

# Mapping a Wild Tomato Introgression Associated with Tomato Yellow Leaf Curl Virus Resistance in a Cultivated Tomato Line

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**ABSTRACT.** Tomato yellow leaf curl virus (TYLCV), a heterogeneous complex of whitefly-vectored geminiviruses, is a serious production constraint of tomato (*Lycopersicon esculentum* Mill.) in Asia, the Middle East, and the Americas. In this study we report on mapping of a DNA fragment introgressed into cultivated tomato presumably from the wild species *L. hirsutum* Humb. and Bonpl. and found to be associated with TYLCV resistance. To locate introgressions of wild tomato alleles in TYLCV-resistant tomato line H24, its DNA was digested with six restriction enzymes and probed with 90 RFLP markers evenly spaced throughout the genome. This polymorphism survey revealed the presence of one wild tomato introgression each on chromosomes 8 and 11. Plants of a F<sub>2</sub> cross between H24 and a susceptible tomato line were probed with randomly amplified polymorphic DNA (RFLP) markers linked to the targeted regions and F<sub>3</sub> families were developed by self-pollination of F<sub>2</sub> plants that carried none, one, or both introgressions in either homozygous or heterozygous states. Plants of F<sub>3</sub> families, parents, and control tomato line Ty52 (homozygous for the *Ty-1* allele for TYLCV tolerance) were exposed to viruliferous whiteflies (*Bemisia tabaci* Gennadius) in greenhouses at the Asian Vegetable Research and Development Center, Taiwan, and the University of Agricultural Sciences, Bangalore, India. Results indicated that F<sub>3</sub> families homozygous for the introgression on chromosome 11 were resistant to TYLCV at both locations. Additional probing showed that the chromosome 11 introgression spanned markers TG36 to TG393, covering a distance of at least 14.6 centimorgans. This is the first report of TYLCV resistance in tomato mapped to chromosome 11.

Tomato yellow leaf curl virus (TYLCV) refers to a heterogeneous complex of geminiviruses (Czosnek and Laterrot, 1997) vectored by the whitefly, *Bemisia tabaci*, that infects tomato (*Lycopersicon esculentum*), pepper (*Capsicum annuum* L. var. *annuum*), potato (*Solanum tuberosum* L. ssp. *tuberosum*), tobacco (*Nicotiana tabacum* L.) and several dicot weed species (Green and Kalloo, 1994; Polston and Anderson, 1997; Ramappa et al., 1998). In tomato, disease symptoms include leaf curling and yellowing, and plant stunting; yield losses can reach 100% if plants are infected in early growth stages (Green and Kalloo, 1994; Picó et al., 1996).

TYLCV is a serious tomato production constraint in tropical and subtropical Asia (Green and Kalloo, 1994; Zeidan et al.,

1999). Symptoms of the disease were first described in India by Vasudeva and Samraj (1948). In the southern Indian state of Karnataka, the major tomato producing region in India, TYLCV can infect tomato crops throughout the year, although disease incidence is highest during the summer crop (February to May) when 100% incidence has been reported (Saikia and Muniyappa, 1989). In Taiwan, the disease has been detected in farmers' fields in all parts of the island (Asian Vegetable Research and Development Center, 1994) but infection is frequent in southern and central counties where tomato cultivation is common year-round close to fields of whitefly hosts such as watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], melon (*Cucumis melo* ssp. *melo*) and vegetable soybean [*Glycine max* (L.) Merr.]. Disease incidence is highest in the late dry season/early wet season (March to June) when high temperatures favor rapid growth of whiteflies. Few TYLCV-resistant tomato cultivars are available in Asia, and attempts by farmers to control the disease through frequent pesticide applications are often ineffective, contaminate the environment, and pose a health risk to themselves and consumers.

High levels of TYLCV resistance have been reported in

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certain accessions of several wild *Lycopersicon* species, including *L. hirsutum*, *L. chilense* Dun., *L. pimpinellifolium* (Jusl.) Mill., *L. cheesmanii* Riley, and *L. peruvianum* (L.) Mill. (Friedmann et al., 1998; Hassan et al., 1984; Kalloo and Banerjee, 1990; Kasrawi et al., 1988; Kasrawi and Mansour, 1994; Laterrot, 1992; Muniyappa et al., 1991; Pilowsky and Cohen, 1974; Scott et al., 1995; Vidavsky and Czosnek, 1998; Zakay et al., 1991). Introgression of TYLCV resistance alleles into cultivated tomato from the wild species started as early as 1974 (Pilowsky and Cohen, 1990) and was completed successfully by several researchers (Friedmann et al., 1998; Kalloo and Banerjee, 1990; Kasrawi et al., 1988; Laterrot, 1992; Scott et al., 1995; Vidavsky and Czosnek, 1998; Zamir et al., 1994). A single partially dominant gene for TYLCV tolerance, *Ty-1*, was introgressed into tomato from *L. chilense* accession LA1969 (Zamir et al., 1994). In India, Kalloo and Banerjee (1990) developed six TYLCV-resistant tomato lines with resistance derived from *L. hirsutum* f. *glabratum* accession 'B 6013'. One of these lines, H24, has shown excellent TYLCV resistance in Taiwan (Green, unpublished data) and south India (Muniyappa, unpublished data). Therefore, the objective of this study was to map TYLCV resistance factors in H24 and to determine their relationship to other mapped TYLCV resistance genes.

### Materials and Methods

TYLCV resistance in H24 is reported to have originated from *L. hirsutum* f. *glabratum* accession 'B6013' (Kalloo and Banerjee, 1990); additional information about B6013 such as the collection site, date of collection, and other passport data are unknown or not available. Line H24 was selected from a population that had undergone four backcrosses to *L. esculentum* TYLCV-susceptible recurrent parent 'Hisar Arun' (Hisar Arun = Sel-7), followed by two generations of inbreeding (Kalloo and Banerjee, 1990; Kalloo and Bhutani, 1993). We hypothesized that the H24 genome retained a few wild tomato fragments in an otherwise *L. esculentum* background, and that these fragments putatively controlled TYLCV resistance. We carried out a three step process to test this hypothesis: 1) localization of wild tomato introgressions in H24; 2) use of restricted fragment length polymorphism (RFLP) markers linked to the putative resistance factors to select F<sub>2:3</sub> (F<sub>3</sub>) families from a segregating population with single or combined introgressions; 3) TYLCV screening of the F<sub>3</sub> families to confirm the effects of *L. hirsutum* introgressions on TYLCV resistance.

**LOCATION OF INTROGRESSIONS IN H24.** The genomes of TYLCV-resistant tomato inbred line H24 and a TYLCV-susceptible AVRDC inbred tomato line CL5915-93D<sub>4</sub>-1-0-3 (CL5915) were surveyed with RFLP markers and compared for polymorphism. RFLP analysis can easily detect wild tomato alleles in a *L. esculentum* background (Chunwongse et al., 1994; Miller and Tanksley, 1990; Young and Tanksley, 1989), though in rare instances polymorphisms between *L. esculentum* sources can also be detected (Miller and Tanksley, 1990). To address this limitation, all polymorphic regions were considered potentially associated with TYLCV resistance.

Plants of H24 and CL5915 were grown in a greenhouse at Cornell University, and DNA was extracted according to the methods described by Bernacchi and Tanksley (1997). DNA samples were digested with restriction enzymes *Hind*III, *Eco*RV, *Xba*I, *Sca*I, *Eco*RI, and *Bst*NI and probed with 90 mapped RFLP markers spaced evenly throughout the genome. A total of 15

replicate filters were made containing the samples for each of the six restriction enzymes.

**IDENTIFICATION OF F<sub>2</sub>/F<sub>3</sub> FAMILIES WITH SPECIFIC INTROGRESSIONS FROM H24.** Genotypes to study the effects of different H24 introgressions on TYLCV resistance were developed by probing an F<sub>2</sub> population from the cross of CL5915 (susceptible) x H24 (resistant) with RFLP markers showing polymorphism between CL5915 and H24. A total of 168 F<sub>2</sub> seedlings were grown in 72-hole trays, under artificial illumination, and subjected to DNA extraction 30 d after sowing. DNA from the F<sub>2</sub> plants was digested, electrophoresed, blotted, and probed with markers corresponding to the two polymorphic regions described previously. Selected F<sub>2</sub> plants with specific genotypic configurations were transplanted to the greenhouse into larger pots, and self-pollinated manually to produce F<sub>3</sub> seed.

**TYLCV SCREENING.** A total of 22 entries, including 19 F<sub>3</sub> families, two parents, and TYLCV-tolerant control line Ty52 (homozygous for the *Ty-1* allele for TYLCV tolerance) were tested for TYLCV reactions at the University of Agricultural Sciences (UAS), Bangalore, India and the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Taiwan. At UAS, TYLCV strains were cultured on plants of susceptible tomato cultivars Arkavikas or Rashmi. Before inoculation, whitefly adults were collected from healthy cotton plants (*Gossypium hirsutum* L.) of the cultivar Laxmi (nonhost of TYLCV) and placed on TYLCV-infected tomato plants for 24 h to acquire the virus. Tomato seedlings 10 to 15 d old were inoculated individually with 10 to 15 viruliferous whiteflies and each plant was covered with plastic or polyvinyl chloride tubes for 48 h. Entries were not replicated and each included 36 plants, except for H24 and Ty52 which had 48 and 23 plants, respectively. Parental lines and Ty52 were sown and inoculated about 15 d after respective sowing and inoculation dates of the F<sub>3</sub> families. Inoculated seedlings were kept in the greenhouse and evaluated for disease incidence at 3, 6, 9, and 11 weeks after whitefly exposure. Disease incidence was determined as the percentage of symptomatic plants in each entry. Symptom severity for each entry was rated as described by Muniyappa et al. (1991): none; mild (light yellowing along the leaf margins); moderate (slight plant stunting, and foliar yellowing and curling); and severe (very severe plant stunting, and leaf size reduction, leaf curling and yellowing).

At the AVRDC, tomato seedlings were grown in 72-cell seedling trays (12 x 6 cells, cell volume = 65 mL). An experimental unit included 12 plants (two adjacent rows of six plants) grown on the same tray. Experimental units were arranged in a randomized complete block design with two replications. Subsequently, the 12-d-old seedlings were moved to a netted screen house and each tray was placed on a bench and surrounded on three sides by TYLCV-infected tomato plants (cultivar TK70) infested with whiteflies. Disease incidence and symptom severity were evaluated as at UAS. AVRDC TYLCV incidence data were analyzed as a randomized complete block design using the General Linear Model (PROC GLM) procedure of the SAS (SAS Institute Inc., Cary, N.C.). Entry means at AVRDC were separated by the Waller-Duncan test ( $k = 100$ ). Comparison of F<sub>3</sub> genotypic group means was carried out by unpaired *t* tests.

Parental lines, Ty52, and four resistant F<sub>3</sub> families were sampled 11 weeks after inoculation and tested for TYLCV infection by dot blot hybridization. Leaf disks ≈ 1 cm in diameter were squashed onto nylon membranes. After denaturation and neutralization, the membranes were treated with 2x sodium chloride and sodium citrate (SSC). After fixing the nucleic acid to the membrane, the membranes were treated with prehybridization solution for 1 h at 65 °C for hybridization and then were

incubated overnight at 65 °C with a digoxigenin-labeled DNA probe. Membranes were washed twice with 2× SSC [0.1% sodium dodecyl sulfate (SDS)] for 5 min at room temperature and 0.1× SSC (0.1% SDS) for 15 min at 65 °C. Digoxigenin detection was carried out according to the supplier's (Roche Molecular Biochemicals, Mannheim, Germany) instructions.

Leaf samples from the AVRDC experiment screening were probed with a 1.5 kb DNA-A probe of TYLCV-TW (GenBank No. U88692). Samples from UAS were first probed with a 1.5 kb probe of TYLCV-SL (Sri Lanka) which shares a 94% sequence homology with, and is regarded as an isolate of TYLCV-Ban2 (GenBank No. Z48182). After probing with TYLCV-SL, membranes from UAS were subsequently probed with a 1.5kb DNA-A probe of TYLCV-Ban3 (GenBank No. U38239). TYLCV-Ban2 and TYLCV-Ban3 are geminiviruses isolated from infected tomato plants in the Bangalore area of south India and identified in previous samples of TYLCV-infected tomato samples from UAS.

## Results

**POLYMORPHIC REGIONS BETWEEN TYLCV-RESISTANT AND TYLCV-SUSCEPTIBLE PARENTS.** Analysis of the 90 RFLP loci assayed revealed polymorphisms between H24 and CL5915 for marker TG302 on chromosome 8, and markers TG36 and TG393 on chromosome 11. These two introgressions in H24 were associated putatively with

TYLCV resistance. Both regions were homozygous in H24 for the wild tomato introgression as shown by the absence of the corresponding *L. esculentum* allele, which if still segregating, would have appeared in the bulk DNA preparation.

**TYLCV REACTIONS.** The percentage of symptomatic plants 6 and 11 weeks after inoculation and symptom severity of parental lines CL5915 and H24, tolerant control Ty52 and 19 F<sub>3</sub> families, evaluated at UAS and AVRDC, are presented in Table 1. Following 11 weeks of exposure to viruliferous whiteflies, almost all plants of susceptible parent CL5915 had developed severe stunting, foliar yellowing, and curling symptoms at both locations. No symptoms were observed on H24 at AVRDC. At UAS, slightly more than half the H24 plants developed mild (light foliar yellowing) or moderate (foliar yellowing and curling and slight plant stunting) by 11 weeks after exposure. Disease incidence of TYLCV-tolerant control Ty52 (homozygous for the TYLCV tolerance allele, *Ty-1*) was 52% at UAS and 36% at AVRDC; symptom severity on symptomatic Ty52 plants was moderate or severe.

**PRODUCTION AND TESTING OF SELECTED F<sub>3</sub> FAMILIES.** After detecting polymorphism between H24 and CL5915 at two genomic regions, 168 F<sub>2</sub> seedlings derived from the cross CL5915 × H24 were probed with TG302, TG36, and TG393. Nineteen F<sub>2</sub> plants were selected on the basis of their RFLP genotypes (Table 1) and self-pollinated in the greenhouse to produce F<sub>3</sub> families for TYLCV screening. Based on disease reactions at UAS and AVRDC,

Table 1. Percentage of symptomatic plants and symptom severity of F<sub>3</sub> families, parental lines, and Ty52 after inoculation with tomato yellow leaf curl viruses at the University of Agricultural Sciences (UAS), Bangalore, India, and the Asian Vegetable Research and Development Center (AVRDC), Taiwan.

Entry	RFLP <sup>z</sup>		UAS			AVRDC		
	TG302	TG36/ TG393	Weeks after exposure		Symptom severity <sup>y</sup>	Weeks after exposure		Symptom severity <sup>y</sup>
			6	11		6	11	
F3-77	EE	EE	83	100	Severe	75 ab <sup>x</sup>	95 a	Severe
F3-7	EH	EE	75	100	Severe	79 ab	100 a	Severe
F3-49	EH	EE	94	100	Severe	76 ab	88 a	Severe
F3-31	EH	EE	97	97	Severe	58 b	88 a	Severe
F3-6	HH	EE	69	100	Severe	83 a	100 a	Severe
F3-19	HH	EE	92	100	Severe	63 ab	88 a	Severe
F3-53	HH	EE	75	100	Severe	83 a	100 a	Severe
F3-3	EE	EH	58	94	Segregating	10 cd	5 fg	Segregating
F3-75	EE	EH	44	52	Segregating	28 c	46 b	Segregating
F3-4	EH	EH	31	86	Segregating	25 c	33 b–d	Segregating
F3-55	EH	EH	17	28	Segregating	17 cd	26 c–e	Segregating
F3-79	HH	EH	22	39	Segregating	13 cd	15 e–g	Segregating
F3-114	EE	EH	14	25	Mild	13 cd	0 g	None
F3-123	EH	EH	36	42	Segregating	17 cd	17 d–f	Segregating
F3-50	EH	HH	6	19	Mild	0 d	0 g	None
F3-24	EE	HH	8	36	Mild	0 d	0 g	None
F3-99	EE	HH	8	33	Mild	0 d	0 g	None
F3-90	HH	HH	3	33	Mild	0 d	0 g	None
F3-91	HH	HH	3	17	Mild	0 d	0 g	None
H24	HH	HH	16	57	Mild	0 d	0 g	None
CL5915	EE	EE	94	94	Severe	65 ab	94 a	Severe
Ty52 <sup>w</sup>	---	---	19	52	Moderate/severe	25 c	36 bc	Moderate

<sup>z</sup>Presence of *L. esculentum* (E) or *L. hirsutum* (H) alleles at that locus or loci in the F<sub>2</sub>. TG302 and TG36/393 are mapped to tomato chromosomes 8 and 11, respectively.

<sup>y</sup>Symptom severity: mild = light yellowing along leaf margins but no curling; moderate = foliar yellowing and curling, slight plant stunting; severe = severe leaf curling and puckering, and plant stunting; segregating = individual plants showing mild, moderate, or severe symptoms.

<sup>x</sup>Mean separation in columns by Waller-Duncan test (*k* = 100).

<sup>w</sup>Ty52 is homozygous for the *Ty-1* allele for TYLCV tolerance (Zamir et al., 1994)

Table 2. Mean percentage of TYLCV-infected plants of F<sub>3</sub> genotypic groups classified according to RFLP markers TG36/TG393, and *t* test comparisons.

Genotype <sup>y</sup>	n	UAS <sup>z</sup>		AVRDC	
		Weeks after exposure		Weeks after exposure	
		6	11	6	11
EE	7	83.6	99.6	73.9	94.1
EH	7	31.7	52.3	17.6	20.3
HH	5	5.6	27.6	0	0
		Difference			
<i>t</i> test					
EE vs. HH		78.0**	72.0**	73.9**	94.1**
EE vs. EH		51.9*	47.3*	56.3*	73.8**
EH vs. HH		26.1 <sup>NS</sup>	24.7 <sup>NS</sup>	17.6 <sup>NS</sup>	20.3 <sup>NS</sup>

<sup>z</sup>UAS = University of Agricultural Sciences, Bangalore, India; AVRDC = Asian Vegetable Research and Development Center, Taiwan.

<sup>y</sup>Presence of *L. esculentum* (E) or *L. hirsutum* (H) alleles at TG36/TG393 in the F<sub>2</sub>. TG36 and TG393 were mapped to tomato chromosome 11.

<sup>NS</sup>, \*\*, \*Nonsignificant or significant at *P* = 0.05 or 0.01, respectively, by unpaired *t* test.

it was possible to classify the families into three groups. One group, consisting of the five, F<sub>3</sub> families homozygous for the chromosome 11 introgression (F<sub>3</sub>-50, F<sub>3</sub>-24, F<sub>3</sub>-99, F<sub>3</sub>-90, F<sub>3</sub>-91), was highly resistant to TYLCV-TW in the AVRDC trial, remained symptomless throughout the experiment, and were not significantly different than H24. At UAS, where virus strains TYLCV-SL (closely related to TYLCV-Ban2) and TYLCV-Ban3 were present, mild symptoms were observed on some plants in each of the above F<sub>3</sub> families and disease incidence at week 11 ranged from 17% to 36%, and the group mean was 27.6% (Table 2). The average disease incidence of F<sub>3</sub> families 90 and 91, homozygous for both introgressions, was 25% at UAS, compared to the average TYLCV incidence of 31% for the three families homozygous for the chromosome 11 introgression but lacking the chromosome 8 introgression. This last result would indicate that the chromosome 8 introgression did not greatly enhance TYLCV resistance at UAS.

A second group, consisting of the seven F<sub>3</sub> families lacking the chromosome 11 introgression (F<sub>3</sub>-77, F<sub>3</sub>-7, F<sub>3</sub>-49, F<sub>3</sub>-31, F<sub>3</sub>-6, F<sub>3</sub>-19, and F<sub>3</sub>-53) developed high TYLCV incidences and severe symptoms by week 6 after whitefly exposure. Eleven weeks after exposure, incidences ranged from 97% to 100% at UAS and 88%-100% at AVRDC. At both UAS and the AVRDC, mean TYLCV incidences 11 weeks after exposure of F<sub>3</sub> families lacking the chromosome 11 introgression (99.6% and 94.1%, respectively) were sig-

nificantly greater (*P* = 0.01) than the mean of those families homozygous for the chromosome 11 introgression (27.6% and 0%, respectively) according to *t* tests (Table 2). Symptoms on the above families were severe and like those of the susceptible parent, CL5915.

The third group included the seven F<sub>3</sub> families (F<sub>3</sub>-3, F<sub>3</sub>-4, F<sub>3</sub>-75, F<sub>3</sub>-55, F<sub>3</sub>-79, F<sub>3</sub>-114, and F<sub>3</sub>-123) derived from F<sub>2</sub> plants heterozygous for the chromosome 11 introgression. Self-pollination of these F<sub>2</sub> plants would have resulted in F<sub>3</sub> families segregating in the expected ratio of 1 HH (homozygous for the wild tomato introgression): 2EH (heterozygous) : 1 EE (homozygous for *L. esculentum* alleles). If resistance is completely dominant, about 25% of the plants would be expected to develop symptoms, while if resistance is recessive, about 75% of the plants in these families would be susceptible.

Dot blot hybridization tests of CL5915 plants sampled at the AVRDC tested positive for infection by TYLCV-TW (Table 3). Resistant parent H24 tested negative for infection by TYLCV-TW, indicating the virus was not present in the samples or virus titer was too low for dot blot detection. About 20% of the Ty52 samples tested positive for TYLCV-TW. Samples taken from F<sub>3</sub>-24, F<sub>3</sub>-99, F<sub>3</sub>-114, F<sub>3</sub>-90, and F<sub>3</sub>-91 11 weeks after exposure also tested negative for TYLCV-TW. At UAS, almost all CL5915 samples were coinfecting by TYLCV-SL and TYLCV-Ban3. Of

Table 3. Nucleic acid hybridization test results of parental lines, TY52, and selected F<sub>3</sub> families for TYLCV infection at the University of Agricultural Sciences (UAS), Bangalore, India, and the Asian Vegetable Research and Development Center (AVRDC), Taiwan.

Entry	UAS			AVRDC		
	Samples (no.)	No. positive			Samples (no.)	No. positive
		SL <sup>z</sup>	Ban3	Both		TW
H24	44	0	16	2	10	0
CL5915	9	1	0	7	10	10
Ty52	21	10	1	1	10	2
F3-24	9	0	0	0	7	0
F3-50	11	2	2	2	NT <sup>y</sup>	---
F3-55	10	3	1	2	NT	---
F3-79	6	1	1	1	NT	---
F3-90	5	1	1	2	8	0
F3-91	8	4	1	1	10	0
F3-99	5	0	2	1	8	0

<sup>z</sup>SL = TYLCV-SL, Ban 3 = TYLCV-Ban3, and TW = TYLCV-Taiwan.

<sup>y</sup>NT = not tested.

the virus-positive H24 samples, most were infected with TYLCV-Ban3 while most of the virus-positive Ty52 samples at UAS were infected with TYLCV-SL. Except for F<sub>3</sub>-24, virus-positive samples were found in each of the F<sub>3</sub> families tested.

**CHROMOSOME 11 INTROGRESSION SIZE.** To estimate the size of the introgression on chromosome 11 in more detail, DNA of resistant parent H24 and susceptible parent CL5915 were cut with 13 restriction enzymes and probed with additional markers mapping to chromosome 11. This introgression spanned the region from TG393 to TG36, a distance of at least 14.6 centimorgans (cM) (Fig. 1). The introgression ends between TG36 and CT107C on the centromere side and may extend beyond TG393 on the telomeric end of the chromosome.

## Discussion

TYLCV resistance in H24 was mapped by locating its wild tomato introgressions via an RFLP survey, followed by tests of F<sub>3</sub> families representing all possible combinations of the detected polymorphisms. We succeeded because: 1) wild tomato introgressions in a *L. esculentum* background can be identified by RFLP; 2) introgressed TYLCV resistance in H24 was fixed stably by selection and inbreeding, which also resulted in elimination of unlinked wild tomato DNA; 3) 'Hisar Arun', the recurrent parent used to develop H24 apparently did not carry introgressions from wild tomato; and 4) no polymorphisms could be detected between the *L. esculentum* components of CL5915 and H24.

Results indicate that a wild tomato DNA fragment, presumably from *L. hirsutum*, introgressed into chromosome 11 of tomato inbred line H24, contains at least one gene conditioning TYLCV resistance or tolerance. Banerjee and Kalloo (1987) studied inheritance of TYLCV resistance in *L. hirsutum* accession B6013 and concluded that two genes acting epistatically conditioned resistance. We did not detect a second TYLCV resistance gene and it is possible that during the backcrossing process, H24 received only one major resistance gene from B6013. Presence of the chromosome 11 fragment was associated with a high level of resistance to the geminiviruses used in this study and it should be useful in development of TYLCV-resistant tomato cultivars in regions where the geminiviruses used in this study or related geminiviruses are present. We observed differences in the degree of resistance between AVRDC and UAS trials. H24 and the six F<sub>3</sub> families homozygous for the chromosome 11 introgression were symptomless 11 weeks after inoculation at the AVRDC, and none of the plants sampled from these F<sub>3</sub> families tested virus-positive for TYLCV-TW infection by dot blot hybridization. In the UAS trial, about half of the H24 plants developed slight yellowing along the leaf margins 11 weeks after inoculation, and 38% of H24 plants tested positive for infection by TYLCV-SL, TYLCV-Ban3, or both. Symptomatic plants in F<sub>3</sub> families homozygous for the chromosome 11 introgression ranged from 17% to 36%. In summary, resistance to TYLCV-TW approached immunity while resistance to TYLCV-SL and TYLCV-Ban3 acted more as tolerance, similar to the expression of *Ty-1* (Zamir et al., 1994). It would appear that gene action for resistance to TYLCV-TW is completely or partially dominant because TYLCV incidence of the seven heterozygous F<sub>3</sub> families ranged from 0% to 46% with a group mean of 20.3%. Disease incidence of the seven heterozygous F<sub>3</sub> families at UAS ranged from 25% to 94% at 11 weeks after exposure. Because more than half the plants of resistant parent H24 showed symptoms, it is difficult to estimate the gene action for TYLCV resistance at UAS.

Entries were not tested for reaction to the TYLCV-TW, TYLCV-SL, and TYLCV-Ban3 in the same experiment under similar environmental conditions; consequently, it is not possible to determine whether differences in expression of resistance between locations were due to differences in environment, geminiviruses, or whitefly populations. At both locations, seedlings of entries were exposed to viruliferous whiteflies in the greenhouse. However, at UAS each seedling was caged with 10 whiteflies, while at the AVRDC a mass inoculation technique was used and very likely inoculum pressure was less than at UAS. Picó et al. (1998) found that whitefly inoculation technique affected expression of TYLCV resistance and high inoculum pressure reduced the level of TYLCV resistance. Genetic variation between geminiviruses at AVRDC and UAS may also have affected resistance. TYLCV-TW, TYLCV-SL, and TYLCV-Ban3 are all monopartite geminiviruses, and in that regard are similar to the tomato-infecting geminiviruses that predominate in the Mediterranean region (Czosnek and Laterrot, 1997). The TYLCV-TW replicase gene (C1) and coat protein gene (V1) share sequence homologies of 82.1% and 77.0%, respectively, with those of TYLCV-Ban3. Replicase gene and coat protein gene sequence homologies between TYLCV-TW and TYLCV-SL have not yet been determined. However, TYLCV-Ban2, closely related to TYLCV-SL, has common C1 and V1 sequence homologies of 79.1% and 73.4%, respectively, with TYLCV-TW (Green, unpublished data). Taxonomically, TYLCV-TW would be considered a different virus species from TYLCV-Ban3 and TYLCV-Ban2 (Czosnek and Laterrot, 1997; Padidam et al., 1995; Polston and Anderson, 1997).

This is the first report of TYLCV resistance in tomato mapped on chromosome 11. TYLCV resistance originating from *L. chilense* accession LA1969 (Zamir et al., 1994), *L. pimpinellifolium* hirsute INRA (Chagué et al., 1997), *L. cheesmanii* (Chagué et al., 1997), and *L. chilense* accessions LA1932 and LA 1938 (Griffiths and Scott, 1998) have been mapped to chromosome 6. Existence of a region of major TYLCV resistance genes on chromosome 6 has been suggested (Chagué et al., 1997). The chromosome 11 introgression identified in this experiment is longer than 14 cM and, consequently, we cannot rule out the possibility that more

than one TYLCV resistance locus resides on this fragment. Additional fine-mapping will be required to determine the number and location of TYLCV resistance loci. Fine-mapping would be helpful to determine the distance of TYLCV resistance loci from the Immunity-1 (*I-1*) and Immunity-2 (*I-2*) loci for resistance to race 2 of the fusarium wilt pathogen [*Fusarium*

## Chromosome 11

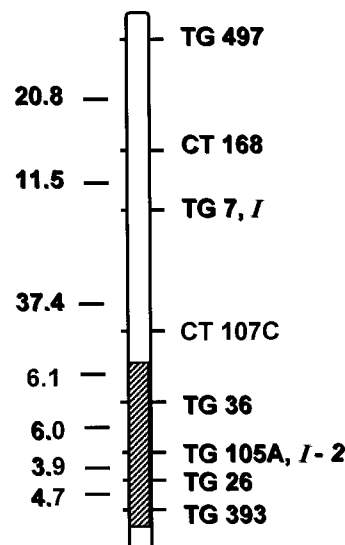


Fig. 1. Location of the introgression associated with TYLCV resistance on tomato chromosome 11. Distances between markers are shown in Kosambi cM units. Loci *I* (Immunity) and *I-2* (Immunity-2) condition resistances to races 1 and 2, respectively, of *Fusarium oxysporum* f. sp. *lycopersici*, the causal agent of tomato fusarium wilt.

*oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen] and linked to TG105A (Tanksley et al. 1992). The chromosome 11 introgression overlaps *I-2* and carries an allele for susceptibility to fusarium wilt race 2. Because fusarium wilt and TYLCV are tropical tomato production constraints, it would be advantageous for tomato cultivars to possess both fusarium wilt and TYLCV resistance.

Ty52, a tomato inbred line homozygous for *Ty-1* (Zamir et al., 1994) for TYLCV tolerance, was not as resistant as H24 in our trials but clearly superior to CL5915 and other susceptible entries. *Ty-1* originated from *L. chilense* accession LA 1969 (Zamir et al., 1994) and conditions tolerance to the Israeli geminivirus, TYLCV-ISR. Pyramiding *Ty-1* and the chromosome 11 introgression identified in this study might broaden resistance against a wider range of geminiviruses or improve the degree of resistance or tolerance (Polston and Anderson, 1997). In this study, the two resistances seemed to complement each other: Ty52 plants showed less infection by TYLCV-SL than H24, while H24 seemed to demonstrate greater tolerance to TYLCV-Ban3 compared to Ty52. These results need confirmation screening with pure cultures of these virus isolates but they may indicate potential benefits of combining these resistance genes. Molecular markers would facilitate pyramiding TYLCV resistance genes at the AVRDC because it would be difficult to distinguish individuals carrying just the chromosome 11 introgression from individuals with both genes based on reaction to TYLCV-TW.

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