

Co-dominant SCAR marker for detection of the begomovirus-resistance *Ty2* locus derived from *Solanum habrochaites* in tomato germplasm

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Introduction: Begomoviruses are a major threat to tomato production in most subtropical and tropical regions (Anderson and Morales, 2005). These geminiviruses are transmitted by the whitefly (*Bemisia tabaci*) complex and thus, many management strategies have focused on use of insecticides. Integrated Pest Management strategies include virus-free seedlings, host-free periods, management of time and location of planting, and begomovirus-resistant hybrids (Salati et al., 2002). Breeding begomovirus-resistant hybrids has involved the introgression of resistance genes from wild tomato species. Hanson et al. (2000) mapped the introgression from *Solanum habrochaites*, which conditioned resistance to *Tomato leaf curl virus*, to the long arm of chromosome 11 between TG36 (84 cM) and TG393 (103 cM) in the breeding line H24. This resistance gene was designated *Ty2* (Hanson et al., 2006) and was reported to be associated with an introgression from TG36 (84 cM) to TG26 (92 cM). Maxwell and Mejía (2002, unpublished data) developed PCR primers from the sequence of the TG105A (90 cM) marker and amplified PCR fragments from the susceptible tomato, M82-1-8 (EU053434), H24 (EU053432) and *S. habrochaites* LA1223 (EU053433). Sequence alignments showed that H24 was nearly identical to the *S. habrochaites* and very different from M82-1-8. Recently, Garcia and Maxwell (unpublished data) sequenced PCR fragments for the marker TG105A for begomovirus-resistant breeding lines with resistance derived from *Solanum chilense* and found that these lines had an introgression in this region (eg., Gc143-2, EU053431). This introgression was very different from the one in H24, and it might be an introgression for the *I2* gene (91.5 cM), which is present in Gc143-2. Because of this potential confusion, it was decided to search for a better marker for the *Ty2* locus. PCR primers from the SGN website for T0302 (89 cM), C2-At5g25760 (89.5 cM), and Hba78A16T7 (89.7 cM) were used. This report describes two co-dominant SCAR markers developed from the sequence of the RFLP marker T0302 for lines with the *Ty2* locus.

PCR methods: DNA was extracted from fresh leaves of plants with PUREGENE® DNA Purification Kit (Gentra Systems, Inc., Minneapolis, MN) and DNA adjusted to approximately 10 ng/µl. PCR parameters were for 25-µl reactions containing 2.5 µl 2.5 mM dNTPs, 5 µl 5x buffer, 2.5 µl 2.5 mM MgCl₂, 0.1 µl (0.5 units) GoTaq DNA polymerase (Promega Corp., Madison, WI), 2.5 µl each forward and reverse primer at 10 µM, 2-5 µl of DNA extract, and water. PCR cycles were 94 C for 4 min, 35 cycles of 94 C for 30 sec, 55 C for 1 min or 53 C for 1.5 min, and 72 C for 1.5 min. These cycles were followed by 72 C for 10 min, and then the reaction was held at 4 C. PCR reactions were performed in the MJ DNA Engine PT200 Thermocycler™ (MJ Research Inc.,

Waltham, MA). PCR-amplified fragments were separated by gel electrophoresis with 1.5 or 2% agarose in 0.5 X TBE buffer and stained with ethidium bromide and visualized with UV light. ssDNA was digested in PCR reactions with shrimp alkaline phosphatase (Promega Corp.) and exonuclease I (Epicentre, Madison, WI), and the PCR-fragments directly sequenced with Big Dye Sequencing Kit™ and analyzed by the Biotechnology Center, University of Wisconsin-Madison. The SGN primers for T0302 (T0302F/T0302R) and TY2R1 were used at extension of 55 C and those for C2-At5g25760 (C2-11-89F/C2-11-89R), and Hba78A16T7 (BAC-11-89F/BAC-11-89R) were used at extension of 53 C. The SGN primers for T0302 are:

TG0302F, 5' TGGCTCATCCTGAAGCTGATAGCGC
 TG0302R, 5' AGTGTACATCCTTGCCATTGACT

A reverse primer was designed from the sequence of the T0302 fragment:

TY2R1, 5' TGAT(T/G)TGATGTTCTC(T/A)TCTCT(C/A)GCCTG

Results and Discussion: Initial comparisons involved the susceptible line, M82-1-8 (*ty2/ty2*), and the resistant line, H24 (*Ty2/Ty2*). The PCR fragment sizes for the markers C2-At5g25760 and Hba78A16T7 were ca. 1.3 kb and 760 bp, respectively. Interestingly, the PCR primers for the T0302 marker gave ca. 800-bp and ca. 900-bp fragments for M82-1-8 and H24, respectively (Fig. 1, lane 2 and 4).

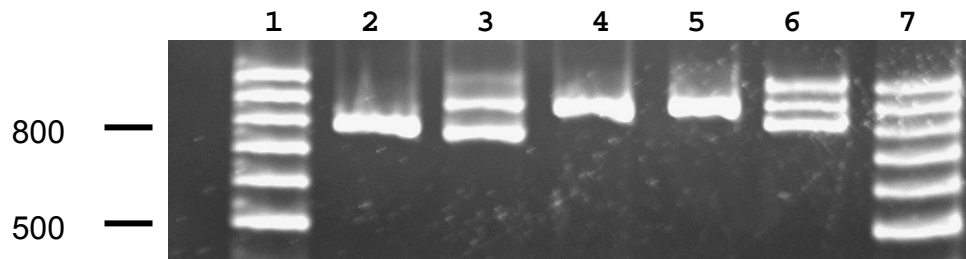


Fig. 1. PCR fragments with the *Ty2* locus primers for the T0302 marker. Lane 1, 100-bp marker (Promega Corp.); lane 2, M82-1-8 (susceptible), lane 3, TyQueen (hybrid, resistant to begomoviruses); lane 4, H24 (resistant to begomoviruses); lane 5, *S. habrochaites* LA0386; lane 6, *S. habrochaites* LA1777; lane 7, 100-bp marker.

The sequences for the three markers (T0302, Hba78A16T7, and C2_At5g25760) for the susceptible line M82-1-8 (EU046613, EU053430, EU053428, respectively) were different from the respective marker sequences from the resistant line H24 (EU046610, EU053429, EU053427, respectively). These results clearly indicated that H24 had an introgression at these three marker locations. Since the marker T0302 seemed to have the greatest potential as a SCAR marker for the *Ty2* locus, PCR fragments from two additional begomovirus-resistant lines (G1h902 and Gc143-2), which had begomovirus-resistance genes (*Ty1* and *Ty3* loci on chromosome VI, Maxwell et al., 2006) most likely derived from *S. chilense*, were sequenced. The sequences for G1h902 and Gc143-2 for this T0302 fragments (792 bp) were identical to those of M82-1-8. This was in contrast to the sequences for the TG105A marker, where the Gc143-2 and G1h902 sequences were identical but different from the sequence for M82-1-8. Both Gc143-2 and G1h902 have tested positive for the *I2* gene and M82-1-8 tested negative for this gene (Salus and Maxwell, pers. com.).

When the sequences for the T0302 PCR fragment for M82-1-8 and H24 (909 bp) were compared, there were 36 SNPs, and four indels (four indels of 1 nt, one of 2 nt,

one of 3 nt, and one of 120 nt). The sequence for the T0302 marker from *S. habrochaites* LA0386 (909 bp, EU046611) was identical to the sequence from H24. The sequence for the T0302 marker (789 bp, EU046612) was also obtained for another accession, *S. chilense* LA2779, that has been used as a source of begomovirus-resistance genes (Agrama and Scott, 2006); and the T0302 sequence for LA2779 was different from those for both M82-1-8 and H24. Most notably, the 120-nt indel associated with the fragments from M82-1-8 and H24 was not present between the M82-1-8 and LA2779 sequences for T0302. These results support the presence of an introgression from *S. habrochaites* in H24 at this marker.

This primer pair T0302F/T0302R produced two bands (800 and 900 bp) for the commercial hybrid TyQueen (Green Seeds Co., Vietnam), which is reported to have *Tomato yellow leaf curl virus*-resistance derived from *Ty2* gene. When tested in Guatemala in October 2006, TyQueen was moderately resistant to begomoviruses. When this primer pair was tested with additional germplasm with the *Ty2* locus from the Asian Vegetable Research and Development Center (AVRDC), CLN2460E gave the 900-bp fragment, and two lines that had shortened introgressions for the *Ty2* locus (P. Hanson, pers. com.) also gave one 900-bp fragment. Fifty-nine breeding lines and hybrids have been evaluated with these primers (T0302F/T0302R) and no unexpected results were obtained.

A reverse PCR primer, TY2R1, was designed from the alignment of the sequences for M82-1-8 and H24 in order to produce smaller fragments for both the susceptible and resistant genotypes. The PCR primers T0302F/TY2R1 gave a 600-bp fragment with two AVRDC lines with the *Ty2* locus, and a 450-bp fragment for three inbred lines and one hybrid all of which lacked the *Ty2* locus. Three F1 plants known to be heterozygous for the *Ty2* locus all gave three PCR fragments, 450, 600, and 700 bp (Fig. 2). The sequences of the 450- and 600-bp fragments were as expected. The 700-bp fragment is most likely a heteroduplex between the 450- and 600-bp fragments.

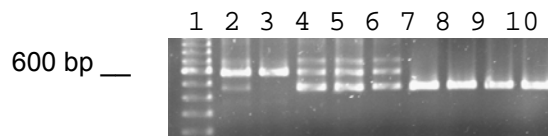


Fig. 2. Lane 1, 100 bp marker (Invitrogen, brighter band is 600 bp); Lane 2, AVRDC line with shortened *Ty2* introgression; Lane 3, H24; Lane 4, TY-Queen (*Ty2/ty2*); Lane 5 and 6, F1 plants, *Ty2/ty2*; Lane 7, susceptible HJJ-VF; Lane 8, TY172 and Lane 9, TY197, Lane 10, Shanty (Hazera Seeds). Note: TY172 and TY197 are resistant to begomoviruses, but lack the *Ty2* locus. HJJ-VF is an inbred line from Hebrew University of Jerusalem.

Conclusions: The two sets of primers, T0302F/T0302R and T0302F/TY2R1, effectively detected the two genotypes, *ty2/ty2* and *Ty2/Ty2*, and the T0302F/TY2R1 primer set also gave clearer bands with the heterozygous plants than the T0302F/T0302R primers. No false positives were detected, when 59 inbred lines and hybrids were evaluated. But it is possible that this marker might not detect all lines that have the *Ty2* gene, since it is not known how closely linked this marker is to the *Ty2* gene. These T0302 primers are a better marker than a TG105A CAPS marker (Graham and Hanson, pers. com.), as the latter marker may also detect an introgression that might be associated with the *I2* gene, and this would result in false positives.

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