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# A Novel Source of Resistance to Tomato Yellow Leaf Curl Virus Exhibiting a Symptomless Reaction to Viral Infection

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Abstract. **Tomato yellow leaf curl virus (TYLCV), transmitted by the tobacco whitefy (*Bemisia tabaci* Genn.), can be devastating to tomato (*Lycopersicon esculentum* L.) crops in tropical and subtropical regions. The development of resistant cultivars is the best option for control of TYLCV. However, all the available resistant commercial cultivars tested at the Volcani Center, when inoculated with TYLCV, developed different levels of disease symptoms. In this study, we report the development of a breeding line, TY172, which is a symptomless carrier of TYLCV. Line TY172, whether infected in the greenhouse with viruliferous whiteflies, or when grown in the field under natural infection, showed no symptoms of the disease. Viral DNA was detected in infected TY172 plants, albeit at much lower levels than a susceptible infected control. In addition, grafting experiments using infected susceptible scions grafted onto TY172 stocks, showed that even when exposed continuously to very high levels of virus, line TY172 did not develop disease symptoms, nor did it accumulate high levels of the virus. When TY172 was crossed with susceptible lines, the hybrids exhibited milder symptoms and lower viral content than the susceptible parent, yet higher than that of TY172, suggesting a partial dominance for the TY172 resistance. Upon inoculation of $F_2$ populations, the amount of symptomless individuals appeared in a ratio of ≈7:64. This suggests that at least three genes may account for the resistance.**

Tomato yellow leaf curl virus (TYLCV) can be devastating to tomato crops in tropical and subtropical regions. The disease is widespread in the Eastern Mediterranean Basin, North and Central Africa, and Southeast Asia (Cohen and Antignus, 1994; Pico et al., 1996). Recently, TYLCV has begun to spread in fields and greenhouses in Southern Europe (Moriones et al., 1993) as well as Central America (Polston et al., 1994). It is transmitted by the tobacco whitefy (*Bemisia tabaci*) whose severe population outbreaks are usually associated with high incidence of the disease. Infection results in cupping of leaves, chlorosis, and prominent stunting of the growing points. Depending on the severity of infection, yield loss can reach 100%. In many tomato-growing areas, TYLCV has become the limiting factor for production in outdoor and protected fields (Pico et al., 1996).

Various strategies have been pursued to control the disease. Chemical control has been ineffective, and in addition, the vector has been shown to develop pesticide resistance (Pico et al., 1996). The use of fine-mesh screens has become widespread as a means of protecting the crop. However, these screens create problems of overheating and poor ventilation (Cohen and Antignus, 1994). The development of resistant tomato varieties is the best alternative for control of TYLCV. Sources of resistance have been described in various wild tomato species (Hassan et al., 1984; Kasrawi et al., 1988; Pilowsky and Cohen, 1974, 1990; Zakay et al., 1991; Zamir et al., 1994), and commercial varieties with varying degrees of resistance to TYLCV have been released (Laterrot, 1993; Pilowsky and Cohen, 1990). However, all the resistant commercial cultivars tested at the Volcani Center, when inoculated with TYLCV, developed different degrees of disease symptoms and sustained yield loss due to viral infection (Lapidot et al., 1997). Moreover, indeterminate cultivars developed for greenhouse production are required to produce fruit over a long growing season, and TYLCV infection, which stunts growth, severely affects yields if the material is not highly resistant to the virus. In this study, we report the development of a breeding line which is a symptomless carrier of TYLCV, making it an ideal source of resistance, especially for greenhouse-type cultivars.

## Materials and Methods

**Plant material.** A susceptible tomato (*Lycopersicon esculentum* L.), line 1630, was crossed with *L. peruvianum* (L.) Mill. accessions PI 126926, PI 126930, PI 390681, and LA 441, that had been previously screened for resistance to TYLCV following a strategy described previously (Pilowsky and Cohen, 1974, 1990). In order to overcome interspecific crossability barriers, the pollen mixture technique was used (Philouze, 1967). The resulting $F_1$ interspecific hybrids were backcrossed to the susceptible parent, and sib crosses were conducted to obtain the $F_2$ populations which were subsequently inoculated with TYLCV as described below. This was continued until the $BC_3F_3$ generation was secured. From these lines, those breeding true for resistance were selected and crosses were made among them. $F_2$ populations derived from these crosses were subjected to TYLCV inoculation. From these segregating populations, a total of 35 resistant plants were selected and their $F_3$ offspring were again inoculated with TYLCV. A highly resistant $F_3$ line exhibiting a symptomless reaction was selected and its $F_4$ offspring were bulked and designated line TY172. Progeny testing showed the line to be fixed for resistance.

**Whitefly maintenance and plant inoculation.** *B. tabaci* were raised on cotton plants (*Gossypium hirsutum* L.) in muslin-covered cages and held in a growth chamber at 26 to 32 °C. The TYLCV used in this study was from the original strain described by Cohen and Nitzany (1966). The whiteflies were given a 48-h acquisition period on TYLCV-infected *Datura stramonium* L. source plants, followed by a 48-h inoculation access on tomato test

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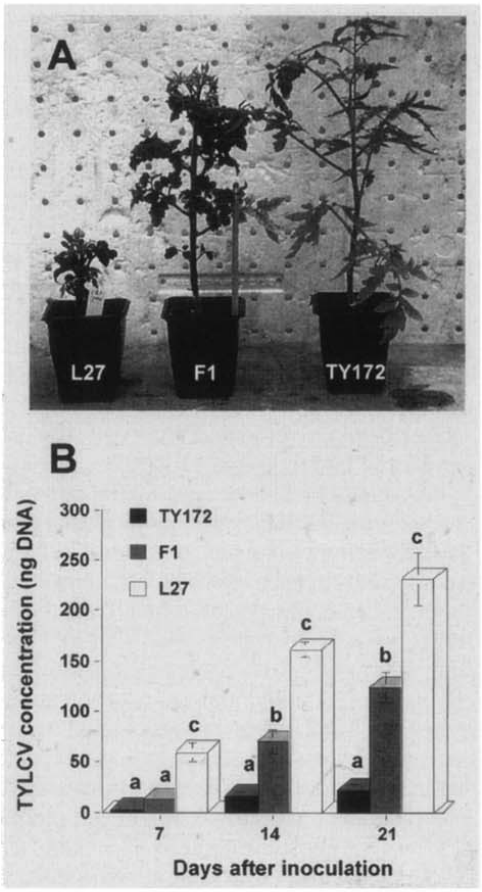


Fig. 1. The $F_1$ hybrid between TY172 (resistant) and L27 (susceptible) shows intermediate symptom development and TYLCV DNA accumulation. The $F_1$ plants were inoculated with TYLCV together with both parent lines and symptoms were appraised after 30 d (**A**). At this time, tissue was collected and viral DNA content determined (**B**). Different letters denote mean separations by one-way ANOVA, $P = 0.05$. Bars = standard error.

plants (first leaf stage,) as described previously (Pilowsky and Cohen, 1990). Thereafter, the plants were sprayed with the systemic pesticide Imidacloprid (Confidor, Bayer, Leverkusen, Germany) and held in an insect-proof greenhouse kept at 26 to 32 °C until symptom development.

**TYLCV SYMPTOM SEVERITY RATING.** Symptom development was evaluated 30 d after inoculation according to the following scale: 0 = no visible symptoms, inoculated plants show same growth and development as noninoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, and pronounced cupping and curling; plants cease to grow.

**GRAFTING EXPERIMENTS.** Two-month-old healthy TY172 plants served as stocks in grafts with infected scions of susceptible plants (line L27) bearing severe symptoms. Healthy susceptible line L27 plants served as control stocks. Plants were kept for up to 3 months for symptom development on the stocks.

**DNA ISOLATION AND QUANTITATION OF VIRUS CONTENT.** DNA was isolated from upper leaves of infected tomato plants and analyzed by dot-blot hybridization as described previously (Lapidot et al., 1997). The blots were hybridized to a $^{32}$P-labelled riboprobe derived from TYLCV cDNA (Antignus and Cohen, 1994). The dot blots were quantitated with a phosphorimager (Bio-imaging Analyzer, BAS-1500, Fujifilm, Tokyo). Data were converted from luminescence units to nanograms viral DNA by comparison to a standard curve, after subtracting background.

**INHERITANCE STUDIES.** Line TY172 was crossed with three susceptible lines (5656, 5692, and line L27) and the $F_1$ offspring were either backcrossed to each parent or selfed to obtain $F_2$ populations. The parent lines, $F_1$ hybrids, $F_2$, and backcross populations were sown and inoculated with TYLCV at the first leaf stage using >100 whiteflies per plant. After 10 d, seedlings were transplanted to small 5-cm$^3$ pots and symptoms were allowed to develop for a period of 30 d. At this time leaf samples were harvested to determine viral content, and the level of symptoms was recorded. Symptoms were reappraised 2 weeks later. In other experiments, symptomless plants were transplanted to the field and symptoms reappraised during their growth.

## Results and Discussion

**TY172 IS A SYMPTOMLESS CARRIER OF TYLCV.** In different tests, involving ≈300 plants of TY172, no disease symptoms developed after inoculation with viruliferous whiteflies in the greenhouse (Fig. 1A). In addition, TY172 plants also remained symptomless when grown under severe TYLCV natural infection in tomato growing areas where the virus is a major problem.

To determine the nature of the resistance, infected TY172 plants were screened for the presence of TYLCV viral DNA. Viral DNA was detected, albeit at much lower levels than a susceptible infected control (Fig. 1B). To determine whether the symptomless reaction was dependent upon the TYLCV-inoculation level, infected susceptible scions from line L27 were grafted onto TY172 stocks and onto L27 stocks (control). Symptoms and viral content were appraised. Whereas the L27 stock developed heavy symptoms 10 d after grafting, and showed high levels of virus content (43% of the amount found in the scion), TY172 remained symptomless even three months after grafting, and the amount of virus was very low (only 6% of the amount found in the scion; Fig. 2). Hence, even when exposed directly to very high

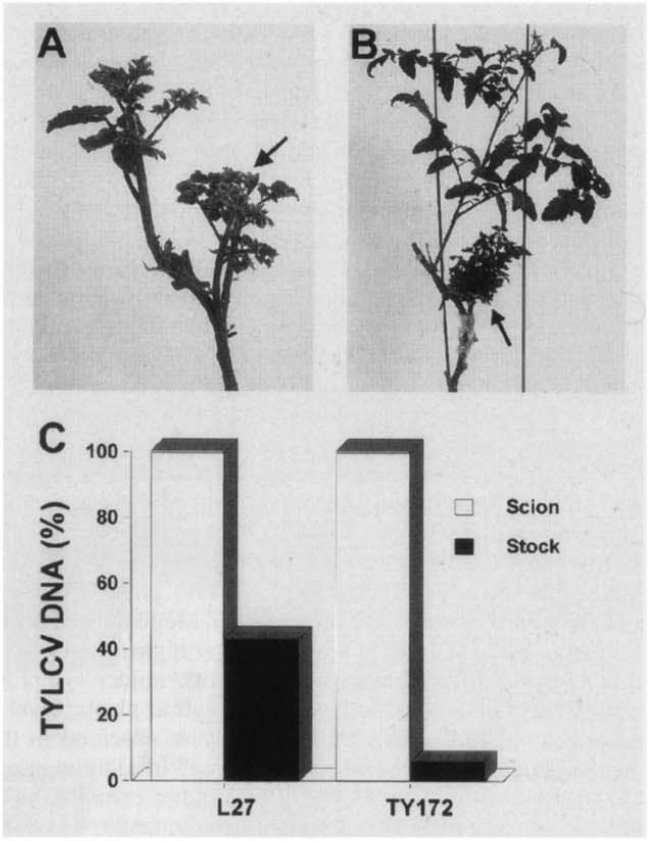


Fig. 2. Effect of grafting TYLCV-infected scions onto susceptible and resistant stocks. TYLCV-infected line L27 branches bearing severe symptoms (shown by arrows) were side-grafted onto healthy (**A**) L27, (**B**) TY172 stocks. Symptoms were appraised after (**A**) 10 d (**B**) 3 months. (**C**) TYLCV DNA content from stock and scion tissue was determined at 10 d after grafting for L27 and 3 months for TY172 as described in Materials and Methods. The data are expressed as the percent of TYLCV DNA content in stock tissue out of DNA content in infected scion tissue.

Table 1. TYLCV symptom severity in segregating $F_2$ populations.

| Symptom level[z] | No. of plants Observed | No. of plants Expected | Expected ratio | $\chi^2$ |
|---|---|---|---|---|
| Experiment 1 | | | | |
| 0 | 44 | 44 | 7:64 | $0^{NS}$ |
| 1 | 31 | | | |
| 2 | 74 | | | |
| 3 | 159 | | | |
| 4 | 97 | | | |
| Total | 405 | | | |
| Experiment 2 | | | | |
| 0 | 435 | 426 | 7:64 | $0.24^{NS}$ |
| 1–4 | 3461 | 3470 | | |
| Total | 3896 | | | |

[z]0 = no visible symptoms, inoculated plants show same growth and development as noninoculated plants; 1 = very slight yellowing of leaflet margins on apical leaf; 2 = some yellowing and minor curling of leaflet ends; 3 = a wide range of leaf yellowing, curling and cupping, with some reduction in size, yet plants continue to develop; and 4 = very severe plant stunting and yellowing, and pronounced cupping and curling; plants cease to grow.
[NS]Nonsignificant deviation from the expected ratio at $P = 0.05$.

levels of virus, line TY172 did not develop disease symptoms, nor did it accumulate high levels of TYLCV virus. Similarly, in the reverse graft experiment, no symptoms developed on TY172 healthy scions when grafted onto infected susceptible stocks.

To address the possibility of whether infected TY172 is capable of transmitting TYLCV, healthy L27 scions were grafted onto infected TY172 stocks and kept protected from whitefly infestation in a insect-proof chamber and sprayed with Confidor. The L27 scions developed symptoms and viral DNA accumulated, although at a slower rate than when grafted onto control infected L27 stocks. In addition, infected TY172 plants were also capable of transmitting TYLCV via whiteflies to susceptible plants.

The data presented here indicates that TY172 is a symptomless carrier of TYLCV. Plants of TY172 remained symptomless under natural heavy infection conditions in the field, after whitefly inoculation in the greenhouse, or by grafting with susceptible infected scions (Figs. 1, 2). Nevertheless, TYLCV DNA was detected in these infected plants. Another source of TYLCV resistance, *L. chilense* Dun. accession LA1969, was reported to be completely resistant to TYLCV (Zakay et al., 1991). However, in contrast to TY172, breeding lines developed from this source and carrying the TY-1 resistance gene did develop mild disease symptoms (Zamir et al., 1994).

**Inheritance of the resistance trait.** When line TY172 was crossed with susceptible lines, the $F_1$ hybrid plants exhibited milder symptoms (level 3, see Materials and Methods) and lower viral content than the susceptible parent, yet higher than that of TY172 (Fig. 1). In preliminary experiments, milder symptom bearing plants (similar to the level in the hybrid plants; level 3 symptoms,) and fully susceptible plants were observed in the backcross populations to the susceptible parent. In addition, when the $F_1$ was backcrossed to TY172, plants either exhibited mild symptoms or were fully symptomless. Consequently, the resistance in TY172 shows partial dominance. However, since symptoms develop in the $F_1$ from a cross between TY172 and a susceptible line, the use of the TY172-derived resistance would necessitate its introgression into both parents of a commercial $F_1$ hybrid.

TYLCV-infected $F_2$ populations showed varying levels of symptom development. The symptoms were classified into five groups ranging from 0 (symptomless) to 4 (fully susceptible). Most plants showed intermediate symptom development. The number of symptomless individuals appeared in a ratio of ≈7:64 (Table 1). Recovery tests indicated that the symptomless plants carried TYLCV. Viral DNA content was determined for all the individuals from each symptom level class. Plants showing no symptoms (level 0) had very low amounts of virus while those showing very mild symptoms (level 1) had slightly higher virus levels, albeit not significantly different from the symptomless plants (Fig. 3). Plants with level 2 symptoms had low amounts of virus, yet significantly higher than those found in the symptomless group. Plants of level 3 had a great variation in the amount of virus, generally lower than, but in some cases reaching amounts as high as level 4 plants (Fig. 3). A screen of a second segregating population gave essentially very similar results and viral content levels fell into the same classes.

As mentioned above, the $F_2$ data showed a segregation pattern in which the frequency of symptomless plants was 7:64. These data fit a model of two genes; one partially dominant (AA), the other recessive (bb), each of which can contribute to the resistance, yet both being controlled dominantly and epistatically by a third, recessive gene (cc). Therefore, TY172 plants are expected to have the genotype AA bb cc, and susceptible individuals aa BB CC. Symptomless individuals in a segregating $F_2$ population could carry any of the following genotypes: AA B_ cc, AA bb cc, aa bb cc, or A_ bb cc, which add up to the expected number of 7:64. In addition, according to the above model, symptomless $F_2$ plants are expected to breed true for lack of symptom development. Indeed, 15 $F_3$ lines (20 plants inoculated per line) derived from symptomless $F_2$ plants tested to date have been themselves symptomless. The majority of the $F_2$ plants showed intermediate symptom development, confirming that there is some level of partial dominance for the TY172 resistance. As seen in Fig. 3, individuals can be classified into discrete groups in terms of symptom develop-

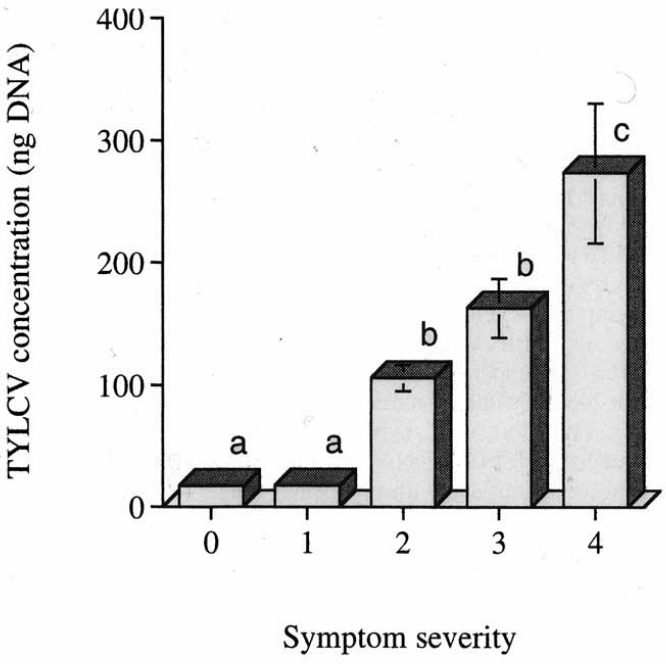


Fig. 3. TYLCV viral DNA content in the $F_2$ population from the cross L27 (susceptible) and TY172 (resistant). Inoculated seedlings were transplanted to 5-$cm^3$ pots and symptoms were appraised after 30 d. Tissue was collected from all the plants and TYLCV virus DNA content was determined as described in Materials and Methods. Different letters denote mean separations by one-way ANOVA, $P = 0.05$. Bars = standard error.

ment and viral DNA accumulation. The segregation ratios of the different symptom severity groups need to be examined in order to elucidate the epistatic effects that lead to the formation of the different symptom levels. It appears that different levels of symptoms develop depending on the genotype of the individual $F_2$ plant for the two to three genes that appear to control most of the resistance.

To date, resistant commercial cultivars suffer significant yield loss under high inoculum pressure (Lapidot et al., 1997; Laterrot, 1993; Pico et al., 1996). It was shown previously that the small amount of virus present in infected TY172 plants did not reduce its potential yield markedly as compared to noninfected plants when resistance was assayed in the field following inoculation with TYLCV (Lapidot et al., 1997). This was in sharp contrast to commercial resistant cultivars, which suffered substantial yield losses as compared to their respective noninfected controls (Lapidot et al., 1997). Moreover, attempts to genetically engineer TYLCV resistance using virus-derived sequences have provided only partial cross-protection or have resulted in undesirable phenotypic effects (Brunetti et al., 1997; Pico et al., 1996). We show here that TY172 showed a symptomless reaction to Israeli TYLCV isolates. Consequently, TY172 holds great promise as a source for the development of TYLCV-resistant tomato cultivars. However, its resistance needs to be ascertained for other TYLCV isolates and closely related geminiviruses.

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