Evaluation of the JB1 marker for detection of the Ty1 introgression in chromosome 6

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Recently, Pérez de Castro et al. (2007) developed a CAPS marker linked to the Ty-1 gene for resistance to *Tomato yellow leaf curl virus*. This marker was designed from the RFLP CT21 (8.6 cM) on chromosome 6. This marker was evaluated with the some of the same lines as used previously for the UWTy1 (TG97) marker. It was of particular interest to see if this marker detected the *S. peruvianum* introgression associated with the Mi/Mi lines (GhT44-2).

Material and Methods

Design of PCR primers: Primers: JB1F 5' aac cat tat ccg gtt cac tc 3' and JB1R 5' ttt cca ttc ctt gtt tct ctg 3' (Pérez et al. 2007).

Germplasm: M82-1-8 (M82, VF1, F. Vidavski, Hebrew University); Heinz 1706 (VF1, R. Ozminkowski, Heinz Seeds); Gh2 (Mi/Mi, Ty1/Ty1, Ty3/Ty3, L. Mejía, San Carlos University, Guatemala); Gc171 (mi/mi, Ty3a/Ty3a, Ty4/Ty4, I2, L. Mejía, San Carlos University); Gc9 (mi/mi, Ty1/Ty1, Ty3/Ty3, I2, L. Mejía, San Carlos University); Marwa (hybrid Syngenta, xxxx, N, F1F2, Ve, tolerant to TYLCV); Llanero (hybrid, Semillas Tropicales SA, Mi/mi, ty1/ty1, I2); Romelia (Semillas Tropicales SA, Mi/mi, Ty1/ty1, I2)

PCR and Restriction Enzyme 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 3 min, the 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). For sequencing, ssDNA (primers) were digested in PCR reactions with shrimp alkaline phosphatase (Progmega 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. PCR-amplified fragments were separated on 2% agarose gels in 0.5 XTBE buffer, stained with ethidium bromide and visualized with UV light.

The PCR program listed in the Pérez et al. (2007) was tried and no bands were obtained. Ana Pérez de Castro was contacted and she suggested that we try 53 C and vary the DNA and $MgCl_2$ concentrations. After this test, the above conditions gave adequate band intensity for direct sequencing.

Results and Discussion

The JB1 primers gave a single, sharp 930-bp fragment with the nine lines tested. These fragments were sequenced (Fig. 1) and three difference sequences were obtained. i) One sequence for *S. lycopersicum* associated with M82-1-8, Gc171, and Heinz 1706; ii) another for TY52 (Ty1 from *S. chilense* LA1969), which was identical for GIh902 and Gc9, and iii) then another sequence for *S. peruvianum* for the GhT44-1 (Mi/Mi) line. So for both TG97 (UWTy1) and JB-1 markers for *Ty-1*, there can be three alleles in this region: *S. lycopersicum*, *S. peruvianum* (from Mi/Mi lines) and *S. chilense* (same as TY52). Gc9, GIh902b and Gh194-1 all have same indels as TY52 and thus have the Ty-1 gene in this region. There were very characteristic regions associated with the sequences for each allele, thus a sequence analysis would be considerably more reliable than the SNP difference detected by the CAPS marker to distinguish ty1, Ty1 and Mi sequences..

For *Taq*I sites, Heinz (S. lyc allele) and GhT44-1 (S. per allele) have two sites and TY52 (S. chil allele) has one site. Thus, the CAPS marker can not distinguish between the *S. lycopersicum* allele and the *S. peruvianum* allele, but can detect the *S. chilense* allele.

Note: The authors are very appreciative of the understanding and patience of Ana Pérez de Castro for her assistance in this research.

Reference:

Pérez de Castro, A., J.M. Blanca, M.J. Díez, and F. N. Viñals. 2007. Identification of a CAPS marker tightly linked to Tomato yellow leaf curl disease resistance gene *Ty-1* in tomato. Eur. J. Plant Pathol. 117:347-356.

Fig. 1. JB1 fragment sequence for Heinz and Gc171(*S. lycopersicum*), TY52 (Ty1/Ty1, *S. chilense*), and GhT44-1 (*Mi/Mi*, *S. peruvianum*).

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Heinz-JB1                                                          CAGATTGCCACTG     17
Gc171-JB1                                                          CAGATTGCCACTG     17
TY52-JB1                                                           CAGATTGCCACTG     13
GhT44-1    aaccattatccggttcactcccacttccaacaaaccattcttcaCaGCAGATTGCCACTG     60
Consensus                                                          cagattgccactg

Heinz-JB1  CTTTACTTGTGGCTCAAACGCCACTTTCTTTTCCACCCATTCATACGAATCTCTCACTAA     77
Gc171-JB1  CTTTACTTGTGGCTCAAACGCCACTTTCTTTTCCACCCATTCATACGAATCTCTCACTAA     77
TY52-JB1   CTTTACTTGTGGCTCAAACGCCACTTTCTTTTCCACCCAnnnnnACGAATCTCTCACTAA     73
GhT44-1    CTTTACTTGTGGCTCAAACGCCACTTTCTTTTCCACCCATTCATACGAATCTCTCACTAA    120
Consensus  ctttacttgtggctcaaacgccactttcttttccaccca     acgaatctctcactaa

Heinz-JB1  CTTCTCATTCCATCCCACTTTCTTAACGTACTCATCACTCGCCCTCGTAAAGAACCCCGC    137
Gc171-JB1  CTTCTCATTCCATCCCACTTTCTTAACGTACTCATCACTCGCCCTCGTAAAGAACCCCGC    137
TY52-JB1   CTTgTCATTCCATCCCACTTTCTTAACGTACTCATCACTCGCCCTCGTAAAGAACCCCGC    133
GhT44-1    CTTgTCATTCCATCCCACTTTCTTAACGTACTCATCACTCGCCCTCGTAAAGAACCCCGC    180
Consensus  ctt tcattccatcccactttcttaacgtactcatcactcgccctcgtaaagaaccccgc

Heinz-JB1  ATTAATAGCTGAACCACCACCTAAGACGCGAGCACGGTGGTTGAAGACACCGTCTGTAGA    197
Gc171-JB1  ATTAATAGCTGAACCACCACCTAAGACGCGAGCACGGTGGTTGAAGACACCGTCTGTAGA    197
TY52-JB1   ATTAATAGCTGAACCACCACCTAAGACGCGAGCACGGTGGTTGAAtACACCGTCTGTAGA    193
GhT44-1    ATTAATAGCTGAACCACCACCTAAGACGCGAGCACGGTGGTTGAAtACACCGTCTGTAGA    240
Consensus  attaatagctgaaccaccacctaagacgcgagcacggtggttgaa acaccgtctgtaga
                                                  AluI
Heinz-JB1  GATGAAAAGTTGCGAGGGAGATGACGGAGAAATGTTAGCTAAGTTGCTTGAGAAACCGTT    257
Gc171-JB1  GATGAAAAGTTGCGAGGGAGATGACGGAGAAATGTTAGCTAAGTTGCTTGAGAAACCGTT    257
TY52-JB1   GATGAAAAGTTGCGAGGGAGATGACGGAGAAATGTTAGCTAAGTTGCTTGAGAAACCGTT    253
GhT44-1    GATGAAAAGTTGCGAGGGAGATGACGGAGAAATGTTgGCTAAGTTGCTTGAGAAACCGTT    300
Consensus  gatgaaaagttgcgagggagatgacggagaaatgtt gctaagttgcttgagaaaccgtt

Heinz-JB1  AATGTTTGTGATGTTTGGGTTTCCATAGGGTAAGTCACCTCTTTCTAATAACAGAACATT    317
Gc171-JB1  AATGTTTGTGATGTTTGGGTTTCCATAGGGTAAGTCACCTCTTTCTAATAACAGAACATT    317
TY52-JB1   AATGTTTGTGATGTTTGGGTTTCCATAGGGTAAGTCACCTCTTTCTAATAACAcAACATT    313
GhT44-1    AATGTTTGTGATGTTTGGGTTTCCATAGGGTAAGTCACCTCTTTCTAATAACAGAACATT    360
Consensus  aatgtttgtgatgtttgggtttccatagggtaagtcacctctttctaataaca aacatt

Heinz-JB1  GAACGATTGTGAGAGTGTTGCTGCTAATGCACAACCAGCAGTTCCTCCTCCTATTATGAT    377
Gc171-JB1  GAACGATTGTGAGAGTGTTGCTGCTAATGCACAACCAGCAGTTCCTCCTCCTATTATGAT    377
TY52-JB1   GAACGATTGTGAGAGTGTTGCTGCTAATGCACAACCAGCAGTTCCTCCTCCTATTATGAT    373
GhT44-1    GAACGATTGTGAGAGTGTTGCTGCTAATGCACAACCAGCAGTTCCTCCTCCTATTATGAT    420
Consensus  gaacgattgtgagagtgttgctgctaatgcacaaccagcagttcctcctcctattatgat
               TaqI                                                TaqI
Heinz-JB1  GTAATCGAACGAAATAACCTTTGGTGATGACGTAGCATCTCTCGCAAACGTCGAATATGG    437
Gc171-JB1  GTAATCGAACGAAATAACCTTTGGTGATGACGTAGCATCTCTCGCAAACGTCGAATATGG    437
TY52-JB1   GTAATCGAACGAAATAACCTTTGGTGATGACGTAGCATCTCTtGCAAACGTgGAATATGG    433
GhT44-1    GTAATCGAACGAAATAACCTTTGGTGATGACGTAGCATCTCTCGCAAACGTCGAATATGG    480
Consensus  gtaatcgaacgaaataacctttggtgatgacgtagcatctct gcaaacgt gaatatgg
              CviR1
Heinz-JB1  GGCTACATTGCATCAAACAATTATTAATATTTTAATTTTATTTATTTGACAAAAACTCTT    497
Gc171-JB1  GGCTACATTGCATCAAACAATTATTAATATTTTAATTTTATTTATTTGACAAAAACTCTT    497
TY52-JB1   GGCTgCATTGCATCAAACAATTATTAATATTTTAATTTTATTTATTTGACAAAAACTCTT    493
GhT44-1    GGCTACATTGCATCAAACAATTATTAATATTTTcATTTTATTTATTTGACAAAAACTtTT    540
Consensus  ggct cattgcatcaaacaattattaatatttt attttatttatttgacaaaaact tt
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Heinz-JB1  TTTTTTTT.....................CTAGATATTCTATGATCATCATAGAATTTGT    536
Gc171-JB1  TTTTTTTT.....................CTAGATATTCTATGATCATCATAGAATTTGn    536
TY52-JB1   TTTTTTTTtaaaaaaaaaaataagagttagCTAGATATTCTATGATCATCATAGAATTTGT    553
GhT44-1    TTaaaaaaataaaataaaataagagttagCTAGATATTCTATGATCATCATAGAATTTGT    600
Consensus  tt                           ctagatattctatgatcatcatagaatttg

Heinz-JB1  GACCCTTTTATTGATTTTGAATTTTTGATAGTAAATTTTTTTTTTTAGCATCAATACATGT    596
Gc171-JB1  GACCCTTTTATTGATTTTGAATTTTTGATAGTAAATTTTTTTTTTTAGCATCAATACATGT    596
TY52-JB1   GACCCTTTTATTGATTTTGAATTTTTGATgGTAAAgaaTTTTTTTAGCATCAATACATtT    613
GhT44-1    GACCCTTTTATTGATTTTGAATTTTTGATAGTAAAgaaTTTTTTTAGCATCAATACATGT    660
Consensus  gacccttttattgattttgaatttttgat gtaaa   ttttttagcatcaatacat t

Heinz-JB1  TAACTTGTCATAGCAAGTTAGTTGTCTTATTTTGAAGTTACCAATTCTATTTACCTTCAA    656
Gc171-JB1  TAACTTGTCATAGCAAGTTAGTTGTCTTATTTTGAAGTTACCAATTCTATTTACCTTCAA    656
TY52-JB1   TAACTTGTtATAGCAAGTTAGTTGTCTTATTTTGAAGTTACCAATTCTATTTACCTTCAA    673
GhT44-1    TAACTTGTtATAGCAAGTTAGTTGTCTTATTTTGAAGTTACCAATTCTATTTACCTTCAA    720
Consensus  taacttgt atagcaagttagttgtcttattttgaagttaccaattctatttaccttcaa

Heinz-JB1  TTGACTTTTAGCTAATTAACTTGAACAATGTAAAACTAAATTCATTTTTCTACCCCATCT    716
Gc171-JB1  TTGACTTTTAGCTAATTAACTTGAACAATGTAAAACTAAATTCATTTTTCTACCCCATCT    716
TY52-JB1   TTGACTTTTAGCTAATTAACTTGAACAATGgAAAACTAAATTCATTTTTCTACCCCATCT    733
GhT44-1    TTGACTTTTAGCTAATTAACTTGAACAATGTAAAACTAAATTCATTTTTCTACCCCATCT    780
Consensus  ttgactttttagctaattaacttgaacaatg aaaactaaattcatttttctaccccatct

Heinz-JB1  TAGTATTATTTTTTTTTAAAAAAAAAATAAATTGCATCTACGTACAAAGTGTATCTTTTAA    776
Gc171-JB1  TAGTATTATTTTTTTTTAAAAAAAAAATAAATTGCATCTACGTACAAAGTGTATCTTTTAA    776
TY52-JB1   TAGTATTtTTTTTTTTtt..AAAAAAgAAATTGCATCTACGTACAAAagGTATCTTTTAA    791
GhT44-1    TAGTATTATTTTTTTTTt...AAAAAAgAAATTGCATCTACGTACAAAaTGTATCTTTTAA    837
Consensus  tagtatt tttttttt    aaaaaa aaattgcatctacgtacaaa  gtatcttttaa

Heinz-JB1  GACAAAAGAAAATGGAAGCAAAGAGAAGTGATTATG                           812
Gc171-JB1  GACAAAAGAAAATGGAAGCAAAGAGAAGTGATTATG                           812
TY52-JB1   GACAAAAGAAAATGGAAGCAAAtAGAAcTGATTATGgaatagaga                 836
GhT44-1    GACAAAAGAAAATGGAAGCAAAGAGAAGTGATTATGgaagagagaagagatcacaattac   897
Consensus  gacaaaagaaaatggaagcaaa agaa tgattatg

GhT44-1    ctttctcagagaaacaaggaatggaaa                                  924
Consensus
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NOTE: The best sequence in both directions was for GhT44-1, which has the *Mi/Mi* phenotype and thus, it is suspected that the introgression for this marker is from *S. peruvianum*. GhT44-1 has an introgression for TG97, which is different than the introgression for TY52 and is different than *S. lycopersicum*. TY52 is Ty-1/Ty-1 and Heinz and Gc171 are both *S. lycopersicum* sequences at this marker and also TG97 and *mi/mi*. Gc171 is Ty3a/Ty3a (25 cM), which is on chromosome 6. This line is very resistance to geminiviruses in Guatemala.