

Chromosome 6, 32 cM

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Previous results from our team has shown that there is an introgression in chromosome 6 associated with resistance to begomoviruses in Guatemala. A RIL population of 80+ families of was created by crossing Gh13 (resistant) and HUIJ-VF and then single seed decent from individual F2 plants. These RIL families were evaluated in the field in replicated blocks and phenotypes determined. The presence or absence of the Ty3-introgression marker at 25 cM on chromosome 6 was determined with PCR primer pair, P6-25F2/R5. Several RILs that had the marker at 25 cM for the Ty3-introgression were susceptible. All RILs that lacked the marker for the Ty3-introgression were susceptible. Because our group had had difficulty getting good sequence with our T0834 primers, new primers were designed for this region. The PCR primers below yielded a PCR fragment which gave excellent sequence when directly sequenced.

Design of Primers from BAC C6Hba0307J13. The marker T0834 is associated with this BAC clone and SGN-E746197 was used to find exons and introns. The following primers were designed to amplify one large intron:

p6-32 F1: GCT GTA GAA TTT GAA TCT GTT TAT GGA G
p6-32 R2: GTC TTC TTG ATT GCG CAA TGT TCC

Annealing Temperature: 53°C, a single PCR fragment was produced, which was directly sequenced.

RIL Families:

Gh13 was the source of resistance for begomoviruses for the RILs, and it is known to have an introgression from about 19 cM to 32 cM. RIL-34 (W312) has the Ty3 introgression marker at 25 cM and is resistant to begomoviruses; and RIL-5 (W316) does not have the Ty3 introgression marker and is susceptible. HUIJ-VF (W168) is the susceptible parent in the RIL families.

Partial Sequence of p6-32 F1/R2 W168 (HUIJ-VF, susceptible to begomoviruses)

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1      GAATTGGGTT TTTCTTCTGG GGTGACTAAA ACTAGTAGAG TGTAACCTTAC AAAATTTAGT
61     CAAATCTTCG TATGGTCACT CCACTGATAC GCTAAGAAAAG TCGCTTTCTT TGTGAAGAA
121    AATCGGAATT AAGAGTAAAG ACGGCTTTTT TGAGATACTA ACTATTAGTT GAGTAACAGT
181    CTAAATAGAA TATTTTGTTF TATTCTTGTT TAAAACTTT CCGTGATGGA TAATAATTTT
241    TGTCTGCAGC ACATGTGTTT GGTGATTAGT GAAGGTTTTG TCTCATATTG AAATGTATT
301    TATTCACTGT CTTTACCTCC AGTAGAAATT GATGGGTCTC TCCCCTTAAG ATTAAGGTAA
361    ACATAATCAT TCCTAATTTT GTGAAAACCTC AGTCGGTGTA CTAAGTTCCT GCTACGTGTG
421    AGGTTCCGAG AGGGACTGAT CACAACCTCA CAAGGTATT GTATGCAGCC TTAATATGCA
481    TTTCTTCAAG AGACGGTCTC CATAGCTCGA ACCCATGATT TGTGCAATAG GCTGTTTCCA
541    ATTTTGTGA TTTGTTTAAA TTGTTGTAAG TTA
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Partial Sequence of P6-32 F1/R2 W316 (Sequence from RIL-5, *S. lycopersicum* sequence, susceptible to begomoviruses)

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1      TGCTGTAGAA ATTTGAATCT GTTTATGGAG GTCAGCATCT TATTTTTTTC TTAATTAGGT
61     TTATGAAATC TGTGTTTTAC CCTTTTTCTT TTGAGGGGTT GAAATGTTTA TTCTGAATTG
121    GGTTTTTCTT CTGGGGTGAC TAAACTAGT AGATTGTAAC TTACAAAATT TAGTCAAATC
181    TTCGTATGGT CACTCCACTG ATACGCTAAG AAAGTCGCTT TCTTTGTTGA AGAAAATCGG
241    AATTAAGAGT AAAGACGGCT TTTTGTGAGT ACTAECTATT AGTTGAGTAA CATTCTAAAT
301    AGAATATTTT GTTTTATCTT TGTGTTAAAA CTTTGCGTGA TGGATAATAA TTTCTGTCTG
361    CAGCACATGT GTTTGGTGAT TAGTGAAGGT TTTGTCTCAT ATTGAAATTG TATTTATTCA
421    CTGTCTTTAC CTCCAGTAGA AATTGATGGG TCTCTCCCTT TAAGATTAAG GTAAACATAA
481    TCATTCCCTAA TTTTGTGAAA ACTCAGTCGG TGTACTAAGT TCCTGCTACG TGTGAGGTTT
541    GGAGAGGGAC TGATCACAAC TTCACAAGG TATTGTATGC AGCCTTACTA TGCATTTCTT
601    CAAGAGACGG TCTCCATAGC TCGAACCAT GATTTGTGCA ATAGGCTGTT TCCAATTTTT
661    GTGATTTGTT TAAATTGTTG TAAGTTATAG TTTTTTACCA ACTCTTCAAT TCGGAACTAA
721    TGTGATCTT  CTCTCTTTA  GGAAGTTATG  AGCGGAGAAC  AAGCATCTTG  G

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Partial Sequence of P6-32 F1/R2 W312 (RIL-34, has Ty3 introgression at 25 cM and is resistant to begomoviruses)

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1      TTGCTGTAGA ATTTGAATCT GTTTATGGAG GTCAGCATCT TATTTTTTCT TAATTAGGTT
61     TATGAATCTG TGTTTTACCC TTTTCTTTT GAGGGGTTGA AATGTTTATT CTGAATTGTG
121    TTTTTTCTTC TGGCGACTA AACTAGTAG ATTGTAAGTT ACAAATTTA GTCAAATCTT
181    CGTATGGTCA CTCAACTGAT ACGCTAAGAA AGTCGCTTTC TTTGGTTGAA GAAAATTGGA
241    ATCAAGAGTA AAGACGACTT TTTTAGATAC TAACTATTAG TTGAGTGACA TTCTAAATAG
301    AATATTTTGT TTTATTTCTT TGTTAAAACT TTGCGTGATG GATTTGGTGA TTAGTGAAGG
361    TTTTGTCTCA TATTGAAATT GTATTTATTC ACTGTCTTTA CCTCCAGTAG AAATTGATGG
421    GTCTCTCCCC TCAAGATAAA GTTAAACATA ATCATTCCCTA ATTTTGTGAA AATTCAGTCG
481    GTGTACTAAG TTCTGCTAT GTGCGAGGTT CGGAGAGGGA CTGATCACA GGGTATTGTA
541    TGCAGCCTTA CTATGCATTT CTTCAAGAGA CGGTCTCCAT AGCTCGAACC CATGATTTGT
601    GCAATAGGCT GTTTCCAATT TTTGTGATTT GTTTAAATTG TTATAACTTA TAGTTTTGTA
661    CCAACTCTTC AATTCGGAAC TAATGTTGAT CTTCTCTTCT TTAGGAAGTT ATGAGC

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Partial Sequence of P6-32 F1/R2 W322 (RIL-73, has introgression at 25 cM and is susceptible to begomoviruses)

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1      TGCTGTAGAA TTTGAATCTG TNATGGAGG TCAGCATCTT ATTTTTTCTT ACTTAGGTTT
61     ATGAATCTGT GTTTTACCCT TTTTCTTTTG AGGGGTTGAA ANGTTTATTG TGAATTGTGT
121    TTTTCTTCTT GGGCGACTAA AACTAGTAGA TTGTAAGTTA CAAAATTTAG TCAAATCTTC
181    GTATGGTCAC TCAACTGATA CGCTAAGAAA GTCGCTTTCT TTGGTTGAAG AAAATTGGAA
241    TCAAGAGTAA AGACGACTTT TTTAGATACT AACTATTAGT TGAGTGACAT TCTAAATAGA
301    ATATTTTGTG TTATTTCTTT GTGAAAACCT TGCGTGATGG ATTTGGTGAT TAGTGAAGGT
361    TTTGTCTCAT ATTGAAATTG TATTTATTCA CTGTCTTTAC CTCCAGTAGA AATTGATGGG
421    TCTCTCCCCT CAAGATAAAG TTAAACATAA TCATTCCCTAA TTTTGTGAAA ATTCAGTCGG
481    TGTACTAAGT TCCTGCTATG TGCGAGGTTT GGAGAGGGAC TGATCACAAG GGTATTGTAT
541    GCAGCCTTAC TATGCATTTT TCAAGAGAC GGTCTCCATA GCTCGAACC ATGATTTGTG
601    CAATAGGCTG TTTCCAATTT TTGTGATTTG TTTAAATTGT TATACTTAT AGTTTTTTAC
661    CAACTCTTCA ATTCGGAAC TATGTTGATC TTCTCTTCTT GAGGAAGTTA TGAGCGGGAG
721    AACAAAGCATC

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Comparison of W312 (Resistant) with W316 (Susceptible)

Upper line: W316, from 1 to 753
Lower line: W312, from 2 to 716

W316:W312 identity= 96.78%(691/714) gap=5.43%(41/755)

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1      .TGCTGTAGAAATTTGAATCTGTTTATGGAGGTCAGCATCTTATTTTTTCTTAATTAGG
      |||
1      TTGCTGTAG.AATTTGAATCTGTTTATGGAGGTCAGCATCTTATTTTTTCTTAATTAGG

60     TTTATGAATCTGTGTTTTACCCTTTTTCTTTTGGGGGTTGAAATGTTTATTCTGAATT
      |||
59     TTTATGAATCTGTGTTTTACCCTTTTTCTTTTGGGGGTTGAAATGTTTATTCTGAATT

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180 CGTATGGTCACTCAACTGATACGCTAAGAAAGTCGCTTTCTTTGGTTGAAGAAAATTGGA
    |||
181 CGTATGGTCACTCAACTGATACGCTAAGAAAGTCGCTTTCTTTGGTTGAAGAAAATTGGA

240 ATCAAGAGTAAAGACGACTTTTTTAGATACTAACTATTAGTTGAGTGACATTCTAAATAG
    |||
241 ATCAAGAGTAAAGACGACTTTTTTAGATACTAACTATTAGTTGAGTGACATTCTAAATAG

300 AATATTTTGTATTCTTTTGTGAAAACCTTTCGCTGATGGATTTGGTGATTAGTGAAGG
    |||
301 AATATTTTGTATTCTTTTGTGAAAACCTTTCGCTGATGGATTTGGTGATTAGTGAAGG

360 TTTTGTCTCATATTGAAATTTGATTTTATCTACTGTCTTTACCTCCAGTAGAAAATTGATGG
    |||
361 TTTTGTCTCATATTGAAATTTGATTTTATCTACTGTCTTTACCTCCAGTAGAAAATTGATGG

420 GTCTCTCCCTCAAGATAAAGTTAAACATAATCATTCCTAATTTTGTGAAAATTCAGTCG
    |||
421 GTCTCTCCCTCAAGATAAAGTTAAACATAATCATTCCTAATTTTGTGAAAATTCAGTCG

480 GTGTACTAAGTTCCTGCTATGTGCGAGGTTCCGAGAGGGACTGATCACAAGGGTATTGTA
    |||
481 GTGTACTAAGTTCCTGCTATGTGCGAGGTTCCGAGAGGGACTGATCACAAGGGTATTGTA

540 TGCAGCCTTACTATGCATTTCTTCAAGAGACGGTCTCCATAGCTCGAACCCATGATTTGT
    |||
541 TGCAGCCTTACTATGCATTTCTTCAAGAGACGGTCTCCATAGCTCGAACCCATGATTTGT

600 GCAATAGGCTGTTTCCAATTTTTGTGATTTGTTTAAATGTTATAACTTATAGTTTTTTA
    |||
601 GCAATAGGCTGTTTCCAATTTTTGTGATTTGTTTAAATGTTATAACTTATAGTTTTTTA

660 CCAACTCTCAATTCGGAACATAATGTTGATCTTCTCTTCTTGGAGGAAGTTATGAGCGGGA
    |||
661 CCAACTCTCAATTCGGAACATAATGTTGATCTTCTCTTCTTGGAGGAAGTTATGAGC

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Blast Search (search sequence= W316, susceptible genotype, *S. lycopersicum* sequence)

Matched nothing with greater than 90% nucleotide identity on NCBI database. Matched the following BAC clone on SGN website: >[C06HBa0307J13.1](#) AC211075.1 htgs_phase:1 submitted_to_sgn_as:C06HBa0307J13 sequenced_by:cbsg upload_account_name:netherlands [organism=Solanum lycopersicum]

Length = 94,250

Score = 1479 bits (746), Expect = 0.0
Identities = 760/762 (99%), Gaps = 2/762 (0%), Frame = +1 / -1

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Query: 10 aattdgaatctgtttatggaggtcagcatcttatttttttcttaattaggtttatgaaat 69
    |||
Sbjct: 26988 aattdgaatctgtttatggaggtcagcatcttatttttt-cttaattaggtttatgaa-t 26931

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```

Query: 70 ctgtgttttaccctttttcttttgaggggtgaaatgtttattctgaattgggtttttct 129
    |||
Sbjct: 26930 ctgtgttttaccctttttcttttgaggggtgaaatgtttattctgaattgggtttttct 26871

```

Query: 130 tctggggtgactaaaactagtagattgtaacttacaaaattagtcaaactcttcgatgg 189
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26870 tctggggtgactaaaactagtagattgtaacttacaaaattagtcaaactcttcgatgg 26811

Query: 190 tcactccactgatacgctaagaaagtcgctttctttggtgaagaaaatcggaattaagag 249
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26810 tcactccactgatacgctaagaaagtcgctttctttggtgaagaaaatcggaattaagag 26751

Query: 250 taaagacggcctttttgagataactaactattagttgagtaacattctaaatagaatattt 309
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26750 taaagacggcctttttgagataactaactattagttgagtaacattctaaatagaatattt 26691

Query: 310 tgttttattcttctgtgttaaaactttgcgctgatggataataatttctgtctgcagcacatg 369
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26690 tgttttattcttctgtgttaaaactttgcgctgatggataataatttctgtctgcagcacatg 26631

Query: 370 tgtttggtgattagtgaaaggttttctctcatattgaaattgtatttattcactgtcttta 429
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26630 tgtttggtgattagtgaaaggttttctctcatattgaaattgtatttattcactgtcttta 26571

Query: 430 cctccagtagaaattgatgggtctctccccttaagattaaggtaaacataatcattccta 489
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26570 cctccagtagaaattgatgggtctctccccttaagattaaggtaaacataatcattccta 26511

Query: 490 attttgtgaaaactcagtcggtgtactaagttcctgctacgtgtgaggttcggagagggga 549
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26510 attttgtgaaaactcagtcggtgtactaagttcctgctacgtgtgaggttcggagagggga 26451

Query: 550 ctgatcacaacttcacaagggtattgtatgcagccttactatgcatttcttcaagagacg 609
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26450 ctgatcacaacttcacaagggtattgtatgcagccttactatgcatttcttcaagagacg 26391

Query: 610 gtctccatagctcgaacccatgatttgtgcaataggctgttccaatttttgtgatttgt 669
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26390 gtctccatagctcgaacccatgatttgtgcaataggctgttccaatttttgtgatttgt 26331

Query: 670 ttaaattggtgtaagttatagttttttaccaactcttcaattcggaactaatgttgatct 729
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26330 ttaaattggtgtaagttatagttttttaccaactcttcaattcggaactaatgttgatct 26271

Query: 730 tctcttcttttaggaagttatgagcgggagaacaagcatcttgg 771
 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
 Sbjct: 26270 tctcttcttttaggaagttatgagcgggagaacaagcatcttgg 26229

>[C06HBa0301C21.1](#) AC211071.1 htgs_phase:1 submitted_to_sgn_as:C06HBa0301C21 sequenced_by:cbssg
 upload_account_name:netherlands [organism=Solanum lycopersicum]
 Length = 148,371

Score = 1479 bits (746), Expect = 0.0
 Identities = 760/762 (99%), Gaps = 2/762 (0%), Frame = +1 / +1

