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Comparison of resistance to *Tomato leaf curl virus* (India) and *Tomato yellow leaf curl virus* (Israel) among *Lycopersicon* wild species, breeding lines and hybrids

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Abstract

The objective of this study was to screen wild and domesticated tomatoes for resistance to *Tomato yellow leaf curl virus*, Israel (TYLCV-Is) and *Tomato leaf curl virus* from Bangalore isolate 4, India (ToLCV-[Ban4]) to find sources of resistance to both viruses. A total of 34 tomato genotypes resistant/tolerant to TYLCV-Is were screened for resistance to ToLCV-[Ban4] under glasshouse and field conditions at the University of Agricultural Sciences, Bangalore, India. Resistance was assessed by criteria like disease incidence, symptom severity and squash-blot hybridization. All the tomato genotypes inoculated with ToLCV-[Ban4] by the whitefly vector *Bemisia tabaci* (Gennadius) produced disease symptoms. In some plants of the lines 902 and 910, however, the virus was not detected by hybridization. The tomato genotypes susceptible to ToLCV-[Ban4] by whitefly-mediated inoculation were also found susceptible to the virus under field conditions. However, there were substantial differences between genotypes in disease incidence, spread, symptom severity and crop yield. Despite early disease incidence, many genotypes produced substantially higher yields than the local hybrid, Avinash-2. Sixteen tomato genotypes from India resistant/tolerant to ToLCV-[Ban4] were also tested for TYLCV-Is resistance at the Hebrew University of Jerusalem, Rehovot, Israel. Accessions of wild species, *Lycopersicon hirsutum* LA 1777 and PI 390659 were the best sources of resistance to both viruses. Lines 902 and 910, which were, resistant to TYLCV-Is were only tolerant to ToLCV-[Ban4] and accession *Lycopersicon peruvianum* CMV Sel. INRA, resistant to ToLCV-[Ban4], was only tolerant to TYLCV-Is. Implications of using the resistant lines in breeding programme is discussed.

Abbreviations: TLCVD – tomato leaf curl virus disease; ToLCV-[Ban4] – *Tomato leaf curl virus* from Bangalore isolate 4, India; TYLCV-Is – *Tomato yellow leaf curl virus*, Israel.

Introduction

Tomato leaf curl virus diseases (TLCVD) have seriously hampered the cultivation and production of tomato in India (Vasudeva and Samraj, 1948; Banerjee and Kalloo, 1987; Saikia and Muniyappa, 1989), the Middle East (Abu-Gharbieh et al., 1978; Kasrawi et al., 1988; Czosnek et al., 1990; Hassan et al., 1991),

Africa (Martelli and Quacqarelli, 1982; Makkouk and Laterrot, 1983) and more recently, in the Western Hemisphere (Polston and Anderson, 1997; Accotto et al., 2000). In India, the disease is widespread in tomato during the summer season in South India (Saikia and Muniyappa, 1989) and autumn in North India (Banerjee and Kalloo, 1987). In Karnataka state, South India, the incidence of TLCVD in susceptible cultivars increases rapidly to 100% causing yield losses exceeding 90% (Saikia and Muniyappa, 1989). In Israel, the disease affects tomato during the summer and autumn seasons causing up to 100% yield loss in susceptible cultivars (Lapidot et al., 1997). The disease is caused by either monopartite (Navot et al., 1991; Hong and Harrison, 1995) or bipartite (Padidam et al., 1995, Srivastava et al., 1995) geminiviruses of the genus *Begomovirus*, family *Geminiviridae.* Despite the great variability in viral genomes, both bipartite and monopartite viruses cause similar symptoms and substantial yield losses (Saikia and Muniyappa, 1989; Czosnek et al., 1990; Muniyappa et al., 1991a). Whitefly vector *Bemisia tabaci* (Gennadius) transmits the viruses causing TLCVD.

TLCVD management measures are based on control of the vector population but simulation modeling has shown that vector control is unlikely to be successful because of the rapid turn-over rate of the vector population in the crop (Holt et al., 1999). A more effective solution is possible by breeding cultivars resistant to TLCVD (Nateshan et al., 1996; Pico et al., 1996; 1998; Lapidot et al., 1997). During the past 20 years, there has been considerable effort to develop *Tomato leaf curl virus* (ToLCV)- and *Tomato yellow leaf curl virus* (TYLCV)-resistant cultivars. Tomato cultivars however, are not completely resistant to TLCVD, therefore wild *Lycopersicon* species have been screened for virus resistance both in India (Nariani and Vasudeva, 1963; Joshi and Choudhury, 1981; Banerjee and Kalloo, 1987; Muniyappa et al., 1991a; Nateshan et al., 1996; Hanson et al., 2000) and Israel (Pilowsky and Cohen, 1974; 1990; Kasrawi et al., 1988; Zakay et al., 1991; Pico et al., 1998). Nevertheless, progress in breeding for TLCVD resistance has been slow (Pilowsky and Cohen, 1974; 1990; Banerjee and Kaloo, 1987; Lapidot et al., 1997) because of the complex genetics of resistance, which probably explain why the cultivars and breeding lines are not as resistant as wild species. The present investigation was performed to determine the level of resistance of TYLCV-Israel (TYLCV-Is)-resistant/tolerant tomato genotypes against a ToLCV Bangalore isolate 4, India (ToLCV-[Ban4]) and *vice versa*. If they were resistant and have acceptable horticultural characteristics, these genotypes will be released to farmers for cultivation.

Materials and methods

Virus culture

The ToLCV-[Ban4] used for whitefly-mediated inoculations under glasshouse conditions was obtained from the original culture described by Muniyappa et al. (1991b). The virus has been cloned and sequenced (Muniyappa et al., 2000). The virus was maintained by whitefly inoculation in the ToLCV-[Ban4]-susceptible tomato variety Arkavikas in an insect-proof glasshouse. The TYLCV-Is (Navot et al., 1991) was the wild type virus found in the experimental fields of the Faculty of Agriculture, Hebrew University of Jerusalem, Israel.

Whitefly culture

Adult *B. tabaci* were collected from horsegram, *Macrotyloma uniflorum*, at the Main Research Station, University of Agricultural Sciences, Hebbal, Bangalore and maintained on cotton (*Gossipium hirsutum* cv. Laxmi) plants in wooden cages ($45 \times 45 \times 30$ cm) covered with 40 mesh size nylon net.

Tomato genotypes

Table 1 lists the genotypes tested in India and their wild tomato genotypes; some are resistant/tolerant to TYLCV-Is and others are susceptible. They include 18 lines from advanced breeding programmes developed at the Hebrew University of Jerusalem, 13 commercial hybrids and three cultivars or hybrids developed in India. Lines 901-1 and 901-2 are F1 hybrids derived from the cross between 902 $\times$ *Lycopersicon esculentum*. Lines 908 and 913 are also F1 hybrids obtained from the cross between 906-7 $\times$ *L. esculentum*, and 910 $\times$ *L. esculentum*, respectively.

Field testing

Field screening of tomato genotypes that showed resistance/tolerance to TYLCV-Is was tested for their

Table 1. TYLCV-Is-resistant/tolerant tomato genotypes tested for resistance to ToLCV-[Ban4] in Bangalore, India

Genotype	Source of resistance[2]	Reaction to TYLCV-Is[3]	Reference/Source
901-1	*L. hirsutum*	T	Vidavski and Czosnek, 1998
901-2	*L. hirsutum*	T	Vidavski and Czosnek, 1998
902	*L. hirsutum*	R	Vidavski and Czosnek, 1998
906-7	*L. hirsutum*	T	Vidavski and Czosnek, 1998
908	*L. hirsutum*	T	Vidavski and Czosnek, 1998
910	*L. hirsutum*	R	Vidavski and Czosnek, 1998
913	*L. hirsutum*	T	Vidavski and Czosnek, 1998
Per 1	*L. peruvianum*	T	Vidavski et al., 1998
Pim 2	*L. pimpinellifolium*	T	Vidavski et al., 1998
Chil 1	*L. chilense*	T	Vidavski et al., 1998
Ty-50	*L. esculentum*	S	Zamir et al., 1994
Ty-52	*L. chilense*	T	Zamir et al., 1994
TY0-2	*L. chilense*	T	Hebrew University of Jerusalem
TY0-5	*L. chilense*	T	Hebrew University of Jerusalem
TY0-15	*L. chilense*	T	Hebrew University of Jerusalem
TY0-23	*L. chilense*, *L. pimpinellifolium*	T	Hebrew University of Jerusalem
FA 9	*L. chilense*	T	Zeraim Gedera Seed Co., Israel
FA 24	*L. chilense*, *L. pimpinellifolium*	T	Zeraim Gedera Seed Co., Israel
FA 38	*L. esculentum*	S	Zeraim Gedera Seed Co., Israel
FA 491	*L. esculentum*	S	Zeraim Gedera Seed Co., Israel
FA 709	*L. chilense*	T	Zeraim Gedera Seed Co., Israel
FA 710	*L. chilense*	T	Zeraim Gedera Seed Co., Israel
FA 712	*L. chilense*, *L. pimpinellifolium*	T	Zeraim Gedera Seed Co., Israel
FA 716	*L. chilense*	T	Zeraim Gedera Seed Co., Israel
FA 736	*L. chilense*	T	Zeraim Gedera Seed Co., Israel
FA 907	*L. chilense*	T	Zeraim Gedera Seed Co., Israel
Fiona	n.k.	T	Sluis and Groot, The Netherlands
Ty King	n.k.	T	Royal Sluis, The Netherlands
3750	*L. chilense*	T	A. B. Seeds, Israel
3761	*L. chilense*	T	A. B. Seeds, Israel
TY-70	*L. peruvianum*	T	Pilowsky and Cohen, 1990
Avinash-2[1]	n.k.	S	Syngenta, India
Rashmi[1]	n.k.	S	Indo-American Hybrid Seeds, India
Arkavikas[1]	n.k.	S	IIHR, Hesaraghatta, India

[1]Susceptible checks; [2]n.k. = not known; [3]R = resistant, T = tolerant, S = susceptible.

level of resistance against ToLCV-[Ban4] during two consecutive summer growing seasons, in 1997 and 1998. Tomato seeds were sown in raised nursery beds (1 m × 3 m) during the first week of April and transplanted (spacing of 2 × 3 feet) to the main field during early May in both the years, when the virus inoculum pressure was at greatest. The whitefly numbers increase rapidly during the summer months of March–July, which coupled with the abundant availability of virus source plants (infected weeds) provides the best season for screening for virus resistance at Bangalore, India. The number of plants tested for each genotype depended on the availability of seedlings. Up to a maximum of 45 tomato plants were tested for some genotypes (Table 2). To facilitate early and continued natural infection, two rows of the TLCVD-susceptible cultivars Arkavikas or Rashmi were planted along the borders of the experimental plots. No pesticides were applied. Plants were examined weekly for TLCVD symptoms. Disease incidence (% diseased plants), spread, symptom severity and yield parameters were recorded. An arbitrary scale was employed to score the disease as described by Muniyappa et al. (1991a), where, resistance (R) = no

Table 2. TYLCV-Is-resistant/tolerant tomato genotypes tested for ToLCV-[Ban4] resistance in the field at Bangalore, India

Genotype	Disease symptoms and presence of virus				Fruit yield		
	No. of plants infected/ planted	Days to first/ final symptom expression	No. of plants contained virus/tested[1,2]	Symptom severity[3]	Mean no. of fruits/plant (±SEM)	Mean fruit yield (g)/plant (±SEM)	Fruit type[4]
901-1	18/18	29–64	10/10 (+)	Mo	26.1 ± 10.7	1076.3 ± 158.1	M, W
901-2	15/15	21–43	4/4 (+)	Mo	6.0 ± 1.7	234.4 ± 41.0	S, R
902	13/13	36–84	8/10 (+/−)	Mo	20.3 ± 3.7	863.2 ± 143.3	M, R
906-7	13/13	21–43	6/10 (+)	Mo	19.2 ± 1.7	1449.6 ± 140.1	L, W
908	18/18	21–43	10/10 (++)	Mo/S	14.3 ± 1.0	1119.3 ± 133.5	M, R
910	7/10	36–84	4/9 (+/−)	M	7.3 ± 1.3	186.6 ± 27.8	S, R
913	18/18	15–43	7/7 (+)	Mo	21.0 ± 2.1	955.7 ± 81.1	L, W
Per 1	23/23	7–29	10/10 (++)	S	7.7 ± 1.3	497.8 ± 66.7	S, WR
Pim 2	13/13	21–43	2/2 (++)	Mo	19.0 ± 4.0	853.0 ± 116.0	S, WR
Chil 1	17/17	15–36	7/7 (+)	S	25.2 ± 7.1	1219.0 ± 175.4	M, R
Ty-50	10/10	14–29	1/1 (+)	S	15.0 ± 1.1	735.2 ± 8.6	M, R
Ty-52	19/19	21–36	9/9 (++)	S	28.8 ± 3.7	608.5 ± 75.5	S, B
TY0-2	10/10	21–36	4/4 (+)	Mo/S	10.5 ± 1.10	466.0 ± 72.96	M, W
TY0-5	12/12	29–50	4/4 (+)	Mo	15.2 ± 2.3	665.3 ± 119.6	M, R
TY0-15	17/17	15–36	7/7 (+)	S	12.4 ± 2.0	671.8 ± 102.5	M, R
TY0-23	10/10	14–29	1/1 (+)	S	21.9 ± 2.6	754.7 ± 86.4	S, R
FA 9	8/9	42–84	n.d.	M/Mo	6.5 ± 2.8	569.0 ± 59.9	M, R
FA 24	4/5	14–84	n.d.	Mo/S	4.2 ± 1.8	544.3 ± 55.2	M, R
FA 38	12/13	21–84	n.d.	Mo/S	5.3 ± 2.8	424.4 ± 41.7	M, R
FA 491	10/10	42–84	n.d.	M	5.4 ± 2.1	565.8 ± 67.0	L, R
FA 709	42/42	21–43	13/13 (++)	Mo/S	17.3 ± 2.2	890.1 ± 109.1	L, R
FA 710	14/14	14–21	11/11 (++)	Mo/S	14.9 ± 1.5	902.6 ± 138.4	M, R
FA 712	22/22	29–43	11/11 (+)	Mo/S	19.1 ± 1.4	1016.2 ± 98.4	M, R
FA 716	24/24	14–50	12/12 (++)	S	12.6 ± 1.3	675.1 ± 40.9	L, R
FA 736	18/18	7–29	8/8 (+++)	S	6.7 ± 1.4	475.2 ± 70.5	M, R
FA 907	42/42	21–50	15/15 (++)	Mo/S	16.6 ± 2.3	1101.6 ± 99.4	L, R
Fiona	11/11	21–50	9/9 (+)	M/Mo	12.1 ± 1.8	741.6 ± 107.7	L, W
Ty-king	19/19	29–43	13/13 (++)	Mo/S	19.9 ± 2.0	1168.9 ± 120.4	L, R
3750	45/45	15–36	10/10 (+)	M	15.2 ± 1.5	1078.0 ± 80.6	M, R
3761	45/45	15–56	10/10 (+)	Mo/S	11.3 ± 1.1	792.2 ± 102.8	M, R
TY-70	29/29	29–43	12/12 (+++)	S	9.2 ± 2.4	553.2 ± 112.7	M, WR
Avinash-2	44/44	14–49	11/11 (+)	Mo	15.5 ± 4.1	887.3 ± 60.1	M, R
Rashmi	161/161	7–42	n.d.	S	9.8 ± 3.4	276.5 ± 45.7	S, R
Arkavikas	48/48	14–29	10/10 (+++)	S	10.5 ± 2.2	222.0 ± 43.5	S, R

[1]Presence of virus as determined by squash-blot hybridization (intensity of hybridization signal measured on a five-point scale); [2]n.d. = not determined; [3]M = mild, Mo = moderate, S = severe; [4]L = large, M = medium, S = small, R = round, W = wrinkled, B = beaked, O = oval, WR = wrinkled to rounded.

symptoms, mild infection (M) = light yellowing but no curling, moderate infection (Mo) = light yellowing, slight curling and stunting, and susceptible (S) = very severe curling, stunting and no or very less yield.

Field screening of 12 ToLCV-[Ban4]-resistant/ tolerant tomato genotypes; *L. peruvianum* CMV Sel. INRA, *L. hirsutum* LA 1777, PI 390659, *L. cheesmani* LA1401, *L. pimpinellifolium* LA 121, LA 1478, Hirsute and *L. esculentum* R 101, R 102, Nema 1400, Nema 1401, Avinash-2 and four susceptible genotypes NS 386, S 41 (Gotya), Arkavikas and Pusaruby, to TYLCV-Is took place during 1997. The experiment was carried out at Rehovot, Israel, essentially as described for ToLCV-[Ban4].

Artificial inoculation

The TYLCV-Is-resistant/tolerant tomato genotypes were screened for resistance to ToLCV-[Ban4] under glasshouse conditions (Muniyappa et al., 1991a). Tomato seeds were sown in nursery pots and 10 days

old seedlings were transplanted into plastic bags filled with soil and compost, which were kept in an insect-proof glasshouse. Whiteflies were released onto ToLCV-[Ban4]-infected tomato plants for a 24 h virus acquisition access period. Meanwhile, individual tomato seedling was caged in a separate plastic tube (2×10 cm) with a provision (hole) to release whiteflies. About 10–15 viruliferous whiteflies were released into the cage and the hole was plugged with cotton wool to allow a 24 h virus inoculation access period. The number of plants inoculated for each tomato genotype is given in Table 3. To ensure that infection was early and complete, the tomato seedlings were inoculated individually. Viruliferous whiteflies were released into the glasshouse at regular intervals thereafter to provide a continuous source of inoculum. Plants were examined weekly for TLCVD symptoms.

Squash-blot hybridization

Tomato plants were assayed for the presence of each virus by the squash-blot method using virus specific-DNA probes (Navot et al., 1989). The second or third fully expanded leaf down the apex of tomato plants either artificially inoculated in the glasshouse or naturally infected in the field were squashed onto dry nylon membranes (Hybond-N, Amersham, UK) after 8 weeks of exposure to virus. Squashes of only one leaf per plant was hybridized initially although the test

Table 3. Whitefly-mediated inoculation of TYLCV-Is-resistant/tolerant tomato genotypes with ToLCV-[Ban4] in the glasshouse

Genotype	No. of plants infected/ inoculated[1]	Days to first/ final symptom expression[2]	Symptom severity[3]	No. of plants contained virus/tested[4]
901-1	12/12	20–90	M	12/12 (+)
901-2	30/30	20–85	Mo	1/20 (+)
902	24/30	45–90	M	2/30 (+/−)
906-7	12/12	20–76	M	7/7 (+)
908	29/29	11–47	S	3/3 (+++)
910	10/19	42–90	M	0/19 (−)
913	18/18	21–75	Mo	3/3 (+)
Per 1	30/30	20–53	S	3/3 (+++)
Pim 2	17/17	20–53	Mo/S	3/3 (+++)
Chil 1	17/17	11–45	Mo	1/3 (+++)
Ty-50	30/30	7–31	S	1/1 (+)
Ty-52	27/27	21–42	S	6/9 (+)
TY0-2	22/22	15–42	Mo/S	4/4 (+)
TY0-5	13/13	26–57	Mo	4/4 (+)
TY0-15	26/26	14–52	Mo/S	11/11 (+)
TY0-23	14/14	14–52	S	10/10 (++)
FA 709	28/28	20–45	S	13/13 (++)
FA 710	22/22	20–29	S	11/11 (++)
FA 712	16/16	31–52	S	11/11 (+)
FA 716	28/28	14–47	S	12/12 (++)
FA 736	17/17	10–21	S	8/8 (+++)
FA 907	20/20	20–50	S	15/15 (++)
Fiona	2/8	47–85	S	8/8 (+++)
Ty King	38/38	33–49	Mo	13/13 (++)
TY-70	30/30	26–47	S	3/3 (+++)
Avinash-2	40/40	21–56	S	11/11 (+)
Rashmi	32/32	10–15	S	3/3 (+++)
Arkavikas	18/18	10–26	S	2/2 (+++)

[1]Number of plants with symptoms/total number of plants inoculated.
[2]First to last day of symptom appearance.
[3]M = mild, Mo = moderate, S = severe.
[4]Presence of virus as determined by squash blot hybridization: number of plants showing a hybridization signal/number of plants tested (intensity of hybridization signal measured on a five-point scale).

was repeated after 12 weeks of virus inoculation for genotypes with weak hybridization signal. Squashes of tomato genotypes screened in Israel were hybridized with a radiolabeled TYLCV-Is specific DNA probe (Navot et al., 1991). Those that were screened in India were hybridized with a radiolabeled ToLCV-[Ban4]-specific DNA probe (Muniyappa et al., 2000). The hybridization signal was measured on a five-point scale; $-$ = no signal (no virus), $+/-$ = signal barely detectable, $+$ = weak signal, $++$ = strong signal, $+++$ = very strong signal (high virus titer) on the autoradiogram.

Results

Field screening for ToLCV-[Ban4] resistance in Bangalore

The tomato lines tested in Bangalore (Table 1) include wild *Lycopersicon* species, advanced breeding lines and commercial hybrids. Of the 34 TYLCV-Is-resistant/tolerant tomato genotypes field screened for ToLCV-[Ban4] resistance, none was found to be resistant, 11 showed mild and/or moderate infections and the remainder were susceptible showing moderate to severe infections (Table 2). The *L. hirsutum* genotype 910 and three commercial hybrids FA 9, FA 24 and FA 38, respectively, showed 70%, 89%, 80% and 93% disease incidence, 12 weeks after transplanting. Spread of the disease differed among tomato genotypes. Initial symptom expression was delayed up to six weeks in hybrids FA 9 and FA 491, and up to five weeks in *L. hirsutum* genotypes 902 and 910 and disease spread slowly in these genotypes (Figure 1A and B). Seven other genotypes showed initial disease symptoms four weeks after transplanting while remaining genotypes were infected within three weeks (Table 2). In general, spread was rapid between three and four weeks of transplanting during both the years and many were totally infected within 6–7 weeks. Squash-blot hybridizations detected ToLCV-[Ban4] in all the tomato genotypes. The virus was, however, detected in only eight out of 10 plants in line 902, four out of nine plants in 910 and six out of 10 plants in line 906-7. The virus was detected in all plants of the remaining genotypes. Because of widespread severe infection, the susceptible variety Arkavikas and hybrid Rashmi were stunted and produced little yield, about 222.0 ± 43.5(SEM) g and 276.5 ± 45.7 g per plant, respectively. In contrast, line 906-7, despite rapid disease spread (Figure 1A),

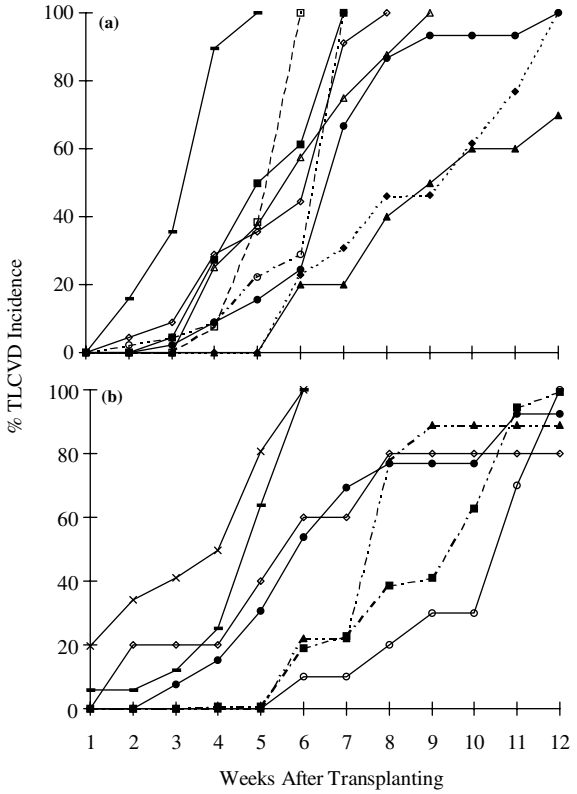

Figure 1. Spread of ToLCV-[Ban4] in selected tomato genotypes under field conditions during summer season of 1997: –△– 901-1; ---♦--- 902; –□– 906-7; –▲– 910; – -⊖- – 3750; –◇– 3761; –●– TY-70; –■– Avinash-2; —— Arkavikas (a), and 1998; –▲– FA 9; –◇– FA 24; –●– FA 38; – -⊖- – FA 491; –■– FA 716; –✳– Rashmi and —— Arkavikas (b). Number of plants tested for each genotype is given in Table 2.

recorded the highest fruit yield of 1449.6 ± 140.1 g per plant followed by the commercial hybrid Ty-King, which produced 1168.9±120.4 g per plant. Most of the genotypes produced medium-sized and rounded fruits (Table 2), which were suitable for the fresh fruit market. Despite high disease incidence, vegetative growth and fruit set were relatively unaffected in many of the genotypes compared to the susceptible checks.

Whitefly-mediated inoculation of ToLCV-[Ban4] in the glasshouse

All of the TYLCV-Is-resistant/tolerant tomato genotypes were susceptible to ToLCV-[Ban4] and developed disease symptoms under glasshouse conditions at Bangalore. However, two lines 902 and 910 derived

from *L. hirsutum*, and the cultivar Fiona showed final disease incidences of 80%, 53% and 25%, respectively. These three genotypes expressed only mild symptoms and these were delayed until six weeks following inoculation. Three other genotypes, which also expressed mild symptoms, were lines 901-1 (F1 hybrid between 902 and *L. esculentum*) and 906-7 derived from *L. hirsutum* and the commercial hybrid 3750 derived from *L. chilense*, but they attained 100% disease incidence and developed symptoms within three weeks of inoculation. The remaining tomato genotypes were susceptible to the disease (Table 3). The squash-blot assay revealed variation in the presence of virus among tomato genotypes (Figure 2). ToLCV-[Ban4] was not detected in line 910 and the virus was detected in only two of 30 plants in line 902, although 53% and 80% of the plants respectively, showed mild symptoms. One out of 20 and one out of three plants of lines 901-2 and Chil 1, respectively, and six out of nine plants of line TY 52 had detectable virus. The virus was detected in all the plants of the remaining genotypes, but at variable concentrations (Table 3).

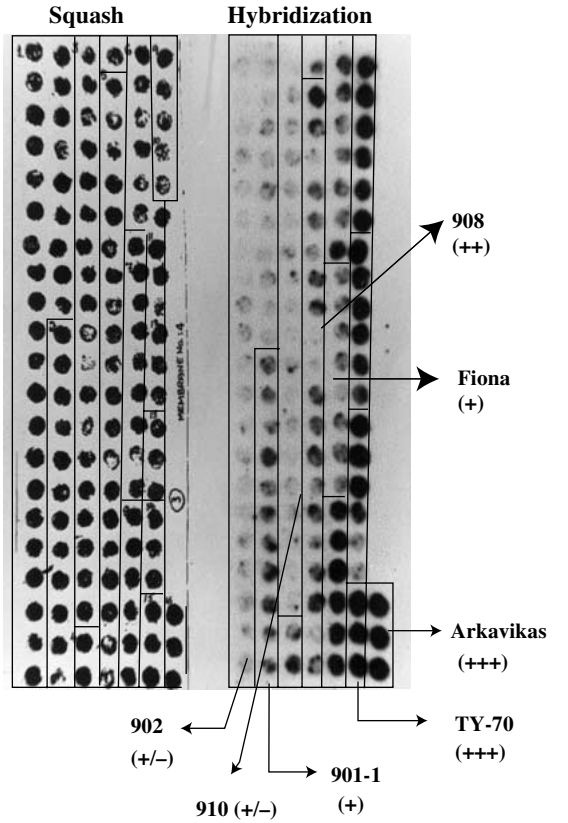

Figure 2. Detection of ToLCV-[Ban4] by squash-blot hybridization. Leaves of plants from selected genotypes were squashed on a membrane (left panel) and hybridized with a ToLCV-[Ban4]-specific DNA probe (right panel). The position of the samples from various tomato genotypes and the intensity of the hybridization signal measured (in parenthesis) are indicated on the membrane.

Field screening for TYLCV-Is resistance in Rehovot

Among 12 ToLCV-[Ban4]-resistant/tolerant tomato genotypes and four susceptible checks screened for TYLCV-Is resistance, accessions of wild species *L. hirsutum* LA 1777 and PI 390659 and *L. peruvianum* CMV Sel. INRA showed mild infection, *L. cheesmani* LA 1401 showed moderate infection and the remaining genotypes including three accessions of *L. pimpinellifolium*, were highly susceptible to the disease (Table 4). One each out of 21 and 12 plants of accessions LA 1777 and PI 390659 were infected accounting for 5% and 9% disease incidence, respectively. However, TYLCV-Is was detected by squash-blots in some symptomless plants of these genotypes in which symptom expression was delayed up to six weeks after transplanting. Accessions CMV Sel. INRA and LA 1401, respectively, attained 41% and 90% disease incidences. TYLCV-Is was detected in most of the symptomatic plants of CMV Sel. INRA and LA 1401, even though the virus was not detected in all the plants showing mild symptoms (data not shown). The virus was detected in all the plants of remaining genotypes except for Hirsute.

Comparison of resistance between TYLCV-Is and ToLCV-[Ban4]

Breeding lines 902 and 910, which were resistant to TYLCV-Is, were found to be only tolerant to ToLCV-[Ban4]. Likewise, wild accessions CMV Sel. INRA, LA 1777 and PI 390659 were resistant to ToLCV-[Ban4], but tolerant to TYLCV-Is. Three TYLCV-Is-tolerant genotypes 908, Pim 2 and Per 1 were susceptible to ToLCV-[Ban4]. Most of the ToLCV-[Ban4]-tolerant genotypes were also susceptible to TYLCV-Is (Table 5), including the widely grown hybrid Avinash-2, which was highly susceptible.

Table 4. ToLCV-[Ban4]-resistant/tolerant tomato genotypes tested for TYLCV-Is resistance in the field at Rehovot, Israel

Source of resistance	Genotype	No. of plants infected/ planted[1]	Days to first/ final symptom appearance[2]	Symptom severity[3]	No. of plants contained virus/tested[4]
L. peruvianum	CMV Sel. INRA	9/22	28–42	M	8/22
L. hirsutum	LA 1777	1/21	42–42	M	6/21
	PI 390659	1/12	42–42	M	4/12
L. cheesmani	LA 1401	18/20	21–42	Mo	10/20
L. pimpinellifolium	LA 121	23/23	14–29	S	23/23
	LA 1478	13/13	14–42	S	13/13
	Hirsute	21/21	14–42	S	18/21
L. esculentum	R 101	13/13	21–42	S	13/13
(commercial cultivars)	R 102	9/9	21–29	S	9/9
	Avinash-2	21/21	14–21	S	21/21
	Nema 1400	21/21	21–35	S	21/21
	Nema 1401	23/23	29–45	S	23/23
	S 41 (Gotya)	23/23	14–42	S	23/23
	NS 386	12/12	29–42	S	12/12
	Arkavikas	22/22	21–42	S	22/22
	Pusaruby	22/22	14–29	S	22/22

[1]Number of plants with symptoms/total number of plants tested.
[2]First to last day of symptom appearance.
[3]M = mild, Mo = moderate, S = severe.
[4]Presence of virus as determined by squash blot hybridization: Number of plants showing hybridization signal/number of plants tested.

Discussion

Success in a disease resistance plant-breeding programme depends on several factors including the precision of the resistance assessment, successful identification of source(s) of resistance and its ready transfer to agronomically superior cultivars (Pico et al., 1998). Breeding for TLCVD resistance is very slow and difficult because of the complex genetics of the resistance (Pilowsky and Cohen, 1974; 1990; Banerjee and Kaloo, 1987; Lapidot et al., 1997). Here, we have screened domestic genotypes, resistant/tolerant to two destructive viral species (ToLCV-[Ban4] and TYLCV-Is). This was done primarily to facilitate the introduction of the readily available and horticulturally acceptable tomato genotypes and hybrids with TLCVD resistance, to reduce the efforts put on the otherwise long and tedious disease resistance breeding programmes. This was considered necessary because there was previously no completely TLCVD resistant cultivar available (Joshi and Choudhury, 1981; Muniyappa et al., 1991a; Nateshan et al., 1996), and to identify new sources of resistance in wild species to further incorporate them in breeding programmes.

The reaction of tomato genotypes to ToLCV-[Ban4] both by artificial inoculation and field testing was similar except for the cultivar Fiona, which showed a disease incidence of 25% by visual observation under whitefly-mediated inoculation but was totally infected in the field. The possibility of escapes was prevented through successful artificial inoculation of all plants of susceptible checks. Moreover, the virus was detected in all the Fiona plants with a strong DNA hybridization signal, and so the genotype was thus considered susceptible. The underlying reasons for some plants of Fiona not showing symptoms in the glasshouse are unknown but could be due to several reasons including the multiple infection with ToLCVs. At least five ToLCV strains/species have been reported in and around the Bangalore area (Muniyappa et al., 2000; Kirthi et al., 2002). Infection of Fiona by more than one virus strain is highly likely, which might explain the expression of clear visible TLCVD symptoms under field conditions. Under glasshouse conditions, however, Fiona was inoculated with the predominantly occurring strain ToLCV-[Ban4], which may not cause clear visible symptoms. Although the possibility of multiple infection to Fiona cannot be determined at this stage, but it is not due to the problems with virus inoculation technique as shown

Table 5. Comparison of the resistance level of some tomato genotypes to ToLCV-[Ban4] and TYLCV-Is

Source of resistance	Genotype	Reaction to	
		ToLCV-[Ban4][1]	TYLCV-Is[2]
L. peruvianum	CMV Sel. INRA	Resistant[3]	Tolerant
L. hirsutum	LA 1777	Resistant[3]	Tolerant
	PI 390659	Resistant[3]	Tolerant
L. cheesmani	LA 1401	Tolerant[3]	Susceptible
L. pimpinellifolium	LA 121	Tolerant[3]	Susceptible
	LA 1478	Tolerant[3]	Susceptible
	Hirsute	Tolerant[3]	Susceptible
L. hirsutum	901-1	Tolerant	Tolerant[4]
	901-2	Tolerant	Resistant[4]
	902	Tolerant	Resistant[4]
	906-7	Tolerant	Resistant[4]
	908	Susceptible	Tolerant
	910	Tolerant	Resistant[4]
	913	Tolerant	Tolerant[4]
L. peruvianum	Per 1	Susceptible	Tolerant[5]
L. pimpinellifolium	Pim 2	Susceptible	Tolerant
L. chilense	Chil 1	Tolerant	Tolerant[5]
L. esculentum	R 101	Tolerant[4]	Susceptible
(commercial cultivars)	R 102	Tolerant[4]	Susceptible
	Avinash-2	Tolerant	Susceptible
	Nema 1400	Tolerant[4]	Susceptible
	Nema 1401	Tolerant[4]	Susceptible
	S 41 (Gotya)	Susceptible[4]	Susceptible
	NS 386	Susceptible[4]	Susceptible
	Arkavikas	Susceptible	Susceptible
	Pusaruby	Susceptible[4]	Susceptible

[1]Tested at the Main Research Station, University of Agricultural Sciences, Bangalore, India.
[2]Tested at the Experimental Station, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, Israel.
[3]Nateshan et al. (1996).
[4]Vidavski and Czosnek (1998).
[5]Vidavski et al. (1998).

by the presence of the virus in this genotype by squash-blot hybridization (Figure 2).

The correlation between visual observation and the DNA hybridization results was valid for all the genotypes (except Fiona) under artificial inoculations. The use of this virus detection method increases the efficiency of selection of resistant genotypes (Zakay et al., 1991) because symptomless carriers can be identified. ToLCV-[Ban4] was detected in all the susceptible tomato genotypes. However, the virus was not detected or a barely detectable hybridization signal was obtained in lines 902 and 910 (Figure 2). This may suggest that these genotypes inhibit the further multiplication of the virus after initial infection and mild symptom expression. These two lines 902 and 910 that are resistant to TYLCV-Is (Vidavski and Czosnek, 1998) sustained rigorous artificial and natural infection of ToLCV-[Ban4], even though line 902 was totally infected in the field but very late in the cropping period. Cultivars Ty-King, 3750 and 3761, and line 906-7 exhibited ToLCV-[Ban4] symptoms, however, their vegetative growth was relatively vigorous. These genotypes had uniform determinate growth characteristics and their yield performance was better than that of the local hybrid Avinash-2. It is probable that the delayed virus infection, for example, by covering the tomato nursery with nylon net (Saikia and Muniyappa, 1989), together with the use of improved agronomic practices could further enhance their yields.

Previously, in a similar attempt to screen TYLCV-Is-resistant/tolerant hybrids to South Indian TLCVD, Nateshan et al. (1996) found F1 Ty-King, F1 and F2

Fiona resistant to TLCVD in Bangalore area. These hybrids were cultivated satisfactorily in South India for some time before they were found susceptible to the disease. In this study, the advanced generations of the above cultivars, and additional breeding lines and hybrids were found tolerant to ToLCV-[Ban4]. Performance of some of these tomato genotypes against high inoculum of ToLCV-[Ban4] was not negligible and prospects for eventual introduction of these cultivars to South Indian farmers appear promising.

Among the accessions of wild species *L. hirsutum* LA 1777 and PI 390659, which are resistant to ToLCV-[Ban4], had very low TYLCV-Is incidence. Accessions of *L. peruvianum* CMV Sel. INRA and *L. cheesmani* LA 1401 were tolerant and *L. pimpinellifolium* LA 121, LA 1478 and Hirsute were highly susceptible to TYLCV-Is. Susceptibility of some of the previously resistant wild species to the present day viruses could be due to several reasons including the emergence of novel and aggressive virus strains/species and/or the vector biotypes. In Bangalore, TLCVD is caused by a complex of at least five ToLCV strains/species, which have emerged through recombinations (Kirthi et al., 2002). In addition, more recently, the B-biotype of the *B. tabaci* was introduced inadvertently into South India and caused an epidemic of TLCVD (Banks et al., 2001). With the threat to tomato cultivation from aggressive viruses and their novel vectors, the wild accessions LA 1777 and PI 390659 were identified as the best source of resistance. These lines are now being used as sources of resistance to TLCVD in an on-going disease resistance-breeding programme.

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