Plant Breeding, 1—7 (2008)

Pyramiding of genes conferring resistance to *Tomato yellow leaf curl virus* from different wild tomato species

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With 3 tables

Received August 13, 2007/Accepted July 1, 2008
Communicated by P. Wehling

Abstract

Tomato (*Solanum lycopersicum*) production in tropical and subtropical regions of the world is limited by the endemic presence of *Tomato yellow leaf curl virus* (TYLCV). Breeding programmes aimed at producing TYLCV-resistant tomato cultivars have utilized resistance sources derived from wild tomato species. So far, all reported breeding programmes have introgressed TYLCV resistance from a single wild tomato source. Here, we tested the hypothesis that pyramiding resistances from different wild tomato species might improve the degree of resistance of the domesticated tomato to TYLCV. We have crossed TYLCV-resistant lines that originated from different wild tomato progenitors, *Solanum chilense*, *Solanum peruvianum*, *Solanum pimpinellifolium*, and *Solanum habrochaites*. The various parental resistant lines and the F_1 hybrids were inoculated in the greenhouse using viruliferous whiteflies. Control, non-inoculated plants of the same lines and hybrids were exposed to non-viruliferous whiteflies. Following inoculation, the plants were scored for disease symptom severity, and transplanted to the field. Resistance was assayed by comparing yield of inoculated plants to those of the control non-inoculated plants of the same variety. Results showed that the F_1 hybrids between the resistant lines and the susceptible line suffered major yield reduction because of infection, but all hybrids were more resistant than the susceptible parent. All F_1 hybrids resulting from a cross between two resistant parents, showed a relatively high level of resistance, which in most cases was similar to that displayed by the more resistant parent. In some cases, the hybrids displayed better levels of resistance than both parents, but the differences were not statistically significant. The F_1 hybrid between a line with resistance from *S. habrochaites* and a line with resistance from *S. peruvianum* (HAB and 72-PER), exhibited the lowest yield loss and the mildest level of symptoms. Although the resistance level of this F_1 hybrid was not statistically different from the level of resistance displayed by the 72-PER parent itself, it was statistically better than the level of resistance displayed by the F_1 hybrids between 72-PER and any other resistant or susceptible line.

Key words: TYLCV — tomato — resistance — virus — whitefly

Introduction

Tomato production worldwide is under the constant threat of geminiviruses (genus *Begomovirus*, family Geminiviridae) transmitted by the whitefly *Bemisia tabaci*. Begomoviruses are characterized by a ca. 20×28 nm twinned isometric particle containing a circular single-stranded DNA molecule of approximately 2800 nucleotides. Some begomoviruses infecting tomato, such as *tomato golden mosaic virus* have their genome split between two different genomic molecules named DNA-A and DNA-B (bipartite), each approximately 2800 nucleotides in size; others such as *Tomato yellow leaf curl virus* (TYLCV) have a single DNA-A like genomic component (monopartite) (Navot et al. 1991).

Tomato yellow leaf curl disease (TYLCD) is a major constraint for open field tomato production in many regions of the world. The disease is induced by a number of begomovirus species and strains, among them, TYLCV, widely spread worldwide (Moriones and Navas-Castillo 2000, Fauquet and Stanley 2005). Management of TYLCD usually consists in spraying large amounts of insecticides to control the population of the whitefly virus vector. Abuse has greatly contributed to the development of pesticide-resistant *B. tabaci* populations (Palumbo et al. 2001).

From the early 1960s, TYLCD has quickly spread to the Middle East and is presently found in many regions of Africa, America and Asia (Abhary et al. 2007). TYLCD has been reported in the mid and late 1970s in Cyprus, Jordan, and Lebanon. It has been identified in Egypt and Turkey in the early 1980s, and in the mid-late 1990s in Iran, the Asian republics of the former USSR, Japan, Saudi Arabia and Yemen. In the early 1990s, TYLCD has been identified in Italy, Spain and Portugal, and later in France and Greece. In Morocco and Tunisia TYLCD was identified in the early 2000s. In East Africa, TYLCD was present in Sudan as early as the late 1970s. In the Réunion Island the disease was detected in the late 1990s. TYLCD has appeared in the Western Hemisphere in the mid 1990s in the Caribbean Islands, first in the Dominican Republic, then Cuba, Jamaica, Puerto Rico and the Bahamas. From there, the disease has reached the USA, identified first in Virginia in the late 1990s, then in Florida, Georgia, Louisiana, North Carolina and Mississipi. TYLCD has been recently identified in several regions of Mexico, in Arizona and in California (Brown and Idris 2006, Abhary et al. 2007, Rojas et al. 2007).

Breeding programmes for resistance to TYLCV have started in the middle 1970s and several commercial varieties with adequate resistance have been released since (Lapidot and Friedmann 2002). Early efforts to identify sources of resistance to TYLCV within the domesticated tomato *Solanum lycopersicum* were unfruitful (Abu Gharbieh et al. 1978, Cohen and

	P B R	1556/PLBR-07-RA-331.R3	B	Dispatch: 21.7.08	Journal: PBR	CE: Bharathy
	Journal Name	Manuscript No.		Author Received:	No. of pages: 7	PE: PUSHPA

Harpaz 1964, Nitzany 1975). It was then observed that some accessions of several wild relatives of tomato, such as *Solanum pimpinellifolium* and *Solanum peruvianum*, possessed a high level of resistance to TYLCV, although they were not immune (Cohen and Nitzany 1966). Crosses between *S. lycopersicum* and *S. pimpinellifolium* accession LA 121 and genetic analyses of F1–3 and backcrosses indicated the existence of incomplete dominance of resistance over susceptibility, suggesting a monogenic control of resistance (Pilowsky and Cohen 1974). A dominant gene (*Tylc*) was later proposed for the resistance gene in *S. pimpinellifolium* accession LA1478 (Kasrawi 1989). The progeny derived from this cross showed only moderate symptoms, but their yield was markedly reduced. Nevertheless, among the *Solanum* species, *S. pimpinellifolium* is one of the most compatible for crossing with *S. lycopersicum* (Picó et al. 1996). In contrast, the inheritance of resistance to TYLCV in *S. peruvianum* (accession PI 126935) is controlled by five recessive factors (Pilowsky and Cohen 1990). This breeding programme initiated in 1977, resulted in the release of the commercial hybrid TY-20, in 1988. This hybrid exhibited delay in symptom expression and viral DNA accumulation in infected plants, resulting in acceptable yields (Pilowsky and Cohen 1990). Other TYLCV-resistant lines generated by this breeding programme (using *S. peruvianum* accessions PI126926, PI 126930, PI 390681 and LA 441) are: TY-172, TY-197, TY-198 and TY-199 (Friedmann et al. 1998, Lapidot et al. 1997, Levy and Lapidot 2008). In the early 1990s, additional accessions from wild tomato species such as *Solanum chilense* and *Solanum habrochaites*, were found to be resistant (low virus amounts and absence of symptoms) in controlled whitefly-mediated inoculation experiments (Zakay et al. 1991). A TYLCV resistance gene from *S. chilense* accession LA 1969 (named *Ty-1*) was mapped on the tomato genome (Michelson et al. 1994, Zamir et al. 1994). TYLCV resistance introgressed from *S. habrochaites* accessions LA1777 and LA386 was found to be under the control of a major dominant gene (Vidavsky and Czosnek 1998). *S. habrochaites* has been back-crossed with *S. lycopersicum*, yielding tolerant and resistant lines. One of the resistant lines was back-crossed with *S. lycopersicum*, to produce the hybrid FAVI-9 (Vidavsky and Czosnek 1998). Another promising species evaluated for TYLCV resistance, *Solanum cheesmanii* (accession LA1401), possesses recessive resistance to TYLCV. Breeding projects in the Mediterranean region have also used *S. cheesmanii*, *S. peruvianum* and *S. pimpinellifolium* to control TYLCV (Laterrot 1992, Laterrot and Moretti 1996). Some of the TYLCV-resistant lines obtained from these projects are: Pimpertylc-J-13 and Chepertylc-92. Interspecific hybrids obtained from crosses between *S. pimpinellifolium* (LA 121), *S. peruvianum* (CMV Sel. INRA), and *S. habrochaites* (H2), showed transgressive segregation for their reaction to TYLCV, suggesting the existence of different, complementary genes (Kasrawi and Mansour 1994). *S. chilense* (accessions LA 1932, LA 1938 and LA 2779) was also used as a source of resistance to the bipartite begomovirus *Tomato mottle virus* (ToMov) (Scott and Schuster 1991). The ToMov-resistance introgressed from LA 1932 was governed by two genes. The genes were additive: a hybrid between a resistant and a susceptible parent (heterozygous resistance) had intermediate resistance. (Griffiths and Scott 2001). Accessions of *S. habrochaites* (PI 390658 and PI 390659) and *S. peruvianum* (PI 127830 and PI 127831) were resistant to another tomato geminivirus, the *Tomato leaf curl virus* (Muniyappa et al. 1991). The resistance mechanism in these wild species was subsequently associated with the presence of exudates from trichome glands on the leaf surface, in which whiteflies became entrapped (Channarayappa et al. 1992). This is one of the few cases where genetic resistance to a viral disease has been achieved indirectly by incorporating genetic traits against *B. tabaci*.

So far, all reported researches and breeding programmes have used resistance to begomovirus from a single wild tomato species at a time. In this report, we have tested the hypothesis that pyramiding resistances from different wild tomato species in a tomato line may improve the degree of resistance or tolerance to TYLCV.

Materials and Methods

Virus and whitefly maintenance: Cultures of the Israeli isolate of TYLCV (Navot et al. 1991) (Genbank Accession number X15656) were maintained in susceptible tomato (cv. 'Rehovot 13') in an insect-proof greenhouse. Whitefly colonies (*B. tabaci*, biotype B) were reared on cotton plants (*Gossypium hirsutum*) grown in muslin-covered cages maintained within an insect-proof greenhouse.

TYLCV inoculation: Adult whiteflies were provided a 48-h acquisition access period on TYLCV-infected tomato source plants, after which they were provided a 48-h inoculation access period (IAP) on tomato test plants, 17–22 days after sowing. To ensure 100% infection, the plants were inoculated with 50 whiteflies per plant. Control, non-inoculated plants of the same genotypes, were exposed to non-viruliferous whiteflies for a 48-h IAP. Following the IAP, whiteflies were discarded by treating plants systemically with imidacloprid (Confidor, Bayer, Leverkusen, Germany). The plants were raised for 3–4 weeks in an insect-proof greenhouse at 26–32°C prior to transplanting to the field.

Plant material and crosses: Eight inbred determinate lines; seven resistant and one susceptible to TYLCV were chosen as parents (Table 1). Lines TY-172 and TY-197 have their resistance introgressed from *S. peruvianum*; both are determinate, yielding red fruits of 40–70 g (Friedmann et al. 1998, Lapidot et al. 1997). Line H-902 has its resistance originating from *S. habrochaites*; it is a determinate plant which yields red fruits of 100–120 g (Vidavsky and Czosnek 1998). The two lines TY-52 and Fla 595-2 have resistance originating from *S. chilense*; TY-52 yields red fruits of 40–60 g (Zamir et al. 1994) and

Table 1: Parent lines used to make a set of non-reciprocal di-alelle crosess

Parent line	Source of resistance	Abbreviation	Reference
TY-172	*Solanum peruvianum*	72-PER	Lapidot et al., 1997; Friedmann et al., 1998
TY-197	*S. peruvianum*	97-PER	Lapidot et al., 1997
H-902	*Solanum habrochaites*	HAB	Vidavsky and Czosnek 1998
TY-52	*Solanum chilense*	52-CHIL	Zamir et al., 1995
Fla-595-2	*S. chilense*	CHIL	Griffiths and Scott, 2001
PIMHIR	*Solanum pimpinellifolium*	PIM	Laterrot, 1992
B-117	None (susceptible)	SUS	Vidavsky, unpublished

Fla 595-2 yields red fruit of 100–120 g (Griffiths and Scott 2001). PIM-HIR has resistance introgressed from *S. Pimpinellifolium*; it yields red fruits of 40–60 g (Laterrot 1992). The susceptible line B-117 yields red fruits of 150 g (Vidavsky, unpublished). They were coined in short 72-PER, 97-PER, HAB, 52-CHIL, CHIL, PIM and SUS, respectively. The seven parents were crossed between them to produce 21 F_1 populations.

Disease severity scoring: Following controlled greenhouse inoculation, the plants were scored three times for disease severity. First scoring was conducted 3–4 weeks following inoculation, just prior to transplanting to the field. The second and third scorings were conducted in the field; the second scoring was conducted 5–6 weeks after inoculation, and the third scoring 5–6 weeks thereafter. Symptom severity was evaluated according to the disease severity index (DSI) described before (Friedmann et al. 1998, Lapidot and Friedmann 2002): 0 = no visible symptoms, inoculated plants show same growth and development as non-inoculated plants; 1 = slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; 4 = very severe plant stunting and yellowing, pronounced leaf cupping and curling, and plant ceased to grow. Three different scoring experiments, which included all F_1 crosses and the parent lines, were conducted during three different seasons. In each experiment, 10 plants from each F_1 hybrid and parent lines were tested.

Field trial for yield estimation: Five parental lines (TY-172, H-902, Fla-595-2, PIM-HIR and B-117, coined respectively 72-PER, HAB, CHIL, PIM and SUS) and all F_1 crosses between those lines were evaluated for yield in the field. Following controlled inoculation in the greenhouse as described above, the plants were treated with imidacloprid and transplanted to the field in April. The plants were grown until July. Plants of each variety were planted in paired rows – inoculated and non-inoculated (control), on 1 m-wide beds, five plants per row. The within-row and between-rows spacing were 0.5 and 1.2 m, respectively. Each pair of rows served as a replicate; a total of 10 randomly distributed replicates for each line or hybrid were planted in the field. Imidacloprid was applied through the drip irrigation system at 4 and 8 weeks after transplanting. Fruits were picked in a single harvest, all mature-red and immature-green fruits were collected. Culls were discarded. Data were taken per row and were averaged for all rows.

Statistical analysis was performed by means of a one-way analysis of variance (ANOVA) test (SAS Institute, Cary, NC, USA).

Results

Disease severity scoring of the resistant parental lines upon whitefly-mediated inoculation of TYLCV

We have chosen six TYLCV-resistant tomato lines, in which the resistance has been introgressed from different wild tomato species (Table 1). The resistant lines were crossed with each other and with a susceptible line in a non-reciprocal diallele crossing. The TYLCV-resistance level of the parental lines and of the resulting F_1 hybrids was evaluated under controlled uniform inoculation. The severity of the disease symptoms (DSI) was evaluated at three different periods following inoculation. The three scoring results were essentially the same. Table 2 shows the mean DSI of the second scoring, conducted 5 weeks following inoculation, approximately a week following transplanting to the field. Lines 72-PER and 97-PER with resistance from *S. peruvianum*, and HAB with resistance from *S. habrochaites* showed the highest level of resistance (DSI of 0.5, 1.0 and 1.2, respectively). Lines CHIL and 52-CHIL with resistance from *S. chilense* showed moderate resistance (DSI of 1.8 and 2.3, respectively). Line PIM with resistance from *S. pimpinellifolium* showed the lowest level of resistance among the resistant lines (DSI of 2.5), followed only by the susceptible line SUS (DSI of 4.0).

Dominance of resistance in the F_1 hybrids

The TYLCV-resistant lines were crossed with the same susceptible line and the resistance level of the resulting F_1 hybrids was estimated (Table 2). Resistance displayed by PIM, 52-CHIL and CHIL was mostly dominant, as the DSI of the F_1 hybrids between the resistant and susceptible line was very close to that of the resistant line itself. The DSI of the hybrid SUS × PIM was 2.8 compared with 2.5 for PIM; it was 2.2 for SUS × 52-CHIL compared with 2.3 for 52-CHIL, and it was 1.8 for SUS × CHIL, the same as the CHIL parent. Resistance from lines HAB and 97-PER was partly dominant, as the F_1 hybrid with the susceptible line showed a lower level of resistance than the resistance parent (DSI for 1.0 for 97-PER compared with 2.5 for the hybrid, and DSI of 1.2 for HAB compared with 2.0 for the hybrid). Resistance of 72-PER showed mainly a recessive character: the DSI of the SUS × 72-PER was 3.2 compared with the DSI of 0.5 for the 72-PER parent.

Pyramiding of resistance in F_1 hybrids between resistant lines

The TYLCV-resistant lines were crossed in a diallelic scheme. Nearly all the F_1 hybrids resulting from crossing two resistant parents were more resistant to TYLCV than the F_1 hybrids resulting from a cross between one of the resistant parents and the susceptible line (Table 2). Only the CHIL × PIM F_1 hybrid, with a DSI of 2.0, exhibited the same level of resistance as the CHIL × SUS F_1 hybrid, with a DSI of 1.8. Moreover, the resistance level of the hybrid was higher than the resistance level shown by at least one of the two resistant parents. Considering the level of resistance (expressed as DSI) of the hybrids compared to the resistance of the most resistant parent, the hybrids could be divided into two categories. The first consisted of hybrids with a DSI that falls between the DSI

Table 2: Disease severity index means of F_1 hybrids and parental lines scored 5 weeks after inoculation with *Tomato yellow leaf curl virus*

Line	SUS	PIM	CHIL	52-CHIL	HAB	72-PER	97-PER
97-PER	2.5 ± 0.3	1.0 ± 0.3	0.7 ± 0.4	0.8 ± 0.3	0.8 ± 0.3	1.3 ± 0.3	1.0 ± 0.3
72-PER	3.2 ± 0.6	1.3 ± 0.2	0.8 ± 0.2	0.7 ± 0.4	0.8 ± 0.3	0.5 ± 0	
HAB	2.0 ± 0.5	1.5 ± 0.3	0.7 ± 0.2	1.0 ± 0.3	1.2 ± 0.3		
52-CHIL	2.2 ± 0.2	1.7 ± 0.2	1.0 ± 0.6	2.3 ± 0.4			
CHIL	1.8 ± 0.2	2.0 ± 0.2	1.8 ± 0.4				
PIM	2.8 ± 0.4	2.5 ± 0.3					
SUS	4.0 ± 0						

Ten infected plants from each parental line or F_1 hybrid were evaluated in each replicate.
± Standard error of means (SEM).

values of each parent; it includes the hybrids of 72-PER with PIM, CHIL and 97-PER (DSI of 1.3, 0.8 and 1.3, respectively) and of HAB with PIM (DSI of 1.5). Included in this group where hybrids with a DSI similar to that of the most resistant parent, such as 72-PER × 52-CHIL, 72-PER × HAB and CHIL × HAB (DSI of 0.7, 0.8 and 0.7, respectively). The second and more interesting group included the hybrids that seem to be more resistant than both parents. This group includes the hybrids from crosses of 97-PER with CHIL (DSI of 1.0, 1.8 and 0.7, respectively), 52-CHIL and HAB (DSI of 2.3, 1.2 and 1.0, respectively) and of 52-CHIL with CHIL (DSI of 2.3, 1.8 and 1.0, respectively). It should be noted that although the standard error of means (SEM) of each DSI value is given in Table 2, the DSI is in essence a qualitative scale, greatly affected by the person performing the scoring, hence, the statistical significance of the correlation between DSI and SEM is not absolute.

TYLCV-induced yield reduction in parental lines and in hybrids between resistant lines

Although symptoms severity is the primary parameter for selecting resistant plants, yield is the most important criteria for growers. In order to estimate yield reduction due to TYLCV infection, a field trial was conducted. Following controlled inoculation of lines and hybrids, the infected plants were transplanted to the field together with control non-infected plants of the same genotypes and of the same age. The plants were scored for disease severity and their yield was measured. The decrease in yield because of virus infection was determined (Table 3). Comparisons of DSI value and yield loss between hybrids and parental lines can tell us about the dominance of the resistance, the compatibility and the additivity of the resistance sources.

To find out whether there is a yield penalty as a result of viral resistance in non-inoculated plants, we first appreciated the yield potential (non-inoculated plants) of the parental lines and of hybrids between these lines. PIM and 72-PER yielded 2 better than the 'SUS' cultivar (respectively 8.8, 7.7 and 5.2 kg/plant), while HAB and CHIL had yields similar to those of SUS (respectively 4.3, 5.4 and 5.2 kg/plant). The hybrids between the resistant lines and the SUS line yielded better than SUS by itself. For example, the hybrids PIM × SUS and CHIL × SUS doubled the yield of SUS (approximately 10 vs. 5 kg/plant). The same phenomenon was observed with crosses between resistant lines. Hence, no yield penalty was associated with the resistant trait. The highest yield was provided by hybrids when PIM was used as a parent (approximately 11–12 kg/plant), higher (but not statistically significant) than PIM alone (8.8) suggesting dominance for yield. 72-PER had also a dominant effect on yield. In most cases (but with CHIL), hybrids with 72-PER had higher yield than the second parent. For example, the yield of the hybrid 72-PERxHAB was twice that of the HAB parent (9.3 vs. 4.3). The same trend was true for the other hybrids, but to a lesser extent (Table 3).

In a second stage, we appreciated the resistance of the parents and hybrids, and the yield loss because of TYLCV infection. The susceptible line lost nearly all its yield because of TYLCV infection. Although the most resistant lines 72-PER and HAB were nearly symptomless upon TYLCV inoculation 8 (both with DSI of 1.3), they lost more than half of their yield because of virus infection (close to 56 and 67%, respectively).

Table 3: TYLCV-induced symptom severity and yield reduction of the different parents and F_1 hybrids

Line/F_1 hybrid	DSI	Average yield kg/plant (SD)[1]	Yield loss (%)[2]
SUS			
Non-inoculated	4.0	5.2 (1.2)	99.2 a
Inoculated		0.04 (0.05)	
P*		< 0.001	
PIM			
Non-inoculated	3.6	8.8 (0.9)	89.7 b
Inoculated		0.9 (0.3)	
P*		< 0.001	
CHIL			
Non-inoculated	2.6	5.4 (1.0)	62.9 c,d
Inoculated		2.0 (0.6)	
P*		< 0.001	
HAB			
Non-inoculated	1.3	4.3 (1.0)	67.4 c,d
Inoculated		1.4 (0.6)	
P*		< 0.001	
72-PER			
Non-inoculated	1.3	7.7 (1.2)	55.8 f,g
Inoculated		3.4 (0.9)	
P*		< 0.001	
SUS × PIM			
Non-inoculated	4.0	10.1 (1.1)	91.1 b
Inoculated		0.9 (0.3)	
P*		< 0.001	
SUS × CHIL			
Non-inoculated	2.7	10.2 (1.8)	81.4 b,e
Inoculated		1.9 (0.7)	
P*		< 0.001	
SUS × HAB			
Non-inoculated	2.2	7.7 (1.0)	77.9d,e
Inoculated		1.7 (0.5)	
P*		< 0.001	
SUS × 72-PER			
Non-inoculated	3.8	8.7 (1.9)	73.6 d,e
Inoculated		2.3 (0.7)	
P*		< 0.001	
PIM × CHIL			
Non-inoculated	1.9	12.2 (2.9)	77.0 d,e
Inoculated		2.8 (0.4)	
P*		< 0.001	
PIM × HAB			
Non-inoculated	1.6	11.1 (2.0)	75.7 d,e
Inoculated		2.7 (0.5)	
P*		< 0.001	
PIM × 72-PER			
Non-inoculated	2.2	11.0 (3.0)	65.5 c,d
Inoculated		3.8 (1.6)	
P*		< 0.001	
CHIL × HAB			
Non-inoculated	2.6	7.3 (2.0)	58.9 c,f
Inoculated		3.0 (0.7)	
P*		< 0.001	
CHIL × 72-PER			
Non-inoculated	2.0	6.9 (2.1)	65.2 c,d
Inoculated		2.4 (1.0)	
P*		< 0.001	
HAB × 72-PER			
Non-inoculated	0.9	9.3 (1.9)	46.2 g
Inoculated		5.0 (1.0)	
P*		< 0.001	

TYLCV, *tomato yellow leaf curl virus*; DSI, disease severity index.
[1]In parenthesis is the standard deviation (SD).
[2]Means with different letters differ significantly at $P < 0.05$ when analyzed by one-way ANOVA (means separated by LSD). Data was transformed by arcsin prior to analysis (Sokal and Rohlf 1981).
*Significance by unpaired *t*-test between inoculated and non-inoculated plants.

The mildly resistant CHIL line (DSI of 2.6) lost close to 63% of its yield upon infection. The poorly resistant PIM line (DSI of 3.6) lost 90% of its yield because of infection. The hybrids between the resistant lines and the susceptible line lost most of their yield because of infection, but all the F_1 hybrids were more resistant than the susceptible parent. Moreover, except for PIM, all the F_1 hybrids between a resistant and a susceptible line gave intermediate levels of TYLCV-resistance when compared to both parents. The yield reduction of the hybrid PIM × SUS was lower than that of the SUS parent but statistically the same as the PIM parent (91.1, 99.2 and 89.7, respectively) indicating that the resistance of PIM is dominant. This was also the case with the hybrid HAB × SUS, indicating that the resistance of HAB is also dominant. Both hybrids of 72-PER and CHIL with SUS gave intermediate levels of yield reduction when compared to both parents, indicating an additive nature of resistance.

Even the more resistant hybrids, such as HAB × SUS and CHIL × SUS, with DSI of 2.2 and 2.7, lost approximately 80% of their yield. The infection-related yield loss of hybrids between resistant lines was variable. However, even the most resistant hybrid 72-PER × HAB, with a DSI of 0.9, lost close to 46% of its yield. In general, there was a correlation between symptom severity (DSI) and yield loss, but not linear (Tables 2 and 3). It should be noted that the yield loss reflected the extremely severe inoculation of all tomato genotypes at a very young age.

These results indicated that all the F_1 hybrids resulting from a cross between two resistant parents, showed a relatively high level of resistance, which in most cases was the same as the level of resistance displayed by the more resistant parent. In some cases, the F_1 displayed a better level of resistance than both parents, but the differences were not statistically significant (Table 3). The highest level of resistance was expressed by the F_1 hybrid between HAB and 72-PER, which exhibited the lowest yield loss and the mildest level of symptoms. Although the resistance level of this F_1 hybrid was not statistically different than the level of resistance displayed by 72-PER itself, it was statistically better than the level of resistance displayed by the F_1 hybrids between 72-PER and any other resistant or susceptible line.

Discussion

Resistance to TYLCV has been found in several wild tomato species. Breeding programmes have used resistant traits mainly from accessions of *S. chilense*, *S. peruvianum* and S. *habrochaites*. The other wild tomato species have not been good resistance sources. Breeding tomato cultivars for resistance consisted of introgressing the resistance traits from one of the wild tomato species into the domesticated tomato. However, it appears that each breeding programme has resistant germplasm with a general combining ability with other resistant sources. In most cases, these lines present excellent agronomical traits (such as yield, fruit size, color, firmness, shelf life, etc.). By combining lines originating from different resistant wild tomato sources, one may shorten the time for breeding commercially valuable tomato resistant to TYLCV, with higher levels of resistance and higher yields than each of the resistant parents.

We have tested TYLCV-resistant lines with resistance originated from four different wild tomato species: *S. chilense*, *S. pimpinellifolium*, *S. peruvianum* and *S. habrochaites*. Two resistant lines presented a dominant effect: HAB (from *S. habrochaites*) and PIM (from *S. pimpinellifolium*). The level of resistance exhibited by hybrids between the resistant and susceptible lines may not be suitable for heavily inoculated area. However, using these hybrids together with integrated crop management practices that reduce virus incidence in region where the disease is not so severe may help the farmer until cultivars with higher level of resistance are available.

The most resistant hybrids in these trials were those which combined more then one source of resistance in one hybrid. All sources of TYLCV resistance in this experiment are complementary to each other. The highest level of resistance was achieved by combining together the resistant lines 72-PER (derived from *S. peruvianum*) and HAB (derived from *S. habrochaites*). The 72-PERxHAB hybrid showed a DSI of 0.9, gave a good yield (9.3 kg/plant) and presented the lowest TYLCV-induced yield loss compared to non-infected plants (46%). Surprisingly, hybrids with the less resistant line PIM (*S. pimpinellifolium*), showed a high level of resistance when combined with HAB, CHIL or 72-PER, and all showed a higher level of resistance than PIM itself, or than the hybrid PIM × SUS. The combination of the resistant lines emphasized the role of major dominant gene in HAB and CHIL lines. Moreover, it showed a surprising combining ability between PIM and 72-PER.

The most important issue in breeding for resistance is yield. In the field test, yield data were estimated per plot and were averaged for all plots. This feature reflected the general ability of the specific genotype to yield, which is a polygenetic trait. Yield loss because of infection was evaluated by comparing yield of infected and non-infected plants from the same genotype, or hybrid, pointing to the contribution of any given source of resistance to the ability of the plants to yield. Overall yield losses reflected the DSI, which agrees with our previous results (Lapidot et al. 2006). However, upon infection plants with mild symptoms may loss as much yield as plants with heavier symptoms. For example, the hybrid HAB × PIM with a DSI of 1.6 suffered a yield loss of 76% while the hybrid HAB × CHIL with a DSI of 2.6 lost only 59% of its yield.

Although 72-PER × HAB was the most resistant hybrid (based on yield loss and disease symptoms), all sources of TYLCV resistance tested in these experiments were complementary to each other. Even the poorly resistant line PIM did not significantly decrease the resistance level of other resistant lines in the F_1 hybrids. Moreover, it contributed to resistance of the PIM × 52-CHI hybrid (DSI of 1.7, compared with DSI of 3.0 and 2.3 for each parent), indicating that PIM possessed an additive dominant component. This feature was confirmed in the field trial: the PIM × 72-PER hybrid suffered a yield loss of 65.5% while PIM itself had a yield loss of 89.7%, 72-PER a yield loss of 55.8%, and the SUS × 72-PER hybrid a yield loss of 73.6%.

In the most resistant hybrid 72-PER × HAB, the decrease of yield was 45%, although the plants were almost symptomless. This finding indicates that highly resistant plants – symptomless upon infection – none the less suffer a yield reduction following inoculation with TYLCV. However, we need to remember that the experiment was conducted in very severe conditions – with plants inoculated at a very early stage with a large number of viruliferous whiteflies. Under the usual field conditions, even in an infested area, inoculation occurs after transplantation (40-days old seedlings), sometimes immediately, in most cases delayed, and escapes

frequently occur (Vidavsky et al. 1998). In this case, the yield loss in the most resistant genotype was between 0% and 20%, depending on the plant age during infection (Levy and Lapidot 2008). The advantage of planting seedlings already inoculated is to select for true and most resistant plants, even if mild resistance plants which might be of interest are discarded. Most important, it prevents selection of plants that escaped infection.

The genotypes selected by using severe inoculation before planting has resulted in the selection of several lines resistant to TYLCV, with resistance introgressed from different wild tomato species. Some of these lines and hybrids with susceptible cultivars or with other resistant lines have been tested successfully in various countries worldwide against monopartite and bipartite begomoviruses (Bian et al. 2007, Maruthi et al. 2003, Mejía et al. 2005). Hence, the genotypes and hybrids present a broad-range of resistance and could be used as genitors in programmes aimed at breeding tomato adapted to local market and consumer preferences.

The diversity, movement and changes of the begomoviruses and of the whitefly vector, compels us to keep improving our understanding of the intricate relationship between the plant the virus and the whitefly and to develop resistance adapted to these rapid changes. Pyramiding the chromosomal regions associated with resistance in the lines from different origins improves the degree of resistance to TYLCV and other begomoviruses. The strategy followed to incorporate high levels of begomovirus resistance in common bean, strictly through the intraspecific recombination and pyramiding of different resistance traits found in diverse gene pools of *Phaseolus vulgaris*, confirms the feasibility of this approach (Blair and Beaver 1993, Singh et al. 2000). However, the important conclusion is that there are both direct and circumstantial evidence indicating the existence of adequate genetic variability in the gene pools of most cultivated species. This genetic variability can be exploited within and between cultivated species and their relatives. Interspecific hybridization in tomato can be practiced not only in search of resistance to begomoviruses, but to other pathogens and pests as well (Debouck 1991, Nichols 1947). In the case of tomato, it is evident that the cultivars with improved TYLCV resistance are also exhibiting resistance to distinct New World begomoviruses infecting tomato in the Americas and Asia (Mejía et al. 2005, Muniyappa et al. 1991, Piven et al. 1995).

The combination of classical breeding together with molecular markers linked to the different sources of resistance will be required in order to allow the pyramiding of the genes. It will help the breeder to distinguish between the different sources of resistant and to combine all TYLCV resistance genes available from the three main resistance source, *S. chilense*, *S. peruvianum* and *S. habrochaites*.

Acknowledgements

The authors wish to thank Dr Ilan Levin (Volcani Center) for critically reading this manuscript. This research was supported by Grant No. M21–037 funded by the US–Israel Cooperative Development Research Program, Bureau for Economic Growth, Agriculture, and Trade, US Agency for International Development, Middle East Research and Cooperation (MERC) Program to H. C. and M. L. Contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel. Number 121/2007.

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