Ty-1 introgression from *S. chilense* – Co-dominant SCAR Marker

LE_HBa0019E05_T7_131902, 5.5cM, Chromosome 6: these primers were designed from the T7 BAC end sequence

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Primer Name	Primer Sequence (5'-3)
P6-6-F1	CAATTTATAGGTGTTTTTGGGACATC
P6-6-R1	GTTCAACACTTGGCCAATGCTTACG

PCR annealing temperature: 53 C

Agarose Gel

Lane	Sample
1	Promega 100 bp Ladder
2	Heinz 1706 (mi/mi, ty1/ty1)
3	Rodeo (Mi/Mi, ty1/ty1)
4	XA577 (Mi/mi, Ty1/ty1)
5	Ty52 (Ty1/Ty1)
6	Llanero (Mi/mi, ty1/ty1)
7	Promega 100 bp Ladder

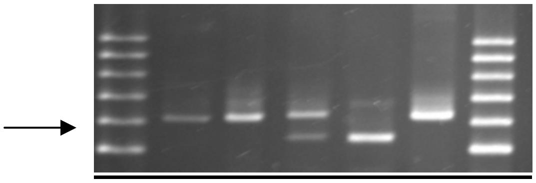

Fig 1. 1.5% agarose gel of the CM3 primers. Arrow points to the marker size of 600bp.

DNA Sequences

Heinz 1706 (ty1/ty1) Sequence P6-6F1-R1: 595 bp;(From SGN site)

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1      CAATTTATAG GTGTTTTTGG GACATCAAAT GCCTCAAATT AAAGTGGTTG ATAACCAATT
61     TTGTAGGGAA TTATTGGTTT TGGTCGTGTG AGATTTCTTT TTAGTTGGTA TGGACAGACT
121    TACTTTACTA AAAACTAGGA ATTATCAATA AATCGAAATC GATAATTTGA ATAAAAAAAA
181    TATTGGATTA TTCATTTAGC GCTTTCGTTT AATGATTTTA TTTATTTTAT TATCGGGATG
241    TTTATTATAC TTAACACGTT AATAGATAAT TCGATAGTAA ATCAATAAAC TATATTTATA
301    TTGTTCGATA TATTACTTTT AGTTTTGGTT ATCTCAAATT TTTGGTTTTT TTTATGTATC
361    AGTGTTTGCT TTTGAACAAG ATACAATCTG TCAACTCATG TGCATGATTC ATTTAGTTTG
421    TCACCTTTTC TCTAAATGAT TTTCAACGTG ATTTTTTGTG TGTGACAAAT TTTAACGAGT
481    AATCGATAAT CGAATCGACA ATGGTCAATA ACTAATAAAA CAATATCTTA ATAGTTCTAT
541    AATGACTAAG TTTTTGTGTT ATTTTATTTT CGTAAGCATT GGCCAAGTGT TGAAC
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Partial sequences from our lab:

HUJ-VF (ty1/ty1) Sequence P6-6R1: 429 bp; (susceptible inbred line from Hebrew University of Jerusalem –partial sequence

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1      CAATTTATAG GTGTTTTTGG GACATCAAAT GCCTCAAATT AAAGTGGTTG ATAACCAATT
61     TTGTAGGGAA TTATTGGTTT TGGTCGTGTG AGATTTCTTT TTAGTTGGTA TGGACAGACT
121    TACTTTACTA AAAACTAGGA ATTATCAATA AATCGAAATC GATAATTTGA ATAAAAAAAA
181    TATTGGATTA TTCATTTAGC GCTTTCGTTT AATGATTTTA TTTATTTTAT TATCGGGATG
241    TTTATTATAC TTAACACGTT AATAGATAAT TCGATAGTAA ATCAATAAAC TATATTTATA
301    TTGTTCGATA TATTACTTTT AGTTTTGGTT ATCTCAAATT TTTGGTTTTT TTTATGTATC
361    AGTGTTTGCT TTTGAACAAG ATACAATCTG TCAACTCATG TGCATGATTC ATTTAGTTTG
421    TCACCTTTT
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Gc9 Sequence (Ty1/Ty1) P6-6F1: 451 bp; (Introgression from LA2779, *S. chilense*) – partial sequence

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1      TTTTTGTGGG AATTATTGGT TGTGGTTCTG TGTGATTTNN NNNNNNNNGG TATTGAGAGA
61     CTTAGTTTAC TAAAAACTAG GAATTATCAA TAAATCGAAA TAGATAATTT GAATAAAAAA
121    ATATTGGTTT ATTCATTTAG CGCTTTCGTT TAATGATTTT ACTTATTTTA TTATTGAGAT
181    GTTCATTATT CTTAACACGT TAATAGATAA TTCGATAGTA AATCAATAAA TTATATTTAT
241    ATCGTTCGAT GTATTACTTT TAGTTTTGGT TGTCTCAAAC TCTTGGTTAT TTTTTTATGT
301    GTCAGTGTTT GCTTTTGAAC AAGATGCAAT CTGTCAACTC ATATACATGA TTCATTTAGT
361    TTGTCACCTT TTCTCTAAAT GATTTTCTAT GTGATTTTTT GTGTGTGACA AATTTTAACG
421    ATTAACCGTA AGCATTGGCC AAGTGTTGAA C
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Gc43 Sequence (Ty1/Ty1) P6-6F1: 451 bp; partial seqeunce

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1      TTTTTGTGGG AATTATTGGT TGTGGTTCTG TGTGATTTNN NNNNNNNNGG TATTGAGAGA
61     CTTAGTTTAC TAAAAACTAG GAATTATCAA TAAATCGAAA TAGATAATTT GAATAAAAAA
121    ATATTGGTTT ATTCATTTAG CGCTTTCGTT TAATGATTTT ACTTATTTTA TTATTGAGAT
181    GTTCATTATT CNNAACACGT TAATAGATAA TTCGATAGTA AATCAATAAA TTATATTTAT
241    ATCGTTCGAT GTATTACTTT TAGTTTTGGT TGTCTCAAAC TCTTGGTTAT TTTTTTATGT
301    GTCAGTGTTT GCTTTTGAAC AAGATGCAAT CTGTCAACTC ATATACATGA TTCATTTAGT
361    TTGTCACCTT TTCTCTAAAT GATTTTCTAT GTGATTTTTT GAGTGTGACA AATTTTAACG
421    ACTAACCGTA AGCATTGGCC AAGTGTTGAA C
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Sequence Alignment

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Heinz_CM3F1-R1 TTTTGTAGGGAATTATTGGTTTTGGTCGTGTGAGATTTCTTTTTAGTTGGTATGGACAGA
HUJBF_CM3-R1   TTTTGTAGGGAATTATTGGTTTTGGTCGTGTGAGATTTCTTTTTAGTTGGTATGGACAGA
Gc43_CM3F1     TTTTtgtGGGAATTATTGGTTgTGGTtcTGTGtGATTTnnnnnnnnnGGTATtGAgAGA
Gc9_CM3F1      TTTTtgtGGGAATTATTGGTTgTGGTtcTGTGtGATTTnnnnnnnnnGGTATtGAgAGA

Heinz_CM3F1-R1 CTTACTTTACTAAAAACTAGGAATTATCAATAAATCGAAATCGATAATTTGAATAAAAAA
HUJBF _CM3-R1  CTTACTTTACTAAAAACTAGGAATTATCAATAAATCGAAATCGATAATTTGAATAAAAAA
Gc43_CM3F1     CTTAgTTTACTAAAAACTAGGAATTATCAATAAATCGAAATaGATAATTTGAATAAAAAA
Gc9_CM3F1      CTTAgTTTACTAAAAACTAGGAATTATCAATAAATCGAAATaGATAATTTGAATAAAAAA

Heinz_CM3F1-R1 AATATTGGATTATTCATTTAGCGCTTTCGTTTAATGATTTTATTTATTTTATTATCGGGA
HUJBF_CM3-R1   AATATTGGATTATTCATTTAGCGCTTTCGTTTAATGATTTTATTTATTTTATTATCGGGA
Gc43_CM3F1     A.TATTGGtTTATTCATTTAGCGCTTTCGTTTAATGATTTTAcTTATTTTATTATtGaGA
Gc9_CM3F1      A.TATTGGtTTATTCATTTAGCGCTTTCGTTTAATGATTTTAcTTATTTTATTATtGaGA

Heinz_CM3F1-R1 TGTTTATTATACTTAACACGTTAATAGATAATTCGATAGTAAATCAATAAACTATATTTA
HUJBF_CM3-R1   TGTTTATTATACTTAACACGTTAATAGATAATTCGATAGTAAATCAATAAACTATATTTA
Gc43_CM3F1     TGTTcATTATtCnnAACACGTTAATAGATAATTCGATAGTAAATCAATAAAtTATATTTA
Gc9_CM3F1      TGTTcATTATtCTTAACACGTTAATAGATAATTCGATAGTAAATCAATAAAtTATATTTA

Heinz_CM3F1-R1 TATTGTTCGATATATTACTTTTAGTTTTGGTTATCTCAAATTTTTGGTT..TTTTTTATG
HUJBF_CM3-R1   TATTGTTCGATATATTACTTTTAGTTTTGGTTATCTCAAATTTTTGGTT..TTTTTTATG
Gc43_CM3F1     TATcGTTCGATgTATTACTTTTAGTTTTGGTTgTCTCAAAcTcTTGGTTatTTTTTTATG
Gc9_CM3F1      TATcGTTCGATgTATTACTTTTAGTTTTGGTTgTCTCAAAcTcTTGGTTatTTTTTTATG

Heinz_CM3F1-R1 TATCAGTGTTTGCTTTTGAACAAGATACAATCTGTCAACTCATGTGCATGATTCATTTAG
HUJBF_CM3-R1   TATCAGTGTTTGCTTTTGAACAAGATACAATCTGTCAACTCATGTGCATGATTCATTTAG
Gc43_CM3F1     TgTCAGTGTTTGCTTTTGAACAAGATgCAATCTGTCAACTCATaTaCATGATTCATTTAG
Gc9_CM3F1      TgTCAGTGTTTGCTTTTGAACAAGATgCAATCTGTCAACTCATaTaCATGATTCATTTAG

Heinz_CM3F1-R1 TTTGTCACCTTTTCTCTAAATGATTTTCAACGTGATTTTTTGTGTGTGACAAATTTTAAC
HUJBF_CM3-R1   TTTGTCACCTTTT...............................................
Gc43_CM3F1     TTTGTCACCTTTTCTCTAAATGATTTTCtAtGTGATTTTTTGaGTGTGACAAATTTTAAC
Gc9_CM3F1      TTTGTCACCTTTTCTCTAAATGATTTTCtAtGTGATTTTTTGTGTGTGACAAATTTTAAC

Heinz_CM3F1-R1 GAGTAATCGATAATCGAATCGACAATGGTCAATAACTAATAAAACAATATCTTAATAGTT
HUJBF_CM3-R1   ............................................................
Gc43_CM3F1     GAcTAA......................................................
Gc9_CM3F1      GAtTAA......................................................


Heinz_CM3F1-R1 CTATAATGACTAAGTTTTTGTGTTATTTTATTTTCGTAAGCATTGGCCAAGTGTTGAAC
HUJBF_CM3-R1   ...........................................................
Gc43_CM3F1     ..................................CGTAAGCATTGGCCAAGTGTTGAAC
Gc9_CM3F1      ..................................CGTAAGCATTGGCCAAGTGTTGAAC
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Summary

These primers were designed to detect the presence of the *Ty1* gene on chromosome 6. Upon PCR amplification, plants with the genotype Ty1/Ty1 gave an agarose gel band size of ca. 530 bp. Plants with a genotype of ty1/ty1 gave a band size of ca. 610 bp. Plants heterozygous for the *Ty1* gene gave a both band sizes (Fig. 1). Some inbred lines, such as TY52 (ty1/Ty1) and

Gc9, have an introgression that extends from at least 5 cM to 9 cM. These primers were designed from a BAC clone mapped to 5.5 cM. This is also the location of the *Mi-1.2 gene* for root-knot nematode resistance. Thus, it valuable to know that plants that have the *Mi-1.2* gene give the susceptible size band. From other results, it is most likely that the *Ty-1* gene is located near 8 cM, so that you could have introgressions of the *Ty-1* locus that would not have the S. chilense sequence at the 5.5 cM region.

Partial sequence was obtained for Gc9, Gc43, and HUJ-VF through standard sequencing procedures in our laboratory. Complete sequence was obtained for Heinz 1706 from the SOL Genomics Network http://www.sgn.cornell.edu/. Upon alignment, Gc9 and Gc43 matched exactly, these two inbred lines have a S. chilense LA2779 introgression in this region. Heinz 1706 and HUJ-VF, both susceptible lines, also matched identically, but differed from Gc9 and Gc43 by 28 SNPs and three INDEL. The 28 SNPs are highlighted in green above. The INDELs are highlighted in yellow. The INDEL account for the large different in band size on the agarose gel.

Thus far (July 25, 2007), our lab has tested 15 breeding lines with these primers. All plant lines derived from crosses with *S. chilense* gave an agarose band size of roughly 530 bp, whereas plant lines derived from *S. lycopersicon* or *S. peruvianum* gave band sizes of roughly 600 bp. Therefore, this primer pair may be useful in tracking the inheritance of Ty1 introgression from the *S. chilense* (LA1969 and LA2779) and could be used in breeding programs for that purpose.