Phenotypic and molecular screening of tomato germplasm for resistance to *Tomato yellow leaf curl virus*

Abdulbaset Azizi[1,2], Javad Mozafari[1*], Masoud Shams-bakhsh[2]

[1]National Plant Gene-Bank, Seed and Plant Improvement Institute, Mahdasht Road, P.O Box 31585-4119, Karaj, Iran [2]Department of Plant Pathology, Faculty of Agriculture,Tarbiat Modares University, P.O. Box 14115-326 Tehran, Iran

Abstract
Tomato yellow leaf curl virus (TYLCV) is a major tomato virus in tropical and subtropical regions. In this study, 134 accessions of *Solanum lycopersicum* and six accessions of *Solanum peruvianum* were assessed for resistance to an Iranian isolate of TYLCV. Plants were inoculated using whiteflies (*Bemisia tabaci*) and the reaction of plants was evaluated based on either disease symptoms or viral DNA amplification. All accessions of *S. lycopersicum* had demonstrated various degrees of disease symptoms. However, all six accessions of *S. peruvianum* were resistant and remained symptomless. Phenotypic evaluation was confirmed by amplification of a 670bp TYLCV DNA fragment in all tested accessions of *S. lycopersicum*. Based on both phenotypic and molecular evaluations, no accession provided complete resistance to TYLCV, whereas nine accessions were assessed as tolerant. The high level of resistance noted in whitefly inoculated accessions of *S. peruvianum* was not observed in graft inoculated plants of these accessions. The TYLCV DNA fragment was detected five weeks post-inoculation when plants were inoculated by grafting. These results suggested that accessions of *S. peruvianum* may be merely resistant to vector inoculation of TYLCV.
Keywords: Molecular screening; Tomato; TYLCV; Virus resistance

INTRODUCTION

Tomato yellow leaf curl virus (TYLCV), a *Begomovirus* in the family *Geminiviridae*, is the most devastating virus of the tomato plant in tropical and subtropical regions including Iran. The family *Geminiviridae* comprises plant viruses that have a circular, single-stranded DNA genome and geminate particles consisting of two incomplete icosahedra (Hull, 2002). Geminoviruses are classified into four genera based on the type of insect vector, host range, and genome organization (Rybicki *et al*., 2000). The genus *Begomovirus* includes species with monopartite or bipartite genomes such as TYLCV that are transmitted by whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Moriones and Navas-Castillo, 2000; Cohen and Nitzany., 1966). *B. tabaci* has developed resistance against insecticides in recent years (Dittrich *et al.*, 1990) and therefore, a few viruliferous whiteflies may be enough for transmitting the virus to a large number of plants.

Chemical control methods as well as integrated pest management (IPM) strategies employed for controlling the vector have not been successful in decreasing the incidence of TYLCV on the tomato crop (Reynaud *et al*., 2003). Under these circumstances, breeding for resistance to TYLCV appears to be a promising and environmentally friendly approach for controlling the disease (Chague *et al*., 1997). Resistance to TYLCV has been reported in wild relatives of the cultivated tomato; *S. peruvianum*, *S. hirsutum*, *S. pimpinellifolium* and *S. cheesmanii* (Kasrawi *et al*., 1988; Geneif, 1984; Hassan *et al*., 1984). However, in some of these highly resistant wild accessions such as *S.peruvianum* LA385, TYLCV was detected by back indexing, hence such a resistance could also be viewed as tolerance (Kasrawi *et al*., 1988). Genetic analyses indicated that tolerance to TYLCV is controlled by five recessive

*Correspondence to: **Javad Mozafari,** Ph.D.
Tel: +98 261 2701260; Fax: +98 261 271-6793
E-mail: jmozafar@yahoo.com

genetic factors (Pilowsky and Cohen, 1990). Zamir *et al*. (1994) mapped a major TYLCV tolerance locus (*TY-1*) in the tomato wild relative *S. chilence* on chromosome 6. Chague *et al*. (1997) reported four Random Amplified Polymorphic DNA (RAPD) markers linked to a quantitative trait locus involved in the resistance to TYLCV. Resistance to TYLCV in *S. pimpinellifolium* IRNA-Hirsute is reported to be against the insect vector rather than the virus. In breeding programs for protection against begomoviruses, tolerant genotypes with low levels of infection but expressing reduced disease incidence may be discarded without full consideration of their epidemiological effects at the population level in the field (Delatte *et al*., 2006). It has also been shown that, acquisition of TYLCV from a tolerant or resistant plant, and its transmission by whiteflies are less efficient than those for a susceptible plant (Lapidot *et al*., 2001). Some sources of resistance to TYLCV may also show resistance to some other viruses such as *Tomato curly stunt virus* (ToCSV) (Pitersen and Smith, 2002).

Although in a breeding program, the evaluation of resistance level should correspond to the effect of infection on total yield and yield components (Lapidot *et al*., 1997), the severity of the infection and the level of viral accumulation can serve as indictors of resistance level (Pico *et al*., 1999). The tomato plant was introduced to Iran several centuries ago and has been subjected to many genetical changes resulting in an interesting diversity, reflecting the various climatic conditions in the country. In this study the Iranian tomato collection was screened for resistance to a newly emerged isolate of TYLCV from Southern Iran (TYLCV-Ir2). Resistance was evaluated based on the severity of the disease symptom and viral DNA amplification. The type of resistance was then analyzed by comparing graft-inoculated and whitefly inoculated *S. peruvianum* plants.

MATERIALS AND METHODS

Virus isolate: The TYLCV isolate used in this study inculation was a newly emerged isolate method known as TYLCV-Ir2 (accession EU085423 in NCBI gene bank) (Azizi, 2007) collected from the infected tomato fields of the Bandar Abbas region, South of Iran. The isolated virus was first identified based on the development of symptoms on tomato, bean (*Phaseolus vulgaris*), and wild tobacco species *Nicotiana benthamiana* and *N. rustica* plants. The TYLCV DNA fragment was then amplified by polymerase chain reaction (PCR), using a pair of TYLCV specific primers (Azizi, 2007). The virus was biologically purified by whitefly transmission and maintained in an insect-proof greenhouse and allowed to propagate in the tomato (*S. lycopersicum* cv. PS111) plants.

Plant material: Iranian collection of tomato germplasm consisting of 125 accessions of *S. lycopersicum* collected from across the country, and nine TYLCV tolerant *S. lycopersicum* accessions introduced by the Asian Vegetable Research and Development Center (AVRDC) and six accession of *S. peruvianum* were evaluated in this study. Eight plants of each accession were grown under greenhouse conditions (25 ± 2ºC, 12 h light and 70-80% relative humidity (RH)) and were tested against TYLCV infection. The experiment was replicated twice.

Whitefly maintenance and plant inoculation: Whiteflies (*Bemisia tabaci*, biotype B) were identified and collected from greenhouse grown potatoes, Karaj, Iran. Whitefly biotype B colonies were established on cotton plants and then transferred, reared and maintained on cabbage (*Brassica oleracea*) plants under a cage in the greenhouse at 25±2ºC. Whitefly mediated mass inoculation technique was used to inoculate plants (Pico *et al*., 1998). The insects were given a 24h acquisition access period to TYLCV- infected tomato source plants. Eight seedlings of each accession at the four-leaf stage were then inoculated by placing the pots of whitefly infected plants between the pots of tomato plants with the appropriate accessions to be examined. This was repeated four times. Plants were periodically shacked to obtain a uniform vector distribution and individually exposed to about 20 viruliferus whiteflies per plant for 10 days. After inoculation, plants were sprayed with the insecticide Imidacloprid (Confidor, Bayer, Germany) and kept in an insect-proof greenhouse for 5 weeks. The experiment was performed twice, during 2005 and 2006. To determine the type of TYLCV resistance, the resistant accessions were inoculated by grafting. After inoculation, plants were kept in an insect-proof greenhouse for 5 weeks. In each experiment, six plants from each resistant accession were tested. PCR detection assay was employed for detection of the virus in inoculated plants three and five weeks post-inoculation.

DNA extraction: Total DNA was extracted according to Dellaporta *et al.* (1983) with minor modifications.

Two apex leaves (0.5 g) were collected from each of eight green house-grown tomato plants of each accession and used for DNA extraction. 0.1 g of fresh leaf tissue was grounded to a fine powder in liquid nitrogen. The homogenate was incubated in 600 µl of extraction buffer (100 mM Tris-HCl (pH 8), 50 mM EDTA, 500 mM NaCl, 10 mM 2-β-mercaptoethanol and 1% (w/v)SDS) at 65ºC for 10 min and mixed with a half volume of Chloroform: Isoamyl alcohol (24:1 v/v). The mixture was centrifuged at 11269 ×*g* for 15 min and the supernatant (500 µl) was transferred to a 1.5 ml microcentrifuge tube and the DNA was precipitated by adding 150 µl of sodium acetate (5 M, pH 5.2) and 600 µl of isopropanol. The pellet was washed with 70%(v/v) ethanol, air dried and dissolved in 100 µl of sterile double distilled water.

PCR amplification of viral DNA: TYLCV specific primers (TYLCV-F and TYLCV-R) amplifying a 670 bp fragment, were designed according to the conserved sequences of TYLCV-sar (EU143757), TYLCV-Is (DQ845787.1) (Pico *et al*., 1999) and TYLCV-Ir2 (EU085423)(Azizi 2007) available in the NCBI GenBank (Table 1). Conserved sequences of 18S rDNA of *S. lycopersicum* were used to design 18S rDNA-specific primers (18S F/18S R) acting as an internal control and amplifying a 406 bp fragment (Table 1). The optimized PCR procedure was carried out in a 25 µl reaction volume containing one unit of *Taq* DNA polymerase (Fermentas), 50 ng of plant DNA, 2.5 µl of 10X PCR buffer (500 mM KCl, Tris-HCl (pH 8.4)), 1.5 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, 25 picomol of each primer and an appropriate volume of deionized H_2O to make up to 25 µl. The optimal conditions for amplification were as follows: Initial denaturation at 94ºC for 4 min, followed by 25 cycles of denaturation at 94ºC for 1 min, annealing at 50ºC for 1 min, extension at 72ºC for 2 min and a final extension at 72ºC for 10 min. PCR was carried out in a thermocycler machine (Mastercycler ep Gradient) supplied by Eppendorff (Germany). PCR products were fractionated and assessed on 1% (w/v)TAE agarose gels.

RESULTS

The incidence of TYLCV infection and the type of observed symptoms in tested tomato accessions are summarized in Table 2. Cultivated tomato accessions (*S. lycopersicum*) exhibited a range of TYLCV symptoms including yellowing (Fig 1a), purple vein (Fig 1b), leaf curling (Fig 1c), stunting (Fig 1d) and reduced leaflet size (Fig 1a and 1b). In contrast, all six accessions of *S. peruvianum* remained symptomless and had normal growth, such as that of healthy plants (Tables 2 and 3). Based on phenotypic evaluation, most of the *S. lycopersicum* accessions lacked resistance and developed severe symptoms. Only nine accessions showed a very weak symptom and were thus considered as tolerant genotypes (Table 3).

Moreover, a DNA fragment of expected size (670 bp) and a 406 bp 18S rDNA fragment were amplified using TYLCV specific primers and specific primers 18SF/18SR, respectively for the accessions of *S. lycopersicum*. However, no viral DNA was detected in any of the six accessions of *S. peruvianum*. (Fig 2).

Three and five weeks following the graft-inoculation of the six *S. peruvianum* accessions with an infected *S. lycopersicum* accession, plants were assessed for the development of disease symptoms and amplification of viral DNA by PCR (Fig 2 and Table 4). Development of the disease symptoms was delayed, and no viral DNA was amplified three weeks post graft-inoculation (Table 4). However, five weeks after graft-inoculation, the expected fragment of 670 bp related to viral DNA along with very weak disease

Table 1. Primer pairs used for PCR detection of TYLCV and amplification of tomato 18S rDNA as internal control.

Primer name	Sequence	Product size
18SF	5'- TTG ACT GGT GAA TCT CTT CCT -3'	406 bp
18SR	5'- CAC CAA TGA GAA GGA CAA GA -3'	
TYLCV-F	5'- CGC CCG TCT CGA AGG TTC-3'	670 bp
TYLCV-R	5'- GCC ATA TAC AAT AAC AAG GC-3'	

Figure 1. Reaction of *S. lycopersicum* to whitefly inoculation of TYLCV: yellowing (a) and purple vein plus small leaflet size (b) in infected plants (right) compared to health plants of the same age (left) and infected plants showing leaf curling (c) and stunting (e-right) compared to symptomless healthy plants (d and e-left).

symptoms including small leaflets and curling were detected in all the tested plants of *S. peruvianum* (Fig and Table 4).

DISCUSSION

In order to identify potential sources of natural resistance to TYLCV, the Iranian collection of cultivated tomato, *S. lycopersicum* and its wild relative, *S. peruvianum*, were evaluated based on symptom development and amplification of viral DNA following whitefly inoculation. Accessions of *S. lycopersicum* exhibited a varying range of disease symptoms and lacked resistance. Most of them were found to be highly susceptible to TYLCV-Ir2 and only nine accessions showed a very mild symptom and considered as tolerant (Table 3). On the contrary the six accessions of *S. peruvianum* tested in this study showed no disease symptom and were assessed as resistant to TYLCV-Ir2 when inoculated with whiteflies. These wild relatives of cultivated tomato have previously shown to be the possible source of resistance against the disease (Morals and Anderson, 2001; Pico *et al*., 1996).

Accessions CLN2114DC1F1-180-31-9-11-12 and CLN2114DC1F1-2-29-20-23-14-0 that have in earlier

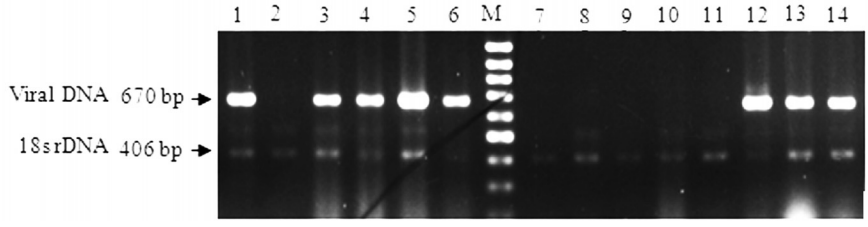

Figure 2. PCR detection of TYLCV specific 670bp and plant 18srDNA specific 406bp DNA fragments in *S. lycopersicum* accessions (lanes 1, 3, 4, 5, 6, 12, 13, 14) and *S. peruvianum* accessions (lanes 2, 7, 8, 9, 10, 11): 1) KC-315091 2) P1 126 944 3) KC-315014 4) KC-315140 5) KC-315072 6) TN-72-165 M) Marker 100bp 7) KC-315037 8) KC-315038 9) KC-315039 10) KC-315040 11) KC-315041 12) KC-315052 13) KC-315087 14) KC-315054.

Table 2. Variation and frequency of TYLCV symptoms observed in the Iranian tomato collection (+ present, - absent).

	Number of accessions	Symptoms				
		Leaf curl	Yellowing	Leaflet	Purple vein	Stunting
S. lycopersicum	8	+	+	+	+	+
	12	+	+	+	+	-
	45	+	+	+	-	-
	21	+	+	-	-	-
	12	+	+	+	-	+
	7	+	+	-	-	+
	7	+	+	-	+	+
	20	+	+	-	+	-
	1	+	-	+	-	+
S. peruvianum	6	-	-	-	-	-

Table 3. Phenotypic evaluation of response to TYLCV in accessions of the Iranian tomato collection.*

Accession	PC	RP	Accession	PC	RP	Accession	PC	RP
S. lycopersicum								
KC-315001	Iran	S	KC-315053	Iran	S	TN-72-142	Iran	S
KC-315002	Iran	S	KC-315054	Iran	S	TN-72-143	Iran	S
KC-315003	Iran	S	KC-315055	Iran	S	TN-72-144	Iran	S
KC-315004	Iran	S	KC-315056	Iran	S	TN-72-145	Iran	S
KC-315005	Iran	S	KC-315057	Iran	S	TN-72-146	Iran	S
KC-315006	Iran	S	KC-315058	Iran	S	TN-72-147	Iran	S
KC-315007	Iran	S	KC-315059	Iran	S	TN-72-148	Iran	S
KC-315008	Iran	S	KC-315060	Iran	S	TN-72-150	Iran	S
KC-315009	Iran	S	KC-315061	Iran	T	TN-72-151	Iran	S
KC-315010	Iran	S	KC-315062	Iran	S	TN-72-152	Iran	S
KC-315011	Iran	S	KC-315063	Iran	S	TN-72-155	Iran	S
KC-315012	Iran	S	KC-315064	Iran	S	TN-72-157	Iran	S
KC-315013	Iran	S	KC-315066	Iran	S	TN-72-159	Iran	S
KC-315014	Iran	S	KC-315067	Iran	S	TN-72-160	Iran	S
KC-315015	Iran	S	KC-315068	Iran	S	TN-72-162	Iran	S
KC-315016	Iran	S	KC-315069	Iran	S	TN-72-164	Iran	S
KC-315017	Iran	S	KC-315070	Iran	S	TN-72-165	Iran	S
KC-315018	Iran	T	KC-315071	Iran	T	TN-72-166	Iran	S
KC-315019	Iran	S	KC-315072	Iran	S	TN-72-167	Iran	S
KC-315020	Iran	S	KC-315073	Iran	S	TN-72-168	Iran	S
KC-315021	Iran	S	KC-315074	Iran	S	TN-72-169	Iran	S
KC-315022	Iran	S	KC-315075	Iran	S	TN-72-170	Iran	S
KC-315023	Iran	S	KC-315076	Iran	S	TN-72-174	Iran	S
KC-315024	Iran	S	KC-315077	Iran	T	TN-72-175	Iran	S
KC-315025	Iran	S	KC-315078	Iran	S	TN-72-180	Iran	S
KC-315026	Iran	S	KC-315079	Iran	S	TN-72-188	Iran	S
KC-315027	Iran	S	KC-315080	Iran	S	TN-72-189	Iran	S
KC-315028	Iran	T	KC-315081	Iran	S	TN-72-190	Iran	S
KC-315029	Iran	S	KC-315082	Iran	T	TN-72-198	Iran	S
KC-315030	Iran	S	KC-315083	Iran	S	TN-72-207	Iran	S
KC-315031	Iran	S	KC-315084	Iran	T	TN-72-208	Iran	S
KC-315032	Iran	S	KC-315085	Iran	S	H24	AVRDC	S
KC-315033	Iran	S	KC-315086	Iran	S	CLN2116DC1F1-180-31-10-25-16	AVRDC	S
KC-315034	Iran	S	KC-315087	Iran	S	CLN2116DC1F1-180-31-10-25-8-0	AVRDC	S
KC-315035	Iran	S	KC-315088	Iran	S	CLN2116DC1F1-180-31-9-24-4-0	AVRDC	S
KC-315036	Iran	S	KC-315089	Iran	S	CLN2116DC1F1-180-31-10-25-22	AVRDC	S
KC-315042	Iran	S	KC-315090	Iran	S	CLN2114DC1F1-2-16-8-2-17-0	AVRDC	S
KC-315043	Iran	S	KC-315091	Iran	S	CLN2114DC1F1-180-31-9-11-12	AVRDC	S
KC-315044	Iran	S	KC-315104	Iran	T	CLN2114DC1F1-2-29-20-23-14-0	AVRDC	S
KC-315045	Iran	T	KC-315110	Iran	S	PS111	Iran	S
KC-315046	Iran	S	KC-315111	Iran	S	S. peruvianum		
KC-315047	Iran	S	KC-315112	Iran	S	KC-315037	Iran	R
KC-315048	Iran	S	KC-315118	Iran	S	KC-315038	Iran	R
KC-315049	Iran	S	KC-315138	Iran	S	KC-315039	Iran	R
KC-315050	Iran	S	KC-315139	Iran	S	KC-315040	Iran	R
KC-315051	Iran	S	KC-315140	Iran	S	KC-315041	Iran	R
KC-315052	Iran	S	TN-72-141	Iran	S	P1 126 944	AVRDC	R

*: Responses presented here are based on the observation of two screening experiments conducted in 2005 and 2006. PC: Place of collection, RP: Response to TYLCV, AVRDC: Asian Vegetable Research and Development Center S: Susceptable (all eigth plants showing severe symptom at least during one growing season). R: Resistance (no symptom and no TYLCV DNA amplification was observed). T: Tolerant (weak or no symptom was observed, but TYLCV DNA amplification was detected).

Table 4. Phenotypic (symptoms) and molecular (PCR) evaluations of TYLCV infection in graft-inoculated *S. peruvianum* accessions as compared to those of *S. lycopersicum* susceptible control, three or five weeks post-inoculation.

Post-inoculation	Type of evaluation	Accessions						
		KC-315037	KC-315038	KC-315039	KC-315040	KC-315041	P1 126 944	*S. lycopersicum* (PS111)
Three weeks	Symptoms	-	-	-	-	-	-	⊕ *
	PCR	-	-	-	-	-	-	+
Five Weeks	Symptoms	⊕	*	*	⊕	*	⊕	⊕ *
	PCR	+	+	+	+	+	+	+

⊕ Leaf curl
* Reduced leaflet size
+ Observed
- Not observed

studies been reported TYLCV tolerant (AVRDC, 2001), were found susceptible in this study. In addition, accession CLN2114DC1F1-2-29-20-23-14-0 showed a much faster development of disease symptoms than other tested accessions. This difference in reaction could be due to the virus strain, vector genotype or altered feeding conditions of the vector (Delatte *et al*., 2006; Navas-Castillo *et al*., 1999). Viruses transmitted by *B. tabaci* are deposited within the phloem through salivation. Therefore, altered feeding behavior could result in a significant decrease in the incidence of several begomoviruses that is usually interpreted as resistance to insect vector. This has been reported in studies with the *Rice ragged stunt virus* transmitted by planthopper *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) to rice (Parejarearn *et al*., 1984) and Maize mosaic virus (MMV) transmitted by the planthopper *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) to maize (Dintinger *et al*., 2005).

The type of resistance expressed by *S. peruvianum* accessions was studied using graft-inoculation of TYLCV and was compared to whitefly inoculations. TYLCV resistance observed in whitefly-inoculated *S. peruvianum* accessions was overcome five weeks after plants were graft-inoculated (Table 4). These results revealed that TYLCV resistance in these accessions can be overcome when plants are infected by graft-inoculation which is not a common method of natural infection in the field. The resistance against vector inoculation of TYLCV in *S. peruvianum* could be in accordance with its acyl sugar content, known to be a whitefly repellent (Liedl *et al*., 1995). Five genomic regions were detected as being associated with acyl sugar production in *S. peruvianum* (Mutschler *et al*., 1996). However, to our knowledge there is no report on the success of transferring these factors into a cultivated *S. lycopersicum* for development of resistance to TYLCV, probably due to unfavorable linkages. Although *S. peruvianum* accessions were not resistant when graft-inoculated, they are still considered major sources of resistance to TYLCV as whitefly transmis-

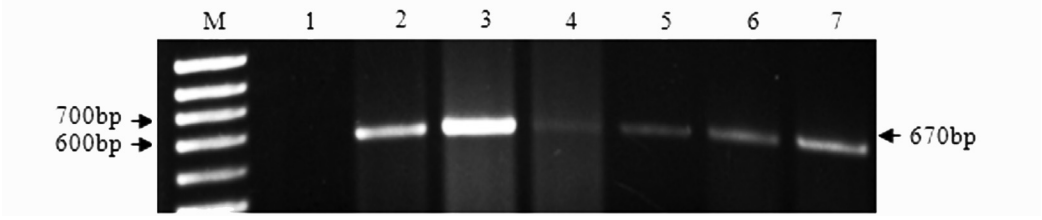

Figure 3. PCR detection of TYLCV in graft inoculated *S. peruvianum* accessions, five weeks post inoculation. M) 100bp Marker DNA 1) Healthy plant 2) KC-315037 3) KC-315038 4) KC-315039 5) KC-315040 6) KC-315041 7) P1 126 944.

Table 5. Classification of different groups of tomato accessions based on their response to TYLCV with respect to phenotypic and molecular evaluations.

Symptom severity	Viral DNA intensity	Accessions
Severe	High	KC-315002, KC-315004, KC-315007, KC-315013, KC-315024, KC-315033, KC-315046, KC-315049, KC-315052, KC-315054, KC-315056, KC-315067, KC-315072, KC-315091, KC-315104, KC-315138, KC-315140, TN-72-141, TN-72-147, TN-72-148, TN-72-150, TN-72-152, TN-72-165, TN-72-168, TN-72-170, TN-72-175, TN-72-189, CLN2116DC1F1-180-31-9-24-4-0, CLN2114DC1F1-2-29-20-23-14-0, PS111
	Low	KC-315005, KC-315008, KC-315014, KC-315016, KC-315019, KC-315023, KC-315025, KC-315026, KC-315030, KC-315035, KC-315043, KC-315050, KC-315057, KC-315063, KC-315069, KC-315083, TN-72-180, H24, CLN2114DC1F1-180-31-9-11-12
	None	-
Weak	High	KC-315045, KC-315084, KC-315087
	Low	KC-315018, KC-315028, KC-315061, KC-315071, KC-315077, KC-315082
	None	-
No symptom	High	-
	Low	-
	None	*S. peruvianum:* KC-315037, KC-315038, KC-315039, KC-315040, KC-315041, P1 126 944

sion is the normal transmission mechanism in the field. TYLCV resistant accessions of *S. peruvianum* have also been reported to be suitable sources of resistance to *Tomato leaf curl virus* (ToLCV) and may show resistance to other tomato begomoviruses as well (Pitersen and Smith, 2002).

Virus titer in plant tissue is an indicator of resistance or susceptibility of plants to the virus. Low levels of virus titer and decreasing virus accumulation rate in plant tissue indicate the presence of a resistance mechanism in the plant (Pico *et al*., 2001; Lapidot *et al*., 1997; Rom *et al*., 1993; Pilowsky and Cohen, 1974). Thus TYLCV accumulation has been used as an indicator for resistance, but not as the sole indicator (Lapidot *et al.*, 1997). In the present study despite the high levels of similarities in symptom development, there were considerable differences in TYLCV concentration among accessions (Table 5). There were also tolerant accessions showing no clear symptoms, but accumulating a high virus titer. Therefore, an assessment of virus titer in the plant along with the phenotypic evaluation of the disease severity is required for evaluation of TYLCV resistance. In general, based on both phenotypic and molecular evaluations, five categories of accession were identified (Table 5): accession with severe symptoms and high concentrations of viral DNA, accessions with severe symptoms but relatively low concentrations of TYLCV, accessions with mild or weak symptoms but high levels of TYLCV concentration and accessions with weak symptoms and low TYLCV concentrations. These two latter groups were considered as tolerant and finally a group of *S. peruvianum* which showed no symptoms and no TYLCV DNA amplification upon whitefly inoculation was considered as resistant. Tolerance and resistance are relative terms, largely related to the rate of virus replication (Pilowsky and Cohen, 1990). Results obtained here, also confirmed this point (Table 5). The accessions reported as tolerant by AVRDC (CLN2114DC1F1-180-31-9-11-12 and CLN2114DC1F1-2-29-20-23-14-0) did have a relatively low virus concentration but three weeks post-inoculation; TYLCV concentration increased in these accessions like in any other susceptible accession. In addition, the accession CLN2114DC1F1-2-29-20-23-14-0 was the fastest among all accessions in showing clear disease symptoms such as yellowing and leaf curling. These differences in reaction can be due to differences in virus strain or vector genotypes which can lead to the susceptibility of a resistant accession (Navas-Castillo *et al*., 1999).

The resistance mechanism in these wild species has been reported to be associated with the presence of exudates from trichom glands on the leaf surface, in which whiteflies become entrapped (Channaryappa and Shivashankar, 1992). A quantitative resistance to vector transmission of TYLCV was also reported in *S. pimpinellifolium* (Delatte *et al*., 2006). The type of resistance in *S. peruvianum* accessions observed in this study may be related to the insect vector as they

become infected by graft-inoculation. The activity of *B. tabaci* on the tomato leaf surface was also studied. Trichomes on the leaf surface of *S. peruvianum* accessions were shorter and much denser compared to the trichomes of *S. lycopersicum*. Whitefly populations were also observed on these resistance accessions in different life stages including egg, nymph and adult whiteflies. Therefore, resistance may not be due to the lack of feeding by the vector, but the change in the feeding behavior. This may affect the virus transmission efficiency via the vector (Delatte *et al*., 2006). Nevertheless, further studies are required to characterize the impact of insect feeding behavior on plant resistance.

Acknowledgments

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