Searching for new resistance sources to tomato yellow leaf curl virus within a highly variable wild *Lycopersicon* genetic pool

Belén Picó, Alicia Sifres, Mónica Elía, Mª José Díez, Fernando Nuez

Department of Biotechnology (Genetics). Polytechnic University of Valencia (Spain). Camino de Vera, 14, 46022. Valencia, SPAIN. E-mail: fnuez@btc.upv.es

Key words: Agroinoculation, Geminivirus, Genetic resources, Tomato, Breeding programs.

Abstract

The high variability found among Tomato yellow leaf curl virus (TYLCV) isolates from different geographical areas makes progress in breeding for TYLCV resistance slow.

By using *Agrobacterium*-mediated inoculation, we have identified several new resistant sources to TYLCV within a extraordinarily variable wild *Lycopersicon* gene pool, collected in semidesert areas of Ecuador and Peru changed into wet by "El Niño". This screening assay revealed a high susceptibility within *L. esculentum* and *L. pennellii*, but different levels of resistance within *L. pimpinellifolium* and *L. hirsutum*. Resistance level was related to the collection place, being concentrated in accessions collected in Northern Peru (Piura province).

Agroinoculation allowed the selection of 4 *Lycopersicon pimpinellifolium* and 2 *Lycopersicon hirsutum* accessions with higher level of resistance than accessions of these species previously reported, avoiding interference due to vector resistance mechanisms reported in both species.

These new resistance sources will be included in pyramiding strategies aimed at obtaining durable resistance to TYLCV.

Introduction

Tomato yellow leaf curl virus (TYLCV) refers to a heterogeneous complex of geminiviruses vectored by the whitefly *Bemisia tabaci* (Czosneck and Laterrot 1997) Disease symptoms include leaf curling and yellowing, and yield losses can reach 100 % if plants are infected in early growth stages (Pico *et al.* 1996). TYLCV was first reported in Spain in 1992. Since then, it has spread to the main vegetable producing regions of South-eastern Spain. Isolates of the TYLCV-Sr (Sardinia) species were found to be involved in these epidemics. More recently, abnormally severe symptoms were reported associated with another species, TYLCV-Is (Israel) (Sanchez-Campos *et al.* 1999).

Different levels of TYLCV resistance were reported in some accessions of several wild *Lycopersicon* species (Laterrot 1995, Pico *et al.* 1996, Vidavsky *et al.* 1998). Most of the tomato breeding lines and the resistant commercial cultivars were derived from few *L. chilense* and *L. peruvianum* accessions (Zamir *et al.* 1994, Lapidot *et al.* 1997, Pico *et al.* 1999). However, some resistance sources identified in specific regions, do not serve in other ones where different virus isolates and epidemiological conditions exist (Pico *et al.* 1998, Sanchez-Campos *et al.* 1999). Pyramiding genes controlling different resistance mechanisms against TYLCV could be an strategy to increase durability and stability of resistance to this geminivirus.

Breeding programs for TYLCV resistance used to date the variability found in international collections of *Lycopersicon* spp. This valuable gene pool has recently been improved by adding a singular, highly variable, collection of *Lycopersicon*. This collection was provided by the expedition of the Centre for Conservation and Breeding of the Agrobiodiversity of the Polytechnic University of Valencia (UPV-Spain) to those areas of Ecuador and Peru affected by the climatic change caused by "El Niño" in 1998.

This current study was conducted to screen this collection, including accessions of *L. esculentum*, *L. esculentum* var *cerasiforme*, *L. pimpinellifolium*,

L. hirsutum, and *L. pennellii*, for their response to agroinoculation with TYLCV-Sr.

Materials and Methods

Plant material

The assayed collection "El Niño 98" included 6 landraces of *L. esculentum*, 1 accession of *L. esculentum* var *cerasiforme*, 40 accessions of *L. pimpinellifolium*, 17 accessions of *L. hirsutum*, and 5 accessions of *L pennelli*. Collections were mainly conducted in Southern Ecuador (Loja and El Oro provinces) and Northern Peru (Piura and Lambayeque provinces), where "El Niño" caused heavy rains in previously semidesert areas. The geographic coordinates of each collect are indicated in tables 1, 2, 3 and 4.

Inoculation

Five to ten plants per accession were inoculated at the 4-6 leaf stage. The tomato breeding line NE-1, developed at the UPV, was included as susceptible control. Plants were agroinoculated by using a culture of *Agrobacterium tumefaciens* LB4404 transformed with a dimeric copy of the isolate TYLCV-Alm (belonging to the TYLCV-Sr species). The bacteria were injected into tomato plants as described before (Kheyr-Pour *et al.* 1994).

Viral DNA detection

The presence of TYLCV DNA in each plant was weekly detected by molecular hybridization. 0.5 cm^2 samples from apex tissues were blotted into a Nylon membrane. The membranes were reacted with a DNA probe spanning the entire genome of the isolate TYLCV-Alm, as reported in Pico *et al.* (1999). After hybridization, membranes were exposed to a phosphorimager screen (Bio-imaging analyser, BAS 1500, Fujifilm, Tokyo, Japan). The densitometric analysis of squash blots was used to compare the levels of viral accumulation among genotypes, scored from 0 (no reaction) to 100 (maximum reaction on the susceptible control). Viral accumulation was weekly screened in the plants during 4 weeks after the first agroinoculation. Negative plants were pruned and new shoots were newly agroinoculated. Plants were then screened for viral accumulation during 1 month after this second inoculation.

Symptom severity was visually scored per plant on a scale of 0 (symptomless) to 4 (severe symptoms).

Data analysis

Statistical analysis were carried out by means of one-way analysis of variance and by a Newman-Keuls multiple range test for means comparison (Statgraphic plus, Statistical Graphics Corp.).

Table.1 Response of *Lycopersicon esculentum* and *L. esculentum* var *cerasiforme* landraces to agroinoculation with TYLCV-Sr.

Accession	Viral accumulation			Symptoms	% Infection	Collection place[3]	Response[4]
	15DAI	30DAI		30DAI [2]	30DAI		
UPV-16986 (var.*cerasiforme*)	38.6	45.3	a[1]	2	100	P-La: 5°-59′-42″ S/79°-43′-03″ W	I
UPV-16895	8.6	50.6	a	3.5	100	E-Lo: 4°-2′-23″ S/79°-47′-19″ W	S
UPV-16897	18.5	57.7	a	3.5	100	P-Lo: 5°-16′-48″ S/79°-28′-17″ W	S
UPV-16902	31.1	45.5	b	4	100		S
UPV-16896	71.2	49.7	bc	3	100	E-Lo: 4°-2′-23″ S/79°-47′-19″ W	S
UPV-16898	20.8	80.4	bc	3.5	100		S
UPV-16907	18.3	91.1	bc	4	100	P-La: 5°-59′-42″ S/79°-43′-03″ W	S
NE-1	100	88.5	c	4	100	UPV	S

[1] Different letters indicate significant differences in maximum viral ssDNA accumulation obtained for each genotype (Newman-Keuls test)
[2] Symptoms scores for each genotype: 0 (symptomless) to 4 (severe symptoms)
[3] Country, P: Peru, E: Ecuador; Province, La: Lambayeque, Lo: Loja; Geographical coordinates, S: South, W: West.
[4] I: intermediate, S: susceptible

Table 2. Response of *Lycopersicon pennellii* accessions to agroinoculation with TYLCV-Sr.

Accession	Viral accumulation			Symptoms	% Infection	Collection place [3]	Response [4]
	15DAI	30DAI		30DAI [2]	30DAI		
UPV-16942	26.3	35.1	a [1]	3	100	P-Pi: 5°-07´-50´´ S/81°-09´-25´´ W	I
UPV-17043	31.3	52.8	AB	3.5	100		S
UPV-16937	18.5	58.2	ab	2.5	100		S
UPV-16941	30.8	59.7	bc	3	100		S
NE-1	100	88.5	cd	4	100	UPV	S
UPV-16945	35.5	117.5	d	3	100	P-Pi: 5°-07´-50´´ S/81°-09´-25´´ W	S

[1] Different letters indicate significant differences in maximum viral ssDNA accumulation obtained for each genotype (Newman-Keuls test)
[2] Symptoms scores for each genotype: 0 (symptomless) to 4 (severe symptoms)
[3] Country, P: Peru ; Province, Pi: Piura; Geographical coordinates, S: South, W: West.
[4] I: intermediate, S: susceptible

Results and Discussion

Disease progress

TYLCV DNA was detected in susceptible controls at 7 days after inoculation (DAI). The highest viral DNA accumulation (VA) was reached from 15 to 30 DAI, decreasing to the end of the assay when plants displayed more severe symptoms (Table 1).

There were significant differences in the level of resistance among the different wild accessions. These wild entries were classified in 3 resistance groups by considering the infection percentage at 30 DAI, the maximum VA, and the symptomatology (Tables 1, 2, 3 and 4):
- Susceptible (S): those accessions with 100 % infection at 30 DAI, a maximum VA 50 % that of the susceptible control, and severe symptoms (3).
- Resistant (R): those accessions with 2 % infection at 30 DAI, a maximum VA % that of the susceptible control, and mild symptoms (.5) after both the first and the second agroinoculation.
- Intermediate (I): those accessions with infection percentages, VA, and symptoms intermediate between R and S plants.

Response of Lycopersicon spp. to TYLCV-Sr

Lycopersicon esculentum

All *L.esculentum* accessions assayed were highly susceptible to TYLCV-Sr (Table 1). Although some of them showed a VA significantly lower than that of the susceptible control (51-91 %), these were the first to produce symptoms, which began with a strong chlorosis and culminated in a complete stunting of growth (3-4). Only the accession *L.esculentum* var. *cerasiforme* UPV-16986 exhibited an intermediate response, with mild symptoms, but high levels of VA.

All tomato commercial cultivars were reported to be extremely susceptible to TYLCV in previous studies conducted in different affected countries (Laterrot 1995, Pico *et al.* 1996, Lapidot *et al.* 1997). Our results agree with the lack of natural resistance to TYLCV in the cultivated tomato, even in naturalized types and landraces collected the tomato area of origin and diversity.

Lycopersicon pennellii

Plants of this species were the next to produce symptoms. The symptoms displayed were milder than those displayed by *L.esculentum* (2-3,5) (Table 2), being then more resistant in terms of symptomatology. However, the phenotypic expression of TYLCV symptoms is highly dependent on the genetic background, as it occurs with most viruses. Indeed, all *L.pennellii* accessions were classified as susceptible, with 100 % infection at 30 DAI, and 35-118 % VA. Therefore, symptomatology is not adequate as sole selection criterion when screening wild accessions against TYLCV.

Previous reports on screening for TYLCV resistance did not include *L.pennellii* (Zakay *et al.* 1991, Pico *et al.* 1996, Lapidot *et al.* 1997, Vidavsky *et al.* 1998). We found a lack of resistance in accessions collected in Northern Peru. However, due to the wide range of distribution of this accession, we cannot discard the existence of resistance in other accessions from different areas.

Table 3. Response of *Lycopersicon pimpinellifolium* accessions to agroinoculation with TYLCV-Sr.

Accession	Viral accumulation			Symptoms	% Infection	Collection place [3]	Response [4]
	15DAI	30/60DAI		30-60DAI [2]	30/60DAI		
UPV-17049	0	5.9/6.7	a [1]	1-1.5	40-50	P-Pi: 4°-48´-37´´ S/80°-17´-46´´ W	R-I
UPV-16954	0	7.3/7.7	a	1.5	60-70	P-Pi: 5°-11´-13´´ S/80°-37´-33´´ W	I
UPV-16953	6.6	8.6/10.1	ab	1.5	40-60	P-Pi: 5°-17´-14´´ S/79°-57´-15´´ W	R-I
UPV-16903	9.9	6.5	abc	1.5	75-100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	I
UPV-16960	0	12.2	abc	1.5	100	P-Pi: 5°-26´-07´´ S/79°-44´-37´´ W	I
UPV-16949	0	13.3/19.9	abcd	0.5-1	20-40	P-Pi: 4°-56´-02´´ S/80°-32´-22´´ W	I
UPV-16991	10.7	35.6/22.1	abcd	1-1.5	20-60	P-Pi: 4°-51´-01´´ S/80°-50´-57´´ W	R-I
UPV-16962	0.5	16.7	abcd	1	100	P-Pi: 5°-16´-54´´ S/80°-41´-03´´ W	I
UPV-16959	0	17.2	abcd	1.5	100	P-Pi: 5°-26´-11´´ S/79°-44´-21´´ W	I
UPV-16958	4.9	23.9	abcd	1.5	100	P-Pi: 5°-20´-46´´ S/79°-50´-57´´ W	I
UPV-16983	6.7	20.1	abcd	1.5	90-100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	I
UPV-16980	0	40.9/21.5	abcd	1	50-75	P-La: 5°-49´-05´´ S/79°-49´-59´´ W	I
UPV-16990	13.6	42.3/23.7	abcde	1-1.5	25-60	P-Pi: 4°-51´-37´´ S/80°-47´-07´´ W	R-I
UPV-16957	0	27.4	abcde	1.0	100	P-Pi: 5°-19´-46´´ S/79°-55´-06´´ W	I
UPV-16952	0	7.7/71.1	abcde	1.5-2	40-70	P-Pi: 5°-17´-14´´ S/79°-57´-24´´ W	I
UPV-16974	6.6	31.7	abcde	1.5	100	P-Pi: 5°-35´-21´´ S/79°-58´-30´´ W	I
UPV-16950	8.5	44.2	abcdef	1.0	100	P-Pi: 5°-09´-15´´ S/80°-10´-05´´ W	I
UPV-16969	3.8	38.9	abcdef	1.5-2	100	P-Pi: 5°-23´-26´´ S/80°-03´-24´´ W	I
UPV-16947	25.2	52.3	abcdef	1.5	100	P-Pi: 4°-55´-00´´ S/80°-20´-35´´ W	I
UPV-16964	36.5	32.3	abcdef	2.5	100	P-Pi: 5°-19´-20´´ S/80°-42´-49´´ W	I
UPV-16970	25,5	49,9	abcdef	2.5	100	P-Pi: 5°-26´-24´´ S/80°-01´-54´´ W	I
UPV-16965	32.3	44.8	abcdef	2.5	100	P-Pi: 5°-26´-03´´ S/80°-45´-35´´ W	I
UPV-17044	8.7	33.4	abcdef	2.5	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	I
UPV-16972	4.3	36.7	abcdef	2	100	P-Pi: 5°-33´-14´´ S/79°-58´-41´´ W	I
UPV-16976	35.6	42.1	abcdef	2.5	100	P-La: 5°-35´-54´´ S/79°-57´-06´´ W	I
UPV-16904	44.9	24.9	abcdef	1.0	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	I
UPV-16961	84.5	33.8	bcdefg	2.5-3	100	P-Pi: 5°-25´-59´´ S/79°-44´-32´´ W	S
UPV-16987	30.5	50.2	bcdefg	2.5-3	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	S
UPV-16966	16.4	34.2/53.4	bcdefg	1.5	40-80	P-Pi: 5°-31´-52´´ S/80°-49´-13´´ W	R-I
UPV-16978	59.4	60.7	cdefg	2	100	P-La: 5°-49´-05´´ S/79°-49´-59´´ W	S
UPV-16968	71.3	52.7	defgh	1.5-3	100	P-Pi: 5°-16´-42´´ S/80°-06´-21´´ W	S
UPV-16989	121.8	62.1	efghi	3	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	S
UPV-16981	110	44.9	fghi	3	100	P-La: 5°-55´-28´´ S/79-46´-29´´ W	S
UPV-16982	112	76.6	fghi	3	100	P-La: 5°-55´-28´´ S/79°-46´-29´´ W	S
UPV-16975	21.0	63.4	ghi	3	100	P-La: 5°-35´-54´´ S/79°-57´-06´´ W	S
UPV-16977	40.3	99.2	ghi	4	100	P-La: 5°-42´-15´´ S/79°-53´-10´´ W	S
UPV-16985	103.2	91.2	ghi	3	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	S
NE-1	100	88.5	ghi	4	100	UPV	S
UPV-17047	120.7	95.8	hi	2.5	100	P-La: 5°-29´-13´´ S/80°-00´-36´´ W	S
UPV-16984	100.8	113.3	hi	4	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	S
UPV-16988	87.5	161.3	i	3	100	P-La: 5°-59´-42´´ S/79°-43´-03´´ W	S

[1] Different letters indicate significant differences in maximum viral ssDNA accumulation obtained for each genotype (Newman-Keuls test)
[2] Symptoms scores for each genotype: 0 (symptomless) to 4 (severe symptoms)
[3] Country, P: Peru; Province, Pi: Piura, La: Lambayeque; Geographical coordinates, S: South, W: West.
[4] R: resistant I: intermediate, S: susceptible

Table 4. Response of *Lycopersicon hirsutum* accessions to agroinoculation with TYLCV-Sr.

Accession	Viral accumulation			Symptoms	% Infection	Collection place [3]	Response [4]
	15DAI	30/60DAI		30-60DAI [2]	30-60DAI		
UPV-16911a	0	0/10.47	a [1]	0.5	0-100	P-Pi: 5°-16′-48″ S/79°-28′-16″ W	R
UPV-16910a	0	0/21.1	ab	0.5	0-100	P-Pi: 5°-12′-53″ S/79°-26′-10″ W	R
UPV-17041	0	27.1	abc	1.5	100	P-Pi: 5°-24′-34″ S/79°-38′-10″ W	I-S
UPV-16933	0	15.7/35.5	bcde	2	20-100	P-Pi: 5°-2′1-53″ S/79°-26′-10″ W	I
UPV-16918	29.3	48.4	bcde	3.5	100	E-Lo: 4-07′-45″ S/79°-55′-10″ W	S
UPV-16941	10.9	17.2/33.2	cdef	2-2.5	60-100	E-Lo: 4°-07′-45″ S/79°-55′-10″ W	S
UPV-16924	63.2	47.8	cdef	2.5	100	E-Lo: 4°-09′-21″ S/79°-54′-20″ W	S
UPV-16920	51.4	50.22	cdef	2.5-3	90-100	E-Lo: 4°-02′-12″ S/79°-11′-20″ W	S
UPV-16934	95.6	53.9	cdef	1.5	100	P-Pi: 5°-12′-53″ S/79°-26′-10″ W	I
UPV-16911b	0	7.9/45.5	cdefg	2	100	P-Pi: 5°-16′-48″ S/79°-28′-16″ W	I-S
UPV-16919	18.1	60.3	cdefg	2.5	100	E-Lo: 4°-10′-00″ S/79°-55′-20″ W	S
UPV-16914	29.1	62.8	defg	3	100	E-Or: 5°-29′-13″ S/80°-00′-36″ W	S
UPV-16930	5.9	47.2/84.8	efgh	2.5-3	100	P-Pi: 5°-24′-45″ S/79°-38′-32″ W	S
UPV-16916	26.5	82.2	efgh	2.5	100	E-Lo: 4°-06′-20″ S/79°-45′-55″ W	S
UPV-16910b	0	76.5	efgh	2.5	100	P-Pi: 5°-12′-53″ S/79°-26′-10″ W	S
UPV-16929	13.1	74.1	fghi	2	100	P-Pi: 5°-26′-07″ S/79°-44′-37″ W	S
UPV-16928	33.9	87.2	ghi	2.5	100	P-Pi: 5°-23′-36″ S/79°-37′-34″ W	S
NE-1	100	88.5	hi	4	100	UPV	S
UPV-16931	42.3	147	i	2	100	P-Pi: 5°-23′-18″ S/79°-36′-48″ W	S
UPV-17046	13.1	117	i	3	100	Ec-Lo: 4°-09′-21″ S/79°-54′-20″ W	S

[1] Different letters indicate significant differences in maximum viral ssDNA accumulation obtained for each genotype (Newman-Keuls test)
[2] Symptoms scores for each genotype: 0 (symptomless) to 4 (severe symptoms)
[3] Country, P: Peru, E: Ecuador; Province, Pi: Piura, La: Lambayeque, Lo: Loja, Or: El Oro; Geographical coordinates, S: South, W: West.
[4] R: resistant I: intermediate, S: susceptible

Lycopersicon pimpinellifolium

A highly variable response to TYLCV-Sr was found in different accessions of *Lycopersicon pimpinellifolium* (Table 3). Some of them segregated, including plants with variable symptoms. Five accessions including resistant plants were selected. Among the others 22 behaved as intermediate and 14 as highly susceptible.

The accessions selected were UPV-17049, UPV-16953, UPV 16991 and UPV-16990. Although others exhibited lower VA, all selected accessions showed low infection percentages after the first agroinoculation (25-40 %). A higher inoculum pressure, second agroinoculation, to overcome their higher resistance level was necessary. VA was also significantly lower than that found in the susceptible control (6.7-23.7 %) even after this second inoculation. This mild infection resulted in mild symptoms (0.5-1.5). Some plants within each accession were completely symptomless, vigorous, and with a healthy appearance through the assay. The more resistant plants of each accession were selected for selfing and backcrossing to *L. esculentum* in order to determine the genetics of resistance, as well as its expression in *L.esculentum* genetic background.

The accession UPV-16966 was also interesting despite it accumulated an intermediate viral DNA amount. Some plants were resistant after the first agroinoculation and some symptomless vigorous plants could be selected after the second agroinoculation.

The resistance level of each accession was related to its collection area. The most resistant were collected in Northern Peru (province of Piura), whereas most of the susceptible were collected in a more southern area (province of Lambayeque).

Partial resistance was previously reported in *L. pimpinellifolium* accessions (collected in southern

provinces of Peru, Lambayeque, La Libertad, and Ancash) (Hassan *et al.* 1982, Kasrawi 1989, Chague *et al.* 1997). Most of them were symptomless, but with high levels of VA. Whitefly-mediated inoculation was used in most assays, so accessions with low levels of resistance to the virus, but with resistance to the vector, were selected.

Among *L. pimpinellifolium* only two accessions, *hirsute* INRA and LA 1478 (collected in Santo Tome, Piura) (Laterrot 1995), were employed to develop breeding lines and commercial tomato cultivars. In previous studies, we reported some levels of vector resistance in *L. pimpinellifolium hirsute* INRA along with intermediate levels of VA. This partial resistance was introgressed in *L.esculentum* by Dr H. Laterrot (INRA, France). The breeding populations derived showed an intermediate VA after *Bemisia tabaci* inoculation when compared with the susceptible control (Pico *et al.* 1998).

Our harsh inoculation conditions, using *Agrobacterium*, allowed the selection of accessions with higher levels of resistance, expressed by considerably lower levels of VA and milder symptomatology. Resistance found in this species are results of a great interest for breeding programs, as it is easily crossed to tomato, compared with the strong incompatibility barriers between *L. chilense* and *L. peruvianum* and tomato.

Lycopersicon hirsutum

Two accessions of *Lycopersicon hirsutum* were resistant (UPV-16910 and UPV-16911) (Table 4), two intermediate (UPV-16933 and UPV-16934), and the other clearly susceptible.

The resistance in this species was expressed as a reduced VA (10.47-21.2 %), higher than that of *L. pimpinellifolium*, but with milder symptoms, thus confirming the symptom expression dependence on the genetic background.

The more resistant plants within each resistant accession were selected for backcrossing to tomato as previously described for *L. pimpinellifolium*.

The more resistant *L. hirsutum* accessions were collected in Peru (region of Huancabamba in the Piura province), being as all the accessions collected in Ecuador highly susceptible. This information, consistent with that previously observed in *Lycopersicon pimpinellifolium*, is valuable for future collecting expeditions.

The *L. hirsutum* accessions previously reported as resistant were collected in more Southern provinces of Peru (Ancash, Cajamarca and Lima) (Hassan *et al.* 1982, Zakay 1991, Picó *et al.* 1996, Hanson *et al.* 2000). Only *L.hirsutum* LA 386 (collected in Cajamarca) and *L.hirsutum* LA 1777 (collected in Ancash) were employed for developing resistant tomato breeding lines. Tomato lines derived by Vidavsky and Czosnek (1998) from these two accessions were completely resistant to whitefly inoculation. However, this resistance was expressed at the whitefly-plant interface, so after graft inoculation the resistant plants became tolerant. Contrarily, other breeding populations derived by Dr. H. Laterrot from LA 1777 showed high levels of VA and severe symptoms after *Bemisia tabaci* inoculation, probably due to the loss of the resistance against the vector in the tomato genetic background (Pico *et al.* 1998). Also, the original source, *L.hirsutum* LA 1777, showed high levels of VA after agroinoculation (Kheyr-Pour *et al.* 1994). Therefore, one of the *L. hirsutum* sources often used in tomato breeding programs does not appear to have resistance to the virus. Also in the case of *L. hirsutum* the selection of resistance sources to TYLCV by agroinoculation ensures a higher level of resistance to the virus along with a possible resistance to the vector, also useful in breeding programs.

Conclusions

Resistance to TYLCV commercially available was mainly derived from *L. chilense* and *L. peruvianum*. These cultivars, of great interest, only exhibit a partial resistance, threatened by the great variability found among the different virus isolates. The resistance sources of *L. pimpinellifolium* and *L. hirsutum* described in this paper have a higher resistance level to TYLCV-Sr than those previously reported. The lack of crossability barriers to cultivated tomato makes their use in breeding programs easy.

At present we are screening this resistance sources against TYLCV-Is isolates. If resistance is held for the selected accessions, these will be most useful

for pyramiding resistance genes in order to get a more durable resistance in tomato to TYLCV.

References

Chague V., Mercier J.C., Guenard M., de Courcel A., Vedel F. 1997. Identification of RAPDs markers linked to a locus involved in quantitative resistance to TYLCV in tomato by bulked segregant analysis. Theor. Appl. Genet. 95:671-677.

Czosnek H., Laterrot H. 1997. A worldwide survey of tomato yellow leaf curl viruses. Arch. Virol. 142:1391-1406.

Hanson P.M., Bernacchi D., Green S., Tanksley S.D., Muniyappa V., Padmaja A.S., Chen H., Kuo G., Fang D., Chen J. 2000. Mapping a wild tomato introgression associated with tomato yellow leaf curl virus resistance in a cultivated tomatoline. J. Amer. Soc. Hort. Sci. 125: 15-22.

Hassan A.A., Mazyad H.M., Moustafa S.E., Nakhla M.K. 1982. Assesment of tomato yellow leaf curl virus resistance in the genus *Lycopersicon*. Egypt. J. Hort. 9:103-116

Kasrawi M.A. 1989. Inheritance of resistance to Tomato yellow leaf curl virus in *Lycopersicon pimpinellifolium*. Plant Dis. 73: 435-437.

Kheyr-Pour A., Gronenborn B., Czosnek H. 1994. Agroinoculation of Tomato yellow leaf curl virus (TYLCV) overcomes the virus resistance of wild *Lycopersicon* species. Plant Breed. 112: 228-233.

Lapidot M., Friedmann M, Lachman O., Yehezkel A, Nahon S., Cohen S., Pilowsky M. 1997. Comparison of resistance level to Tomato yellow leaf curl virus among commercial cultivars and breeding lines. Plant Dis. 81: 1425-1428.

Laterrot H. 1995. Breeding network to create tomato varieties resistant to tomato yellow leaf crul virus (TYLCV). Fruits 50: 439-444.

Pico B., Díez M.J., Nuez F. 1996. Viral Diseases causing the greatest economic losses to the tomato crop. II. The Tomato Yellow Leaf Curl Virus-a review. Sci. Hortic. 67:151-196.

Pico B., Díez M.J., Nuez F. 1998. Resistencia al TYLCV en tomate derivada de *Lycopersicon spp*. Actas de Horticultura 22: 78-88.

Pico B., Ferriol M., Díez M.J., Nuez F. 1999. Developing tomato breeding lines resistant to tomato yellow leaf curl virus. Plant Breed. 118: 537-542.

Sanchez-Campos S., Navas-Castillo J., Camero R., Soria C., Díaz J.A., Moriones E. 1999. Displacement of tomato yellow leaf curl virus (TYLCV)-Sr by TYLCV-Is in tomato epidemics in Spain. Phytopatology 89: 1038-1043.

Vidavsky F., Czosnek H. 1998.Tomato breeding lines resistant and tolerant to Tomato yellow leaf curl virus issued from *Lycopersicon hirsutum*. Phytopathology 88: 910-914.

Vidavsky F., Leviatov S., Milo J., Rabinowitch, H.D., Kedar N., Czosnek H., 1998. Response of tolerant breeding lines of tomato *Lycopersicon esculentum*, originating from three different sources *L. peruvianum, L. pimpinellifolium* and *L. chilense* to early controlled inoculation by Tomato yellow leaf curl virus (TYLCV). Plant Breed. 117: 165-169.

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

Zamir D., Ekstein-Michelson I., Zakay Y., Navot N., Zeidan M., Sarfatti M., Eshed Y., Harel E., Pleben H., Van-Oss H., Kedar N., Rabinowitch H.D, Czosnek H. 1994. Mapping and introgression of a Tomato yellow leaf curl virus tolerance gene, *Ty-1*. Theor. Appl. Genet. 88: 141-146.