Co-dominant SCAR Marker, P6-25, for Detection of the *ty-3*, *Ty-3,* and *Ty-3a* alleles at 25 cM of Chromosome 6 of Tomato

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Breeding for resistance to begomoviruses in tomato can be aided by the availability of PCR-based markers for the resistance loci. Four begomovirus-resistance loci or regions have been mapped to chromosome 6 (Agrama and Scott, 2006; Chagué et al., 1997; Ji and Scott, 2006b; Ji et al., 2007; Zamir et al., 1994). The *Ty-1* locus, which is part of the introgression derived from *Solanum chilense* LA1969, is located between markers TG297 (4 cM) and TG97 (8.6 cM) (Zamir et al., 1994). Agrama and Scott (2006) reported three regions that contributed to resistance in breeding lines with introgressions from *S. chilense* LA2779 or LA1932. One region corresponded to the region having the *Ty-1* locus. Another region was the *Ty-3* locus, which was mapped to a region between cLEG-31-P16 (20 cM) and T1079 (27 cM) (Ji and Scott, 2006b; Ji et al., 2007). The third region was near the self-pruning (*sp*) and potato leaf (*c*) loci. Another begomovirus-resistance QTL, derived from an introgression from *Solanum pimpinellifolium*, was mapped near the marker TG153 (33 cM; Chagué et al., 1997). This is very near the *Ty-3* locus from LA2779-derived lines.

Previously, Ji and Scott (2006a, 2006b; Ji et al., 2007) reported that the *Ty-3* locus mapped to a region that included the *FER* locus (25 cM, BAC clone 56B23, AY678298). Jensen and Maxwell (Maxwell et al., 2007) found that the sequences for the G8 gene of the BAC clone 56B23 are different for lines derived from LA2779 and LA1932. To differentiate the two introgressions, the one from LA2779 is designated *Ty-3* and the one from LA1932, *Ty-3a*. The co-dominant SCAR marker, FLUW25, will distinguish between the *ty-3* and *Ty-3* alleles, but the FLUW25 marker will not detect the *Ty-3a* allele (see FLUW25 SCAR marker, this web site). This report describes a set of PCR primers that provide co-dominant SCAR markers for detection of the *ty-3*, *Ty-3* and *Ty-3a* alleles.

Primer Design:

Since the FLUW25 primer pair did not give a fragment with LA1932-derived lines, a new set of primers was designed using the sequences from the FLUW-25 fragments for *S. lycopersicum* and the *S. chilense* introgression and the BAC 56B23 as a starting point. Also, van Betteray and Smeets had determined that it was the FLUW25R primer that did not anneal to the LA1932 sequence. This new primer pair was designed to give smaller PCR fragments than the FLUW25 primer pair: forward primer, P6-25-F2, 5' GGT AGT GGA AAT GAT GCT GCT C, and reverse primer, P6-25-R5, 5' GCT CTG CCT ATT GTC CCA TAT ATA ACC. The P6-25-F2/P6-25-R5 primers were expected to give fragment sizes for *S. lycopersicum* and the *S. chilense* LA2779 introgression of 320 bp and 453 bp, respectively.

DNA Extraction and 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 $MgCl_2$, 0.1 µl (0.5 units) Go*Taq* 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, 53 C for 1 min, and 72 C for 1 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% agarose in 0.5 X TBE buffer, 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 were directly sequenced with Big Dye Sequencing Kit™ and analyzed by the Biotechnology Center, University of Wisconsin-Madison.

Results and Discussion:

The primer set, P6-25-F2 and P6-25-R5, was designed to include the 143-nt *ty-3/Ty-3* indel and to give smaller fragments than the FLUW25 primer set (Fig. 1). With begomovirus-resistant breeding lines derived from either the *S. chilense* LA2779 source, Gc9, or the Ih902 line (Vidavsky and Czosnek, 1998), the expected 450-bp *Ty-3* fragment was obtained. A 320-bp *ty-3* fragment was amplified from breeding lines lacking the introgression from either of these two begomovirus-resistance sources. A 630-bp *Ty-3a* fragment was obtained from lines derived from *S. chilense* LA1932, such as Gc171. Heterozygous hybrids were easily detected with these primers, which amplified two fragments corresponding to the *S. lycopersicum ty-3* fragment (320 bp) and either the *Ty-3* (450 bp) or the *Ty-3a* (630 bp) fragment (Fig. 2). No F1 hybrids were available to test for fragments with the *Ty3/Ty3a* genotype, but it is expected that this primer pair would also amplify two fragments (450 and 630 bp) with this genotype.

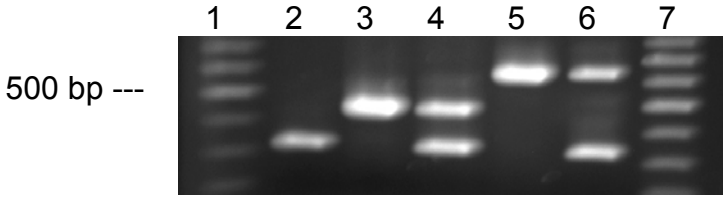

Fig. 1. PCR fragments with primers P6-25-F2/P6-25-R5. Lane 1, 100-bp Brenchtop DNA ladder, Promega; Lane 2, M82-1-8 (ty-3/ty-3); Lane 3, Gc9 (Ty-3/Ty-3); Lane 4, Romelia, F1 hybrid, (Ty-3/ty-3); Lane 5, Gc171 (Ty-3a/Ty-3a); Lane 6, GTc191-3, F1 hybrid, (Ty-3a/ty-3); Lane 7, 100-bp marker.

The three sizes of the P6-25-F2/P6-25-R5 fragments were sequenced (see below). The 320-bp and the 450-bp fragments corresponded to the sequences of *S. lycopersicum* and of the *Ty-3* locus associated with lines derived from *S. chilense* LA2779, respectively. The 650-bp fragment from Gc171 had one large insert, when compared with the *S. lycopersicum* sequence.

Conclusions: This set of primers detect co-dominant SCAR marker, P6-25, for the *ty-3*, *Ty-3* and *Ty-3a* alleles at the FER locus, 25 cM. It is not known how closely these markers are to the functional *Ty-3* gene (Ji et al., 2007), so it is possible that some breeding lines would give false

negative or false positive results. Also, it is not known how this primer pair will function with introgressions from other accessions of wild species.

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Sequences: (PCR fragments form primer pair P6-25-F2/R5)

HUJ-VF, *S. lycopersicum* (susceptible), 320 bp

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1      GGTAGTGGAA ATGATGCTGC TCAAATTATT GTGTGAACAT ATTATGAGAG GTAGGATTAA
61     GAATGAAGTT ATATAAGATA AAGTGGAAGT TACTTTTCGA AAAAAAAAGA AAGACGAAAA
121    AAATGAGATT GAAATGGATT GAATACGTGA AGAAGAGATG CATGGGTTCA CCAATAAAAA
181    GGTTTGAGAG TTTGACTTAA GAAGAGGTAG AAGTAGGTTG AAAAACAACT AGGTAAAGTT
241    TTACTTTTAG TTTTGTTTTG ATTGCACATT TTTTTAGTCG AAATAGAAAC AGAGGTTATA
301    TATGGGACAA TAGGCAGAGC
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Gc143-2, inbred line with resistance to begomoviruses from LA2779, *S. chilense*, 453 bp;

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1      GGTAGTGGAA ATGATGCTGC TCAAATTAAT GTGTGAACAT GAGAGGTAGG ATTAGAAATG
61     AAGTTATATA AGATAAAGTG GAAGTAACTT CCAATAAAAA AAGACGAAAA AAATGAGATT
121    GAAATGGGTT GAATACGTGA AGAAGAGATG CATGGATTCA CCAATAAAAA GGTATGAGAG
181    TTTGACTTAA GAAGATGTAG AAGTAGGTTG AAAAAAAACT ACGTAAAGAT GATTAGATAA
241    GATATATCAC GAGGACACGA CTATAGCAAG ATATGGCAGC AGAGTTTTGT CGTATTGTTA
301    CATGGAAGAG GTAAGGGACT TGTCTCTGCT TTTCATGCAC ATTGCTTCAA TTTACTTTGT
361    TAGACTTGTT ATTTTACTTT TAGTTCTGTT TTGATTGCAC ATTTTTTTAG TCGAAATAGA
421    AACAGAGGTT ATATATGGGA CAATAGGCAG AGC
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Gc171, inbred line with resistance to begomoviruses from LA1932, *S. chilense*, 623 bp;

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1      GGTAGCGGAA ATTATGCTGC TCAAATTAAT GTGTGAACAT ATTATGAGAG GTAGGATTAG
61     AAATGAAGAT ATGTAAGATA AAGTGGGTGA CTTTCAAAAA AAAAAAAGAC GAAAAAAATG
121    AGATTGAAAT GGATTGAATA CGTGAAGAAG AGATGCATGG ATTCATCAAT AAAGAGGTGT
181    GAGAGTTTCA CTTAAGAAGA AATAGAAGTA GGTTGAAGAA CAACTGAAGA TGATTAGATA
241    AGATATATCA CAATTTCAAT AAATGAGGAC ACGACTATAG CAAGATATGG TAGCAGAGTT
301    TTATCGTATT GTTACGTGGA AGAGGTAAGG TACTTGTCTC TACTTTTCAT GCACATTGCT
361    TCAGTTTACT TTGTTAGACT TGTTATTTTA ATGAGATTCG AACCTTGGTA CAACAATATT
421    AAAAGAGTTT TACCCATCTT AGAATCATTG GGTCAACTAT ATGATTTATT CATTAGCTGC
481    TTTATGTTAA TTTTATACAA ATATCTATCG ATTTCTACAT AGATATATAT ATTTCGTAAC
541    AAAGTTAATG AGTGCTCGAG CACCCAGCGG ACAACACGTG GGTCCGCCCT GACAGAGGTT
601    ATATATGGGA CAATAGGCAG AGC
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Sequence of P6-25-F2/P6-25-R5 fragments at 25 cM of chromosome VI

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HUJ-VF (ty3/ty3); Gc143-2 (Ty3/Ty3); Gc171 (Ty3a/Ty3a)

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HUJ-VF     GGTAGTGGAAATGATGCTGCTCAAATTATTGTGTGAACATATTATGAGAGGTAGGATTAA     60
Gc143-2    GGTAGTGGAAATGATGCTGCTCAAATTAaTGTGTGAACAT.....GAGAGGTAGGATTAg     55
Gc171      GGTAGcGGAAATtATGCTGCTCAAATTAaTGTGTGAACATATTATGAGAGGTAGGATTAg     60
Consensus  ggtag ggaaat atgctgctcaaatta tgtgtgaacat     gagaggtaggatta

HUJ-VF     GAATGAAGTTATATAAGATAAAGTGGAAGTTACTTTTCGAAAAAAAAAAGAAAGACGAAAA    120
Gc143-2    aAATGAAGTTATATAAGATAAAGTGGAAGTaACTTcc.....AAtAAAaAAAGACGAAAA    110
Gc171      aAATGAAGaTATgTAAGATAAAGTGG..GTgACTTTc...AAAAAAAAaAAAGACGAAAA    115
Consensus   aatgaag tat taagataaagtgg  gt actt       aa aaa aaagacgaaaa

HUJ-VF     AAATGAGATTGAAATGGATTGAATACGTGAAGAAGAGATGCATGGGTTCACCAATAAAAA    180
Gc143-2    AAATGAGATTGAAATGGgTTGAATACGTGAAGAAGAGATGCATGGaTTCACCAATAAAAA    170
Gc171      AAATGAGATTGAAATGGATTGAATACGTGAAGAAGAGATGCATGGaTTCAtCAATAAAgA    175
Consensus  aaatgagattgaaatgg ttgaatacgtgaagaagagatgcatgg ttca caataaa a

HUJ-VF     GGTTTGAGAGTTTGACTTAAGAAGAGGTAGAAGTAGGTTGAAAAACAACTAGGTAAAG..    238
Gc143-2    GGTaTGAGAGTTTGACTTAAGAAGAtGTAGAAGTAGGTTGAAAAAaAACTAcGTAAAGat    230
Gc171      GGTgTGAGAGTTTcACTTAAGAAGAaaTAGAAGTAGGTTGAAgAACAACTg....AAGat    231
Consensus  ggt tgagagttt acttaagaaga  tagaagtaggttgaa aa aact     aag

HUJ-VF     ............................................................    238
Gc143-2    gattagataagatatatcac.............gaggacacgactatagcaagatatggc    277
Gc171      gattagataagatatatcacaatttcaataaatgaggacacgactatagcaagatatggt    291
Consensus

HUJ-VF     ............................................................    238
Gc143-2    agcagagttttgtcgtattgttacatggaagaggtaagggacttgtctctgcttttcatg    337
Gc171      agcagagttttatcgtattgttacgtggaagaggtaaggtacttgtctctacttttcatg    351
Consensus

HUJ-VF     ............................................................    238
Gc143-2    cacattgcttcaatttactttgttagacttgtta..........................    371
Gc171      cacattgcttcagtttactttgttagacttgttattttaatgagattcgaaccttggtac    411
Consensus

HUJ-VF     ............................................................    238
Gc143-2    ............................................................    371
Gc171      aacaatattaaaagagttttacccatcttagaatcattgggtcaactatatgatttattc    471
Consensus

HUJ-VF     ............................................................    238
Gc143-2    ............................................................    371
Gc171      attagctgctttatgttaattttatacaaatatctatcgatttctacatagatatatata    531
Consensus

HUJ-VF     ..........TTTTACTTTTAGTTTTGTTTTGATTGCACATTTTTTTAGTCGAAATAGAA    288
Gc143-2    ..........TTTTACTTTTAGTTcTGTTTTGATTGCACATTTTTTTAGTCGAAATAGAA    421
Gc171      tttcgtaacaaagTtaaTgagtgcTcGagcacccaGCggAcaacacgtGggtccgccctg    591
Consensus               t   t        g        gc  a        g

HUJ-VF     ACAGAGGTTATATATGGGACAATAGGCAGAGC                                320
Gc143-2    ACAGAGGTTATATATGGGACAATAGGCAGAGC                                453
Gc171      ACAGAGGTTATATATGGGACAATAGGCAGAGC                                623
Consensus  acagaggttatatatgggacaataggcagagc
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