

## Detection methods for the *Ty-1* gene for resistance to begomoviruses on chromosome 6 of tomato

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The first major gene to be mapped for resistance to begomoviruses in tomato was the *Ty-1* gene introgressed from the wild tomato *Solanum chilense* LA1969. The introgression containing this gene was mapped to chromosome 6 between TG297 (4.0 cM Tomato-EXPEN 2000 and 6.0 cM Tomato-EXPEN 1992) and TG97 (8.6 cM Tomato-EXPEN 1992) (Zamir et al., 1994). There has been no published fine mapping for this gene. Most resistant commercial cultivars to Tomato leaf curl disease have the *Ty-1* gene (Pérez de Castro et al., 2007). For example, Garcia and Maxwell (unpublished data) detected *Ty-1* gene in the cv. Marwa and Pérez de Castro et al., 2007) reported the presence of *Ty-1* gene in cv. Boludo and Anastasia. Thus, a method to detect *Ty-1* gene in breeding lines would be advantages.

The *Ty-1* gene is located in a “hot-spot” for resistance genes as described by Pérez de Castro et al. (2007). This “hot-spot” is now to have genes for resistance to *Alfalfa mosaic virus* (*Am* gene, Parrella et al., 2004), powdery mildew (*OI-1* gene, Huang et al., 2000), *Cladosporium fulvum* (*Cf-4* gene, Thomas et al., 1997), *Ralstonia solanacearum* (Wang et al., 2000), and *Meloidogyne* spp. (*Mi-1* gene, see Sean et al., 2007). Since the *Mi-1* gene for root-knot nematode resistance and the *Ty-1* gene have been mapped to this “hot-spot”; and thus, it was expected that it would be difficult to obtain *Mi-1* and *Ty-1* gene in phase. However, Seminis Vegetable Seeds filed a patent in December 2005 for plants that have *Mi-1* and *Ty-1* in coupling phase (denied as of May 2007). In the tomato breeding program at San Carlos University, Guatemala, Gh2 was found to have the *Mi-1* and *Ty-1* loci in phase (Mejía et al., 2005, unpublished data). Several lines of evidence indicate that *Mi-1* gene is near 6 cM and *Ty-1* gene is near 8 cM on the Tomato-EXPEN 2000 map (SGN web site). One complication in developing PCR-based assays for either of these resistance genes is that the introgression associated with *Mi-1* gene (*S. peruvianum*) and that associated with the *Ty-1* gene (*S. chilense*) can overlap each other in this region of chromosome 6 for some germplasm. This is evident as indicated by sequence analysis for the *Mi-1* gene CAPS marker REX-1 (El Mehrach et al., 2005; Williamson et al., 1994). The REX-1 sequence from Motelle (*Mi/Mi*) is *S. peruvianum* and for TY52 (*Ty-1/Ty-1*, LA3473) is *S. chilense* (El Mehrach et al., 2005). Messeguer et al. (1991) reported the introgression associated with the *Mi-1* locus to be between TG297 and TG231 (6.0 cM and 8.6 cM, respectively, Tomato-EXPEN 1992) for VFNT cherry and VFN 8, and this is essentially the same region reported by Zamir et al. (1994) for the introgression for *Ty-1* gene.

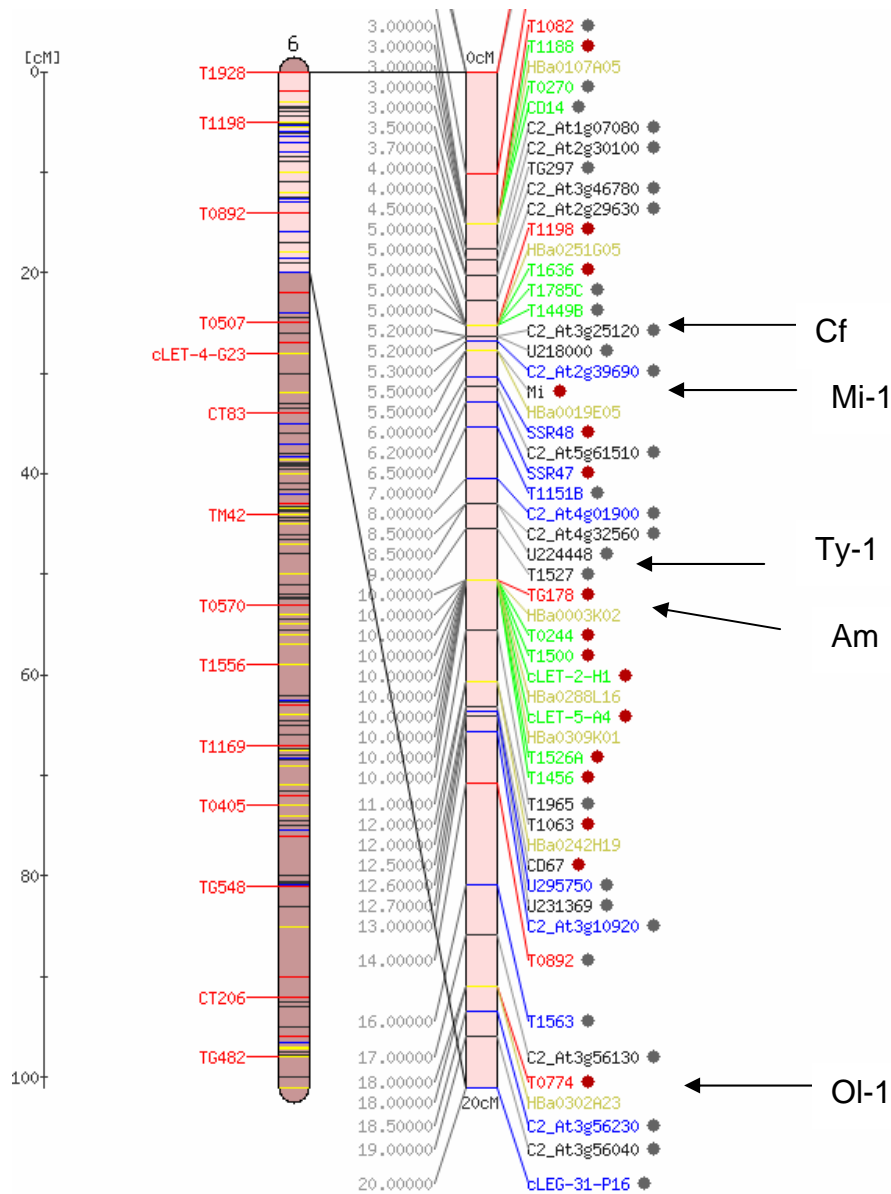
The first report of a PCR-based marker for *Ty-1* gene was by Milo (2001) and involved the *REX-1* locus, where three bands were obtained with a *TaqI* digestion of the PCR fragment instead of two bands as for the *Mi-1* locus (*S. peruvianum*). No *TaqI* sites are associated with *S. lycopersicum* sequence for the *REX-1* locus. The *REX-1* locus (may be 5 cM) appears to be telomeric to the *Mi-1* locus (Maxwell, unpublished data). Pérez de Castro et al. (2007) reported the use of a *TaqI* CAPS marker for CT21 (8.6 cM) using primers JB1F and JB1R, and this marker might avoid some of the problems associated with a marker closer to the *Mi-1* gene, such as REX-1.

The following reports compare four methods for the detection of *Ty-1* locus in breeding lines from the tomato breeding program at San Carlos University, Guatemala (Mejía et al., 2005). The first (Protocol I) is a TG97 CAPS marker (ca. 8 cM) (developed from information from H. Czosnek and F. Akad, unpublished). The second (Protocol II) is a co-dominant SCAR marker, P6-6 (ca. 6 cM). The third (Protocol III) CAPS marker uses the Mi23 co-dominant SCAR marker for the *Mi-1* locus (ca. 6 cM). Finally, there is an evaluation of the use primers developed by Pérez de Castro et al. (2007) for the JB-1 locus (CT21, ca 8 cM) ((Protocol IV).

As of August 2007, a co-dominant SCAR marker for the region near 8-9 cM has not been found.

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Chromosome 6 from the SGN web site for EXPEN 2000 map of tomato. The *Ty-3* locus is near 25 cM and the Bacterial Wilt locus is near 35 cM. Also another locus for begomovirus resistance is below 35 cM.