

Chromosome 3, 71 cM

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BACKGROUND: Dr. Y. Ji, University of Florida, indicated that there was an introgression associated with Ty4 gene on chromosome 3 near 76-85 cM. Originally, Ty4 was thought to be on chromosome 6 between 40-80 cM. Our UW-team scanned this region at 5-cM intervals and did not find any evidence for an introgression in Gc171, which gives the SCAR marker for Ty4. With this new information and the information from Dr. Ji, our group starting scanning chr. 3 from 66 - 85 cM to check for an introgression. Begomovirus resistant inbred used was G70, which was a selection from Gc171 by a susceptible hybrid. The susceptible germplasm was HUI-VF (lab code, W168, an inbred from Hebrew University of Jerusalem, F. Vidavski and H. Czosnek) and M82.

Primers

P3-71a F1: GAGTTTTACCTGAGATATTACGTGG

P3-71a R1: GCCAGTTGTTATCATCTTCCTTC

Annealing temperature: 53 C.

Partial Sequence of G70 (Resistant Inbred), p3-71a

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1      GAATGATACA ATGATTTCGCA AGGAAGTCTT CCTTACCCTT TCTGTCTCTCA AAGAATGCCG
61     CCGCATTGTT GCTGATAGCG AGGTTTTPTTA CCCTCTCCCT TTTTTCGAC  TGCATTTTGT
121    TGTTTTGGCT  TACAATTAGG GTTTGGGTCA AACTCGCTTT CTCCTTTCAA TTTGGTTTTA
181    AGGGTTTGCA GGTGATTTTT GCTACATGTT ATTTATGGGT TTTACTTTGA TTAGTGCAAT
241    GCATCGTCAG TCCAGAGCAC GTTTGCAGGA TGAGTAGTTT TAACTATGAT GTGGAGGATT
301    CATATAGCTA GATAGCCTCA GTTAATGTGA GAATGAGGCA TAATGATTG  ATTGATCGTC
361    TATGGGGTGC TGTCCATTAG CTTTCGGTTG TTACTTATGT TCTGATTTAG GAAAAATCCA
421    AATTATTTGT AGGTCAATGA TTGTGGGAAG GTCAATGAGA AGACTGGAAT TCCGATTTTT
481    AGAGCCATTA CCTAAGAATT GGNAGTAAAG AGCCAAAATT CTCATCCAG  GAGAAAAGATA
541    ATCACCATAT TTTGGGCAAT TTTGGTGCAA CATTCATTGC TGATAAGCAA AATTATCATT
601    TTGATAGCAT GGAAGTAGGT CTACTTTTCT AGATTCAATT AGGTGTAAT  TTCTAGGAAT
661    CAATTTGGTG AAAAGAAGAT TTTTCCTTGT TTCATTCATT TGAATCGTTT TCCTAGGAAG
721    ACTTCCTTCA GAAGGAGAGA TGTAGCTGCG GTGGAGGGTT GGGTTTTTTC TTTTTTCATG
781    ACTAGCTTTC GCTTGTACT  TGTGTCCTGA TTCTGCCAAA ATCGGAGTCT CTTTTTTTTT
841    TGACGACAAG GAAAACCCGC AGCCGCTACC CTTTGGGTG  CGCACAGGGT AAAACCCCGC
901    TCCTATGGGA AGACCAATGA AAAGAGAGGA ATACCAATTT TTAGAGCCAT TTGGATCTAA
961    TAATGTTAGT GAAGAAGCAA AACTGTCATA TCAGAAGAAA GAGAACCATG TTATTTGGAG
1021   CAATTTTGGG GTTGACGTTT ATTGCAACGA AGCAAATTA  TAATTTGAT  GACATGTAAA
1081   TTCGTCTATA AAAAACGAGA TGTTAAATTT CTAGTTATCA CATAGATGAA CTTTCTTGT
1141   TTGAATTGTT GTCCAAGGAA GGGTTCCTTT AGGAGGGGAG AGAGGTGCAG CGGAGGGTTG
1201   GGGTTTGTTT TGTTACAGTG TGATGTAATG TGTGTTTTAT GCAACTCCGA CTTTGTATCT
1261   CTTCCACCT
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Partial Sequence of M82 (Susceptible), p3-71a

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1      AAGAATGATA CAATGATTTC CAAGGAAGTC TTCCTTACCC CTTCTGTTCT CAAAGAATGC
61     CGCCGCATTG TTGCTGATAG CGAGGTTTTT TATCCTCTCC CTTTTTTTCG ACTGCATTTT
121    GTTGTTTTGG CTTACAATTA GGGTTTGGGT CAAACTCGCT TTCTCCTTTG GTTTGCAGGT
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181  GATTTTTGCT ACATGTTATT TAGGGGTTTT ACTTTGATTA GTGCAATGCA TCGTCAGTCC
241  AGAACACGTT TGCTGGATGA TTAGTTTTAA CTATGATGTG GAGGATTCAT ATAGCTAGAT
301  AGCCTCAGTT AATGTGAGAA TGAGGCATAA TTGATTGATT GATTGTCTAT GGGGTGCCGT
361  CCAATAGCTT TCGGTTGTTA CTTATGTTCT GATTTAGGAA AAATCCAAAT TATTTGTAGG
421  TCAATGATTG TGGGAAGGTC AATGAGAAGA CTGGAATTCC AATTTTTAGA GCCATTACCT
481  AAGAATTTGGT AGTAAAGAGC CAAAATTCCTC ATCCCAGGAG AAAGATAATC ACCATATTTT
541  GGGCAATTTT GGTGCAACAT TCATTGCTGA TAAGCAAAAT TATCATTTTA ATAGCATGGG
601  AACTAGGTCA AATTTTCTAG ATTCAAATAG GTGTAAATTT CTAGGTATCA ATTTGTCAAA
661  AAGAAGATTT TTCCCTGTTT CATTCAATTTG AATCGTTTTT CTAGGAAGAC TTCCTTAAGA
721  AGGAGAGAAG CAGCTGCGGT GGAGGGTTGG GGTTTTTTCTT TTTTCATGAC TAGCTTTCGC
781  TTGTTACTTA TGTTCTGATT CTGCCAAAAT CAGAGTCTTT TTTTTTTTTT TTATGACAAG
841  GGAAACCCGC AGCCTCTACC CTTTGGGTGC GAACAGGATA AAACCCCGCT CCTATGGGAA
901  GACCAATGAA AAGAGAGGAA TACCAACTTT TAGAGCCATT TGGATCTAAT AATGTTAGTG
961  AAGAGGCAAA ACTGTCATAT CAGAAGAAAG ATAACCATGT TATTTGGAGC AATTTTGGGG
1021 TTAACGTTCA TTGCAACGAA GCAAAATAT TATTTTGATA ACATAAAAAA ACGAGATGTA
1081 AAATTTCTAG TTATCACATA AATGAACTTT TCTTGTTTGA ATTGTTGTCC AAGTAAGGGT
1141 TCTTTTAGGA GGGGAGAGAG TTGTAGCGGA GGGCTGGGGT TTGTTTTGTT CAGTGTGATG
1201 TAATGTGTGA TTTTTTGCAA CTCCGACTTT GTATCTCTTC CACCT

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Partial Sequence of W168 (Susceptible), p3-71a

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1    TACAAGAATG ATACAATGAT TCGCAAGGAA GTCTTCCTTA CCCCTTCTGT TCTCAAAGAA
61   TGCCCGCCGA TTGTTGCTGA TAGCGAGGTT TTTTATCCTC TCCCTTTTTT TCGACTGCAT
121  TTTGTTGTTT TGGCTTACAA TTAGGTTTGG GGTCAAACCTC GCTTCTCCTT TTGTTTGCA
181  GGTGATTTT GCTACATGTT ATTTAGGGGT TTTACTTTGA TTAGTGCAAT GCATCGTCAG
241  TCCAGAACAC GTTTGCTGGA TGATTAGTTT TAACATGAT GTGGAGGATT CATATAGCTA
301  GATAGCCTCA GTTAATGTGA GAATGAGGCA TAATTGATTG ATTGATTGTC TATGGGGTGC
361  CGTCCAATAG CTTTCGGTTG TTACTTATGT TCTGATTTAG GAAAAATCCA AATATTTTGT
421  AGGTCAATGA TTGTGGGAAG GTCAATGAGA AGACTGGAAT TCCAATTTTT AGAGCCATTA
481  CCTAAGAATT GGTAGTAAAG AGCCAAAAT CTCATCCCAG GAGAAAAGATA ATCACCATAT
541  TTTGGGCAAT TTTGGTGCAA CATTCAATTGC TGATAAGCAA AATTATCATT TTAATAGCAT
601  GGGAACTAGG TCAAATTTTC TAGATTCAAT TAGGTGTAAG TTTCTAGGTA TCAAATTTGTC
661  AAAAAGAAGA TTTTTCCTTG TTTTCATTCAT TTGAATCGTT TTCCTAGGAA GACTTCCTTA
721  AGAAGGAGAG AAGCAGCTGC GGTGGAGGGT TGGGGTTTTT CTTTTTTCAT GACTAGCTTT
781  CGCTTGTTAC TTATGTTCTG ATTCCTGCCA AATCAGAGTC TTTTTTTTTT TTTTATGAC
841  AAGGGAAACC CGCAGCCTCT ACCCTTTGGG TGCGAACAGG ATAAAACCCC GTCCTATGG
901  GAAGACCAAT GAAAAGAGAG GAATACCAAC TTTTAGAGCC ATTTGGATCT AATAATGTTA
961  TGGAAGAGC AAAACTGTCA TATCAGAAGA AAGATAACCA TGTATTTGG AGCAATTTG
1021 GGGTTAACGT TCATTGCAAC GAAGCAAAAT TATTATTTTG ATAACATAAA AAAACGAGAT
1081 GTAAAATTT TAGTTATCAC ATAAATGAAC TTTTCTGTG TGAATTGTTG TCCAAGTAAG
1141 GGTTCTTTTA GGAGGGGAGA GAGTTGTAGC GGAGGGCTGG GGTTTGTTTT GTTCAGTGTG
1201 ATGTAATGTG TGTTTTTTGC AACTCCGACT TTGTATCTCT TCCACCTCT

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Comparison of M82 (upper line) with G70 (lower line)

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1    AAGAATGATACAATGATTTCGCAAGGAAGTCTTCCTTACCCCTTCTGTTCTCAAAGAATGC
    |||
1    . . GAATGATACAATGATTTCGCAAGGAAGTCTTCCTTACCCCTTCTGTTCTCAAAGAATGC

61   CGCCGCATTGTTGCTGATAGCGAGGTTTTTTATCCTCTCCCTTTTTTTTCGACTGCATTTT
    |||
59   CGCCGCATTGTTGCTGATAGCGAGGTTTTTTACCCTCTCCCTTTTTTTTCGACTGCATTTT

121  GTTGTTTTGGCTTACAATTAGGGTTTGGGTCAAACCTCGCTTCTCCTTT . . . . .
    |||
119  GTTGTTTTGGCTTACAATTAGGGTTTGGGTCAAACCTCGCTTCTCCTTTCAATTTGGTTT

170  . . . . GGTTTGCAGGTGATTTTGTACATGTTATTTAGGGTTTTACTTTGATTAGTGCA
    |||
179  TAAGGGTTTGCAGGTGATTTTGTACATGTTATTTATGGGTTTTACTTTGATTAGTGCA

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706  AGGAAGACTTCCTTAAGAAGGAGAGAAGCAGCTGCGGTGGAGGGTTGGGGTTTTTCCTTTT
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715  AGGAAGACTTCCTTCAGAAGGAGAGATGTAGCTGCGGTGGAGGGTTGGGGTTTTTCCTTTT

766  TTCATGACTAGCTTTTCGCTTGTACTTATGTTCTGATTCTGCCAAAATCAGAGTCTTTTT
    |||
775  TTCATGACTAGCTTTTCGCTTGTACTTGTGTCTGATTCTGCCAAAATCGGAGTCTC . . T

826  TTTTTTTTTTATGACAAGGGAAACCCGCAGCCTCTACCC . TTTGGGTGCGAACAGGATAA
    |||
833  TTTTTTTTTTGACGACAAGGGAAACCCGCAGCCGCTACCCTTTTGGGTGCGCACAGGGTAA

885  AACCCCGCTCCTATGGGAAGACCAATGAAAAGAGAGGAATACCAACTTTTAGAGCCATTT
    |||
893  AACCCCGCTCCTATGGGAAGACCAATGAAAAGAGAGGAATACCAATTTTAGAGCCATTT

945  GGATCTAATAATGTTAGTGAAGAGGCAAACTGTCATATCAGAAGAAAGATAACCATGTT
    |||
953  GGATCTAATAATGTTAGTGAAGAAGCAAACTGTCATATCAGAAGAAAGAGAACCATGTT

1005  ATTTGGAGCAATTTTGGGGTTAACGTTTCATTGCAACGAAGCAAATATATTTTGATAA
    |||
1013  ATTTGGAGCAATTTTGGGGTTGACGTTTCATTGCAACGAAGCAAATATATAATTTTGATGA

1065  CATAAAAAACGAGATGTAAAATTTCTAGTTATCACATAAATGAACTTTCTTGTGTTGAA
    |||
1073  CATGTAAATTCGTCAT . . AAAAAACGAGATGTTAAATTTCTAGTTATCACATAGATGAA

1125  TTGTTGTCCAAGTAAGGGTTCTTTTAGGAGGGGAGAGAGTTGTAGCGGAGGGCTGGGGTT
    |||
1131  CTTTTCTTGTGTTGAATTGTTGTCCAAGGAAGGGTTCTTTTAGGAGGGGAGAGAGGTGCAG

1185  TGTGTTGTTTCAGTGTGATGTAATGTGTGTTTTTTGCAACTCCGACTTTGTATCTCTTCCA
    |||
1191  CGGAGGGTTGGGGTTTGTGTTTGTTCACGTGTGATGTAATGTGTGTTTTATGCAACTCCGA

1245  CCTCT
    ||
1251  CTTTGTATCTCTTCCACCT

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Blast Search

Matched nothing on NCBI database greater than 90% on February 11, 2009.
Matched with greater than 90 % the following from SOL genomics database:

[SGN-M3864](#) C2_At1g02140 [cosii_markers]

Length = 1,923

Score = 155 bits (78), Expect = 1e-37
Identities = 78/78 (100%), Frame = +1 / +1

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Query: 5  gatacaatgattcgcaaggaagtcttctacccttctgttctcaaagaatgccgccgc 64
    |||
Sbjct: 199 gatacaatgattcgcaaggaagtcttctacccttctgttctcaaagaatgccgccgc 258

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Query: 65  attgttgctgatagcgag 82
    |||
Sbjct: 259 attgttgctgatagcgag 276

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Summary

The data for p3-71a came out exceptionally well. All three samples (G70, M82 and W168 had excellent sequence that was easy to piece together. When a comparison was done between G70 (resistant inbred) and either susceptible plant (M82 or W168), the results showed a lot of differences. There is one large indel, and many single nucleotide polymorphisms (SNPs). Therefore, our conclusion is that there is an introgression at 71 cM.