolates; this study had 40% of the plants free of infection using TSWV6 on PI 128657 (24,33,34). Finding that 40% of the plants resisted TSWV6 suggests the existence of alleles differing *Sw-1*, or it is quantitatively inherited. Our results, in combination with the past studies of *Sw-1*, do suggest that a given accession may harbor multiple genes for resistance to TSWV.

The tomato lines UPV 1 and UPV 32 were derived from *L. peruvianum* accession PE-18 (46). UPV 1 is purported to have a single dominant unnamed TSWV resistance gene suggesting allelism to *Sw-5* (46). UPV 32 was identified as having a single resistance gene not allelic to *Sw-5* named "*Sw-6*" (45). We did not have PE-18 to screen; however, this *L. peruvianum* accession was reported to be from Huallanca, Ancash, Peru, where three accessions used in our study originated (LA 1982, LA 2561, and LA 2562; Table 2). Two of these accessions (LA 2561 and LA 2562) were tested with our isolates, resulting in three to four times the number of plants with resistance to An_{wa}-1 than to TSWV6. LA 1982 was tested only with TSWV6 and it had little resistance (2%) similar to the 3 and 5% found in LA 2561 and LA 2562, respectively. Thus, all three of the accessions from Huallanca, Ancash, Peru indicate little resistance to TSWV6, the isolate which completely overcomes *Sw-5*. These data suggest agreement with the results of PE-18 (45-47) with an *Sw-5* like response. We have tested UPV 32 with TSWV5 from Hawaii and found it fully susceptible (*unpublished data*). UPV 1 has not been tested in our program.

The last of the reported cultivar with purported *L. peruvianum*-derived TSWV resistance is Stevens (53,57) . This cultivar is one of two separately derived sources of

Sw-5 (5). There is evidence that *Sw-5* (from Stevens) originated from one of the *L. peruvianum* accessions PI 126928, PI 126929, PI 126944, PI 128645, PI 128654, or PI 129109 (57). We were able to include all except PI 129109 in our study. PI 126928, PI 126929, PI 126944, and PI 128645 were tested with both isolates, while PI 128654 was tested only with TSWV6. Interestingly, all of these accessions were among the most resistant accessions tested (Table 2). PI 128654 is considered a strong candidate as the source of *Sw-5* (57). However, our data provide an alternative candidate accession, PI 126929, which did not indicate resistance to TSWV6, although it had 44% of the plants indicate An_{wa}-1 resistance. Even with two lines of evidence for the possible origin of *Sw-5*, it is possible that all six accessions have *Sw-5*. Interestingly, at least five of the six accessions have differing resistance relative to *Sw-5*, in that a substantial number of plants were resistant to TSWV6 in those five accessions.

The value of the *Sw-5* gene

There is clear evidence that variant isolates of TSWV can overcome *Sw-5* (1,7,47,61). Furthermore, studies have demonstrated that tospoviruses can readily overcome both pathogen-derived resistance and natural resistance through reassortment of the TSWV genome (17,36,37). With these two lines of evidence in mind, the question arises as to why *Sw-5* has still been used worldwide, providing a high level of resistance in most areas where utilized. In regions where a TSWV isolate has overcome *Sw-5*, two related conditions have been noted. First, TSWV6 came from a situation where virtually 100% of the $Sw\text{-}5^{+}/Sw\text{-}5^{+}$ genotypes were naturally infected; thus, the disease pressure was extremely high. Second, where clear case histories can be identified; there often

have been year-around cropping of *Sw-5* genotype tomato with consistently "high" *Tospovirus* pressure. Curiously, TSWV6-like isolates have not been found in Hawaii since *Sw-5* was no longer used in the area. In fact, tomato plants with *Sw-5* were planted again several years later in the same region and *Sw-5* provided its normal level of resistance (John Cho, *personal communication*). This "*Sw-5* rest" allowed the indigenous tospovirus to revert to an "environmentally fit" tospovirus that did not include the ability to readily overcome *Sw-5*.

These anecdotal observations, coupled with the data we obtained when using An_{wa}-1, opens the question to the environmental "fitness" of TSWV isolates that overcome *Sw-5*. An_{wa}-1 was used in the experiments, demonstrating that TSWV isolates could be selected to overcome TSWV resistance (25). If isolates overcoming *Sw-5* are generally less "environmentally fit", it would explain why growers who seasonally utilize *Sw-5* genotype cultivars where TSWV pressure is high expect as much as 20% of their crop to be infected with the virus. However, regions that have consistently high TSWV pressure and never allow a rest from *Sw-5* are purported to be areas having troubles with TSWV overcoming this resistance. In considering the evidence that tospoviruses can quickly and easily "reshuffle" their genome, developing stable TSWV resistance may be a challenge (17,36,37). However, the slow adaptation of the virus to overcome *Sw-5* coupled with information that this event is documented mostly in areas where TSWV is infecting nearly 100% of known susceptible lines is in complete harmony with Leach et al. (26). Their results suggest that environmental fitness of this pathogen is directly related to the durability of resistance genes. The study we are reporting here was

conducted to search for additional genes equal to or even of greater value than *Sw-5* in the *L. peruvianum* germplasm.

Our results demonstrate that there is additional tospovirus resistance to be found in the *L. peruvianum* germplasm. It is unclear as to how much of this resistance is quantitative or qualitative in nature. However, there is evidence that both inheritance patterns for TSWV resistance can be found within *L. peruvianum* germplasm. *Sw-5* was identified from this species (57) and, in an unpublished study, we recently have endeavored to introgress resistance to TSWV6 from PI 128660 into tomato. We found that the high level of tospovirus resistance seems to be quantitative in nature. It becomes clear that mining additional TSWV resistance from *L. peruvianum* is realistic but should be approached with full awareness that both successful introgression of resistance and failure of that objective is a possible outcome.

Making interspecific crosses with *L. peruvianum* is challenging (16,35). Therefore, an optimal strategy that may produce functional, transferable, TSWV resistance would be to focus on accessions that appear to provide resistance to one isolate and not the other. For example, LA 2808 indicated resistance to An_{wa}-1 and G 30027 to TSWV6, but neither accession suggested resistant to the opposite isolates (Table 2). Even with the obvious hazard of incorporating isolate-specific resistance, it may be that this resistance is more heritable and different from each other. Attempts have been made to introgress the resistance of PI 128660 and other previously reported accessions into tomato by several breeding programs, resulting in no known long-term usable resistance incorporated into elite cultivars. Identifying more single genes such as *Sw-5* for breeding potentially can provide a suite of resistant genes. Thus, searching for accessions

demonstrating clear segregating type resistance may produce genes more readily introgressed with more utility than the resistance introgressions previously attempted.

Although there is clear evidence that tospoviruses potentially can readily overcome various types of resistance (1,17,25,36,37), the environmental fitness of such a tospovirus may prove difficult for an epidemic type of behavior of a new isolate (26). Nevertheless, carefully planned deployment of tospovirus resistance, either natural or pathogen derived, should be part of the overall management of a crop in conditions where this virus is prevalent.

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Table 1. Summary of *L. peruvianum Tomato spotted wilt virus* (TSWV) resistance response to the Hawaiian (TSWV6) and Australian Anemone (An_{wa}-1) isolates across all accessions, individual plants, and *L. esculentum* (89S and 89R) controls[a]

	TSWV isolates[b]											
	Number of				With resistant reaction							
	Accessions tested		Plants tested		Plants (no.)		Plants (%)		Accessions (no.)		Accessions (%)	
Accessions	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1
TGRC Core	35	24	767	962	24	51	3	5	12	10	34	42
TGRC	129	111	5,559	3,575	497	426	9	12	81	66	63	57
USDA	116	42	4,308	1,164	916	387	21	33	75	30	65	71
Totals	280	177	10,634	5,701	1,437	864	14	15	168	106	60	59
Controls[c]												
89S			1,404	495	0	23	0	5				
89R			1456	574	0	153	0	27				
Totals			2,860	1,069	0	176	0	16				

[a]In total, 285 *L. peruvianum* accessions screened. Of those, 169 were from Tomato Genetics Resource Center (TGRC), University of California, Davis, 38 of which belong to the core collection (TGRC Core), and 116 from the United States Department of Agriculture (USDA) Research Service collection located at the Cornell University Geneva, NY campus.
[b]TSWV isolate TSWV6 infects tomato with a *Sw-5*/*Sw-5* genotype and An_{wa}-1 partially infects the same genotype.
[c]Near-isogenic lines 89S (*Sw-5*$^+$/*Sw-5*$^+$) and 89R (*Sw-5*/*Sw-5*) were used as controls.

Table 2. All *Lycopersicon peruvianum* accessions tested and ranked according to average percent of apparent resistant plants to *Tomato spotted wilt virus* (TSWV) isolates TSWV6 and anemone (An_{wa}-1)[a]

		Plants tested with TSWV isolates[b]						
		Resistant (%)		Resistant (no.)		Tested (no.)		
Accession no.	Avg. (%)	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1	TSWV6	An_{wa}-1	Origin of the accession[c]
PI 126946[de]	68	72	64	33	23	46	36	Culebras, Perú*
PI 128648[f]	63	80	45	24	10	30	22	Azapa Valley, Chile*
LA 3636	63	63	63	19	27	30	43	Coayllo, Lima, Perú**
PI 128646[f]	57	42	71	16	17	38	24	Lluta/Arica, Chile*
PI 128649[f]	57	43	70	17	19	40	27	Arica, Chile*
LA 2742[f]	56	67	45	31	13	46	29	Camarones-Guancarane, Tarapacá, Chile**
PI 126930[de]	56	56	55	24	12	43	22	Chancay, Perú*
PI 127832	55	52	57	25	25	48	44	Sacabaya, Perú*
PI 128650[f]	54	39	68	15	27	38	40	Arica, Chile*
PI 128652[fg]	54	48	60	23	25	48	42	Arica, Chile*
PI 128643	53	80	26	28	10	35	39	Lima, Perú*
LA 3900	52	67	36	32	16	48	45	Canta, Lima, Perú**
PI 126441[h]	52	61	42	22	10	36	24	Canta, Lima, Perú*
PI 127831	51	55	46	21	19	38	41	Sacabandía, Perú*
PI 128645[fi]	49	55	43	26	16	47	37	Lluta/Arica, Chile*
PI 128647[f]	49	48	50	10	9	21	18	Arica, Chile*
PI 126945	49	36	62	12	23	33	37	Culebras, Perú*
LA 1677	48	23	73	5	30	22	41	Fundo Huaquina to Topara, Lima, Perú**
PI 126944[deghij]	48	39	57	11	21	28	37	Culebras, Perú*
PI 126928[dei]	47	54	39	26	19	48	49	Pacasmayo, Perú*
PI 126935[h]	47	51	43	19	13	37	30	Saña, Perú*
LA 3640	41	54	28	26	10	48	36	Mexico City, Mexico**
LA 2745[f]	40	42	38	20	9	48	24	Pan de Azúcar (Azapa), Tarapacá, Chile**
LA 3858	39	40	38	19	15	48	40	Canta, Lima, Perú**
PI 127830	39	20	58	9	23	44	40	Mollendo, Arequipa, Perú*
LA 2770[f]	36	38	33	15	7	39	21	Lluta, Tarapacá, Chile**
G 30046	35	46	23	22	9	48	40	Perú*
LA 1975	33	28	37	13	10	47	27	Desvio Santo Domingo, Lima, Perú**

LA 2717	33	44	21	16	6	36	28	Chilca, Lima, Perú**
PI 128651[f]	33	35	30	17	14	48	47	Arica, Chile*
LA 98	31	23	38	13	18	56	48	Chilca, Lima, Perú**
LA 2959[f]	31	31	31	13	12	42	39	Chaca-Vitor, Tarapacá, Chile**
PI 126926	31	44	18	21	5	48	28	Pacasmayo, Perú*
LA 2808	26	0	52	0	15	48	29	Huaylas, Ancash, Perú**
LA 3639	25	21	29	7	7	34	24	Catac, Lima, Perú**
LA 3799	25	38	11	18	4	47	37	Rio Pativilca, Ancash, Perú**
G 30027	25	48	2	23	1	48	43	Perú*
LA 2581[f]	24	22)	26	9	5	41	19	Chacarilla (4x), Tarapacá, Chile**
LA 3637	24	19	28	9	8	48	29	Coayllo, Lima, Perú**
PI 326173	23	0	45	0	10	24	22	South America*
LA 1675	22	2	42	1	16	41	38	Toparilla Cañón, Lima, Perú**
PI 126929[i]	22	0	44	0	11	13	25	Pacasmayo, Perú*
LA 1513	21	34	7	11	1	32	14	Atiquipa, Arequipa, Perú**
LA 1647	21	16	25	6	10	37	40	Huaquina, Topara, Ica, Perú**
LA 153	20	8	31	1	15	12	48	Culebras, Ancash, Perú***
LA 464[f]	20	0	40	0	19	52]	48	Hacienda Rosario, Tarapacá, Chile**
LA 2962	20	19	21	9	6	47	29	Chancay, Arequipa, Perú**
LA 2744[f]	18	9	26	3	12	32	47	Sobraya (Azapa), Tarapacá, Chile***
LA 1373	17	10	24	4	9	40	37	Asia, Lima, Perú**
LA 1475	16	15	17	6	7	40	42	Fundo "Los Anitos", Lima, Perú**
LA 1692	16	12	19	3	5	25	27	Putinza, Lima, Perú**
PI 126444[k]	16	23	9	11	4	48	43	Canta, Perú*
PI 306811	16	0	32	0	7	24	22	United States*
LA 444[ejl]	15	14	15	3	7	22	47	Chincha #1, Ica, Perú***
LA 1300	15	0	29	0	14	53	48	Santa Rosa de Quives, Lima, Perú**
LA 2721	15	29	0	13	0	45]	17	Putinza, Lima, Perú**
LA 2724	15	30	0	12	0	40	13	Huaynilla, Lima, Perú**
LA 1270	14	2	26	1	12	42	46	Pisiquillo, Lima, Perú**
LA 2068	14	27	0	10	0	37	27	Chasquitambo, Ancash, Perú**
LA 2732[f]	14	8	19	1	9	13	48	Moquella, Tarapaca, Chile***
LA 3795	14	28	0	13	0	46	53	Alta Fortaleza, Ancash, Perú**
LA 1473	12	19	5	9	2	48	39	Callahuanca, Santa Eulalia, Lima, Perú**
LA 1552	12	21	3	8	1	39	34	San Mateo, Lima, Perú**

LA 2562[m]	12	5	19	2	4	42	21	Huallanca, Ancash, Perú**
LA 3797	12	18	6	7	2	39	34	Anca Marca, Ancash. Perú**
LA 111[n]	11	1	21	1	10	122	48	Zupe, Lima, Perú**
LA 1616	11	0	21	0	3	13	14	La Molina, La Rinconada, Lima, Perú**
LA 1694	11	3	18	1	16	33	91	Cacachuhuasin, Lima, Perú**
PI 126439	11	22	0	10	0	46	17	Canta, Perú*
LA 1305	10	17	2	5	1	30	48	Ticrapo, Huancavelica, Perú***
LA 1537	10	14	5	6	1	42	19	Probably Perú**
LA 1646	10	19	0	6	0	32	23	Canta, Lima, Perú**
LA 1358	9	13	5	4	2	31	41	Yautan, Ancash, Perú**
LA 1368	9	13	5	3	2	24	37	San Jose de Palla, Lima, Perú**
LA 1935	9	0	18	0	5	33	28	Lomas de Atiquipa, Arequipa, Perú**
LA 1133	9	0	17	0	8	58	48	Huachipa, Lima, Perú**
LA 2563	9	7	10	3	3	43	31	Canon del Pato, Ancash, Perú**
LA 2575	9	0	17	0	6	47	36	Valle de Casma, Ancash, Perú**
LA 2566	8	15	0	6	0	40	29	Pomacocha-Llameyin, Rio Pocha, Ancash, Perú**
LA 2573	8	2	14	1	5	48	36	Valle de Casma, Ancash, Perú**
G 30036	8	4	11	2	2	48	19	Perú*
LA 1292	7	8	5	1	1	13	22	San Mateo, Lima, Perú***
LA 2561[m]	7	3	10	1	2	39	20	Huallanca, Ancash, Perú**
LA 2331	7	13	0	6	0	47	43	Agayapampa, La Libertad, Perú**
LA 3664	7	14	0	1	0	7	13	Nazca grande, Ica, Perú**
G 30040	7	13	0	6	0	48	20	Perú*
G 30044	7	0	13	0	1	42	8	Perú*
LA 1369	6	10	2	4	1	39	45	San Gerónimo, Lima, Perú**
LA 1377	6	5	6	1	2	20	36	Navan, Lima, Perú**
LA 1517	6	8	3	2	1	26	36	Irrigación Santa Rosa, Lima, Perú**
LA 1556	6	5	6	2	2	39	34	Hacienda Higuereta, Lima, Perú**
LA 2164	6	2	10	1	4	47	40	Cuyca, Cajamarca, Perú**
PI 127829	6	11	0	5	0	46	28	San Juán/ Magdalena, Perú*
G 30043	6	6	5	2	1	32	21	Perú*
LA 366	5	0	10	0	5	79	48	Canta, Lima, Perú**
LA 446	5	0	10	0	5	23	48	Atiquipa, Arequipa, Perú**
LA 1161	5	2	8	1	4	42	48	Huachipa, Lima, Perú**
LA 1626	5	10	0	2	0	21	27	Mouth of Rio Rupac, Ancash, Perú***

LA 1977	5	9	0	4	0	47	23	Orcocoto, Lima, Perú**
LA 1981	5	6	3	3	1	48	40	Vocatoma, Ancash, Perú**
LA 2326	5	9	0	4	0	47]	25	Above Balsas, Amazonas, Perú**
LA 2333	5	10	0	5	0	48	32	Casmiche, La Libertad, Perú**
LA 2388	5	2	8	1	3	48	39	Cochabamba to Huambos, Cajamarca, Perú**
LA 2555	5	9	0	3	0	34	41	Mariscal Castilla, La Libertad, Perú**
LA 3218	5	4	6	1	2	24	31	Quebrada Guerrero, Arequipa, Perú**
LA 3219	5	9	0	1	0	11	12	Catarindo, Arequipa, Perú**
LA 1554	4	8	0	3	0	36	24	Huaral to Cerro de Pasco, Lima, Perú**
LA 1913	4	3	5	1	1	39	22	Tinguiyo, Ica, Perú**
LA 2809	4	0	7	0	2	26	30	Huaylas, Ancash, Perú**
LA 2834	4	0	7	0	1	6	15	Hacienda Asiento, Ica, Perú**
LA 3154	4	2	6	1	2	48	34	Otora-Puente Jahuay, Moquegua, Perú**
LA 448	3	0	6	0	3	72	48	Chala, Arequipa, Perú**
LA 1294	3	5	0	1	0	19	47	Surco, Lima, Perú**
LA 1304	3	5	0	3	0	62	47	Pámpano, Huancavelica, Perú**
LA 1983	3	5	0	2	0	42	22	Rio Manta, Ancash, Perú**
LA 2327	3	6	0	3	0	48	33	Aguas Calientes, Cajamarca, Perú**
LA 2565	3	0	5	0	1	48	22	Potrero de Pomacocha, Ancash, Perú**
LA 3156	3	5	0	1	0	21	15	Omate Valley, Moquegua, Perú**
PI 127828	3	5	0	1	0	22	25	San Juán, Cajamarca, Perú*
G 30034	3	0	6	0	1	46	18	Perú*
LA 1337	2	1	2	1	1	81	48	Atiquipa, Arequipa, Perú**
LA 1379	2	0	4	0	2	36	47	Cavajo, Lima, Perú***
LA 1653	2	0	3	0	1	36	37	Uchumayo, Arequipa, Perú**
LA 1937	2	4	0	1	0	27	8	Quebrada Torrecillas, Arequipa, Perú***
LA 1949	2	3	0	1	0	34	12	Las Calaveritas, Arequipa, Perú**
LA 1951	2	3	0	1	0	32	39	Ocoña, Arequipa, Perú***
LA 455	1	1	0	1	0	132	44	Tambo, Arequipa, Perú**
LA 752	1	0	2	0	1	48	48	Sisacaya, Lima, Perú**
LA 1280	1	1	0	1	0	102	48	Chaciacayo, Lima, Perú**
LA 1364	1	0	2	0	1	9	48	Alta Fortaleza, Ancash, Perú***
LA 1551	1	2	0	1	0	42	19	Surco, Lima, Perú**
LA 1984	1	0	2	0	1	11	41	Otuzco, La Libertad, Perú***
G 30039	1	2	0	1	0	41	12	Perú*

LA 378	0	0	0	0	0	110	47	Cascas, Cajamarca, Perú**
LA 451	0	0	0	0	0	36	47	Arequipa, Arequipa, Perú**
LA 453	0	0	0	0	0	57	46	Yura, Arequipa, Perú**
LA 462[ef]	0	0	0	0	0	60	35	Sobraya, Tarapaca, Chile**
LA 1032	0	0	0	0	0	27	47	Aricampa, La Libertad, Perú**
LA 1278	0	0	0	0	0	55	8	Trapiche, Lima, Perú**
LA 1331	0	0	0	0	0	21	40	Nazca, Ica, Perú***
LA 1394	0	0	0	0	0	43	38	Balsas, Amazonas, Perú**
LA 1396	0	0	0	0	0	46	30	Balsas (Chachapoyas), Amazonas, Perú**
LA 1474	0	0	0	0	0	8	48	Lomas, de Camaná, Arequipa, Perú***
LA 1708	0	0	0	0	0	39	9	Chamaya to Jaen, Cajamarca, Perú**
LA 1722	0	0	0	0	0	18	22	Ticrapo Viejo, Huancavelica, Perú**
LA 1723	0	0	0	0	0	3	3	La Quinga, Ica, Perú**
LA 1744	0	0	0	0	0	42	20	Putinza, Lima, Perú**
LA 1910	0	0	0	0	0	36	3	Tambillo, Huancavelica, Perú***
LA 1929	0	0	0	0	0	13	48	La Yapana, Ica, Perú***
LA 1944	0	0	0	0	0	41	19	Rio Atico, Arequipa, Perú**
LA 1945	0	0	0	0	0	7	36	Caravelí, Arequipa, Perú***
LA 1947	0	0	0	0	0	21	21	Puerto Atico, Arequipa, Perú**
LA 1955	0	0	0	0	0	46	24	Matarani, Arequipa, Perú**
LA 1985	0	0	0	0	0	47	35	Casmiche, La Libertad, Perú**
LA 2163	0	0	0	0	0	45	45	Cochabamba to Yamaluc, Cajamarca, Perú***
LA 2185	0	0	0	0	0	37	44	Pongo de Rentema, Amazonas, Perú***
LA 2330	0	0	0	0	0	45	20	Chagual, La Libertad, Perú**
LA 2964	0	0	0	0	0	49	48	Quebrada de Burros, Tacna, Perú***
LA 2981-B	0	0	0	0	0	47	47	Torata to Chilligua, Moquegua, Perú***
LA 3220	0	0	0	0	0	5	15	Cocachacra-Quebrada Cachendo,Arequipa, Perú**
LA 3666	0	0	0	0	0	1	24	La Yapa, Ica, Perú**
LA 3781	0	0	0	0	0	7	27	Quebrada Oscollo, Arequipa, Perú**
LA 3783	0	0	0	0	0	11	16	Chapana, Arequipa, Perú**
LA 3787	0	0	0	0	0	26	21	Alta Chaparra, Arequipa, Perú**
LA 3790	0	0	0	0	0	16	33	Caravelí, Arequipa, Perú**
G 30028	0	0	0	0	0	47	25	Perú*
G 30029	0	0	0	0	0	34	19	Perú*
G 30032	0	0	0	0	0	45	22	Perú*

G 30033	0	0	0	0	0	46	23	Perú*
G 30035	0	0	0	0	0	44	8	Perú*
G 30037	0	0	0	0	0	30	13	Perú*
G 30045	0	0	0	0	0	48	7	Perú*
PI 251307	---	74	---	25	---	34	0	Cascas/La Libertad, Perú*
PI 128660[dfg]	---	60	---	28	---	47	0	Tacna, Perú*
PI 251306	---	59	---	23	---	39	0	Culebras/Ancash, Perú*
PI 143679[h]	---	54	---	25	---	46	0	Manabi, Ecuador*
PI 128653[f]	---	53	---	24	---	45	0	Arica, Chile*
PI 129147	---	48	---	23	---	48	0	Guayaquil/Salinas, Ecuador*
PI 129152	---	46	---	22	---	48	0	Guayaquil, Ecuador*
PI 129145	---	45	---	20	---	44	0	Tambo, Perú*
PI 128654[fi]	---	44	---	14	---	32	0	Arica, Chile*
PI 128656[f]	---	41	---	12	---	29	0	Characarilla/Tarapaca, Chile*
PI 128657[defo]	---	40	---	19	---	47	0	Tacna, Perú*
PI 246586 97GI	---	39	---	16	---	41	0	Atico/Arequipa, Perú*
PI 128655[f]	---	38	---	10	---	26	0	Chacarilla/Tarapaca, Chile*
PI 128663	---	35	---	17	---	48	0	Moquegua, Perú*
PI 129149	----	33	---	10	---	30	0	Guayaquil, Ecuador*
PI 390671	---	32	---	9	---	28	0	Lima, Perú*
PI 270435	---	30	---	14	---	47	0	Mexico City, Mexico*
PI 251311[h]	---	25	---	10	---	40	0	Arequipa, Perú*
PI 251308	---	24	---	7	---	29	0	Atiquipa/Arequipa, Perú*
PI 128661[f]	---	23	---	10	---	43	0	Tacna, Perú*
PI 390682	---	23	---	11	---	48	0	Lima, Perú*
PI 390681	---	21	---	6	---	28	0	Lima, Perú*
LA 1609	---	20	---	2	---	10	0	Asia-El Pinon, Lima, Perú***
PI 251309	---	19	---	8	---	42	0	Chala/Arequipa, Perú*
PI 251310	---	19	---	4	---	21	0	Atico, Perú*
PI 266375	---	19	---	5	---	26	0	Lima, Perú*
PI 390672	---	18	---	8	---	44	0	Lima, Perú*
PI 390670	---	17	---	7	---	42	0	Lima, Perú*
PI 246586 60OI	---	16	---	5	---	31	0	Atico/Arequipa, Perú*
PI 128659[dfg]	---	13	---	6	---	45	0	Tacna, Perú*
LA 1365	---	12	---	3	---	25	0	Caranquillo, Ancash, Perú***

PI 129146[de]	---	12	---	2	---	17	0	Tambo, Perú*
PI 365956	---	12	---	3	---	25	0	Lima, Perú*
PI 128658[f]	---	11	---	5	---	45	0	Tacna, Perú*
PI 365942	---	11	---	2	---	18	0	Lima, Perú*
PI 246585	---	9	---	3	---	32	0	Ayacucho, Perú*
PI 390684	---	8	---	3	---	36	0	Lima, Perú*
LA 1350	---	8	---	1	---	12	0	Chauna, Cajamarca, Perú**
LA 110	---	7	---	9	---	123	0	Cajacay, Ancash, Perú**
PI 379029	---	7	---	3	---	44	0	Tambo, Perú*
PI 390680	---	7	---	3	---	43	0	Lima, Perú*
LA 445	---	6	---	3	---	51	0	Chincha #2, Ica, Perú**
PI 390664	---	6	---	2	---	32	0	Paramonga, Perú*
LA 364	---	5	---	1	---	22	0	Canta, Lima, Perú**
LA 1293	---	5	---	2	---	43	0	Matucana, Lima, Perú**
PI 303814	---	5	---	2	---	43	0	Idaho, United States*
PI 365946	---	5	---	1	---	20	0	Puquino, Perú*
LA 370	---	4	---	3	---	79	0	Hacienda Huampaní, Lima, Perú**
PI 365943	---	4	---	2	---	48	0	Lima, Perú*
PI 390669	---	4	---	2	---	48	0	Lima, Perú*
LA 372[n]	---	3	---	3	---	91	0	Culebras #1, Ancash, Perú**
LA 1271	---	3	---	1	---	37	0	Horcón, Lima, Perú**
PI 379015	---	3	---	1	---	40	0	Ticrapo, Perú*
LA 392	---	2	---	1	---	45	0	Llallan, Cajamarca, Perú**
LA 1982[m]	---	2	---	1	---	42	0	Huallanca, Ancash, Perú***
LA 1283	---	2	---	1	---	41	0	Sta.Cruz de Laya, Lima, Perú**
PI 365947	---	2	---	1	---	47	0	Arequipa, Perú*
PI 365950	---	2	---	1	---	48	0	Caciche, Perú*
LA 371[e]	---	0	---	0	---	79	0	Zupe, Lima, Perú**
LA 374	---	0	---	0	---	107	0	Culebras #2, Ancash, Perú**
LA 441[p]	---	0	---	0	---	3	0	Cerro Campana, La Libertad, Perú***
LA 454	---	0	---	0	---	15	0	Tambo, Arequipa, Perú***
LA 1027	---	0	---	0	---	13	0	Cajamarca, Perú**
LA 1031	---	0	---	0	---	92	0	Balsas, Amazonas, Perú**
LA 1274	---	0	---	0	---	2	0	Pocabamba, Lima, Perú***
LA 1284	---	0	---	0	---	30	0	Espiritu Santo, Lima, Perú**

LA 1296	---	0	---	0	---	8	0	Tornamesa, Lima, Perú**
LA 1333	---	0	---	0	---	76	0	Loma Camana, Arequipa, Perú**
LA 1336	---	0	---	0	---	39	0	Atico, Arequipa, Perú**
LA 1339	---	0	---	0	---	35	0	Capillucas, Lima, Perú***
LA 1346	---	0	---	0	---	51	0	Caciche, La Libertad, Perú**
LA 1351	---	0	---	0	---	6	0	Rupe, Cajamarca, Perú***
LA 1360	---	0	---	0	---	29	0	Pariacoto, Ancash, Perú***
LA 1395	---	0	---	0	---	26	0	Chachapoyas, Amazonas, Perú***
LA 1973	---	0	---	0	---	15	0	Yura, Arequipa, Perú***
LA 2152	---	0	---	0	---	2	0	Cospán, Cajamarca, Perú***
LA 2172	---	0	---	0	---	35	0	Cuyca, Cajamarca, Perú***
LA 2328	---	0	---	0	---	1	0	Aricapampa, La Libertad, Perú***
LA 3853	---	0	---	0	---	22	0	Moyopampa, La Libertad, Perú**
PI 251314	---	0	---	0	---	24	0	The Rio Jequetepeque, Perú*
PI 266376	---	0	---	0	---	14	0	Magdalena/Cajamarca, Perú*
PI 365938	---	0	---	0	---	15	0	Pocabamba/Lima, Perú*
PI 365939	---	0	---	0	---	31	0	Pocabamba, Perú*
PI 365940	---	0	---	0	---	23	0	Pocabamba, Perú*
PI 365941	---	0	---	0	---	4	0	Quilca/Lima, Perú*
PI 365948	---	0	---	0	---	45	0	Carretera, Perú*
PI 365951	---	0	---	0	---	17	0	Contumazá, Perú*
PI 365952	---	0	---	0	---	13	0	Huaras, Perú*
PI 365953	---	0	---	0	---	24	0	Caranquillo, Perú*
PI 365955	---	0	---	0	---	17	0	Lima, Perú*
PI 365968	---	0	---	0	---	30	0	Cuzco, Perú*
PI 365969	---	0	---	0	---	33	0	Yaca, Perú*
PI 379016	---	0	---	0	---	30	0	Chauna, Perú*
PI 379017	---	0	---	0	---	15	0	Lima, Perú*
PI 379018	---	0	---	0	---	48	0	Lima, Perú*
PI 390665	---	0	---	0	---	48	0	Camaná, Perú*
PI 390666	---	0	---	0	---	44	0	Lima, Perú*
PI 390667	---	0	---	0	---	47	0	Lima, Perú*
PI 390668	---	0	---	0	---	24	0	Lima, Perú*
PI 390673	---	0	---	0	---	42	0	Lima, Perú*
PI 390674	---	0	---	0	---	42	0	Lima, Perú*

PI 390675	---	0	---	0	---	48	0	Lima, Perú*
PI 390676	---	0	---	0	---	48	0	Lima, Perú*
PI 390678	---	0	---	0	---	48	0	Lima, Perú*
PI 390679	---	0	---	0	---	48	0	Lima, Perú*
PI 390683	---	0	---	0	---	48	0	La Libertad, Perú*
PI 390685	---	0	---	0	---	48	0	Cerro de Pasco, Perú*
PI 390687	---	0	---	0	---	22	0	Canchaque, Perú*
LA 107	---	---	4	---	2	0	47	Hacienda San Isidro, Lima, Perú***
LA 385[n]	---	---	0	---	0	0	48	San Juán, Cajamarca, Perú**
LA 1989	---	---	0	---	0	0	4	**
LA 2157	---	---	0	---	0	0	38	Tunel Chotano, Cajamarca, Perú***
LA 2553	---	---	0	---	0	0	48	Balconcillo de San Marcos, Cajamarca, Perú***

[a] The TSWV6 isolate overcomes *Sw-5*/*Sw-5* genotypes where An_{wa}-1 partially overcomes the same genotype. In total, 285 *L. peruvianum* accessions were screened. Of those, 169 were from theTomato Genetics Resource Center (TGRC), University of California, Davis), 38 of which belong to the core collection (TGRC Core), and 116 from the (United States Department of Agriculture (USDA) Research Service collection located at the Cornell University Geneva, NY campus.

[b] TSWV isolate TSWV6 infects *Sw-5*/*Sw-5* and *Sw-5*$^{+}$/*Sw-5* genotypes and An_{wa}-1 genotypes partially infects these genotypes. Avg. (%) = percent average resistant.

[c] Asterisks *, **, and *** indicate collections from the USDA, TGRC, and TGRC Core, respectively.

[d] Eight *L. peruvianum* accessions that Smith and Paterson et al. reported on TSWV resistance in 1944 and 1989, respectively (34,52).

[e] Nine accessions reported as being resistant to TSWV by Segeren et al. in 1993 (49,50).

[f] Twenty-six accessions from the southern tip of Peru and northern Chile, with the majority of the accessions associated with a high percentage of TSWV resistance (Fig. 1).

[g] Five *L. peruvianum* accessions identified by Cho et al. in 1996 (7) as being resistant to TSWV6.

[h] Five of the *L. peruvianum* accessions reported with TSWV resistance by Roselló et al. in 1999 (47).

[i] Five of the *L. peruvianum* accessions mentioned in the development of cv. Stevens as a possible source of *Sw-5* (57).

[j] *L. peruvianum* accession studied for TSWV resistance by Jordá et al. in 1993 (22).

[k] *L. peruvianum* accession studied for TSWV resistance by Stevens et al. in 1994 (58).

[l] *L. peruvianum* accession studied and reported on for possible TSWV resistance by Boiteux et al. 1992 (4).

[m] *L. peruvianum* accessions originating from Huallanca, Ancash, Peru, where PE-18 originated; PE-18 is the source of *Sw-6* (45-47).

[n] Three *L. peruvianum* accessions studied and reported as having a resistance response by Iizuka et al. in 1993 (20).

[o] *L. peruvianum* accession used by Gilbert and Tanaka in 1971 (15) for the development of 'Anahu' and also studied for TSWV resistance and reported on by Kumar et al. 1993 (24).

[p] *L. peruvianum* accession studied for TSWV resistance by Maluf et al. in 1991 (27).

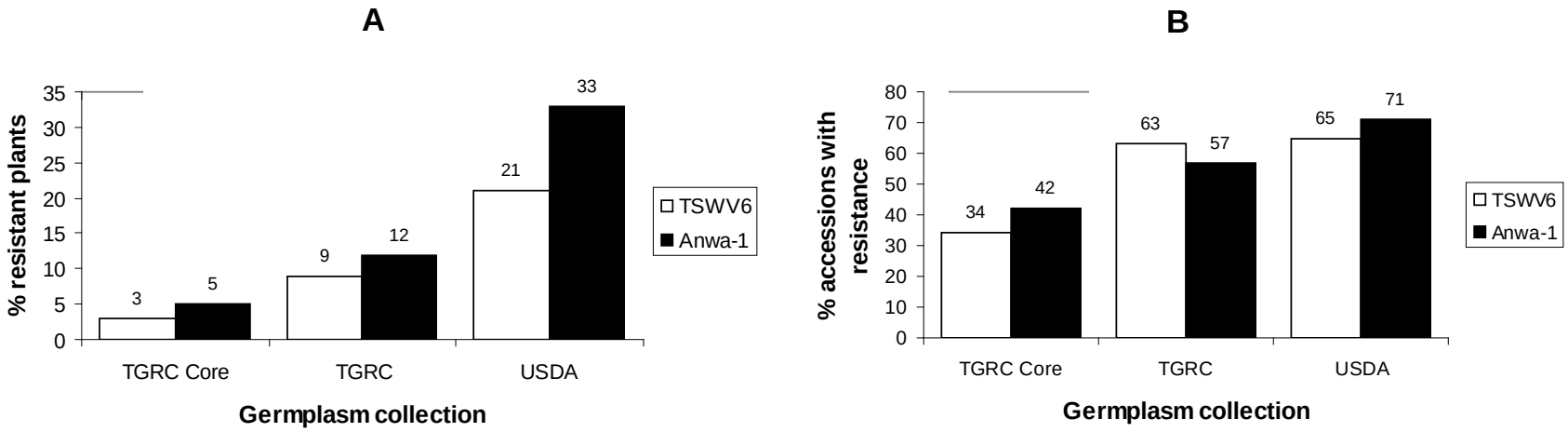
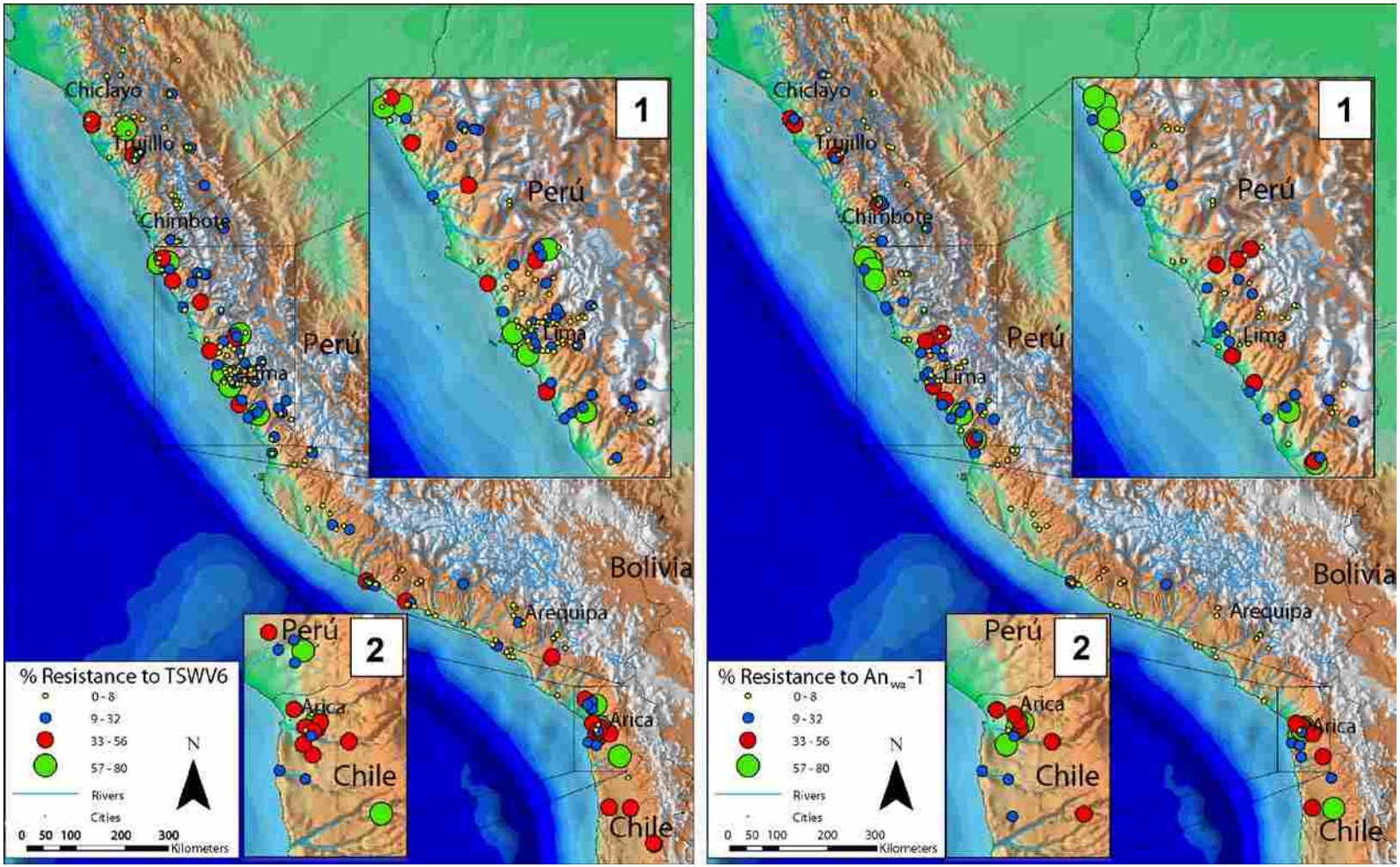


Fig. 1. *Tomato spotted wilt virus* (TSWV) resistance response of the accessions from the United States Department of Agriculture (USDA) Research Service located at the Cornell University Geneva, NY campus, Tomato Genetics Resource Center (TGRC), University of California, Davis and TGRC-Core collections to the Hawaiian isolate (TSWV6) and to the anemone isolate (An_{wa}-1) expressed as a percentage of plants free of infection.

Fig. 2. Maps of Peru and Chile created with the *Arc/GIS* program showing the spatial distribution of the accessions collected according to the global positioning coordinates. There is sufficient passport data to map 256 accessions tested with the **A,** *Tomato spotted wilt virus* (TSWV) Hawaiian isolate (TSWV6) and **B,** 158 tested with anemone isolate (An_{wa}-1) for a total of 260 between the two isolates. These accessions are maintained at the United States Department of Agriculture Research Service (USDA) located at the Cornell University Geneva, NY campus), Tomato Genetics Resource Center (TGRC), University of California, Davis, and TGRC Core accessions. Accessions are presented with their percent resistance (dot size and color coded). Specific accessions indicated on this map are those with city or region as well as country included in its source information in Table 2.

Fig. 2A TSWV6 Isolate

Fig. 2B An_{wa}-1 Isolate



CHAPTER THREE

Loss of Resistance when Breeding TSWV6 Resistance in Tomato (*Solanum lycopersicum* L.) Derived from *Solanum peruvianum* (L.)

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Abstract

Sw-5 derived from *Solanum peruvianum* (L.) confers resistance to *Tomato spotted wilt virus* (TSWV). A unique TSWV isolate from Hawaii (TSWV6) overcame *Sw-5* in this work when tested on near isogenic lines (NILs) controls 89R (*Sw-5/Sw-5*), and 89S ($Sw\text{-}5^{+}/Sw\text{-}5^{+}$). Both lines were 100% infected. Our TWSV6 resistance screening trials of *S. peruvianum* accessions indicate resistance in line PI 128660, although repeated testing of cuttings of putatively resistant plants suggests less than 100% penetrance. We have developed interspecific hybrids and some backcrosses between *S. lycopersicum* (L.) and TSWV6 resistant PI 128660 lines. To circumvent the postzygotic genetic barrier, we used embryo rescue to obtain the F_1 and the BC_1P_1 generations. We screened the F_1, BC_1P_1, BC_2P_1, and BC_3P_1 generations by mechanically inoculating them with TSWV6. Apparently uninfected plants were selected and tested for the presence/absence of TSWV6 by enzyme-linked immunosorbent assay (ELISA). Fewer plants indicating TSWV6 resistance in the BC_2P_1 and BC_3P_1 to the point where there were no significant differences between the TSWV susceptible controls and BC_3P_1 when tested in either the greenhouse or in the field with common isolates of TSWV or the TSWV6 isolate.

Introduction

Tomato spotted wilt virus (TSWV), classified as a tospovirus, infects cultivated tomato [*Solanum lycopersicum* (L.)], causing plant stunting, necrotic leaf spotting, mottling, epinasty, plant and fruit deformity, and in severe cases, death (Aramburu et al. 2000; Cho et al. 1989). Thrips are the natural vectors of tospoviruses with species of the genera *Frankliniella* and *Thrips* being the most common (Cho et al. 1989, 1986; Parrella et al. 2003; Riley and Pappu 2004). Because of the globalization of trade these insects have spread worldwide along with their vectored disease. TSWV has been reported in Europe, Asia, Africa, the Caribbean, South Africa, Australia, and in Oceania (McMichael et al. 2000; Thomas-Carroll and Jones 2003; Thompson and van Zijl 1996).

Tospoviruses infect at least fifteen monocotyledonous and sixty-nine dicotyledonous families of which the Asteraceae (Compositae), Solanaceae, and Leguminoseae are the most commonly affected. More than 1,090 plant species have been reported to be infected by this virus including many crops of economic importance with vegetable yield losses worldwide estimated at $1.0 billion annually (Parrella et al. 2003). In the 2000 growing season, TSWV epidemics in tomato were estimated to cause losses totaling $8.8 million in Georgia, USA (Riley and Pappu 2004). Cho et al. (1996) reported that yield losses in Hawaii have been high enough to preclude tomato production in some areas due to TSWV. In Brazil, Argentina, Spain, Portugal, and Italy, it is becoming the most limiting factor for tomato production (Aramburu et al. 2000; Aramburu and Martí 2003; Boiteux and Jordano 1992; Resende et al. 2000; Sialer et al. 2002; Williams et al. 2001).

Thrips are minute winged insects that migrate into fields infecting plants before insecticides have time to control them effectively. Growers have tried to control TSWV by

limiting thrips reproduction through different approaches such as crop rotation, fallowing, eliminating crop residue, pesticides, weed control, reflective plastic mulch, and combinations of these methods. Unfortunately, these methods have not been sufficiently effective in controlling the thrips vectoring the virus (Cho et al. 1989). Natural resistance found in the wild relatives of tomato provides a means to reduce TSWV damage (Cho et al. 1989; Riley and Pappu 2004).

Stevens et al. (1994) screened 188 accessions of seven different *Solanum* species for resistance to isolates of TSWV, finding that *S. peruvianum* and *S. chilense* (L.) (formerly, *Lycopersicon esculentum* and *L. chilense* Dun., respectively), provided the highest level of resistance to TSWV. Although they screened all available *S. chilense* accessions in the US germplasm collections, only twelve *S. peruvianum* accessions were screened (Stevens 1993; Stevens et al. 1994). Additionally, a number of other reports (Boiteux and Giordano 1992; Cupertino et al. 1986; Finlay 1952, 1953; Gilbert and Tanaka 1971; Hutton and Peak 1949; Iizuka et al. 1993; Kumar and Irulapan 1992; Kumar et al. 1993; Maluf et al. 1991; Norris 1946; Paterson et al. 1989; Roselló et al. 1999; Segeren et al. 1993; Smith 1944; Stevens 1964; van Zijl et al. 1986; Watterson 1993) have identified a high level of TSWV resistance in *S. peruvianum* beginning as early as 1939 (Wenholz 1939). Interestingly Norris (1946) reported that, in relationship to TSWV, *S. peruvianum* possesses true resistance amounting almost to immunity. The *Sw-5* gene has been introgressed into the cultivated tomato from this species (Cho et al. 1996;Stevens et al. 1992; Thompson and van Zjl 1996).

New TSWV isolates overcoming *Sw-5* have been identified in several parts of the world (Aramburu and Martí 2003; Roselló et al. 1999;Thompson and Van Zijl 1996).

Furthermore, Latham and Jones (1998) deliberately developed two TSWV isolates, under controlled conditions, which completely infected plants homozygous for *Sw-5*.

Recently Gordillo et al. (2008), screened 285 *S. peruvianum* accessions from the United States Department of Agriculture (USDA) Research Service, Cornell University, Geneva, NY and the Tomato Genetics Resource Center (TGRC) at the University of California Davis for TSWV resistance to the isolate TSWV6 that completely overwhelmed *Sw-5*, and to the An_{wa}-1 isolate that partially overcame *Sw-5*.

In this work, the Hawaiian isolate TSWV6 that overcame *Sw-5*/*Sw-5* genotypes in previous studies, was used in our breeding program to select for resistance in our F_1, BC_1P_1, BC_2P_1, and BC_3P_1 progenies. In our TSWV6 resistance screening trials of *S. peruvianum* accessions, we found that PI 128660 (Table 1**)** showed resistance to this new isolate from Hawaii. We used it to obtain these progenies by crossing it with the cultivated tomato.

Materials and methods

Plant materials

Six cultivated tomato breeding lines were used as pistillate parents (P_1): 1= UCT5, 2 = Florida 7613, 3 = Florida 8044 (a heat tolerant plant), 4 = Florida 8021B (the Florida lines kindly provided by J.W. Scott at the University of Florida), 5 = NC84173 PVP, and 6 = NCEBR8 (Table 1).

Putatively resistant cuttings of accession PI 128660 (*S. peruvianum*) and the F_1 and F_2 of *S. peruvianum* x EPP1 (kindly provided by Dr. John Cho) were used as male parents (P_2): A = PI 128660C, B = F_2 (EPP1 x PI 128660A)1 plant #6, C = F_2 (EPP1 x PI 128660A)1 plant #20, D = F_1 (EPP1 x PI 128660A)2 (was a poor pollen producer), and E = F_2 (EPP1 x PI 128660A)1

plant #18 (Table 1); [numbers 1 and 2 after parenthesis refer to the group of seeds that germinated first or secondly], were rooted in a misting system. EPP1 is a hybrid peruvianum-like test line obtained by crossing the *S. lycopersicum* cultivar Pardue 812 with *S. peruvianum* accession PI 128648-6 interspecific hybrids and selected for good crossability to *S. lycopersicum* and used as a bridge line for breeding tomato (Poysa 1990).

Growth conditions of plant stock

Male and female plants were grown in greenhouse conditions at approximately 18-30^{o} C and maintained in 19 L containers. All plants were grown in a soil-less peat mix media, fertilized alternatively at each watering with either 15-15-15 Peter's commercial fertilizer or $Ca(NO_3)_2$ applied with a Syphonex injector at a rate of 100 ppm N. Near isogenic lines (NILs) 89R (*Sw-5/Sw-5*) and 89S (*Sw-5^{+}/Sw-5^{+}*) were used as control plants. We tested a total of 997 plants of 89R and 970 plants of 89S with TSWV6. Both NILs were *S. lycopersicum* (L.), where 89R carries the *Sw-5* gene resistance to TSWV and 89S is a susceptible tomato line (Table 1).

Inoculation, maintenance, use, and infection evaluation of TSWV6 isolate

Isolate TSWV6 was identified in Hawaii, infecting (*Sw-5/Sw-5*) tomato plants (Cho et al. 1996; Gordillo et al. 2008). Isolate maintenance and inoculations were conducted in controlled-access greenhouses at Brigham Young University, Provo, Utah. TWSV6 isolate was maintained in *Nicotiana rustica* (L.) and used to screen our crosses to select resistant plants (Gordillo et al. 2008). Male parents, the F_1, BC_1P_1, BC_2P_1, and BC_3P_1 progenies were inoculated twice, one week apart, and resistant survivors were selected and tested using ELISA.

Two weeks after the second inoculation, visual symptoms were used to eliminate infected plants. Plants without TSWV6 infection were evaluated by peroxidase double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (Agdia Inc., Elkhart, IN). Optical density values used by Stevens et al. (1992) were used to discriminate healthy from infected plants. Plants that were free of infection both visually and by ELISA, were selected for the next breeding stage.

Hybridization procedure and embryo rescue

Male parents (P_2) A = PI 128660C and D = F_1 (EPPI x PI 128660A)2 were selected as pollen donors because they showed the highest percentage of infection-free plants (see results, Table 1). Flowers of female parents were emasculated daily, by hand, removing the anthers before anthesis and pollinated twenty-four hours later. Pollen from the male parents was collected in gelatin capsules with an electric toothbrush and stored until use at -20^{o} C in airtight plastic vials containing Drierite (anhydrous $CaSO_4$) (W.A. Hammond Drierite Company, Xenia, OH). Fresh pollen was collected regularly and used preferentially for immediate pollination. Stigmas were pollinated with a homemade cotton fiber brush twenty-four hours after emasculation and tagged accordingly, with emasculation/pollination dates and cross information.

Fruit was harvested at pink to red (ripe) stage and surface sterilized for twenty minutes in a 50% solution of common bleach (sodium hypochlorite 5.25%), and soaked in distilled water for five minutes. Immature F_1 seeds were dissected for embryo rescue (Barbano and Topoleski, 1984) and placed in Petri dishes with Murashige and Skoog media and maintained at 24^{0} C until germination. F_1 hybrids obtained from embryo rescue were acclimated and transplanted to pony-packs in the greenhouse and later transplanted to 19 L containers. This procedure was repeated

for the BC_1P_1 generation since the post-zygotic genetic barriers were still in effect. BC_2P_1 and BC_3P_1 progeny set seeds normally; hence, embryo rescue was not necessary for these crosses (Barbano and Topoleski 1984; Nettancourt et al. 1974; Segeren et al. 1993; Thomas and Pratt 1981; Takashina et al. 1997).

Hybrid identification

Morphological traits such as fruit characteristics, leaf and flower morphology, growth habit, and hairiness were observed, as well as the suigeneris scent of the leaves and stems of all interspecific hybrids, which resembled mostly the *S. peruvianum* plants. Some hybrids were weak, and died in a few days. As a result of using the above observations, hybrids were easily distinguished from self-pollinated tomato seedlings (Takashina et al. 1997; Thomas and Pratt 1981).

Results

Control lines 89R and 89S were 100% overcome by TSWV6 (997 and 970 plants, respectively). 89R plants were more virulently attacked by TSWV6 per symptoms observed on infected plants as visually compared with 89 S. Male parents A = PI 128660C, and D = F_1 (EPPI x PI 128660A)2 had a resistant response of 100% to TSWV6, while B = F_2 (EPP1 x PI 128660A)1 plant #6, C = F_2 (EPP1 x PI 128660A)1 plant #20, and E = F_2 (EPP1 x PI 128660A)1 plant #18 had a resistance response of 90% to TSWV6 (Table 1). Therefore, lines A = PI 128660C and D = F_1 (EPPI x PI 128660A)2 were chosen as male parents (P_2) to produce our interspecific hybrid crosses.

Interspecific F_1 Hybrids and backcrosses

Interspecific hybrids ranged from 74% (1A = UCT5 x A) to 33% (2A = Fla 7613 x A) resistance response to TSWV6 (Table 1, Fig. 1). From a 100% free of infection response in the resistant parents (P_2) we regressed to 33%. The lowest resistance response were hybrids 2A = Fla 7613 x A and 1D = UCT5 x D with 4 resistant plants out of 12 (33%) and 62 resistant plants out of 138 (45%) respectively. Backcrosses to the recurrent tomato susceptible parent (P_1) with these hybrids were performed to obtain the BC_1P_1 generation. The BC_1P_1 generation had plants free of infection values ranging from 77% [3 x 2A = Fla 8044 x (Fla 7613 x A)] to 0% [3 x 1A = Fla 8044 x (UCT5 x A) and 2 x 1D = Fla 7613 x (UCT5 x D)] control plants were all infected by TSWV6 (Table 1, Fig. 2). We selected some of these crosses to obtain the BC_2P_1 progeny. In the BC_2P_1 generation, the resistance response plummeted to range from 6% {4h = Fla 8021B x [UCT5 x (UCT5 x A)] and 5h = NC84173 x [UCT5 x (UCT5 x A)]} to 0% {3g = Fla 8044 x [UCT5 x (UCT5 x D)], 5g = NC84173 x [UCT5 x (UCT5 x D)], 1h = UCT5 x [UCT5 x (UCT5 x A)], and 6h = NCEBR8 x [UCT5 x (UCT5 x A)]}(Table 1, Fig. 3).

BC_3P_1 generation

Our third backcrossed progeny showed no resistance. 1 x 3g = UCT5 x {Fla 8044 x [UCT5 x (UCT5 x D)]} with a sample size of 240 plants, and 1 x 4h = UCT5 x {Fla 8021B x [UCT5 x (UCT5 x A)]} with a sample size of 383 plants, both had a zero resistance response (Table 1, Fig. 4). Backcross 1 x 3g, which has the D resistant parent as background, already had lost resistance in the BC_2P_1 generation. While working on the third backcross it was unknown to us that the second backcross 3g = Fla 8044 x [UCT5 x (UCT5 x D)] with a D background cross

was going to totally lose its resistance response (Fig. 3). Nonetheless, we used it to obtain our BC_3P_1 generation.

Response of some backcrossed plants in the field

Table 2 and Fig. 5 show the BC_2P_1 cross 3h = Fla 8044 x [UCT5 x (UCT5 x A)] with a slightly higher resistance response (84%) to TSWV6 than the BC_3P_1 generation. When compared to the controls carrying the *Sw-5* gene (Q2E and Sweet Tan), the BC_3P_1 generation resulted in no significant differences in resistance response (two-proportions Z test, P = 0.38). Thus, field-testing yielded the same result obtained under greenhouse conditions where the BC_3P_1 generation lost its resistance response to TSWV6 (Fig. 4).

Average resistance response from all the crosses

The F_1 generation showed a 56.6% resistance response to TSWV6 with a sample size of 201 plants (Fig. 6). The BC_1P_1 generation showed a 29.41% resistance response with a sample size of 686 plants, the BC_2P_1 generation presents a 2.15% response of plants free of infection with a sample size of 3522 plants, and the BC_3P_1 progeny had zero resistance response to TSWV6 with a population of 623 plants. These data show that the resistance response was lost across the hybrid crosses the different backcrosses.

Discussion

Other researchers have found this phenomenon of dilution or loss of resistance in a few crops, but mostly in wheat. Aghaee-Sarbarzeh et al. (2001) and Ma et al. (1995) found that when breeding wheat for resistance most of the genes conditioning leaf rust and stripe rust resistance from C and U genomes under field conditions were suppressed by the A and/or B genomes of

Triticum durum in amphiploids. Assefa and Fehrmann (2004), Kerber (1983), Kerber and Green (1980), and Knott (2000) also reported a significant decrease or complete loss of stem rust resistance in synthetic hexaploid wheat due to the presence of suppressor genes in tetraploid durum wheat parents. They also encountered similar results from other colleagues.

When breeding wheat, loss of resistance to powdery mildew, to the Russian Wheat Aphid, and to Tan Spot caused by *Pyrenophora tritici-repentis* (Died) has been reported (Bennett 1984; Hanusová et al. 1996; Lage et al. 2003; Potgieter et al. 1991; Siedler et al. 1994; Zeller and Hsam 1996). When breeding for *Orobanche crenata* Forsk resistance in faba bean (*Vicia faba* L.), resistance was lost under hot, dry spring weather conditions (Cubero and Hernández 1991). Kornegay and Cardona (1991) also reported loss of resistance to *Acanthoscelides obtectus* (bean weevil) when breeding beans.

Bernacchi et al. (1998) created twenty-three near isogenic lines (NILs) of tomato for fifteen genomic regions predicted to contain twenty-five quantitative trait factors to improve seven agronomic traits such as total yield, red yield, soluble solids, viscosity, fruit color, and firmness. They accomplished this by using a molecular breeding strategy of advanced backcross QTL (AB-QTL) analysis. Such NILs contain introgressions of wild alleles from *S. habrochaites* and *S. pimpinellifolium*. Some of these NILs performed as expected, but others did not. Some NILs containing a longer segment of foreign DNA showed the predicted effect, while others with shorter introgressions did not differ significantly from the control in any location. Another factor is the environment that affects the expression of the genotype. These same authors found that the performance of NILs was variable across different environments. Forty percent of the NILs showed the predicted phenotypic improvement over the control while sixty percent did not show the predicted response, or partially showed it at different levels at different locations.

This unpredicted response may be caused by epistatic interactions among the introgressed foreign alleles and the cultivated tomato that is not detected by QTL mapping, becoming rate limiting when the rest of the genome is replaced by the cultivated tomato alleles, as happens in these NILs. This experiment suggests that desired improvement may not occur, sometimes the effects could be negative (meaning that we may get a phenotype that performs lower than the control), and sometimes a positive improvement of the expected traits could result (Tanksley and Nelson 1996).

Gorguet et al. (2008) mapped and characterized novel parthenocarpy QTLs in tomato using a cross of *S. lycopersicum* with *S. habrochaites* followed by several backcrosses and at least one selfed generation. Of these plants, 44% did not express the gene for parthenocarpy, suggesting that it may have been due to the mode of action of another locus. This QTL region where the parthenocarpy gene is supposed to be located was tightly linked to the molecular marker T0635, but the gene/genes was/were not there.

Conclusion

In this study, when breeding for resistance to TSWV6, resistance was lost from the F_1 to the BC_1P_1, to the BC_2P_1, and to the BC_3P_1. This resistance was lost in a consistent manner from 100% resistance in resistant parental lines to 56.6%, to 29.41%, to 2.15% and finally to 0% (Fig. 6). This observation suggests that several QTLs containing the resistance alleles were lost through dilution in backcrossing.

The fact that resistance was lost to TSWV6 when transferring this resistance derived from *S. peruvianum* into the cultivated tomato does not mean that it cannot be done. Many breeders have been able to transfer QTLs from wild tomato to cultivated tomato, including

the *Sw-5* gene that confers resistance against TSWV. The tomato lines or cultivars Anahu, UPV 1, UPV 32, and Stevens are some examples (Cho et al. 1996; deVicente and Tanksley 1993; Foolad 2007; Gilbert and Tanaka 1971; Lander and Botstein 1989; Langella et al. 2004; Roselló et al. 2001, 1999; Stevens et al. 1992; Thompson and van Zijl 1996).

More research is needed to decipher the unanswered questions regarding the genetics of tomato breeding. The results of this study are interesting but difficult to interpret and provide an ongoing challenge to discover this loss of resistance mechanism. With the help of marker assisted selection, fine QTL mapping (mapping polygenes), new findings about gene expression, epigenetics, gene silencing, and interfering RNAs processes (Meister et al. 2004; Ribeiro et al. 2007) and mapping of the tomato genome, may help us to better understand the intricacies in tomato genome gene expression. *S. peruvianum* is an excellent source of resistance genes, and QTLs controlling many agronomic traits and several of them have been introgressed, and many more have the potential to be introgressed into the cultivated tomato (Barone and Frusciante 2007; Brouwer et al. 2003; Causse et al. 2002; Foolad 2007; Naz et al. 2008).

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Table 1 Resistance reactions to TSWV6 isolate[a] (totally overwhelms *Sw-5*) of the P_1 (*S. Lycopersicon* susceptible parent tested with the Anemone isolate[b] that partially overwhelms *Sw-5*), P_2 (*S. peruvianum*, resistant parent), F_1 hybrid, BC_1P_1 (first backcross to the susceptible parent), BC_2P_1 (second backcross to the susceptible parent), BC_3P_1 (third backcross to the susceptible parent), $BC_1P_1 \otimes$ (self-pollinated first backcross to the susceptible parent) and 89S (*Sw-5*$^+$/ *Sw-5*$^+$) and 89R (*Sw-5/ Sw-5*), susceptible and resistant controls respectively, based on visual and ELISA results.

Progeny	Number of Plants (%)			Ratios R: S		X^2	df	P
	Resistant	Susceptible	Total	Resist: Susceptible				
P_1 Plants (tested with Anemone isolate)[b]				Observed: Expected				
1 = UCT5	0 (0%)	16 (100%)	16	0:1	0:1			
2 = Fla 7613	0 (0%)	12 (100%)	12	0:1	0:1			
3 = Fla 8044	0 (0%)	16 (100%)	16	0:1	0:1			
4 = Fla 8021B	0 (0%)	16 (100%)	16	0:1	0:1			
5 = NC84173	0 (0%)	16 (100%)	16	0:1	0:1			
6 = NCEBR8.	0 (0%)	16 (100%)	16	0:1	0:1			
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	8 (100%)	8	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	8 (100%)	8	0:1	----			
P_2 Cuttings								
A = PI 128660C (*S. peruvianum*)[c]	10 (100%)	0 (0%)	10	1:0	1:0			
B = F_2 (EPP1 x PI 128660A)1[d] plant #6	9 (90%)	1 (10%)	10	9:1	3:1			
C = F_2 (EPP1 x PI 128660A)1[d] plant #20	9 (90%)	1 (10%)	10	9:1	3:1			
D = F_1 (EPP1 x PI 128660A)2[c,d]	10 (100%)	0 (0%)	10	1:0	1:0			
E = F_2 (EPP1 x PI 128660A)1[d] plant #18	9 (90%)	1 (10%)	10	9:1	3:1			
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	6 (100%)	6	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	6 (100%)	6	0:1	0:1			
F_1 hybrids								
1A = UCT5 x A	23 (74%)	8 (26%)	31	3:1	1:0			
2A = Fla 7613 x A	4 (33%)	8 (67%)	12	1:2	1:0			
1D = UCT5 x D	62 (45%)	76 (55%)	138	0.82:1	1:1	1.42029	1	0.1 < P < 0.5
2D = Fla 7613 x D[e]	9 (64%)	5 (36%)	14	1.80:1	1:1	1.11428	1	0.1 < P < 0.5
3D = Fla 8044 x D[e]	4 (67%)	2 (33%)	6	2:1	1:1	0.66666	1	0.1 < P < 0.5
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	21 (100%)	21	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	21 (100%)	21	0:1	0:1			
BC_1P_1 Plants								
h = 1 x 1A = UCT5 x (UCT5 x A)[f]	23 (40%)	35 (60%)	58	0.66:1	1:1	2.48275	1	0.1 < P < 0.5
2 x 1A = Fla 7613 x (UCT5 x A)	7 (1.5%)	477 98.5%)	484	0.02:1	1:1	456.405	1	P < 0.005
3 x 1A = Fla 8044 x (UCT5 x A)	0 (0%)	24 (100%)	24	0:24	1:1	24	1	P < 0.005
4 x 1A = Fla 8021B x (UCT5 x A)	3 (12.5%)	21 87.5%)	24	0.14:1	1:1	13.5	1	P < 0.005
1 x 2A = UCT5 x (Fla 7613 x A)	11 (58%)	8 (42%)	19	1.4:1	1:1	0.47368	1	0.1 < P < 0.5
3 x 2A = Fla 8044 x (Fla 7613 x A)	10 (77%)	3 (23%)	13	3.22:1	1:1	3.76923	1	0.05 < P < 0.1

4 x 2A = Fla 8021B x (Fla 7613 x A)	2 (67%)	1 (33%)	3	2:1	1:1	0.33333	1	0.5 < P < 0.9
g = 1 x 1D = UCT5 x (UCT5 x D)[f]	18 (37.5%)	30 62.5%)	48	0.6:1	1:1	3	1	0.05 < P < 0.1
2 x 1D = Fla 7613 x (UCT5 x D)	0 (0%)	1 (100%)	1	0:1	1:1	1	1	0.1 < P < 0.5
3 x 1D = Fla 8044 x (UCT5 x D)	3 (30%)	7 (70%)	10	0.43:1	1:1	1.6	1	0.1 < P < 0.5
4 x 1D = Fla 8021B x (UCT5 x D)	0 (0%)	2 (100%)	2	0:2	1:1	2	1	0.1 < P < 0.5
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	72 100%)	72	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	70 100%)	70	0:1	0:1			
BC_2P_1 Plants								
3g = Fla 8044 x [UCT5 x (UCT5 x D)][g]	0 (0%)	22 (100%)	22	0:22	1:1	22	1	P < 0.005
5g = NC84173 x [UCT5 x (UCT5 x D)]	0 (0%)	3 (100%)	3	0:3	1:1	3	1	0.05 < P < 0.1
1h = UCT5 x [UCT5 x (UCT5 x A)]	0 (0%)	765 (100%)	765	0:765	1:1	765	1	P < 0.005
2h = Fla 7613 x [UCT5 x (UCT5 x A)]	5 (1.2%)	397(98.8%)	402	0.01:1	1:1	382.248	1	P < 0.005
3h = Fla 8044 x [UCT5 x (UCT5 x A)][h]	31 (4%)	729 (96%)	760	0.04:1	1:1	641.057	1	P < 0.005
4h = Fla 8021B x [UCT5 x (UCT5 x A)][i]	40 (6%)	647 (94%)	687	0.06:1	1:1	536.315	1	P < 0.005
5h = NC84173 x [UCT5 x (UCT5 x A)]	36 (6%)	533 (94%)	569	0.07:1	1:1	434.110	1	P < 0.005
6h = NCEBR8 x [UCT5 x (UCT5 x A)]	0 (0%)	314 (100%)	314	0:314	1:1		1	P < 0.005
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	348 (100%)	348	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	341 (100%)	341	0:1	0:1			
BC_3P_1 Plants[j]								
1 x 3g = UCT5 x [Fla 8044 x (UCT5 x 1D)]	0 (0%)	240 (100%)	240	0:240	1:1	240	1	P < 0.005
1 x 4h = UCT5 x [Fla 8021B x (UCT5 x 1A)]	0 (0%)	383 (100%)	383	0:383	1:1	383	1	P < 0.005
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	52 (100%)	52	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	48 (100%)	48	0:1	0:1			
Self-Pollinated Plants ⊗								
BC_1P_1 ⊗ Plants h = [UCT5 x (UCT5 x A)] ⊗	25 (45%)	31 (55%)	56	0.8:1 (3:1, 2:1, or 1:1)		0.64285	1	0.1 < P < 0.5
89S (*Sw-5*$^+$/ *Sw-5*$^+$), susceptible control	0 (0%)	4 (100%)	4	0:1	0:1			
89R (*Sw-5/ Sw-5*), resistant control	0 (0%)	4 (100%)	4	0:1	0:1			

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[a]TSWV6 isolate from Hawaii infecting *Sw-5/ Sw-5* genotypes.
[b]Anemone isolate (An_{wa}-1) from Western Australia partially overwhelms *Sw-5* gene. It is less virulent than the Hawaiian isolate TSWV-6.
[c]A and D with 100% Virus-free plants were chosen as male parents for our F_1 generation in our breeding program.
[d]Number 1 and 2 after parenthesis indicates the first and second group to germinate of the (male parents) F_1 and F_2 plants.
[e]2D and 3D were lost by accident, not being available for crosses. We used 1D instead.
[f]h and g were used as male parents for our BC_2 generation.
[g]3g lost its resistance coming from g in our BC_1. One 3g visually healthy (ELISA infected) and robust plant was used for our BC_3.
[h]3h was evaluated in the field under TSWV6 pressure.
[i]4h was chosen as male parent for our BC_3 generation.
[j]BC_3 generation lost resistance.

Table 2 Number of plants field tested with TSWV6 and visually evaluated at an Hawaiian field[a].

Progeny	Number of Plants (%)		
	Resistant	Susceptible	Total
BC_2P_1 Plants[b]			
3h = Fla 8044 x [UCT5 x (UCT5 x A)]	82 (84%)	16 (16%)	98
BC_3P_1 Plants[b]			
4 x 3g = Fla 8044 x [Fla 8044 x (UCT5 x 1D)]	78 (74%)	28 (26%)	106
5 x 3g = NCPVP x [Fla 8044 x (UCT5 x 1D)]	43 (67%)	21 (33%)	64
3 x 4g = Fla 8044 x [Fla 8021 x (UCT5 x 1D)]	66 (79%)	18 (21%)	84
4 x 4h = Fla 8021B x [Fla 8021B x (1 x 1A)]	83 (78%)	23 (22%)	106
5 x 4h = NC84173 x [Fla 8021B x (1 x 1A)]	83 (74%)	29 (26%)	112
Controls[b]			
Q2E-1A *Sw-5* resistant control	84 (78%)	24 (22%)	108
Sweet tan *Sw-5* resistant control[c]	80 (77%)	24 (23%)	104

[a]Plants were evaluated for TSWV6 at 34 and 44 days after transplanting into the soil.
[b]There are no significant differences between the backcrosses and the controls with the test of two proportions. P-value=0.379746.
[b]Previously shown resistance was lost or did not express under these conditions.
[c]Sweet Tangerine is a commercial hybrid with *Sw-5* introgressed by Dr. John Cho.

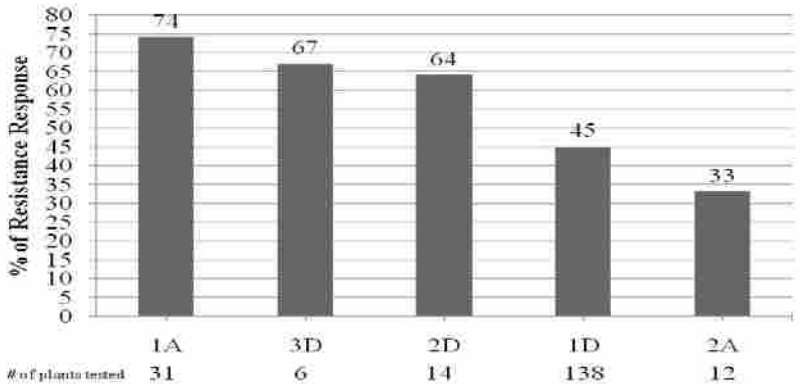

Fig. 1 % of interspecific hybrids resistance response to TSWV6 isolate

1A = UCT5 x PI 128660C; 2A = Fla 7613 x PI 128660C; 1D = UCT5 x [F_1 (EPP1 x PI 128660A)2];
2D = Fla 7613 x [F_1 (EPP1 x PI 128660A)2]; 3D = Fla 8044 x [F_1 (EPP1 x PI 128660A)2]

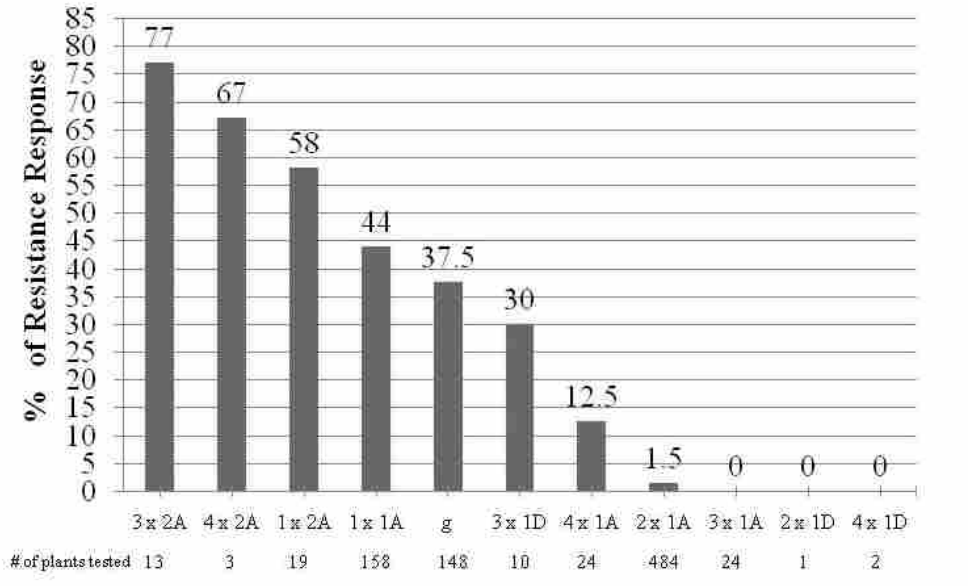

Fig. 2 % of the BC_1P_1 progeny resistance response to TSWV6 isolate

h = 1 x 1A = UCT5 x (UCT5 x PI 128660C); 2 x 1A = Fla 7613 x (UCT5 x PI 128660C);
3 x 1A = Fla 8044 x (UCT5 x PI 128660C); 4 x 1A = Fla 8021B x (UCT5 x PI 128660C);
1 x 2A = UCT5 x (Fla 7613 x PI 128660C); 3 x 2A = Fla 8044 x (Fla 7613 x PI 128660C);
4 x 2A = Fla 8021B x (Fla 7613 x PI 128660C);
g = 1 x 1D = UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2];
2 x 1D = Fla 7613 x [(UCT5 x F_1(EPP1 x PI 128660A)2]
3 x 1D = Fla 8044 x [(UCT5 x F_1(EPP1 x PI 128660A)2])
4 x 1D = Fla 8021B x [(UCT5 x F_1(EPP1 x PI 128660A)2]

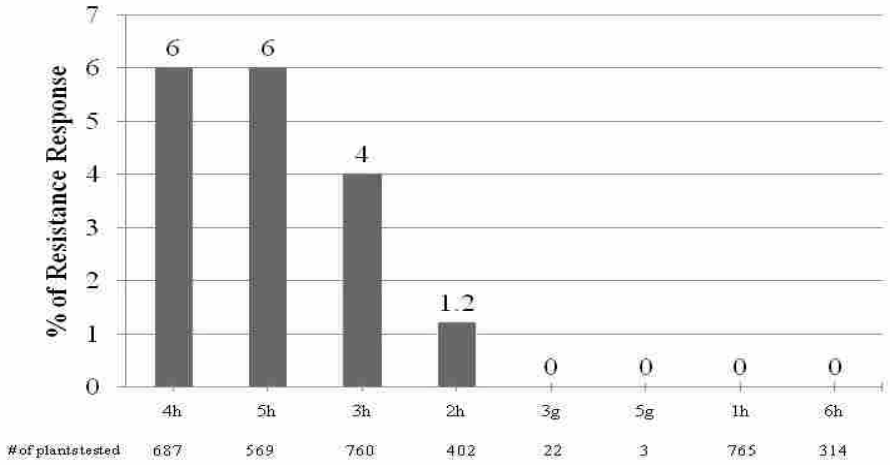

Fig. 3 % of the BC_2P_1 progeny resistance response to TSWV6 isolate

3g = Fla 8044 x (1 x 1D) = Fla 8044 x {UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2]};
5g = NC84173 x (1 x 1D) = NC84173 x {UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2]};
1h = UCT5 x (1 x 1A) = UCT5 x [UCT5 x (UCT5 x PI 128660C)];
2h = Fla 7613 x (1 x 1A) =Fla 7613 x [UCT5 x (UCT5 x PI 128660C)];
3h = Fla 8044 x (1 x 1A) = Fla 8044 x [UCT5 x (UCT5 x PI 128660C)];
4h = Fla 8021B x (1 x 1A) = Fla 8021B x [UCT5 x (UCT5 x PI 128660C)];
5h = NC84173 x (1 x1A) = NC84173 x [UCT5 x (UCT5 x PI 128660C)];
6h = NCEBR8 x (1 x 1A) = NCEBR8 x [UCT5 x (UCT5 x PI 128660C)]

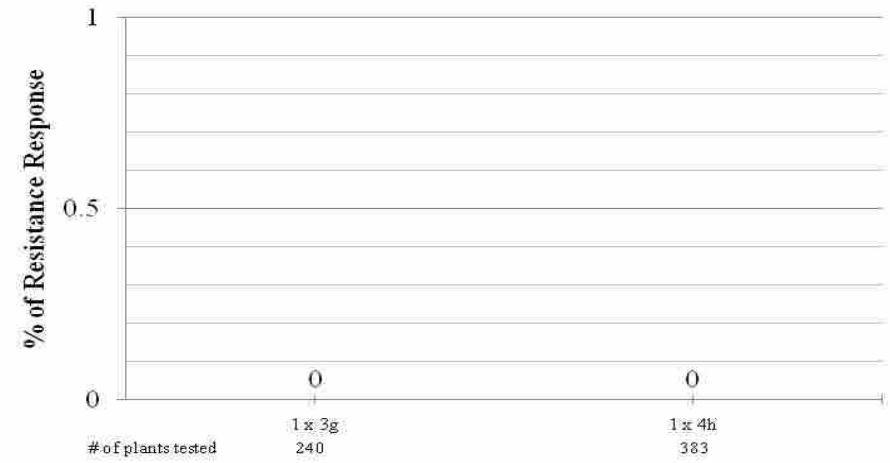

Fig. 4 % of the BC_3P_1 resistance response to TSWV6 isolate

1 x 3g = UCT5 x [Fla 8044 x (UCT5 x 1D)] = UCT5 x {Fla 8044 x [UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2]}
1 x 4h = UCT5 x [Fla 8021B x (UCT5 x 1A) = UCT5 x {Fla 8021B x [UCT5 x [UCT5 x (UCT5 x PI 128660C)]}

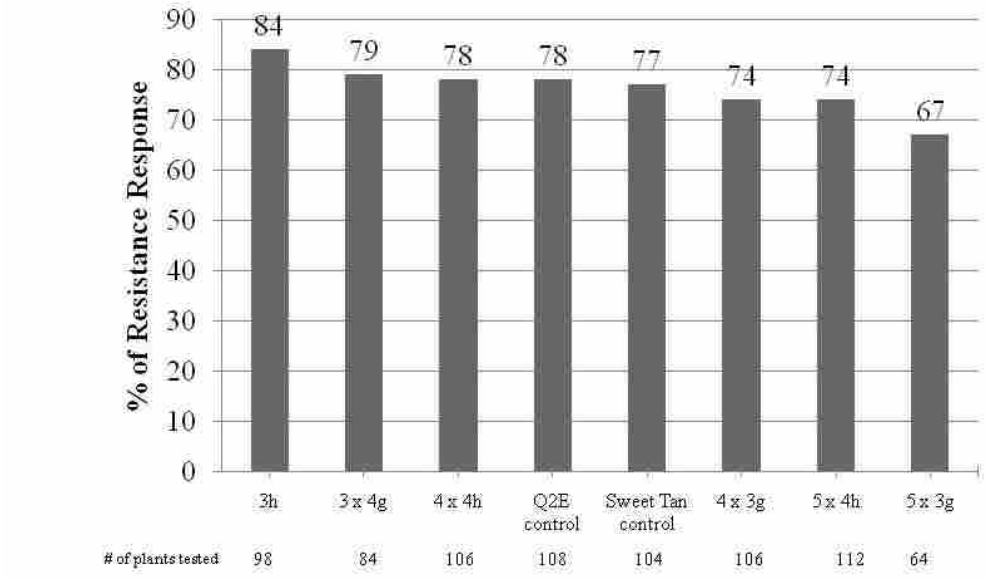

Fig. 5 % of the BC_3P_1 resistance response to TSWV6 field-tested. When compared to the controls carrying the *Sw-5* gene (Q2E and Sweet tan), the BC_3P_1 generation resulted in no significant differences in resistance response (two-proportions Z test, P = 0.38)

3h (BC_2P_1 generation) = Fla 8044 x (1 x 1A) = Fla 8044 x [UCT5 x (UCT5 x PI 128660C)];
BC_2P_1 generation:
4 x 3g = Fla 8044 x {Fla 8044 x [UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2]}
5 x 3g = NCPVP x {Fla 8044 x [UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2]}
3 x 4g = Fla 8044 x {Fla 8021B x [UCT5 x [UCT5 x F_1(EPP1 x PI 128660A)2]}
4 x 4h = Fla 8021B x {Fla 8021B x [UCT5 x (UCT5 x PI 128660C)]}
5 x 4h = NC84173 x {Fla 8021B x [UCT5 x (UCT5 x PI 128660C)]}
Controls:
Q2E-1A *Sw-5* resistant control
Sweet tan *Sw-5* resistant control

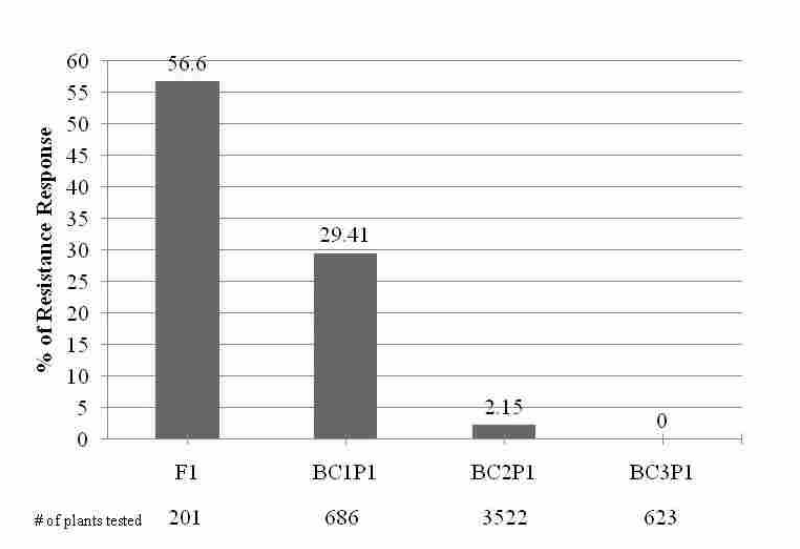

Fig. 6 Average of Percentages of the F_1, BC_1P_1, BC_2P_1 and the BC_3P_1 resistance response to TSWV6