Screening *Lycopersicon* Accessions for Resistance to Tomato Yellow Leaf Curl Virus: Presence of Viral DNA and Symptom Development

Y. ZAKAY, N. NAVOT, M. ZEIDAN, N. KEDAR, H. RABINOWITCH, H. CZOSNEK, and D. ZAMIR, Department of Field and Vegetable Crops and the Otto Warburg Center for Biotechnology in Agriculture, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, P.O. Box 12, Israel

ABSTRACT

Zakay, Y., Navot, N., Zeidan, M., Kedar, N., Rabinowitch, H., Czosnek, H., and Zamir, D. 1991. Screening *Lycopersicon* accessions for resistance to tomato yellow leaf curl virus: Presence of viral DNA and symptom development. Plant Dis. 75:279-281.

Twenty-three *Lycopersicon* accessions representing five tomato species were screened for resistance to the tomato yellow leaf curl virus (TYLCV). Plants were grown in a field naturally infested with *Bemisia tabaci*, the natural vector of this geminiviral disease. The screened genotypes were examined for the presence of viral DNA and symptom development at 2-wk intervals. Tomato cultivars harbored the virus and developed symptoms. Accessions of the wild species *L. pimpinellifolium*, *L. hirsutum*, and *L. peruvianum* showed variance in their response to infection. An accession of *L. chilense* presented the highest level of resistance: Only two of 58 plants contained viral DNA and none developed symptoms.

The tomato yellow leaf curl virus (TYLCV) is responsible for a severe disease of tomato crops. The virus, which is transmitted by the whitefly *Bemisia tabaci* (Gennadius), affects tomatoes during the summer and autumn in Eastern Mediterranean countries and North and Central Africa (7,8). Control measures in infected regions are based on limitation of vector population and have not proven very successful. A more effective solution might be offered by the genetic approach of breeding cultivars tolerant or resistant to TYLCV (12).

Because all tomato cultivars are extremely susceptible to TYLCV, wild *Lycopersicon* species have been screened for their response to the virus. Plants were grown in infected fields and the occurrence of disease symptoms was recorded. Certain accessions of *L. pimpinellifolium* (L.) Mill., *L. cheesmanii* Riley, *L. hirsutum* Humb. & Bonpl., *L. peruvianum* (L.) Mill., and *L. chilense* Dunal were found to be resistant (3,6). In some cases, healthy scions were grafted on diseased stocks in order to determine whether selected plants contained the virus.

Although the genetic basis for most of the apparent resistances in the wild species was not fully defined, it appears to range from a single incomplete dominant gene in the case of *L. pimpinellifolium* (5,10) to a polygenic pattern (recessive in *L. cheesmanii* and dominant in *L. hirsutum*) (4). TY20, a TYLCV-tolerant tomato variety, was recently released in Israel (12). *L. peruvianum* was the source for the tolerance, which was polygenic and recessive (11).

Accepted for publication 26 July 1990 (submitted for electronic processing).

TYLCV is a member of the geminiviruses, which are characterized by twinned particle morphology with a genome of circular single-stranded DNA (2). In a previous study, the replicative form of the TYLCV genome was cloned and a simple method for rapid detection of the viral DNA in plants was developed (9). Briefly, infected shoot tips are squashed onto a nylon membrane which is hybridized to radiolabeled TYLCV DNA. After autoradiographic exposure, the presence of the virus can be detected. The method is sensitive enough to detect viral DNA present in amounts corresponding to 1–2% of the quantity in a squash of a plant with typical disease symptoms. In a controlled infection experiment, by means of whitefly-mediated inoculations, we found that the first disease symptoms in *L. esculentum* Mill. appeared 2 wk postinoculation, whereas viral DNA could be detected 7–10 days earlier (1). The incorporation of this virus detection method can increase the efficiency of selection of resistant genotypes, because a large number of plants can be screened in a short time.

In the present study, we screened *Lycopersicon* accessions for resistance to TYLCV by monitoring the presence of viral DNA and symptom development.

MATERIALS AND METHODS

Field testing. Screening for sources of resistance to TYLCV took place during two successive years. The accession numbers of *L. esculentum*, *L. pimpinellifolium*, *L. hirsutum*, *L. peruvianum*, and *L. chilense* analyzed in 1988 and the number of plants in each case are listed in Table 1. Seed was germinated in seedling trays, and 1-mo-old seedlings were planted in September 1988 in the Gilgal Field Experiment Station in the Jordan Valley. In this region, 100% infection of susceptible genotypes is routinely obtained because of the dense population of whiteflies carrying TYLCV. Plants were scored independently by two people for symptom development 22, 36, 50, 63, and 84 days after planting. In 1989, 43 *L. chilense* plants and 40 *L. esculentum* 'M82' plants were grown and tested for resistance in the Gilgal Field Experiment Station.

Controlled inoculation. Ten *L. chilense* 'LA1969' and 20 *L. esculentum* 'M82' plants were inoculated with viruliferous whiteflies in insect-proof cages. Whiteflies (*B. tabaci*) were maintained on cotton plants (*Gossypium hirsutum* L.) in insect-proof cages kept at 30 C. Virus cultures were maintained in tomato plants (cv. M82). Virus was acquired by the whitefly vector *B. tabaci* after an access period of 48 hr on tomatoes infected with TYLCV. Healthy tomato plants were kept in contact with viruliferous whiteflies for 48 hr (about 10 insects per plant) to inoculate them at the four-leaf stage. The tomato plants were then sprayed with 0.3% senprotathrin (Smash) and grown in an insect-proof growth chamber.

Squash blots. Squash blots (9) were obtained on the same days that plants were visually scored for symptoms. The shoot apex from each of the 148 assayed plants was squashed onto dry nylon membrane (50 cm^2) (Hybond-N, Amersham, Arlington Heights, IL) and fixed by UV irradiation for 2 min. Squashed blots were hybridized for 18 hr at 42 C with 0.1 μg of cloned TYLCV-DNA probe radiolabeled by nick translation with [^{32}P]-dCTP. The blots were washed at 65 C for 2 × 20 min in 150 mM NaCl and 15 mM trisodium citrate (1 × standard saline citrate [SSC]) and exposed at −80 C to film for 18 hr.

RESULTS

Accumulation of viral DNA. Use of the squash-blot method to detect the presence of TYLCV nucleic acids in the plants (Fig. 1) revealed variation in the presence of virus among different accessions (Table 1). Thirty-six days after planting, all plants of susceptible cvs. M82 and MS10 contained viral DNA.

The cv. TY20 showed a higher degree of tolerance; squash-blot assays showed that 22 days after planting, none of the TY20 plants contained viral DNA, whereas 50 days after planting, all plants tested positive for presence of the virus.

L. pimpinellifolium resembled *L. esculentum* with respect to the viral DNA assay, because 50 days after planting, 95% of the plants contained viral DNA and 1 mo later, all plants were infected.

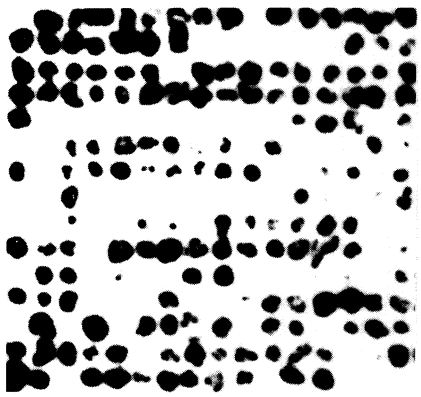

Fig. 1. Squash blot detection of TYLCV DNA in field-grown tomato plants. A stem cross-section from each plant was squashed onto a grid drawn on a Hybond-N membrane, which was later hybridized to a TYLCV-specific probe. Each dot is the autoradiographic signal from a single TYLCV-infected plant; an absent dot on the grid indicates a healthy plant.

In *L. hirsutum* accession LA1777, only one of the five plants tested contained viral DNA, whereas the response of the INRA accession resembled that of *L. pimpinellifolium*. Little variation was observed in virus accumulation among the *L. peruvianum* accessions; 84 days after planting, viral DNA was detected in 80% of the plants. *L. chilense* accession LA1969 was the most tolerant. At the conclusion of the experiment, 84 days after planting, only one of the 15 plants contained viral DNA.

In 1989, *L. chilense* 'LA1969' plants were again grown in the Jordan Valley in order to verify the resistance of this accession. In squash-blot assays performed 75 days after planting, only one of the 43 plants was found to contain viral DNA, whereas all of the control plants were infected. Ten *L. chilense* and 20 *L. esculentum* plants were also inoculated with viruliferous whiteflies in insect-proof cages. After 31 days, none of the plants of the wild species were found to contain viral DNA, whereas all but one of the control plants were infected.

Symptom development. All of the *L. esculentum* cultivars developed symptoms of disease; however, in the tolerant cv. TY20, symptom development was slightly delayed (Table 1). Among the 11 *L. pimpinellifolium* accessions tested, variation in symptom development was observed—accessions 69-187 and 75-298 were symptomless. In *L. hirsutum*, none of the LA1777 plants developed symptoms, whereas in the INRA line, all of the plants did. *L. peruvianum* differed markedly from the other accessions in that only one of the 33 assayed plants showed symptoms. In the field studies conducted in 1988 and 1989 and in the controlled inoculation experiment, none of the plants of *L. chilense* accession showed disease symptoms.

DISCUSSION

One of the difficulties in selecting a source for resistance to TYLCV in the wild *Lycopersicon* germ plasm stems from the fact that in the different accessions, the disease may be expressed with varying degrees of severity. Symptoms in the wild species are generally much weaker than in the cultivated tomato (6). The difficulties in visual scoring for resistance, in addition to the year-to-year variation in the degree of the TYLCV epidemic, can produce conflicting results regarding the response of the accessions to TYLCV. Kasrawi (5) found *L. pimpinellifolium* accession LA1478 to be completely resistant, whereas in our experiment, which was conducted 1 yr later in the Jordan Valley, symptoms were observed in 75% of the plants of the same accession. The putative existence of different strains of TYLCV may also

Table 1. The number of plants containing viral DNA and showing tomato yellow leaf curl symptoms at different days after planting in the Gilgal Field Experiment Station in the Jordan Valley

Species	Accession	Seed source[x]	Number of plants[y]										Total no. of plants tested
			Day 22		Day 36		Day 50		Day 63		Day 84		
			V	S	V	S	V	S	V	S	V	S	
L. esculentum													
	M82	A	2	0	6	4	6	6	6	6	6	6	6
	MS10	A	2	0	10	9	10	10	10	10	10	10	10
	TY20	A	0	0	9	1	12	10	12	10	12	12	12
L. pimpinellifolium													
	LA1478	C	3	0	8	0	8	6	8	6	8	6	8
	1318	D	3	0	7	0	8	1	8	4	8	4	8
	1519	D	1	0	3	0	3	0	3	1	3	1	3
	3150	D	8	0	8	1	8	6	8	6	8	6	8
	3408	D	0	0	2	0	3	3	4	4	4	4	4
	3465	D	0	0	1	0	3	0	3	0	4	2	4
	69-187	D	3	0	8	0	8	0	8	0	8	0	8
	75-298	D	1	0	3	0	5	0	5	0	6	0	6
	78-183	D	1	0	6	0	7	2	7	2	7	2	7
	82-2541	D	1	0	4	0	5	3	5	3	5	3	5
	83-2876	D	0	0	3	2	3	3	3	3	3	3	3
L. hirsutum													
	LA1777	C	1	0	1	0	1	0	1	0	1	0	5
	H2-INRA	B	0	0	2	0	2	2	3	3	3	3	3
L. peruvianum													
	3407	D	1	0	2	0	3	0	4	0	4	0	5
	78-1556	D	0	0	1	0	3	0	4	0	4	0	4
	81-2274	D	0	0	0	0	4	0	6	0	6	0	7
	PI 127831	B	0	0	2	0	2	1	3	1	3	1	4
	PI 127832	B	0	0	0	0	2	0	2	0	2	0	5
	CMV-INRA	B	0	0	1	0	1	0	7	0	7	0	8
L. chilense													
	LA1969[z]	C	0	0	0	0	0	0	0	0	2	0	58

[x] A = Commercially available; B = H. Laterrot, INRA, Avignon, France; C = C. M. Rick, University of California, Davis, USA; D = D. Zamir, Rehovot, Israel.

[y] V = Plants containing viral DNA, S = plants showing tomato yellow leaf curl virus.

[z] Results pooled from tests in 1988 and 1989.

explain these discrepancies.

The squash-blot procedure (9) provides a rapid and simple means of employing molecular hybridization techniques to detect viral DNA in infected plants. The results obtained upon screening of the different accessions up to 84 days after planting indicate that there are three possible types of response in *Lycopersicon* to TYLCV infection: 1) susceptibility—plants contain viral DNA and develop symptoms of the disease, 2) tolerance—plants contain detectable amounts of viral DNA but are symptomless, and 3) resistance—plants show neither the presence of the virus in squash blots nor the symptoms of the disease. The latter may be attributable to the inability of the whitefly vector to feed on the host plants or may result from interference of the plant with the life cycle of the virus.

L. chilense 'LA1969' appears to be the best available source for breeding tomato lines resistant to TYLCV, because in 1988 none of the plants developed symptoms and only one plant acquired the virus 84 days after planting. A similar response was observed in 1989 both in the field and in the greenhouse after controlled inoculations. The fact that the viruliferous whiteflies were observed to be feeding on the host leaves suggests that in *L. chilense*, although virus might be introduced into the plants, it neither spreads nor replicates within it. Accordingly, this accession has been selected as our base population for breeding tomatoes resistant to TYLCV.

ACKNOWLEDGMENTS

We thank Sara Ovadia and Tzili Pleban for their excellent technical assistance. We also thank Shirley Smith for editing the manuscript. This research was supported by BARD Project I-1110-86 and by Yissum.

LITERATURE CITED

1. Ber, R., Navot, N., Zamir, D., Antignus, Y., Cohen, S., and Czosnek, H. 1990. Infection of tomato by the tomato yellow leaf curl virus: Susceptibility to infection, symptom development and accumulation of viral DNA. Arch. Virol. 112:169-180.
2. Czosnek, H., Ber, R., Antignus, Y., Cohen, S., Navot, N., and Zamir, D. 1988. Isolation of tomato yellow leaf curl virus, a geminivirus. Phytopathology 78:508-512.
3. Hassan, A. A., Mazayd, H. M., Moustafa, S. H., and Nakhla, M. K. 1982. Assessment of tomato yellow leaf curl virus resistance in the genus *Lycopersicon*. Egypt. J. Hortic. 9:103-116.
4. Hassan, A. A., Mazayd, H. M., Moustafa, S. H., Nassar, S. H., Nakhla, M. K., and Sims, W. L. 1984. Inheritance of resistance to tomato yellow leaf curl virus derived from *Lycopersicon cheesmanii* and *Lycopersicon hirsutum*. HortScience 19:574-575.
5. Kasrawi, M. A. 1989. Inheritance of resistance to tomato yellow leaf curl virus (TYLCV) in *Lycopersicon pimpinellifolium*. Plant Dis. 73:435-437.
6. Kasrawi, M. A., Suwwan, M. A., and Mansour, A. 1988. Sources of resistance to tomato yellow leaf curl virus in *Lycopersicon* species. Euphytica 37:61-64.
7. Makkouk, K. M., and Laterrot, H. 1983. Epidemiology and control of tomato yellow leaf curl virus. Pages 315-321 in: Plant Virus Epidemiology. R. T. Plumb and J. M. Tresh, eds. Blackwell Scientific Publications, Oxford.
8. Martelli, G. P., and Quacqarelli, A. 1982. The present status of tomato and pepper viruses. Acta Hortic. 127:39-63.
9. Navot, N., Ber, R., and Czosnek, H. 1989. Rapid detection of tomato yellow leaf curl virus in squashes of plants and insect vectors. Phytopathology 79:562-567.
10. Pilowsky, M., and Cohen, S. 1974. Inheritance of resistance to tomato yellow leaf curl virus in tomatoes. Phytopathology 64:632-635.
11. Pilowsky, M., and Cohen, S. 1990. Tolerance to tomato yellow leaf curl virus derived from *Lycopersicon peruvianum* Plant Dis. 74:248-250.
12. Pilowsky, M., Cohen, S., Ben Joseph, R., Shlomo, A., Chen, L., Nahon, S., and Krikun, J. 1989. TY20 A tomato cultivar tolerant to tomato yellow leaf curl virus. Hassadeh 69:1212-1215.