Tolerance to Tomato Yellow Leaf Curl Virus Derived from *Lycopersicon peruvianum*

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ABSTRACT

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The inheritance of tolerance to tomato yellow leaf curl virus (TYLCV), deriving from the wild tomato *Lycopersicon peruvianum*, was determined by crossing the cultivated tomato (*L. esculentum*) line M-60 (TYLCV-tolerant) with line 10 (TYLCV-susceptible). Inoculations were made in F_1, F_2, and backcross populations by means of the vector, the tobacco whitefly (*Bemisia tabaci*). Genetic data indicated that tolerance was controlled by five recessive genetic factors. Transmission tests by means of whiteflies to *Datura stramonium* indicated that the tolerant F_1 hybrid commercial cultivar TY-20 was as good a source of the virus as was the susceptible F_1 hybrid cultivar Naama.

Tomato yellow leaf curl virus (TYLCV), a geminivirus transmitted by the tobacco whitefly (*Bemisia tabaci* Genn.), is a serious problem in tomatoes (*Lycopersicon esculentum* Mill.) in the Middle East (1,5,6,10,12,13,22), Senegal, and Tunisia (7). In Israel, TYLCV is the causal agent of a major disease affecting tomatoes in the summer and autumn, causing severe damage to fresh market tomatoes grown for local consumption and export. Affected plants are markedly stunted, and their branches and petioles tend to assume an erect position, Leaflets are rolled upward and inward, showing severe interveinal chlorosis, and are smaller than those of healthy plants. Fruit set is greatly reduced, and infected young plants produce almost no marketable yield.

Attempts to reduce the incidence of TYLCV by chemical control of the vector (19), soil mulching with straw or yellow polyethylene sheets to attract and kill the whiteflies (3,4), and physical barriers (2) often are ineffective, particularly when peak whitefly populations occur. Consequently, resistance is an attractive option.

Resistance to TYLCV has been reported in wild relatives of the cultivated tomato (8,9,11,12,15). Breeding for resistance to TYLCV was initiated at The Volcani Center in 1974, using accession LA121 of *L. pimpinellifolium* (Jusl.) Mill. as the source of resistance (15). LA121 plants exhibited strong vegetative growth; when grown in the Jordan Valley, no difference was noted between LA121 plants infected by TYLCV and those that had escaped infection. When tomato breeding lines involving LA121 were grown in the field, they showed moderate disease symptoms, but growth and yield were markedly reduced. Therefore, a new breeding program was begun in 1977 to incorporate tolerance from accession PI 126935 of *L. peruvianum* (L.) Mill. (Pilowsky and Cohen, *unpublished*). This program led to the development of F_1 hybrid TY-20, which was released in 1988 for commercial cultivation in the open field (16). When infected with TYLCV, the leaves of young TY-20 plants exhibit a mild interveinal chlorosis. In mature plants, leaflets usually become slightly cupped, but the plants give an acceptable yield in spite of the infection.

This study was conducted to determine the genetic basis of tolerance to TYLCV and the efficiency of tolerant and susceptible plants as TYLCV sources for whiteflies. We defined tolerance as the characteristic of a host genotype to show less severe disease symptoms and to suffer less damage (in terms of yield and quality) than a susceptible genotype (18).

MATERIALS AND METHODS

Development of tolerant tomato line M-60. M-60 was a F_6 inbred in the third backcross generation (BC_3F_6) from the cross between a susceptible tomato line and the TYLCV-tolerant accession PI 126935 of *L. peruvianum*. Seeds of PI 126395 were obtained from the North Central Regional Plant Introduction Station, Ames, Iowa. Plants of PI 126935 showed a light interveinal chlorosis on the leaves soon after inoculation, followed by a slight cupping of the new growth. The interspecific hybridization was made using the cultivated tomato as the female parent, since a unilateral incompatibility prevents the cultivated species from serving as the male parent in crosses with the wild species. When *L. peruvianum* is the male parent, the cross is normally prevented by embryo abortion. Fruits are set, but no viable seeds are formed (17). The mixture-of-pollen technique was used to overcome this difficulty (14).

A tomato line homozygous for the recessive gene netted-virescent (*nv*) was used as the female parent in crosses with *L. peruvianum*. Cotyledons and leaves of plants of genotype *nv*/*nv* are greenish yellow (21). Pollen was collected from flowers of the tomato and *L. peruvianum* (normal green). A 1:1 (v/v) mixture of pollen of these lines was used to pollinate flowers of the female parent. Seeds were extracted from mature fruits and planted in flats containing a 2:1 (v/v) mixture of peat and vermiculite. Sixty-six of 3,255 seedlings were normal green; these were derived from the interspecific cross. The remaining seedlings exhibited the netted-virescent characteristic of genotype *nv*/*nv*, indicating that they originated from the intraspecific cross or were produced by accidental self-fertilization. These seedlings were discarded.

The interspecific hybrids were self-incompatible, a characteristic typical of *L. peruvianum*. The fertility relations of these hybrids with *L. esculentum* and *L. peruvianum* were exactly those expected of *L. esculentum* × *L. peruvianum* hybrids (17).

Twenty-five interspecific hybrids (F_1) at the one-leaf stage were inoculated with TYLCV by means of the vector whitefly, using the procedure we reported in an earlier study (15). All of these plants became heavily infected and were classified as susceptible.

Of 8,250 F_2 plants obtained from crosses between healthy interspecific hybrids, only six were found to be tolerant. Two of these plants were of genotype *nv*/*nv* and were discarded. Crosses were made among pairs of the four normal green F_2 plants. The resulting six F_3 populations were subsequently inoculated (60 plants per population) with TYLCV. All six populations were found to contain only tolerant plants, suggesting that the F_2 plants were homozygous for tolerance. One of the six F_3 populations segregated for normal green and netted-virescent classes in a 3:1 ratio and therefore was obtained from the cross between two plants heterozygous for *nv* (genotype *nv*/+). Five additional F_3 populations contained only normal green plants, confirming that the two other F_2 plants were

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homozygous for normal green (genotype +/+).

F_4 progenies derived from the cross between the two normal green F_2 plants served as the male parent to produce the first backcross to tomato. The mixture-of-pollen technique was used to produce seeds of the backcross, and the netted-virescent character was again used as a marker to select backcross progenies. Crosses were made between backcross progenies to obtain seeds of the BC_1F_2 generation, from which tolerant plants were selected following TYLCV inoculation. These tolerant plants were backcrossed to a normal green tomato (line 10). No difficulty was encountered in obtaining normal, viable seeds of this backcross (BC_2F_1 generation). The resulting self-pollinated population (BC_2F_2) was inoculated, and tolerant plants were selected. An additional backcross to line 10 was made to obtain BC_3F_1 progenies. Seeds of a BC_3F_5 line tolerant to TYLCV were harvested, bulked, and designated line M-60.

Inheritance studies. The inheritance of tolerance to TYLCV was studied in an insect-proof greenhouse that was sprayed or fumigated regularly with insecticides. Crosses were made between the susceptible line 10 and the tolerant line M-60 to obtain F_1, F_2, and F_3 generations. Line 10 was the female parent in each cross. The reciprocal F_1 cross of line M-60 × line 10 was unnecessary, because both parental lines possessed the same cytoplasm. The F_1 hybrid was backcrossed to line 10 and to line M-60. Seeds from selfing progenies of the backcross to line M-60 (BC_1F_2 populations) and parental selfs of line 10 and line M-60 were also obtained.

TYLCV was maintained on *Datura stramonium* L. plants, which were also used as a source of inoculum. Stock cultures were renewed every 3–4 wk. Colonies of nonviruliferous *B. tabaci* were maintained on cotton plants.

When tomato plants reached the one-leaf stage, they were inoculated by means of *B. tabaci*, using the procedure described previously (15). At least 20 viruliferous whitefly females were allowed to feed on each tomato plant, after which the plants were sprayed with an insecticide. The final observations were made 12 wk after inoculation. Plants that remained free from symptoms were assayed for the presence of the virus using *D. stramonium* plants. Genetic data were analyzed by the chi-square test.

Transmission tests. Using *D. stramonium* plants, we examined possible differences in rates of virus transmission by whiteflies using the virus source plant, tolerant F_1 hybrid TY-20, and susceptible F_1 hybrid Naama. Ten plants with chronic TYLCV infections were selected from the two genotypes 8 wk after inoculation for each of a total of five transmission tests. Three virus-free whitefly females enclosed in a leaf cage were allowed to feed for 48 hr on each infected tomato plant. The leaf cage was removed, and the whiteflies were placed singly on healthy *D. stramonium* plants for an inoculation period of 48 hr. Three additional transfers to new test plants were made at 48-hr intervals. The results were analyzed by the sign test (20).

RESULTS

About 95% of the inoculated plants of the different groups developed disease symptoms; the remaining plants were free from symptoms. Recovery tests from these symptomless plants to *D. stramonium* revealed that they had escaped infection. TYLCV-infected plants of line 10 were stunted, exhibiting smaller leaflets with severe interveinal chlorosis and upward cupping. Conversely, those of the tolerant parent line M-60 were affected by only a slight interveinal chlorosis and were slightly shorter than noninoculated control plants. Periodic assays of tolerant plants to *D. stramonium* indicated the presence of TYLCV.

F_1 plants and progenies of the backcross to the susceptible parent were all susceptible (Table 1). In the F_2 and backcross to the tolerant parent, only plants reacting similarly to the tolerant parent line M-60 were classified as tolerant. All other F_2 and BC_1F_1 populations developed different degrees of symptoms 3–4 wk after inoculation. Subsequently, however, these plants developed severe symptoms similar to those of the susceptible parent and therefore were counted as susceptible.

Only eight of 8,240 F_2 plants were classified as tolerant; this segregation agreed statistically with a 1:1,023 ratio of tolerant to susceptible plants. Progenies of the backcross to the tolerant parent segregated into a ratio of 1 tolerant:31 susceptible plants. The genetic data suggest that TYLCV tolerance is inherited recessively and is conditioned by five factors.

Eight F_2 plants and 13 backcross progenies to the tolerant parent were classified as tolerant following TYLCV inoculation (Table 1). To obtain further evidence that these plants would breed true for tolerance, they were allowed to self-pollinate. Seeds were harvested separately from each plant. The resulting F_3 and BC_1F_2 lines were inoculated (40–50 plants per line) with TYLCV and found to contain only tolerant plants. These results support the hypothesis that the F_3 and BC_1F_2 lines were derived from F_2 and BC_1F_1 plants that were homozygous for tolerance.

The results of the transmission tests (Table 2) show that there were no significant differences in rates of TYLCV transmission by whiteflies when tolerant and susceptible plants were used as virus sources. There was no evidence that the relative rates by which infectious TYLCV by whiteflies was recovered from tolerant and susceptible plants was related to severity of disease symptoms.

DISCUSSION

In our study, tolerance to TYLCV was inherited as a recessive trait. The segregation pattern obtained from the F_2 and backcross progenies to tolerant parent line M-60 fit the hypothesis that tolerance is controlled by five genetic factors (Table 1). Therefore, a breeding program designed to incorporate TYLCV tolerance into improved germ plasm will require testing of a large number of F_2 plants followed by backcrosses to the recurrent parent and self-pollinating to obtain tolerant plants. The expected ratio of tolerants to susceptibles is 1:1,023. So far, about 150,000 plants of the above-mentioned populations have been tested for tolerance at The Volcani Center. Segregation of tolerant and susceptible plants ranged between 1:1,000 and 1:1,030, agreeing statistically with the expected ratio.

Although the evidence supports the hypothesis that TYLCV is conditioned by five genetic factors, the susceptible population in the F_2 was not uniform. Differences in degree of disease severity were observed in this population 3–4 wk after inoculation. Plants subsequently became heavily infected, however, making impracticable any classification of these plants into definite groups. Nevertheless, tolerant plants have been recovered after self-pollination of susceptible F_2 plants showing intermediate degrees of disease severity 3–4 wk after inoculation.

F_1 hybrid plants of the cross susceptible × tolerant were found to be susceptible to TYLCV. Consequently, in the

Table 1. Reaction of F_1, F_2, and backcross (BC) tomato plants from the cross between line 10 (susceptible) and line M-60 (tolerant) to tomato yellow leaf curl virus[a]

Generation	Parental line or cross	Number of plants		Expected T:S ratio	Chi-square	*P* value
		Tolerant	Susceptible			
P_1	Line 10	0	55	...	...	...
P_2	Line M-60	60	0	...	...	...
F_1	$P_1 \times P_2$	0	96	...	...	...
F_2	$(P_1 \times P_2)$ selfing	8	8,232	1:1,023	0.0003	0.95–0.99
BC_1F_1	$P_1 \times (P_1 \times P_2)$	0	432	...	...	...
BC_1F_1	$P_2 \times (P_1 \times P_2)$	13	436	1:31	0.0782	0.70–0.80

[a] Plants were inoculated at the first-leaf stage by means of the vector, *Bemisia tabaci*.

Table 2. Transmission of tomato yellow leaf curl virus by whiteflies after feeding on tolerant (cv. TY-20) or susceptible (cv. Naama) tomato plants[a]

Virus source	Transmission 2 days after feeding		Transmission 4 days after feeding		Transmission 6 days after feeding		Transmission 8 days after feeding	
	Rate[b]	Percentage	Rate	Percentage	Rate	Percentage	Rate	Percentage
TY-20	91/131	69	54/95	57	45/72	63	23/58	40
Naama	81/129	63	52/86	60	54/73	74	33/63	52

[a] In each test, 10 plants of each tomato cultivar served as virus sources. Three virus-free whiteflies were allowed to feed on each tomato plant for 48 hr and then were transferred singly four times to *Datura stramonium* plants at 48-hr intervals.

[b] Results are totals of five tests and are expressed as number of plants infected divided by number of plants inoculated. Differences are not significant ($P = 0.05$) by the sign test.

production of TYLCV-tolerant F_1 hybrid tomatoes, both parents of the hybrid would have to be tolerant.

Virus titer is not necessarily reduced in tolerant types, although virus tolerance and resistance to virus multiplication are often associated (18). Our previous work (15) suggested that lower titers have been present in TYLCV-resistant plants of accession LA121 of *L. pimpinellifolium* than in susceptible plants. In the present study, no differences were found in rates of TYLCV transmission by whiteflies when susceptible and tolerant plants were used as virus sources. These results suggest that similar virus titers may have been present in tomato plants of the two genotypes.

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