

Introduction. Five breeding lines are released that have begomovirus resistance gene *Ty-3* which provides resistance to tomato yellow leaf curl virus (TYLCV), the new world virus tomato mottle virus (ToMoV), and up to 7 other bipartite geminiviruses in Guatemala. We have found *Ty-3* in breeding lines derived from 2 wild species (*S. chilense*) accessions, LA1932 and LA2779. We have a co-dominant CAPS molecular marker linked very tightly to the resistance gene that works well for both sources of resistance and that we feel is the best marker we have tested over several years of research in this region of the tomato genome. Data on disease severity along with some yield information (from spring 2007) is provided (Table 1). All data are from plants inoculated with viruliferous whiteflies for 14 days when they were 3 weeks past the cotyledon stage. Also provided is the PCR banding pattern that results when the CAPS marker is used for both resistance sources (Fig. 1).

The begomovirus resistance in these lines is from *Ty-3* and a second resistance gene based on data we have obtained over the years. In plum line Fla. 726 the second gene is *Ty-4*, a gene we have recently mapped to chromosome 3. A manuscript is in preparation on this gene. A robust SCAR marker P137A (Fig. 2) is tightly linked to this gene. Incorporation of *Ty-3* and *Ty-4* from Fla. 726 can be done using marker assisted selection (MAS). Resistance levels were high with these 2 genes combined in various combinations of heterozygosity and homozygosity (Table 2). The ability to attain this resistance by MAS is considered to be a huge breakthrough that should greatly facilitate the utilization of these genes by themselves or in conjunction with other genes such as *Ty-1* and/or *Ty-2*. Other resistances of the five breeding lines are summarized (Table 3). A fusarium wilt race 1 test was not done but it is assumed that all are resistant to this race. Information on verticillium wilt race 1 for Fla. 678 is underway (11/13/08) and will be provided to companies that acquire this material.

Also in the contract we mention that we will provide royalty-free markers tightly linked to the *Ty-1* and *Ty-2* genes. The 2 markers are designated P197B and P137A, respectively. Both are SCAR markers.

Table 1. Begomovirus resistant; tomato yellow leaf curl virus (TYLCV) and tomato mottle virus (ToMoV), tomato breeding lines with large round, plum, or cherry fruit types from two resistance sources. All have the *Ty-3* resistant gene and linked molecular marker.

Designation	Resistance Source	Begomovirus resistance ^z				Yield (kg/plant)	Fruit size (g)
		TYLCV		ToMoV			
		Spring '07	Fall '07	Spring '07	Fall '07		
<u>Large Fruited</u>							
Fla. 678	LA2779	1.62 c ^y	0.0	0.15 f ^y	0.0	3.39 b ^x	147.3
Fla. 701	LA1932	1.52 c	0.5	0.45 d-f	0.38	4.94 a	168.5
<u>Plum</u>							
Fla. 685	LA2799	1.33 cd	1.38	0.71 e	0.25	4.28 a ^x	71.6
Fla. 726 ^w	LA1932	0.91 e	0.72	1.5f	0.81	1.29 b	61.8
<u>Cherry</u>							
Fla. 707	LA1932	0.5 f	0.88	0.77 c-e	0.5	2.54	13.1

^z Rated on a 0-4 scale where 0 = no disease, 1 = slight symptoms visible only on close inspection, 2 – symptoms on part of plant visible from 2/3 m, 3 = severe symptoms on entire plant, and 4 = severe symptoms and stunting. In the tests our susceptible controls were >3.

^y Mean separation to column by Duncan's Multiple Range test at $P \leq 0.05$ based on a larger number of genotypes.

^x Mean separation as in ^y except in column within fruit type, not entire column.

^w Fla. 726 was segregating for the *Ty-4* gene when these data were obtained.

Table 2. ANOVA for the association between markers C2_At4g17300 on chromosome 3 (Ty-4) or P6-25 on chromosome 6 (Ty-3) and the average disease severity of progeny from a plant heterozygous for both *S. chilense* introgressions on chromosome 3 and 6, respectively, which were inoculated with tomato yellow leaf curl virus (TYLCV)

Genotype ^y		Number observed	Disease Severity ^z	
C2_At4g17300	P6-25 ^x		Mean	Range
-	-	16	2.66	2-3.5
-	±	23	0.96	0-2.0
-	+	9	0.72	0-1.5
±	-	21	1.83	0-3.0
±	±	64	0.41	0-2.0
±	+	21	0.07	0-0.5
+	-	13	1.85	1-2.5
+	±	23	0.43	0-1.5
+	+	11	0.23	0-1.5

^z Rated 58 days after inoculation began on same scale as Table 1.

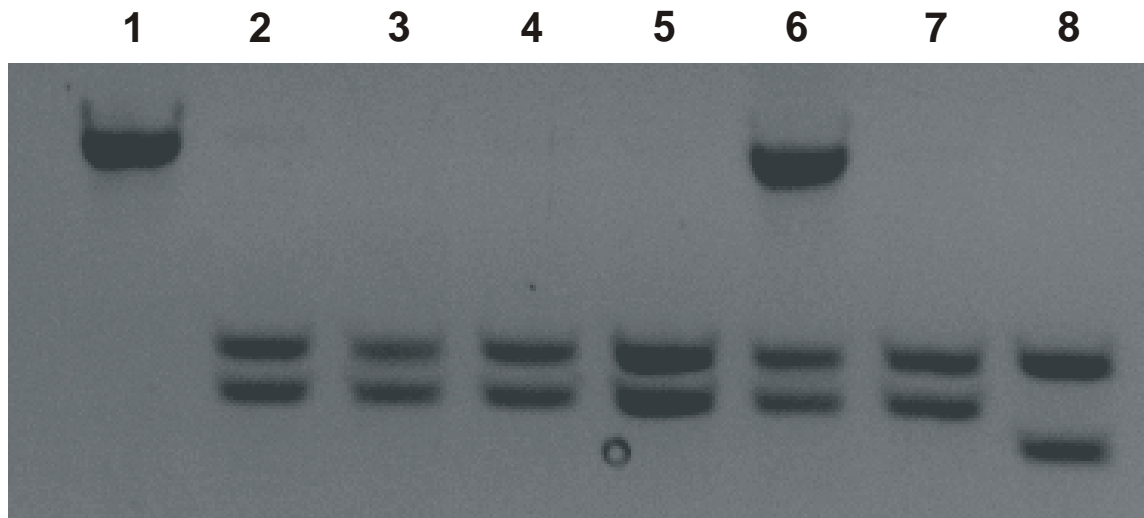
^y +, Homozygous for *Solanum chilense* allele; -, homozygous for the *S. lycopersicum* allele; ±, heterozygous

^x A SCAR marker designed from the BAC clone 56B23, forward, GGTAGTGGAAATGATGCTGCTC, reverse, GCTCTGCCTATTGTCCCATATATAACC (Ji *et al.* 2007a)

Table 3. Disease resistance statue of TYLCV breeding line releases with the Ty-3 gene.

Line	Disease		
	Fusarium wilt race 2	Verticillium wilt	Gray leafspot
Fla. 678	R ^z	S	R
Fla. 701	R	R	R
Fla. 685	R	R	R
Fla. 726	R	S	R
Fla. 707	G	R	R

^z R= resistant, S = susceptible, G = Segregating



Figure

1. PCR fragments with primer TY3-CAPS: lane 1 is susceptible tomato line Fla. 7781; lane 3 is *S. chilense* accession LA1932; lanes 2, 4, 5, and 7 are breeding lines derived from LA1932, lane 6 is a heterozygous hybrid with a LA1932 derived resistant parent, and lane 8 is a breeding line derived from LA2779. Band sizes are 950bp for susceptible tomato, 500 and 350bp for LA2779 lines, and 500 and 450bp for LA1932 derived lines.

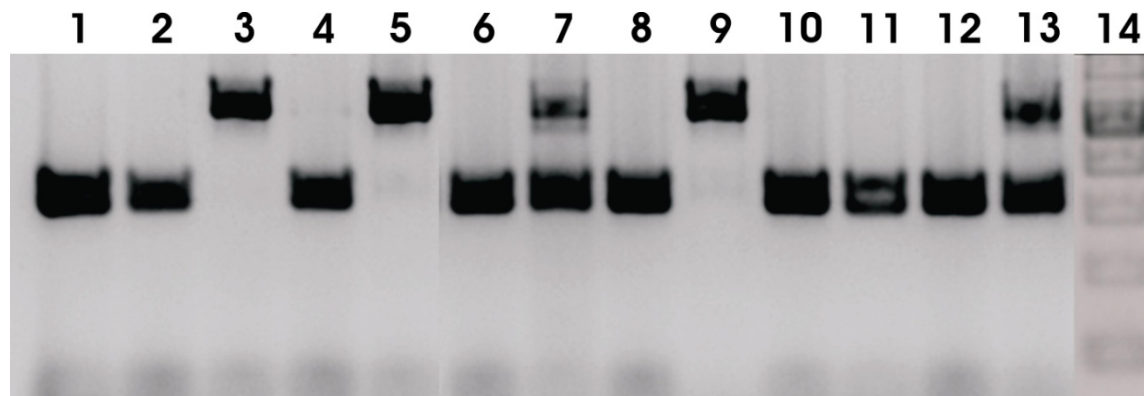


Figure 2. PCR fragments with marker P137A. Band sizes are ~350 bp for *S. lycopersicum* line 6013 (Lane 1) and ~550 bp for *S. chilense* accessions LA2779 (lane 3) and LA1932 (Lane 5), respectively. Lanes 7, 9, 11, and 13 are advanced breeding lines derived from LA2779/LA1932 [note they do not all carry *Ty-4*], and the lanes with an even number are advanced breeding lines derived from LA1938/Tyking except for lane 14, which is 100 bp DNA Ladder (New England Biolab, USA).

Information on Markers linked to begomovirus resistance genes.

Ty-3 refers to the resistance allele from LA2779 and Ty-3a to the resistance allele from LA1932 (see Ji et al., 2007 Rept. Tomato Genetics Coop. 57:25-28). P6-25 (not being licensed) is a scar marker derived from the left end of BAC 56B23, and TY3-CAPS (being licensed) is a CAPS marker derived from a potential gene sequence (#8) in BAC 56B23. Both markers can differentiate among tomato (*ty-3*), LA2779 (*Ty-3*) and LA1932 (*Ty-3a*). TY3-CAPS can detect

all TYLCV resistant lines derived from LA1932, but some of the LA1932-derived resistant lines show the susceptible tomato allele for the P6-25 marker. Both markers can detect all LA2779-derived resistant lines.

Lane 8 of Figure 1 is a LA2779-derived line carrying the Ty-3 allele and lanes 2-7 are lines derived from LA1932 carrying the Ty-3a allele (to be consistent with the terminology published for P6-25 in TGC 2007 Volume 57).

TY3-CAPS is at ~25cM and the *Ty-3* gene is most likely between T0774 (18 cM) and cLEG-31-P16 (20cM) according to the most recent data (The RIL experiments and some data from Sam). So this marker is ~5 cM away from the *Ty-3* gene. But between TY3-CAPS and cLEG-31-P16, only one recombinant was observed among 940 F2 progenies. So if talking distance in the introgression, it should be 1/1880 cM. When introgression segment turns smaller, the recombination will be even much more reduced as shown from one of my study from UCDAVIS. P137A is for *Ty-4* gene on chromosome 3 and it is about 3.3 cM away from the *Ty-4* gene.