Present Status of Begomovirus Resistance Breeding Efforts at UF

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UF UNIVERSITY of FLORIDA IFAS

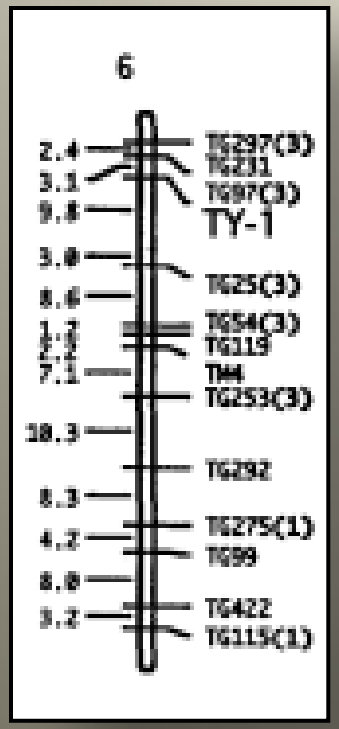
Begomovirus Resistance

- Major focus at University of Florida since 1990

- Resistance sources
  - *S. chilense* LA 2779
  - *S. chilense* LA 1932
  - *S. chilense* LA 1938
  - *S. lycopersicum* cv. 'Tyking'

- Inoculations
  - TYLCV presently
  - ToMoV – through 2006

Ty-1



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Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *TY-1*

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Abstract The whitefly-transmitted tomato yellow-leaf curl gemini-virus (TYLCV) is a major pathogen of tomatoes. The wild tomato species *Lycopersicon chilense*, which is resistant to the virus, was crossed to the cultivated tomato, *L. esculentum*. The backcross-1 selfed (BC1S1) generation was inoculated and a symptomless plant was selected. This plant was analyzed using 61 molecular markers, which span the tomato genome, to determine which *L. chilense* chromosome segments were introgressed. A BC2S1 population was cage-inoculated with viroliferous whiteflies (*Bemisia tabaci*), the natural insect vector of the virus, and subjected to RFLP analysis. Markers on chromosomes 3 and 6 were significantly associated with the level of tolerance; the association of chromosome-6 markers was further substantiated in two additional BC2S1 populations. A tolerant BC2S1 plant which was homozygous for *L. chilense* introgressions in chromosomes 3, 6 and 7 was crossed to generate a BC3S1 population which was planted in an infested field. A TYLCV-tolerance gene with partial dominance, *TY-1*, was mapped to chromosome 6; two modifier genes were mapped to chromosomes 3 and 7. Field and whitefly-mediated cage inoculations of nearly-isogenic lines in BC3S3 supported our conclusion that *TY-1* is the major TYLCV-tolerance locus.

Key words *Lycopersicon* · Tomato yellow leaf curl virus · Gemini-virus · Tolerance · RFLP-assisted selection

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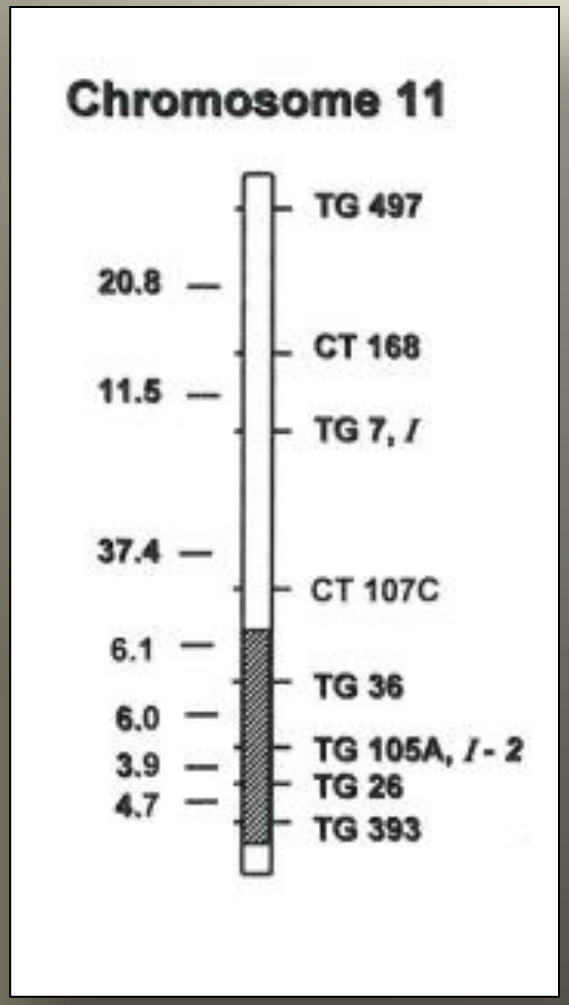
Introduction

The tomato yellow leaf curl virus (TYLCV; gemini-virus) is responsible for severe disease of tomatoes (Cohen and Harpaz 1964; Czosnek et al. 1988). The virus, which is transmitted by the whitefly *Bemisia tabaci* Genn., infects tomato varieties during the summer and autumn in the Eastern Mediterranean, North and Central Africa and Southeast Asia (Czosnek et al. 1990). TYLCV is the first reported whitefly-transmitted gemini-virus to possess a single genomic molecule (Kheyr-Pour et al. 1991; Navot et al. 1991). Its genome is composed of 2787 nucleotides and encodes six open reading frames (Navot et al. 1991). Related gemini-viruses which infect tomato were identified in Mexico (Brown and Nelson 1988), Florida (Scott and Schuster 1991) and Australia (Dry et al. 1993).

The first step in classical breeding for TYLCV resistance was to screen species in the genus *Lycopersicon* for resistance to TYLCV (Zakay et al. 1991). In field tests the wild species *L. Pimpinellifolium*, *L. hirsutum* and *L. peruvianum* all showed partial tolerance. The green-fruited species *L. chilense* (LA 1969), which has severe crossability barriers with the cultivated tomato (Rick 1979), was completely resistant and therefore was selected as the source for our breeding project.

Mapping genes in tomato has been facilitated by the development of a saturated restriction fragment length polymorphism (RFLP) map (Tanksley et al. 1992). In tomato the following disease-resistance genes have been mapped using molecular markers: *Tm-2* (tobacco mosaic virus; Young et al. 1988), *Tm-1* (Levesque et al. 1990), *I-2* (*Fusarium oxysporum* f. sp. *lycopersici* race 2; Sarfatti et al. 1989), *I-3* (*Fusarium oxysporum* f. sp. *lycopersici* race 3; Bournival et al. 1989), *Mi* (root knot nematode; Klein-Lankhorst et al. 1991; Messeguer et al. 1991), *Pto* (*Pseudomonas tomato:* Martin et al. 1991), *Sm* (*Stemphylium*; Behare et al. 1990) and *Cf-2* and *Cf-9* (*Cladosporium fulvum*; Jones et al. 1991). The mapping strategy in the above studies was based on previous

Ty-2



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

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Additional index words. geminivirus, plant breeding, *Lycopersicon* sp.

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_2 cross between H24 and a susceptible tomato line were probed with randomly amplified polymorphic DNA (RFLP) markers linked to the targeted regions and F_3 families were developed by self-pollination of F_2 plants that carried none, one, or both introgressions in either homozygous or heterozygous states. Plants of F_3 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_3 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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Ty-3

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Ty-3, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus *Ty-1* on chromosome 6 of tomato

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Abstract Resistance to begomoviruses including bipartite *Tomato mottle virus* (ToMoV) and monopartite *Tomato yellow leaf curl virus* (TYLCV) has been introgressed to cultivated tomato (*Solanum lycopersicum*) from *S. chilense* accessions LA1932, LA2779, and LA1938. Previous research demonstrated that three regions on chromosome 6 were associated with the resistance, two of which were required for a line to provide a high level of resistance. In the present study, we identified a large *S. chilense* introgression spanning markers from C2_At2g39690 to T0834 in LA2779-derived advanced breeding lines resistant to both TYLCV and ToMoV. A begomovirus resistance locus, *Ty-3*, was mapped to the marker interval between cLEG-31-P16 and T1079 on the long arm of chromosome 6. In addition to the *Ty-3* locus, the large introgression also spans the *Ty-1* region near the *Mi* gene, suggesting the possible coexistence and linkage of resistance alleles at both *Ty-1* and *Ty-3* loci in these lines. In contrast, LA1932-derived advanced breeding lines possess a much shorter introgression from cLEG-31-P16 to C2_At5g41480, which also carries a begomovirus resistance locus that is probably allelic at the *Ty-3* locus. The PCR-based markers tightly linked to the *Ty-3* locus as we markers near the *Ty-1* region have been u breeding program for efficient selection of virus resistance in the past three growin and will be useful resources for tomato around the world.

Keywords Disease resistance · *Lycopersicon esculentum* · *Solanum chilen* Tomato mottle virus · Tomato yellow leaf

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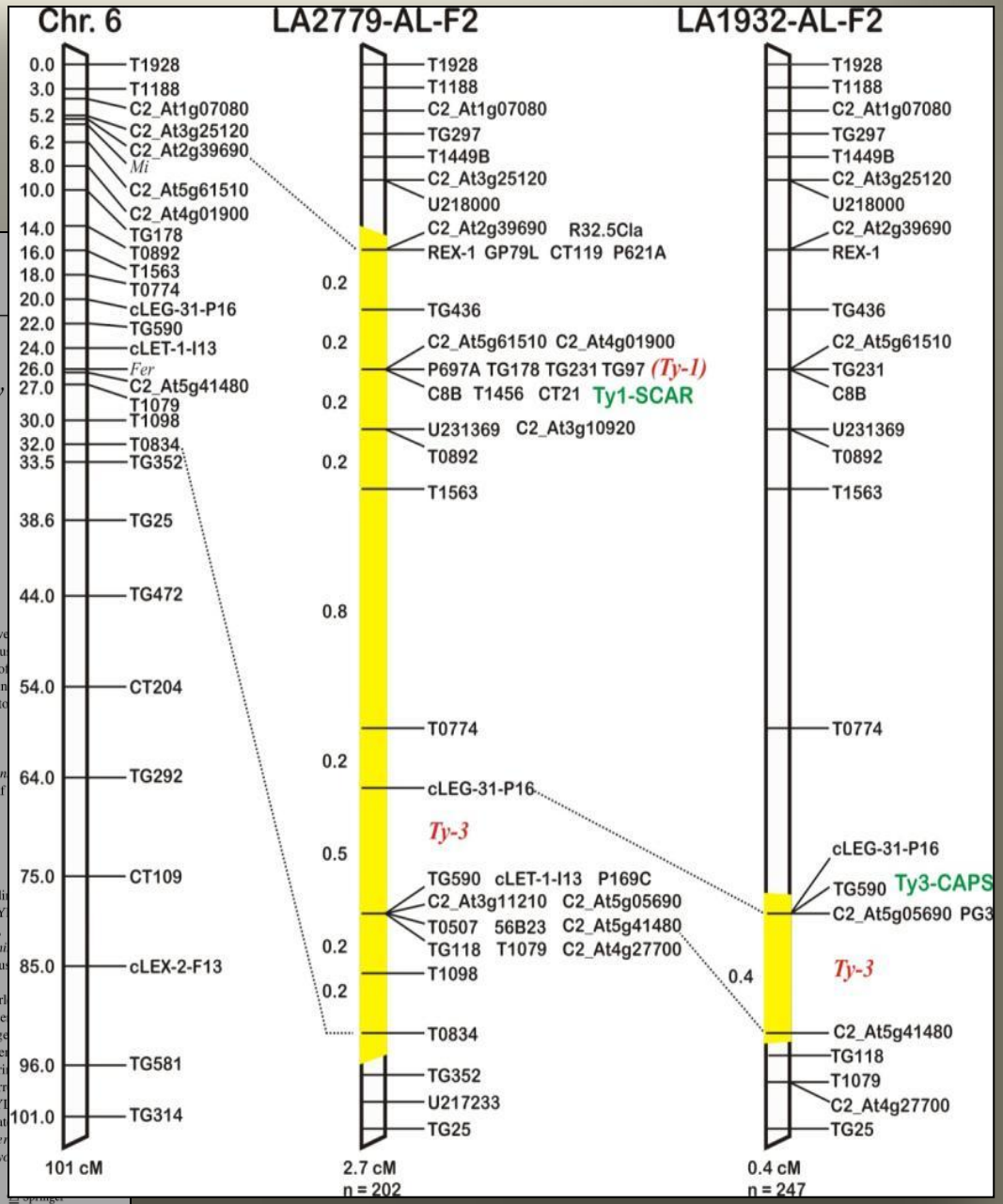
Introduction

Tomato-infecting begomoviruses, includi partite *Tomato yellow leaf curl virus* (TY bipartite *Tomato mottle virus* (ToMoV), mitted by the sweetpotato whitefly, *Bemi* (Genn.). These viruses have caused serious tomato production in the United States tropical and subtropical regions in the worl and Anderson 1997). No resistance has bee date in the *Solanum lycopersicum* ge although some tomato varieties have bee to be less susceptible than others duri epidemics (Hassan et al. 1991; Laterr Fortunately, sources of resistance to TYL been discovered in numerous wild tomat including *S. pimpinellifolium*, *S. per* *S. chilense*, *S. habrochaites* (formerly *Lyc*



Ty-4

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Molecular Mapping of *Ty-4*, a New Tomato Yellow Leaf Curl Virus Resistance Locus on Chromosome 3 of Tomato

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Additional index words. *Lycopersicon esculentum*, *Solanum chilense*, begomovirus, geminivirus, marker-assisted selection, QTL

Abstract. Resistance to begomoviruses, including bipartite tomato mottle virus (ToMoV) and monopartite tomato yellow leaf curl virus (TYLCV), has been introgressed to cultivated tomato (*Solanum lycopersicum*) from *Solanum chilense* accessions LA1932 and LA2779. A major gene, *Ty-3*, responsible for resistance to ToMoV and TYLCV was previously mapped on the long arm of chromosome 6. In the present study, we identified a 14-cM *S. chilense* introgression on the long arm of chromosome 3 in some resistant breeding lines derived from LA1932. A new begomovirus resistance locus, *Ty-4*, was mapped to the 2.3-cM marker interval between C2_At4g17300 and C2_At5g60160 in the introgression. Analysis of a population segregating for *Ty-3* and *Ty-4* demonstrated that *Ty-3* accounted for 59.6% of the variance, while *Ty-4* only accounted for 15.7%, suggesting that *Ty-4* confers a lesser effect on TYLCV resistance. Recombinant inbred lines (RILs) with *Ty-3* and *Ty-4* had the highest level of TYLCV resistance. The PCR-based markers tightly linked to the *Ty-4* locus as well as the *Ty-3* locus have been recently used in our breeding program for efficient selection of high-levels of begomovirus resistance and now allow for efficient breeding by marker-assisted selection.

Tomato-infecting begomoviruses, including monopartite tomato yellow leaf curl virus (TYLCV) and numerous bipartite viruses including tomato mottle virus (ToMoV), are transmitted by the sweetpotato whitefly (*Bemisia tabaci*), the B biotype of which is also known as the silverleaf whitefly (*B. argentifollii*). These viruses have caused serious losses to tomato production in many tropical and subtropical regions in the world (Polston and Anderson, 1997). Although the use of insecticides for whitefly control can limit disease, epidemics can still occur, and whitefly resistance to the chemicals has been reported (Omer et al., 1993). Therefore, breeding resistant cultivars would be an effective method for control of this disease. Thus far, TYLCV resistance has not been found in *Solanum lycopersicum* germplasm, but has been discovered in several tomato wild species, including *S. pimpinellifolium*, *S. peruvianum*, *S. chilense*, *S. habrochaites*, and *S. cheesmaniae* (Ji et al., 2007b; Pico et al., 1996; Scott, 2007). Early breeding efforts involved the introgression of resistance genes from the wild accessions into cultivated tomato using traditional breeding approaches (Scott et al., 1996), a process that usually takes many years to breed a resistant cultivar with acceptable horticultural characteristics. Marker-assisted selection (MAS) provides a tool to improve the efficiency of cultivar development. There has been significant progress in the development of markers for important tomato resistance genes (Foolad and Sharma, 2005), including numerous TYLCV resistance genes (Ji et al., 2007b). The first such gene, *Ty-1*, which originated from *S. chilense* accession LA1969, was mapped near a molecular marker (TG97) on chromosome 6 of tomato (Zamir et al., 1994). PCR-based markers closely linked to the *Ty-1* gene have been developed and can be used for MAS (de Castro et al., 2007; Ji et al., 2007a). A second TYLCV resistance gene, *Ty-2* (Hanson et al., 2006), which originated from *S. habrochaites* (Kalloo and Banerjee, 1990), was mapped to the long arm of chromosome 11, delimited by restriction fragment length polymorphism markers TG393 and TG36 (Hanson et al., 2000). This gene was further delimited to a 4.5-cM marker interval (Ji et al., 2009). We recently identified a third begomovirus resistance gene, *Ty-3*, in *S. chilense* accessions LA1932- and LA2779-derived resistant lines, the former carrying a 6-cM introgression and the latter ≈27-cM on the long arm of chromosome 6. *Ty-3* was mapped to the same marker interval in the two introgressions, ≈15 cM away from *Ty-1* (Ji et al., 2007a). *Ty-3* differs from the first two genes mainly in two aspects. First, unlike *Ty-1* and *Ty-2*, which are effective against only monopartite TYLCV, *Ty-3* is effective against TYLCV and the bipartite begomovirus ToMoV (Agrama and Scott, 2006; Ji et al., 2007a). In addition, *Ty-1* and *Ty-2* genes express complete or nearly complete dominance (Hanson et al., 2000; Ji et al., 2009; Zamir et al., 1994), while *Ty-3* is more additive (Ji et al., 2007a). *Ty-3* is a major locus explaining a high degree of the observed phenotypic variance from the two accessions, but additional resistance loci are required to obtain the highest levels of resistance

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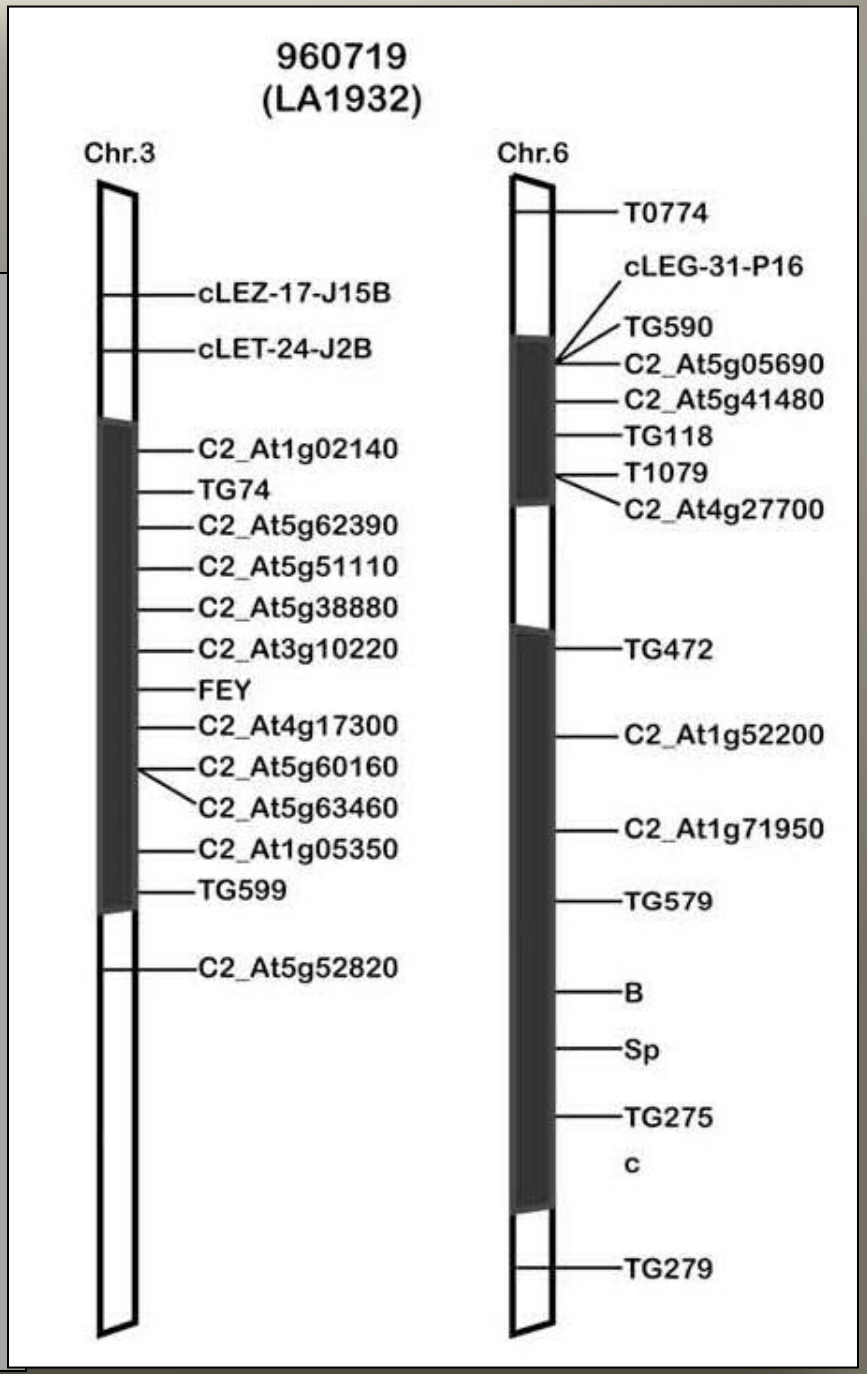
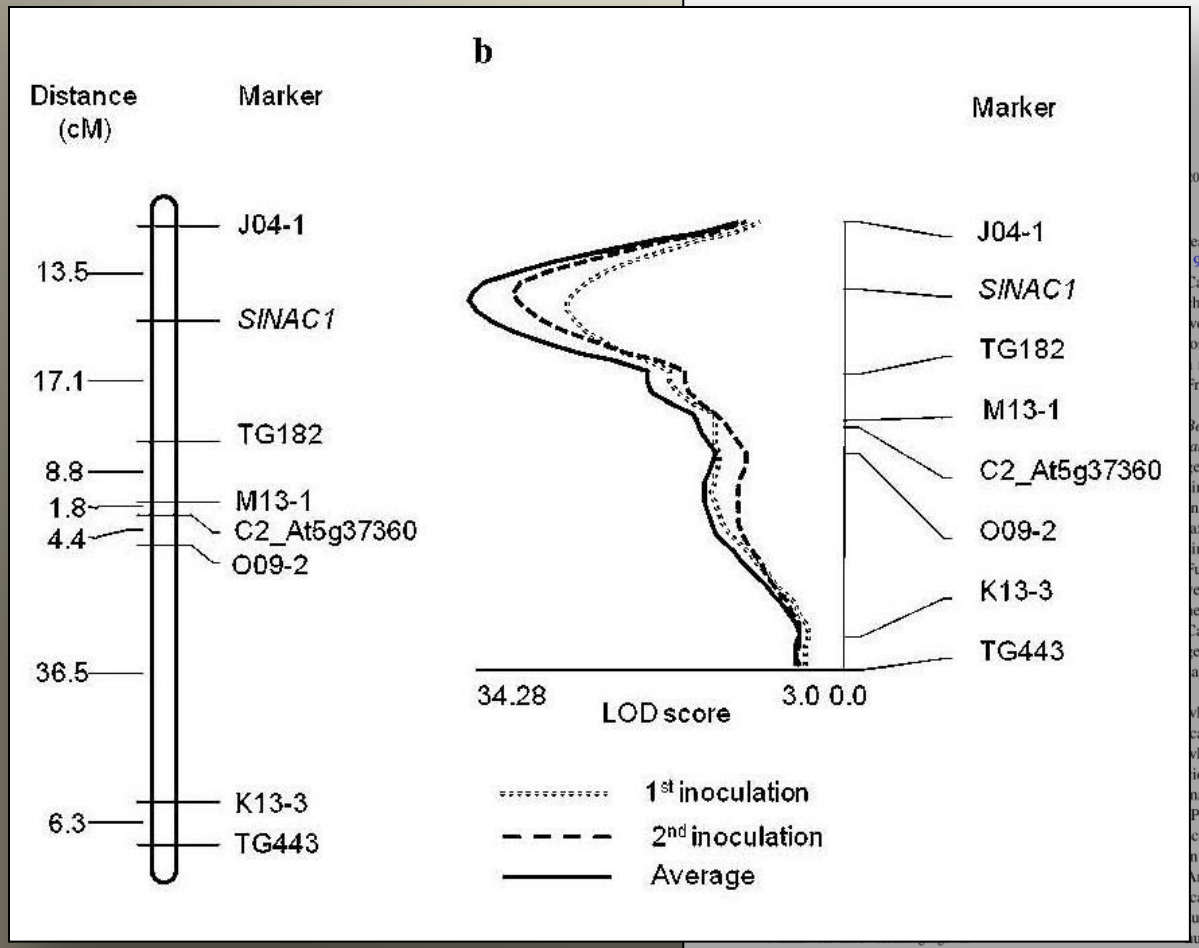


Ty-5



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ORIGINAL PAPER

Molecular dissection of *Tomato leaf curl virus* resistance in tomato line TY172 derived from *Solanum peruvianum*

0 May 2009

eached worldwide distribution (Czosnek and Laterrot 997; Polston and Anderson 1997; Moriones and Navas-Castillo 2000). TYLCV induces a severe tomato disease haracterized by yellowing and cupping of apex leaves as well as stunted plant growth coupled with significant yield osses. In many tomato-growing areas, TYLCV has become major limiting factor in tomato production (Lapidot and Friedmann 2002).

TYLCV belongs to the family *Geminiviridae*, genus *Begomovirus*, and transmitted by the whitefly *Bemisia abaci* in a circulative and persistent manner. The virus enome is composed of a single (monopartite) circular ingle-stranded DNA molecule of about 2,800 nucleotides. n the past, there has been some confusion regarding the axonomy of TYLCV. Several begomoviruses, inducing imilar symptoms in tomato, were all named TYLCV. Further analyses of these viruses showed that the tomato ellow leaf curl disease (TYLCD) is induced by a heteroge-eous complex of begomoviruses (Moriones and Navas-Castillo 2000). Most of the isolates have a monopartite enome, and recently a TYLCV isolate containing a DNA-β atellite was identified (Khan et al. 2008).

The management of TYLCV is difficult because its hitefly vector populations can reach high numbers. Chem-cal control methods have been only partially effective, hile raising concerns that the vector may develop pesti-ide resistance and that intense application of pesticides ay have deleterious environmental consequences Palumbo et al. 2001). Physical barriers such as fine-mesh creens and UV-absorbing plastic sheets or screens are used n the Mediterranean region to protect crops (Cohen and Antignus 1994; Antignus et al. 2001). However, such phys-cal barriers add to production costs and may also result in uboptimal light conditions, overheating, and increased umidity, which can hamper appropriate plant growth and

Selective genotyping at the *SlNAC1* locus of resistant and susceptible F_2 tomato plants grown in Spring 2009 and test for transmission disequilibrium

Family	Phenotype	DSI	Total no.	Plant nos. at the *SlNAC1* locus[z]			Transmission Disequilibrium *(P)*
				+/+	+/-	-/-	
(7655B x 8753) F_2	Resistant	0.5 2.0	21	14	7	0	
(8248 x 8753) F_2		1.0 – 2.0	12	11	0	1	
Total			33	25	7	1	
							<.0001
(7655B x 8753) F_2	Susceptible	3.5 4.0	21	0	11	10	
(8248 x 8753) F_2		3.5	9	0	6	3	
Total			30	0	17	13	

[z] +/+ homozygous resistant, +/- heterozygous, -/- homozygous susceptible; *DSI* disease severity index.

Spring 2011 Survey

- 53 advanced lines
- CRBD
  - 2 reps
  - 4 plant plots
- Seedling TYLCV inoc.
- Genotyped at resistance loci
- Lines evaluations:
  - Viral disease severity
  - Non viral foliar disease severity

Line	TY Gene			
8259				*6?*
8299			5	*6?*
8300			5	*6?*
8302				*6?*
8347	3	4		
8352	3			*6?*
8353	3			*6?*
8379	3			*6?*
8383				*6?*
8387			5	*6?*
8467			5	*6?*
8468				*6?*
8469			5	*6?*
8470			5	*6?*
8472			5	*6?*
8480	3			*6?*
8501	3			*6?*
8503				*6?*
8600				*6?*
8601	3		5	
8602	3			*6?*
8603				*6?*
8604			5	*6?*
8624				*6?*
8625			5	*6?*
8635				*6?*
8636	3			*6?*

Line	TY Gene			
8637				*6?*
8638			5	*6?*
8655			5	*6?*
8656			5	*6?*
8657	3			*6?*
8680	3			*6?*
8737			5	*6?*
8738	3			*6?*
8739	3			*6?*
8741				*6?*
8753			5	*6?*
8779			5	*6?*
8823	3			*6?*
8824	3			*6?*
8825	3			*6?*
8826	3			*6?*
8827				*6?*
8840			5	*6?*
8841				*6?*
8843				*6?*
8844	3			*6?*
8845			5	*6?*
Fla678	3			*6?*
Fla685	3			*6?*
Fla707	3			*6?*
Fla726	3	4		

Mean non-viral foliar disease severity of advanced TYLCV-resistant breeding lines as affected by genotype at three resistance loci.

Genotype[1]	Mean Foliar DSI[2]		
	Ty-3	***Ty-4***	***Ty-5***
+/+	6.1 a (*n* = 130)	4.6 b (*n* = 16)	5.4 a (*n* = 174)
+/-	5.7 b (*n* = 46)	6.0 a (*n* = 5)	4.4 b (*n* = 15)
-/-	5.3 c (*n* = 346)	5.6 a (*n* = 501)	5.7 a (*n* = 333)

[1] +/+ homozygous resistant; +/- heterozygous; -/- homozygous susceptible
[2] *DSI* disease severity index of non-viral infections (primarily bacterial spot and early blight) based on the Horsfall-Barratt scale, lower number means less disease; *n* number of plants in each group; means in column with same letter are not significantly different at $P \leq 0.05$ based on Duncan's multiple range test

Ty-5-based resistance
Ty-3-based resistance

Ty-3 Fine Mapping

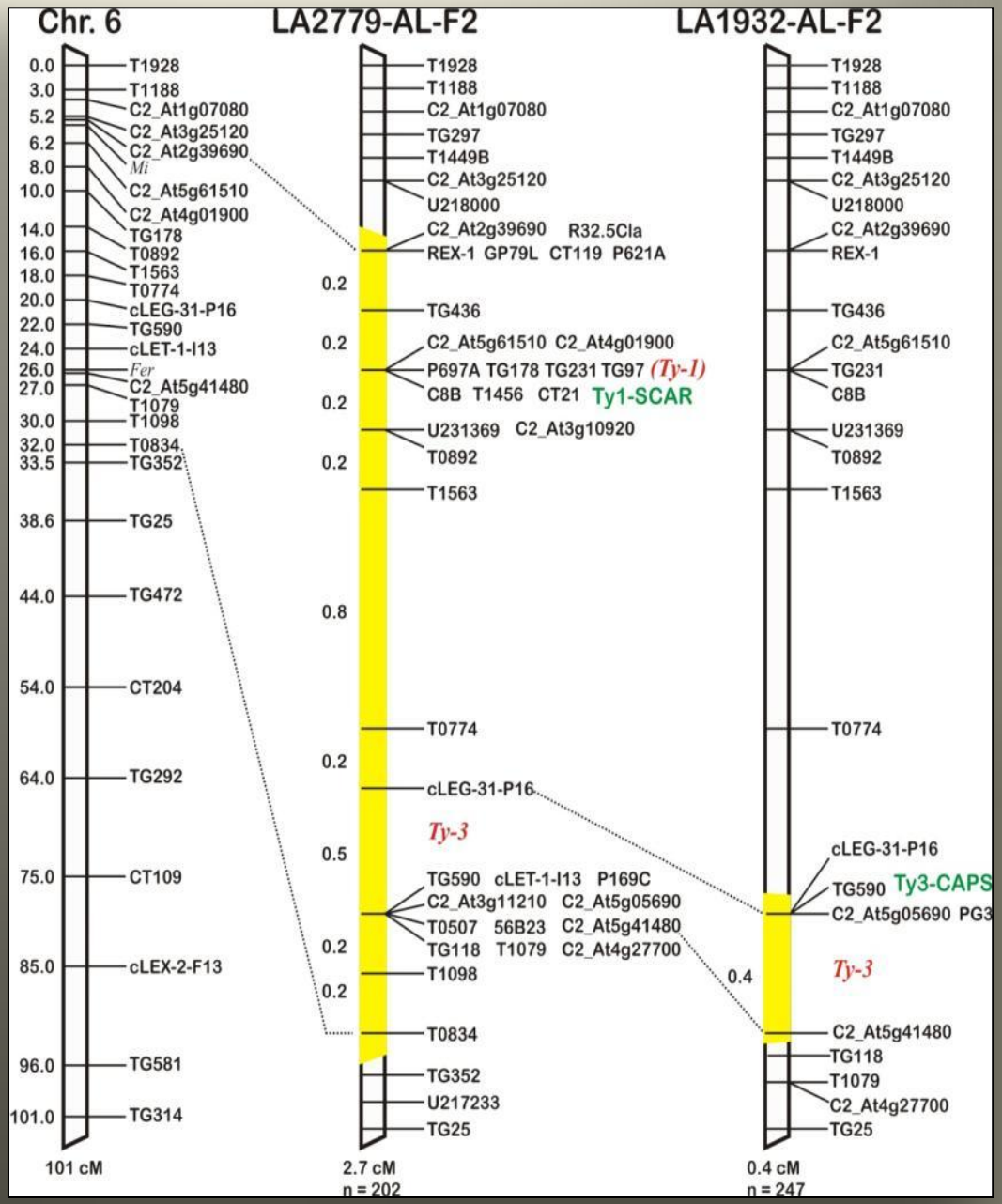
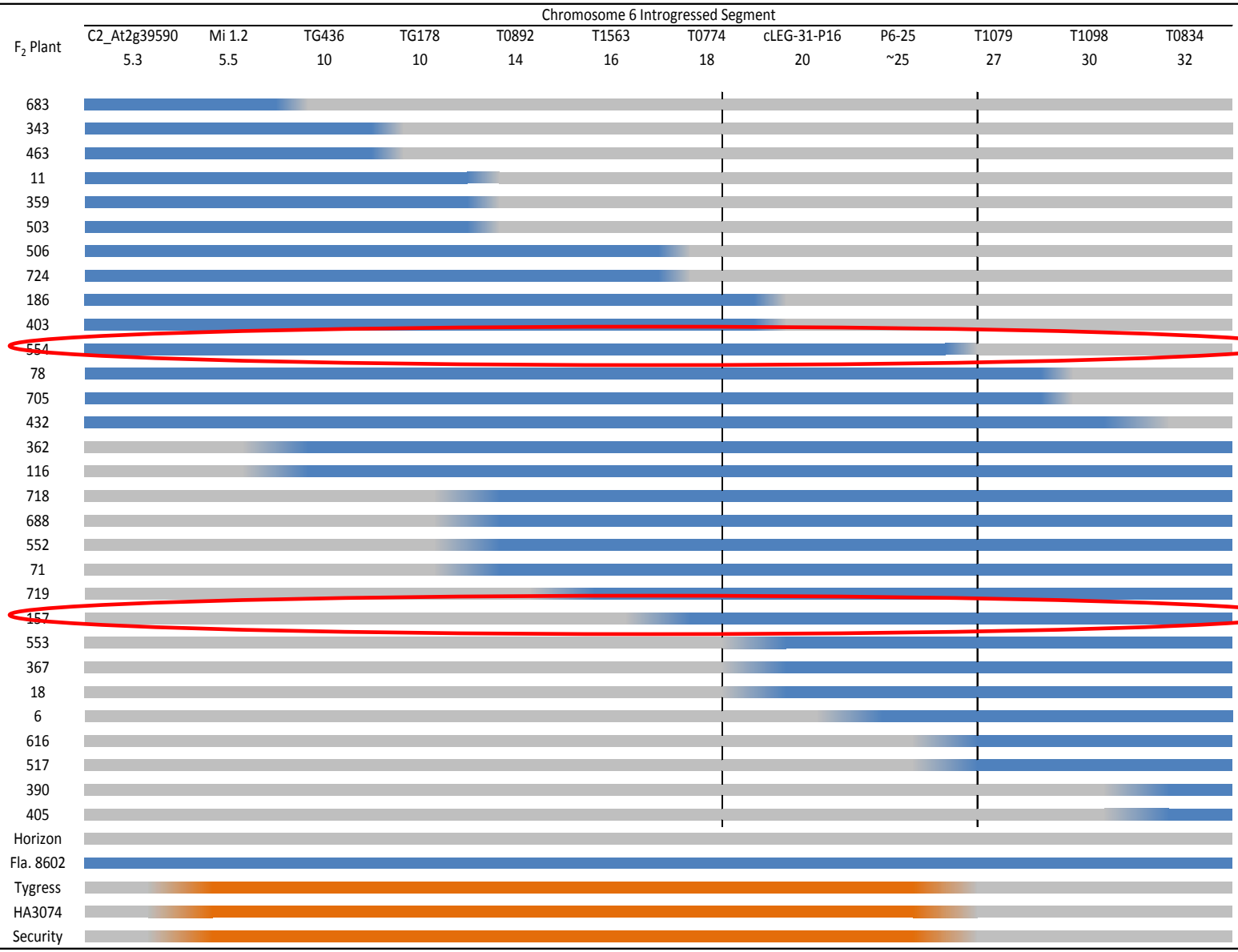


Table 1. Recombinant inbred lines (RILs) and their genotypes for the *Ty-3* region of chromosome 6.



F_2 Plant	Chromosome 6 Introgressed Segment												Category[z]	Mean RIL Ratings	
	C2_At2g39590	Mi 1.2	TG436	TG178	T0892	T1563	T0774	cLEG-31-P16	P6-25	T1079	T1098	T0834		Fall 2007	Spring 2008
	5.3	5.5	10	10	14	16	18	20	~25	27	30	32			
683													S		3.4 ab
343													TS[y]	1.8 f-i	2.5 c-e
463													S		3.3 ab
11													S	3.0 b	3.7 a
359													S	3.2 ab	3.9 a
503													RS[y]	1.2 k-n	3.0 bc
506													TS[y]	2.4 cd	3.0 bc
724													TS[y]	2.3 de	2.7 c-e
186													S	3.0 b	3.7 a
403													T	2.0 d-g	2.1 e-h
554													RT	2.1 d-f	0.9 o-r
78													R	1.3 j-m	1.0 m-r
705													RT[y]	1.1 k-n	2.1 e-h
432													R	0.8 n-p	1.6 h-n
362													RT[y]	0.6 o-q	1.6 h-m
116													R	0.6 o-r	0.9 o-r
718													RT[y]	1.3 j-l	1.9 f-j
688													R	1.1 l-m	0.9 o-r
552													RT[y]	1.2 k-n	2.0 f-i
71													R		1.5 h-o
719													R	1.0 l-o	1.1 l-r
157													T	1.8 f-i	2.4 d-g
553													R	0.9 m-o	1.5 h-o
367													RT[y]	0.9 m-o	2.0 f-h
18													RT[y]	1.0 l-o	1.6 h-m
6															
616													S	3.1 b	3.7 a
517													S	2.8 bc	
390													S	3.5 a	2.9 b-d
405													TS[y]	1.9 e-h	2.7 c-e
Horizon													S	2.8 bc	2.9 b-d
Fla. 8602													T	1.7 g-j	
Tygress													T		1.9 g-k
HA3074													R	0.4 p-s	
Security													T		1.5 h-o

[z] Resistant (R), tolerant (T), susceptible (S)

[y] Conflicting data between seasons

Ty-3 Fine Mapping

- Spring 2009: Screened ~10,500 F_2 plants for recombination

  ~300 total recombinants

  ~100 informative recombinants

Ty-3 Fine Mapping

- Spring 2009:

  Mapped within ~2.5 cM

  between M1 and M8

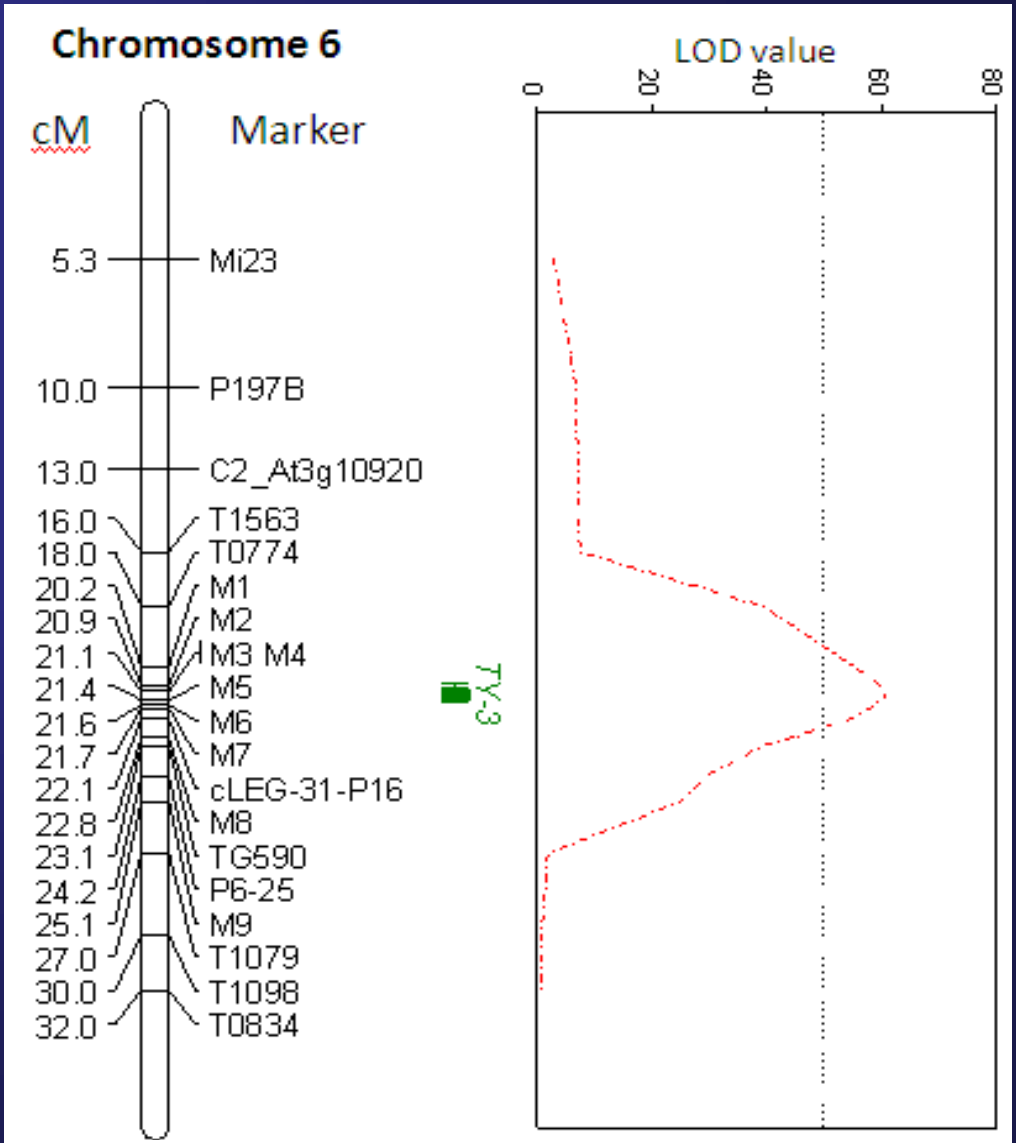


Figure 2. Mapping and interval analysis of *Ty-3* (map distance in cM) among the markers analyzed on chromosome 6; Interval analysis displaying LOD scores along Chromosome 6 for disease severity of approximately 300 recombinant plants. Map positions between 18 and 26 cM are based on recombination data from the present population.

Ty-3 Fine Mapping

- Spring 2010:

  Confirmed within 0.2 cM between M3 and M5

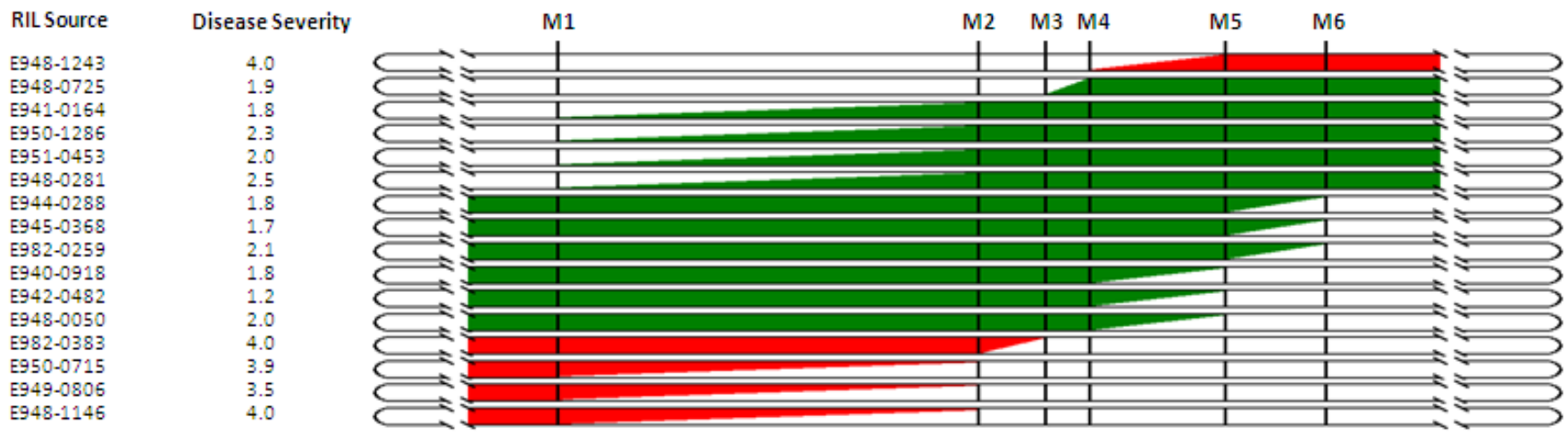


Figure 3. Evaluation of 16 RILs with recombination between markers M1 and M6. Each RIL is identified by the F_2 recombinant from which it was derived. Average disease severity for each RIL is presented. Introgression patterns are shown in green for resistant RILs and in red for susceptible RILs. Diagonally shaded intervals represent regions in which the crossover occurred.

Ty-3 Fine Mapping

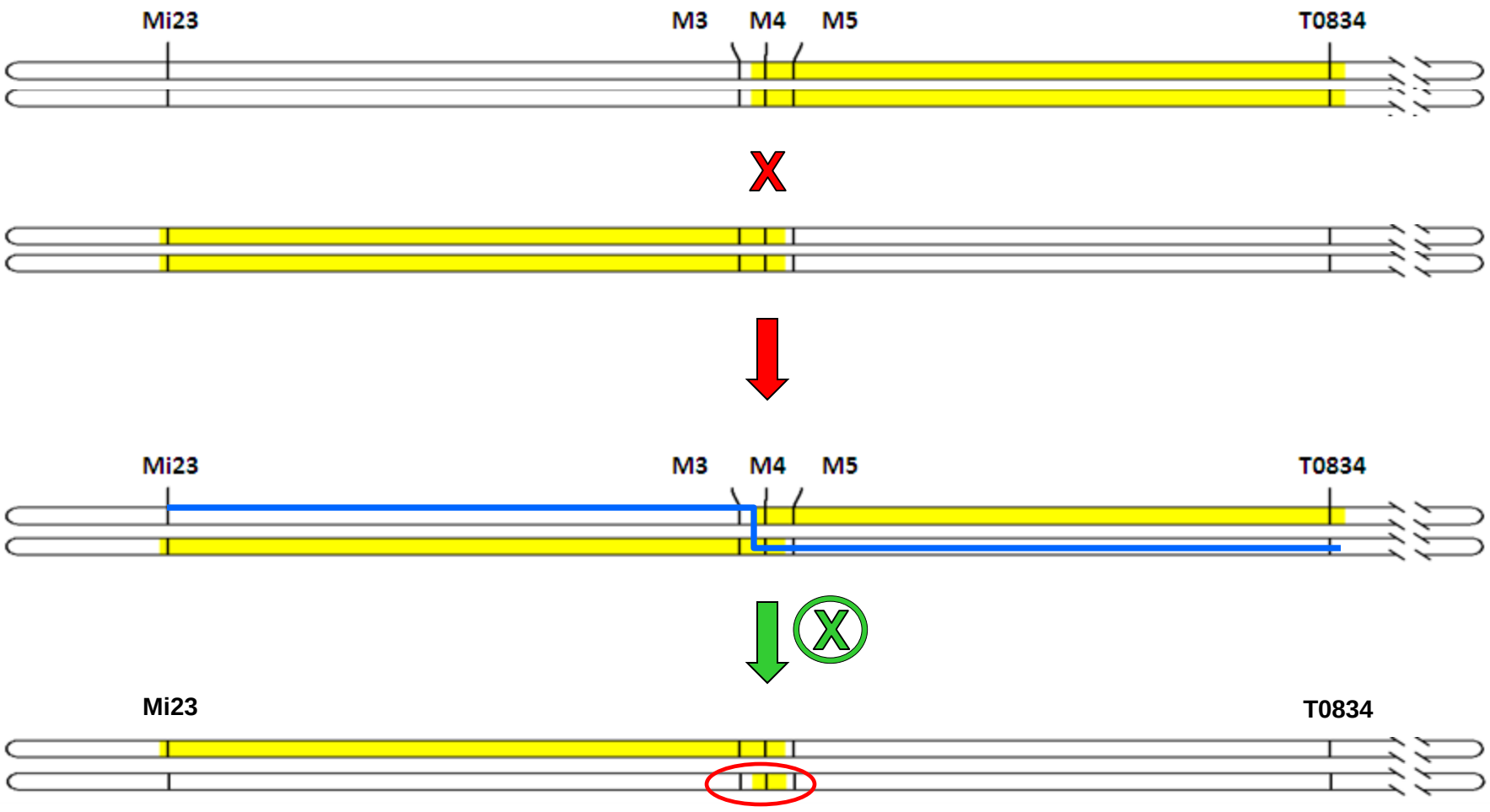
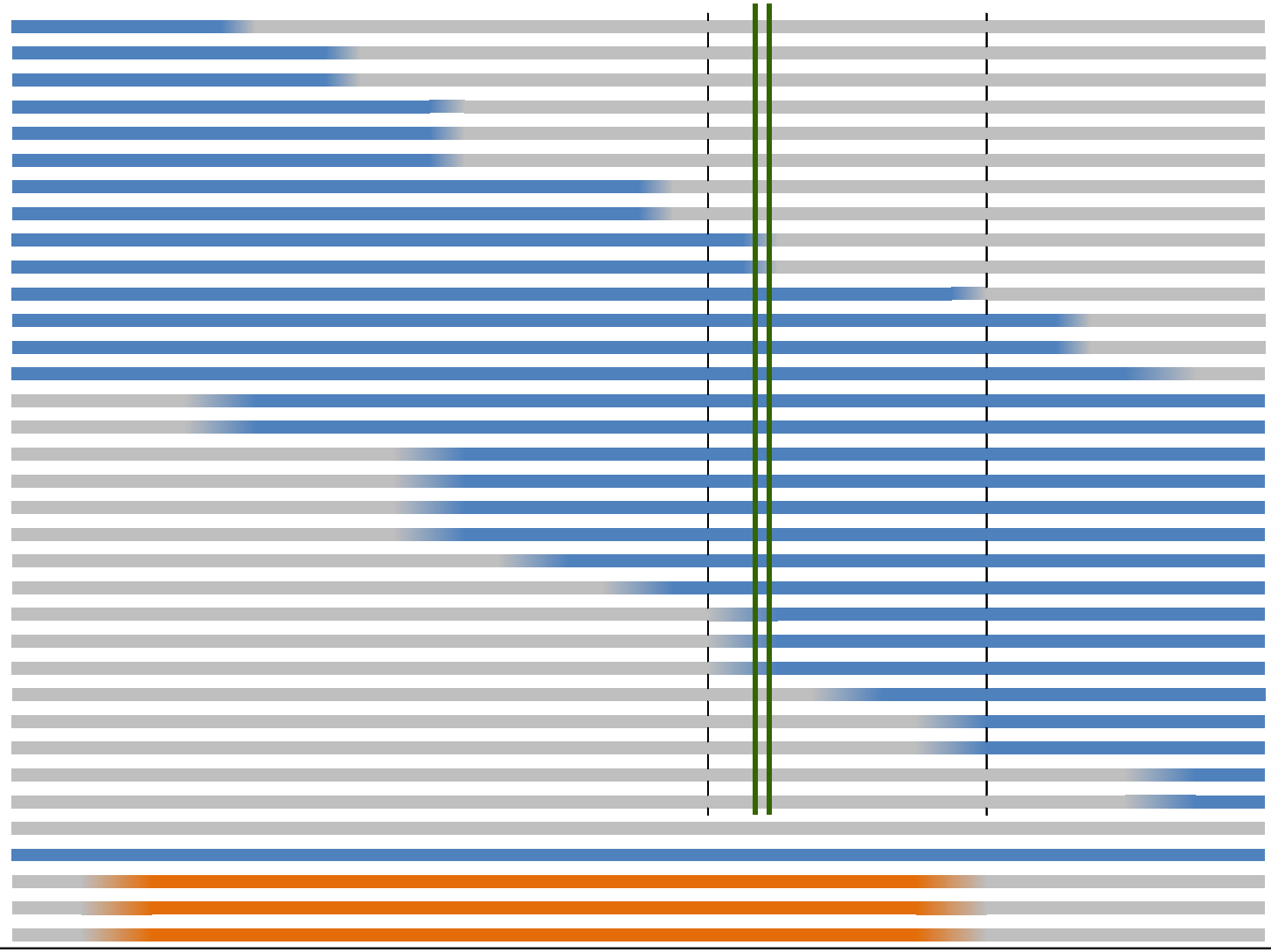


Table 1. Recombinant inbred lines (RILs) and their genotypes for the *Ty-3* region of chromosome 6.



F_2 Plant	Category[z]	Mean RIL Ratings Fall 2007	Mean RIL Ratings Spring 2008
683	S		3.4 ab
343	TS[y]	1.8 f-i	2.5 c-e
463	S		3.3 ab
11	S	3.0 b	3.7 a
359	S	3.2 ab	3.9 a
503	RS[y]	1.2 k-n	3.0 bc
506	TS[y]	2.4 cd	3.0 bc
724	TS[y]	2.3 de	2.7 c-e
186	S	3.0 b	3.7 a
403	T	2.0 d-g	2.1 e-h
554	RT[y]	2.1 d-f	0.9 o-r
78	R	1.3 j-m	1.0 m-r
705	RT[y]	1.1 k-n	2.1 e-h
432	R	0.8 n-p	1.6 h-n
362	RT[y]	0.6 o-q	1.6 h-m
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718	RT[y]	1.3 j-l	1.9 f-j
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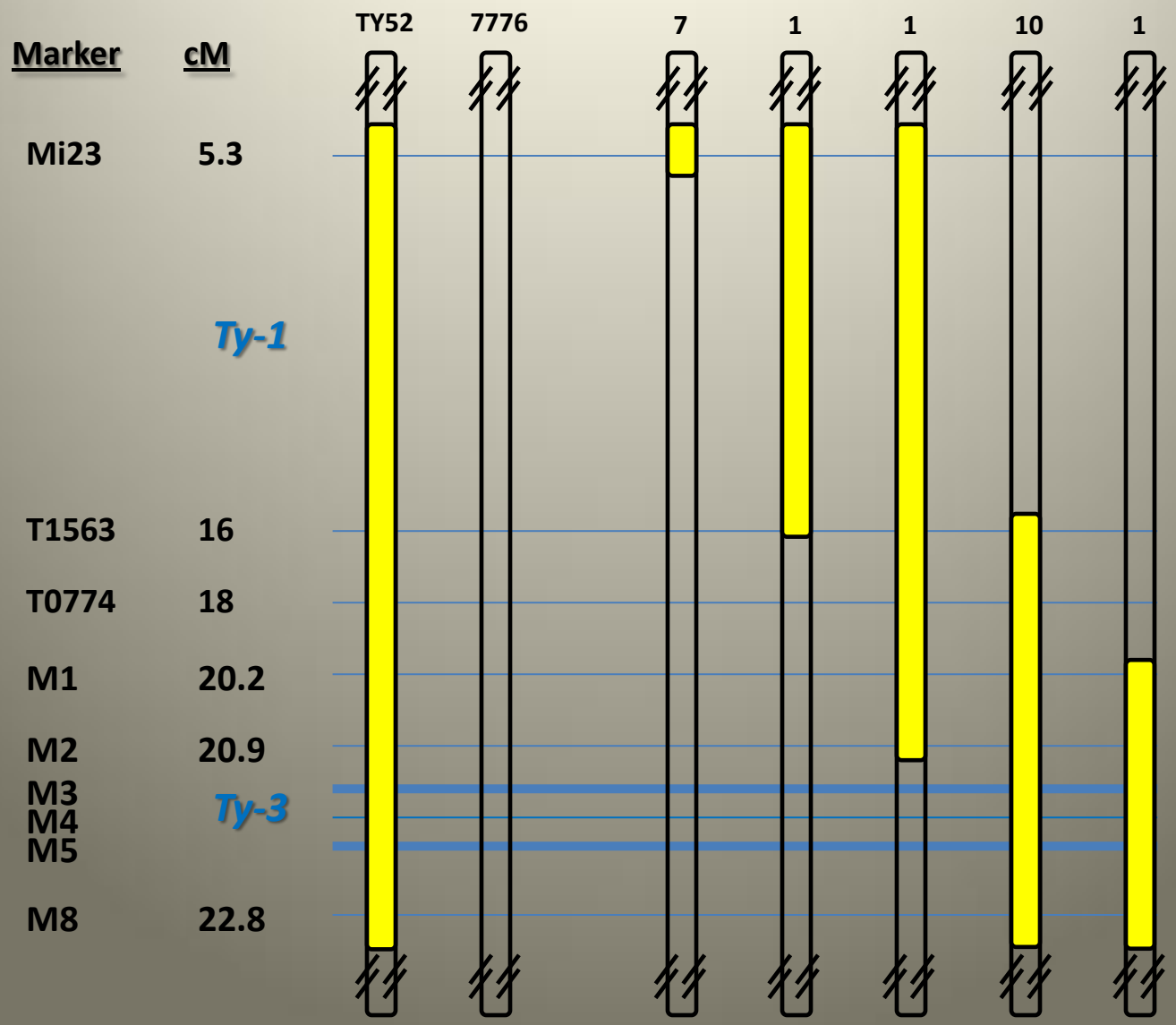
552	RT[y]	1.2 k-n	2.0 f-i
71	R		1.5 h-o
719	R	1.0 l-o	1.1 l-r
157	T	1.8 f-i	2.4 d-g
553	R	0.9 m-o	1.5 h-o
367	RT[y]	0.9 m-o	2.0 f-h
18	RT[y]	1.0 l-o	1.6 h-m
6			
616	S	3.1 b	3.7 a
517	S	2.8 bc	
390	S	3.5 a	2.9 b-d
405	TS[y]	1.9 e-h	2.7 c-e
Horizon	S	2.8 bc	2.9 b-d
Fla. 8602	T	1.7 g-j	
Tygress	T		1.9 g-k
HA3074	R	0.4 p-s	
Security	T		1.5 h-o

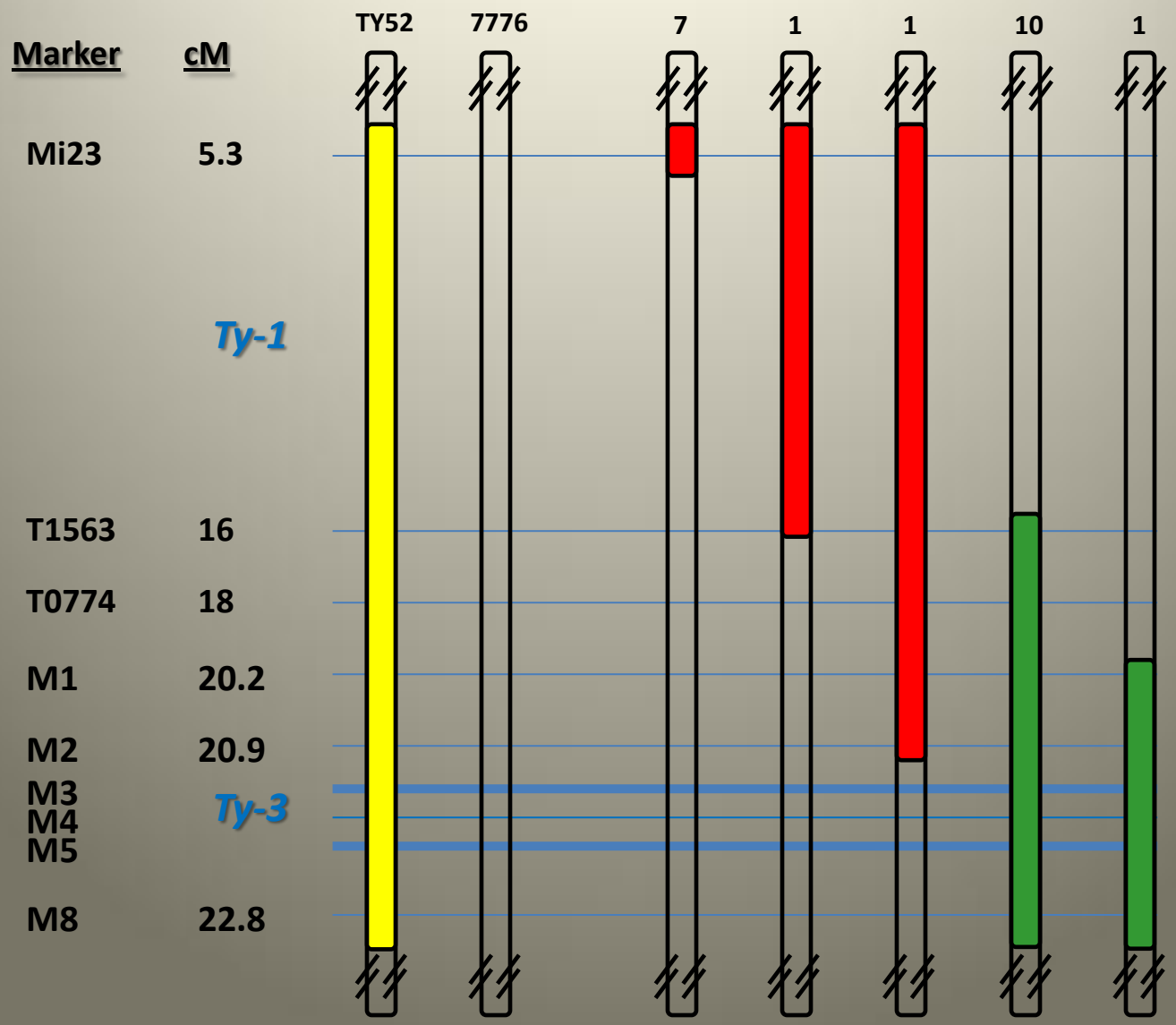
[z] Resistant (R), tolerant (T), susceptible (S)

[y] Conflicting data between seasons

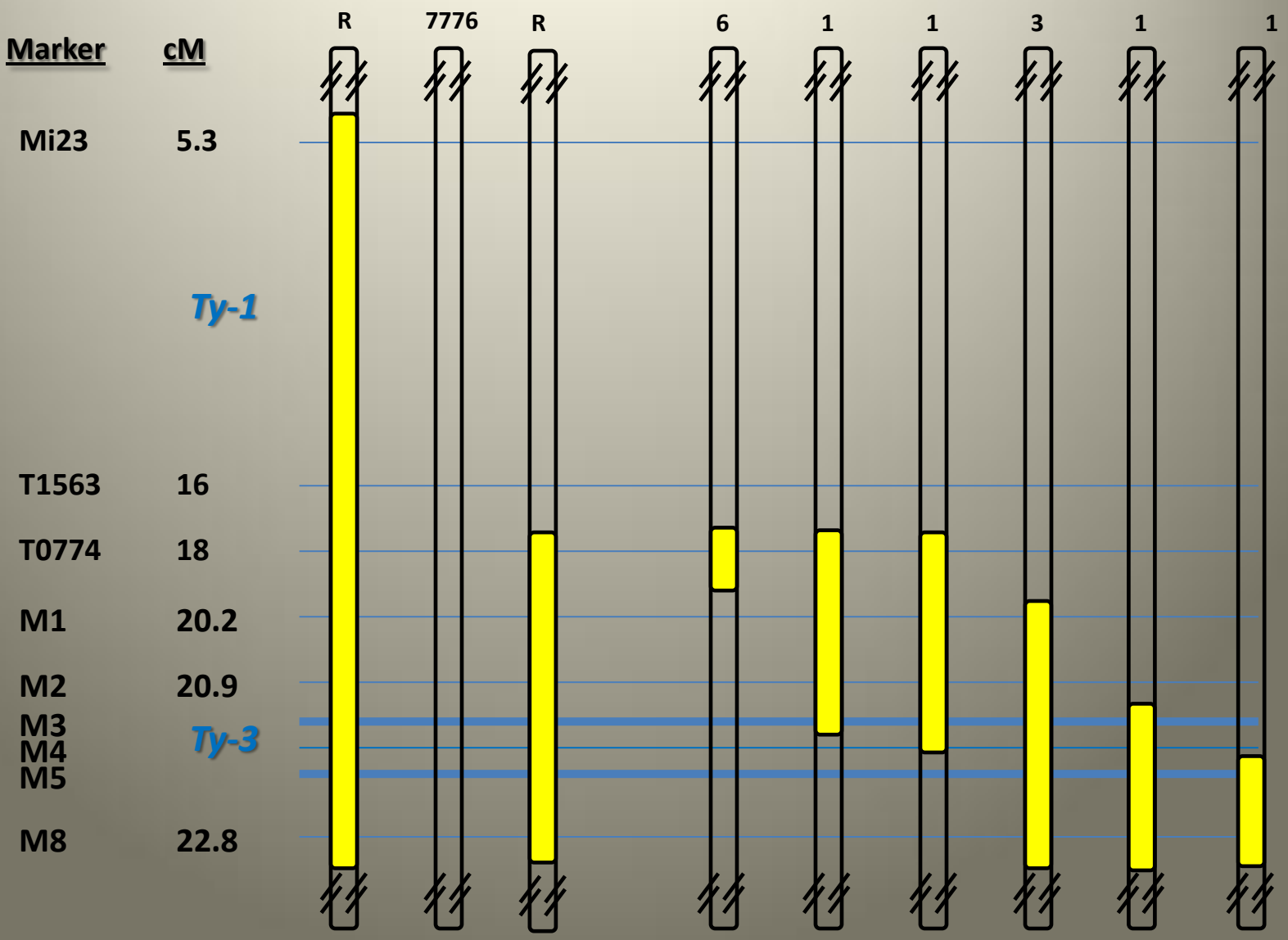
Ty-1 RILs (Ty52)



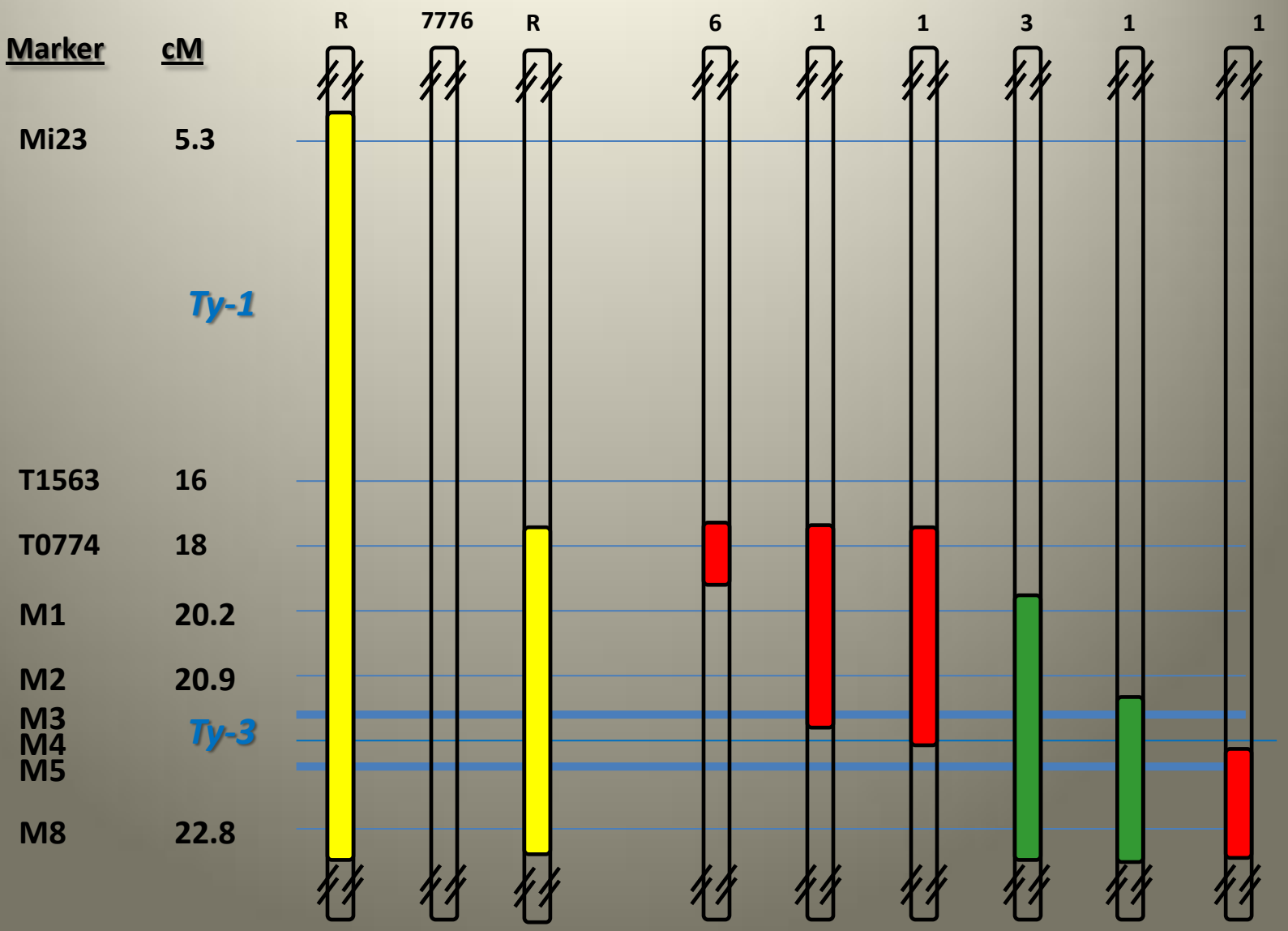
Ty-1 RILs (Ty52)



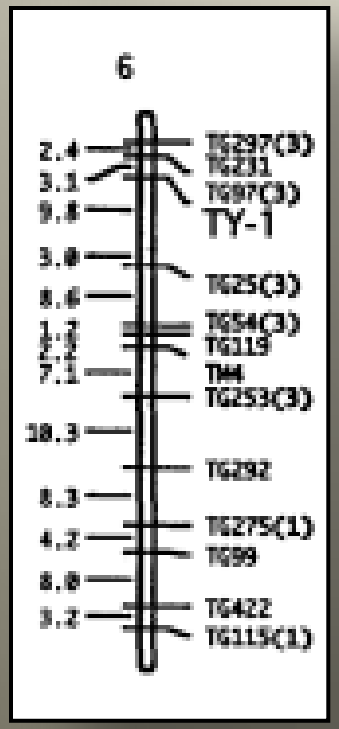
Ty-1 RILs (Commercial Hybrid)



Ty-1 RILs (Commercial Hybrid)



Ty-1



Discussion

The inheritance of TYLCV tolerance was studied extensively in classical breeding studies. The genetic basis for the tolerance in the wild species ranges from a single incompletely-dominant gene (Hassan et al. 1984; Kasrawi 1989) to a polygenic recessive pattern (Pilowsky and Cohen 1974, 1990). Our results indicate the existence of a major incompletely-dominant gene for TYLCV tolerance, (*Ty-1*), which maps to chromosome 6 and was consistently associated with the tolerance response in all the populations examined. Minor loci, whose effects were detected only in certain experiments, were mapped to chromosomes 3 and 7. The term tolerance, rather than resistance, was chosen since *Ty-1* homozygous plants can develop mild disease symptoms. The existence of a major tolerance locus on chromosome 6 is also supported by a study of a segregating population involving another tolerant wild species, *L. cheesmanii* (Zamir, unpublished).

The gene *Mi* (nematode resistance), which was introgressed into the cultivated tomato from the wild green-fruited species *L. peruvianum*, maps very close to *Ty-1*. Since *L. peruviannum* was shown to have tolerance to TYLCV we examined different nematode-resistant varieties for their TYLCV response. Lines with the *L. peruvianum* introgression spanning from TG297 to TG97 (Messeguer et al. 1991) were as susceptible as the control (data not shown). This observation, together with the results presented in this paper, suggest that *Ty-1* is located near TG97 on the centromeric side.

RFLP markers tightly linked to disease resistance genes can be used for multiple screening of genotypes in breeding programs without resorting to inoculations with the pathogens. The availability of markers for the major disease resistances in tomato (see Introduction) and the efforts directed at mapping additional genes will undoubtedly have an impact on the performance of future tomato varieties.

In recent years a molecular approach has been used to engineer viral resistances in plants. Transgenic plants expressing viral genes in sense or antisense orientations

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llow leaf curl virus

troduction

he tomato yellow leaf curl virus (TYLCV; geminirus) is responsible for severe disease of tomatoes (Con and Harpaz 1964; Czosnek et al. 1988). The virus, hich is transmitted by the whitefly *Bemisia tabaci* enn., infects tomato varieties during the summer and tumn in the Eastern Mediterranean, North and Cenl Africa and Southeast Asia (Czosnek et al. 1990). YLCV is the first reported whitefly-transmitted geni-virus to possess a single genomic molecule (Kheyrour et al. 1991; Navot et al. 1991). Its genome is comsed of 2787 nucleotides and encodes six open reading mes (Navot et al. 1991). Related gemini-viruses which fect tomato were identified in Mexico (Brown and elson 1988), Florida (Scott and Schuster 1991) and ustralia (Dry et al. 1993).

The first step in classical breeding for TYLCV resistce was to screen species in the genus *Lycopersicon* for sistance to TYLCV (Zakay et al. 1991). In field tests e wild species *L. Pimpinellifolium*, *L. hirsutum* and *L. ruvianum* all showed partial tolerance. The greenited species *L. chilense* (LA 1969), which has severe ossability barriers with the cultivated tomato (Rick 79), was completely resistant and therefore was seted as the source for our breeding project.

Mapping genes in tomato has been facilitated by the velopment of a saturated restriction fragment length lymorphism (RFLP) map (Tanksley et al. 1992). In mato the following disease-resistance genes have been apped using molecular markers: *Tm-2* (tobacco moc virus; Young et al. 1988), *Tm-1* (Levesque et al. 90), *I-2* (*Fusarium oxysporum* f. sp. *lycopersici* race 2; rfatti et al. 1989), *I-3* (*Fusarium oxysporum* f. sp. *opersici* race 3; Bournival et al. 1989), *Mi* (root knot matode; Klein-Lankhorst et al. 1991; Messeguer et al. 91), *Pto* (*Pseudomonas tomato:* Martin et al. 1991), *Sm emphylium*; Behare et al. 1990) and *Cf-2* and *Cf-9 ladosporium fulvum*; Jones et al. 1991). The mapping ategy in the above studies was based on previous

Other Projects

- BARD
  - Fine map / clone *Ty-3*
  - Fine map *Ty-4*
  - Fine map / clone *Ty-5*
  - Evaluate all combinations of *Ty-3*, *Ty-4* and *Ty-5* for effect against monopartite (TYLCV) and bipartite (ToMoV) begomoviruses
- Backcrossing
  - Parallel backcrossing of *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5* (*Ty-6*) into >40 parents
- Search for *Ty-6*
- Fine mapping of *Ty-2*

Thanks!