

COSII Marker C2_At1g07960, 82.50 cM

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Fig.1: Map of the top of Chr. 11. (Modified from Solanaceae Genomics Network, 2006).

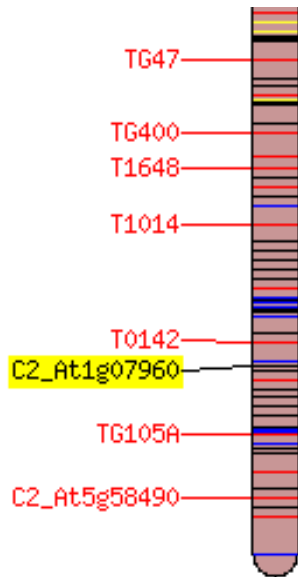


Table 1: Primers from C2_At1g07960 on Chr. 11

Primer Name	Sequence (5' to 3')
DM11 - F11	ATGGTTTGTCAAATTTTGTGTTC
DM11 - R11	AAGAGTTTGAATGTAGGGTATGAATG

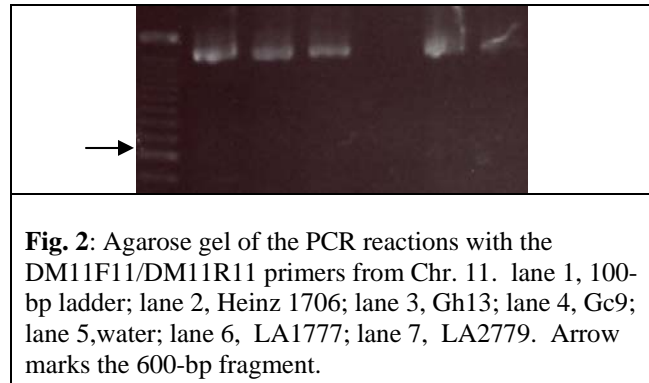


Fig. 2: Agarose gel of the PCR reactions with the DM11F11/DM11R11 primers from Chr. 11. lane 1, 100-bp ladder; lane 2, Heinz 1706; lane 3, Gh13; lane 4, Gc9; lane 5, water; lane 6, LA1777; lane 7, LA2779. Arrow marks the 600-bp fragment.

Background: The purpose of this project was to locate molecular markers for disease resistance in tomato. To accomplish this goal, primers were obtained from the Solanaceae Genomics Network (SGN) website (Solanaceae Genomics Network, 2006), and used with five different tomato lines.

We used the tomato breeding lines, Gh13 and Gc9 which are resistant to the bipartite begomoviruses in Guatemala (Mejía *et al.*, 2004; Nakhla *et al.*, 2004). Gh13 is the F7 generation and is a homogeneous breeding line with resistance derived from *L. hirsutum*. Gc9 is at least an F8 breeding line with resistance genes introgressed from *L. chilense* by J. W. Scott (Scott *et al.*, 1995). LA1777 is the *L. hirsutum* parent, and is thought to be the source of the introgression in Gh13. LA2779 is the *L. chilense* parent and is thought to be the source of the introgression in Gc9 (Maxwell, D., pers. com.)

As a control, we used the breeding line Heinz 1706. Heinz 1706 is the tomato cultivar being sequenced in an international sequencing project (Budiman *et al.*, 2000; Ozminowski, 2004), and is susceptible to geminiviruses (Hapidat, M., pers. com.). The susceptibility of Heinz 1706 to geminiviruses was confirmed through testing with *Tomato Yellow Leaf Curl Virus*, which is a begomovirus (Maxwell, D., pers. com.).

The begomovirus resistant lines, Gh13 and Gc9 were supplied by Dr. L. Mejía, Universidad de San Carlos, Guatemala City. The susceptible line, Heinz 1706, was supplied by Dr. R. Ozminowski, Heinz Seed Co., Stockton, CA.

Polymerase Chain Reaction (PCR): PCR fragments from each set of primers, for each of the five genotypes, were obtained using methods developed in the Maxwell lab (Czosnek *et al.*, 2004). PCR parameters were for 50- μ l reactions containing: 5- μ l 2.5mM deoxynucleotide triphosphates (dNTPs), 5- μ l 10X buffer, 5- μ l 25 mM MgCl₂, 0.2- μ l *Taq* DNA polymerase, 5- μ l each forward and reverse sense primer at 10 μ M, 5-7 μ l of DNA extract, and H₂O. Some PCR reactions were run with 25- μ l reactions. When this was the case, the concentrations of all chemicals were exactly half of what appeared in the 50- μ l reactions. PCR cycle parameters for fragment amplification were as follows: denaturation at 94°C for 3 min, then 35 cycles at 94°C for 30 sec each, annealing at 53°C for 1 min, and extension at 72°C for 1 min. These cycles were followed by a reaction at 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).

The PCR-amplified DNA was run on an electrophoresis gel of 1.5% Seakem LE™ agarose (BioWhittaker Molecular Applications Rockland, ME) in 0.5X TBE buffer, stained with ethidium bromide, and visualized with a Kodak Gel Logic 200 Imaging System.

DM11F11-R11 Results: The DM11F11/DM11R11 primer pair was chosen from the list of COSII primers on the SGN website (Solanaceae Genomics Network, 2006). The primer pair produced a single band with all tested samples at greater than 1400bp (Fig. 2). This PCR product was directly sequenced with both the forward and reverse primer. Gc9, Gh13, and LA2779 gave sequence with both the forward and reverse primers. Heinz 1706 gave sequence with only the forward primer and LA1777 gave sequence with only the reverse. Upon alignment, there were no SNP or INDEL that distinguished the begomovirus resistant breeding lines from the susceptible Heinz 1706. LA1777 differed from Heinz 1706 by greater than 10 SNP although the sequence is not of a high enough quality to determine an exact number. LA2779 differed from Heinz 1706 by at least 15 SNP and 2 INDEL. Thus, there is no indication that a molecular marker for begomovirus resistance can be found at this location.

SEQ Gh13_DM11F11-R11, Genbank Accession DQ855117, 1443 bp;

ORIGIN

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1      GCAGCATCTG CTTCTAGTA AAATCATGAA TTGGTAGTAA TCCGTTATAA CCATTTGGAA
61     ACTAGGCTGA AATGTTTGTG TTCTGAAAAG ACAAGTGCAG CTTAGTGTTC ATTGCTATAT
121    AAGTCTATTA TCAGGATCTA AAAGATAACC AATTAGGGCA CAAATCTTTG AAAGGGATTTC
181    TTGTAACATG GTTCAAAGCC ATTGTTACCG TCTCCGTGCT AAGCAGCACC AAACATTCTT
241    CTGTTAGCAA TACACATAAA GAATTCTCTT GGTCAAATGA AGGAAAGTTT AAAGCGCGTT
301    TTATATTACA TGCTAATTAC CATTATTAGC ATATTGCACA CTTTCTTTTG TCTAGACTAC
361    AATGTAGCAG AAGATGATCC TCATTCTCCC CTGCTTCCTC CTTGTATAGG TAACACCAAC
421    TAATGCAAAC AACTTTATGT TTCCTAAGGT TTAGGTTTTT CACCTTCAAA ATCACCCCTC
481    CTAGTAGCTG ACTATGTGAA AGAAACACCT TTGTTGCCAC CATAGTGATC CAAACCGAGT
541    TCCAATGGAT AACAATCCTC CTCCTAATA AAAACCTTTT CATAAGAGGC CTTACAGAG
601    AAAACTTTTT TGTTATTATC GTTTTTCGATT GGTTAGGTAG AAAACTTTGT TATTCCCCAA
661    CTCCCAACTC AAAGCCTCAA GTCTACCAGC TGGCTACCA TGCTTATGAA GTAAAGCAAG
721    CAATCTTTGA AACTCATTCA TCTCCCAATC TTGGAAATCC CTCCTGTAAC CCAAGTCCCA
781    AAACGTCTTT CCCCCTAGA TTTCCCCTGA TTTGCTGGAC AAATAAGTCC CTTTGGCATG
841    AACTCTATG CCTACATGGA AACTCATCCT CTTACAGAAG TCCAATGCCA ACGTAATGGT
901    TTCTAGTGGG AATACATCGT GAACCCATGT ATATATCATC TGCTAATAGA CAGCTAAAAG
961    TCCTCCACAC CACTTATGGC TCCAAAAATT TACTCTCCTC CCATCTCCAA CTCTAAAAGA
1021   AATGTTACCA TAAAAATCGT GCCAACCTT CATAATATTC CTCCACTC CACACTCAA
1081   CGGCATGGTG ACATCATTTG TTCTCCACAT ATGAGTCTAT ATCCGACTTG ATCCCTGAAG
1141   GCTTTTCTTG ATTTTCCAAA TTGAATTCC TGAAGGTGCC TTGATTCTCT GAACAACCTG
1201   TAGTGCATGA ATGTGGCTTG CCTCAAAAAA AAAATAATGG AAAGGATGAT AACTGTTTTA
1261   CCTCTGGTAT TTCTTCATAT ACATCAGAAT TTTAGTCATT CATATGTTCA TTTCTGTATT
1321   TTCCTCGCAA GGGGCTTCTG TTTTGCTAAT TCTGAAACCT TTTTTTCAGC AAAAACCTGG
1381   GAACACTTTG GGATGATTTG GGGAAACCAA GGAGGAGGGA GGATGAGATA GAGGTGGGAC
1441   AAG

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SEQ LA2779_DM11F11-R11, Genbank Accession DQ855118, 1348 bp;

ORIGIN

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1      TTAGCAGCAT CTGCGTTCCT AGTAAAATCA TGAATTGGTA GTAATCTGTT ATAACCATTT
61     GGAAACTAGG CTGAAATGTT TGNCTTCTGA AAAGACAAGT GCAGCTTAGT GTTTATTGCT
121    ATATAAGTCT ATTATCAGGA TCTAAACGAT AACCAATTAG GGCAAAAATC TTTGAAAGGG
181    ATTCTTGTA CATGGTTCAT AGCCATTGTT ACCGTCTCCG TGCTAAGCAG CACCAAACAT
241    TTTTCTGTGA GCAATGCACA TAAAGAATTC TCTTGGTCAA ATGCATGAAA GTCTATAGCG
301    CGTTTTTATAT ACATGCTAAT TACCATTATT AGCATATTGC AACTTTTCTT TTGTCTAGAC
361    TACAATGTAG CAGAAGATGA TCCTCATTCT CCCCTGCTTC CTCCTTGAT AGGTAACACC
421    AACTAAGCAG ACAACTTTAT GTTTCCTAAG GTTAGGTTTT CCACCNTCAA AATCACCCCT
481    CCTAGTAGCT GACTATGTGA AAGAAACACC TTCCTTGCCA CCATAGTGAT CCAAACCGAG
541    TTCCAATGGA TAACAATCCT CCTCCCTAAT AAAAACTTT TCATAAGAGG CCTTCACAGA

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601 GAAAACCTTTG TTGTTATTAT CGTTTTTCGAT TGGTTAGGTA GAGAACCTTTG TTATTCCCCA
661 ACTCCCAACT CAAAGCCTCA AGTCTACCAG CTGGCTCACC ACGCTTATGA AGTAAAGCAA
721 GCAATCTTTG AAACCTCATT ATCTCCCAAT CTTGGAAATC CCTCCTGTAA CTCAAGTCCC
781 AAAACGTCCT TCCCCCCTTT CCCCTGATTT GCTGGTCAGA TAAGTCCCTT TGGCATGAAA
841 CTCTATGCAT ACATGGAAAC TCATCCTCTT ACAGAAAGTCC AATGCCAACG TCATGGTTTT
901 TAGTGGGAAT ACATCGTGAA CCCATGTATA TATCATCTGC TAATACACAG CCAAAGTCC
961 CCCNCNCCAC TTATGGCTCT AAAAATTTAC TCTTCTCCCA TCTCCAACCTC TAAAAGAAAT
1021 GTTACCATAA AAATCGTGCC ACCCCTTCAT AATATTCCTC CACACTCAA CCGCACGGNG
1081 ACATTATTTG TTCTCCACAT ATGAGTTCTG TATCCGACTT GATCCGTGAA GGCTTTTTCTT
1141 GATTTTCCAA ATTTGAATTC CTGAAGGTGC CTTGATTCTC TGAACAACCT GTAGTGCATG
1201 AATGTGGCTT ACCTCAAAAA AAAAATTAAT GGAAAGGATG ATAACCTGTTT TACCTCTGTT
1261 TTTTATTCAT ATACATCAGA ATCTTAGTCA TTGATATGTT CATTTCTGTA TTTTCCTCGC
1321 AAGGGGCNTC TGTTTTGCTA ATTCTGAA

SEQ LA1777 DM11R11: 1102 bp:

ORIGIN

1 CCTTCNTTCT TCTTGGTNGG GGAACCCCN NNNNAANGNCA ANCNNTTTTT TTTTCNTAAG
61 GGTTTGGTTT TTCCACAATN AAAATNCCCC CNTNNNGNAG CGGGNAAGGG AAAGAAANCC
121 CTTCCNTGCC NCCATAGNGA TCCAANCCNA GTNGCCAANG GATAACCCAT CCTCCTCCNA
181 AATAAANNCC TTTGCNTNAG AGGCCNTCAC AGAGAAAANT TTGTTGTTAT TNCGTTTTG
241 GGANGGGNGA GGAAGAAAAN TTTGTTATTC CCCAACTCCC AACTCAAAGC CTCAAGTNTT
301 CCAGCTGGCT CNCCCANGNT TANGANGTAA AGCAAGCNAN CTTTGAAACT CANTCATCTC
361 CCNANATTGG AAATCCCTCC CGTAACTCAA GTCCCAAANC GTCCTTCCCC CNTAGATTTT
421 CATTGNGNNG CNGGACAGAT AAGTCCCTNT GGCATGAAAC TCTATGCNNA CATGGAAACT
481 CACCCTCTTN CAGAAGTCCA ATGCCAAGNG TCATGGTTNG GTAGTGGGAA TACATCCNTG
541 TGAACCCACG TATATATCAT NTGATAATAA ACNGCCAAAA GTCCCCACG CCNCCTANGG
601 ATCCAAAAAT TTACNCTTCT NTCATCTCCA ANTANANAAG CCAATGTTAC CATANACANC
661 CCGCCACCCC TTCATAACAC TCCTCCCCAC NCAAGGNCA GGGTGNCCNC ATTGGTTCNC
721 NCCATGGGNG TTCNATTTCN GNCTNGANCC GGGANGGCTT TTCTGGNTTT NCCAAATTGG
781 AATNCCGGAN GGNCCTGGA TTNTNGGANC ANCTNGNNGN NCAGGAANGG GGCTTNCNC
841 AAAAAAAAAA NTAATGGAAA GGATGATAAC TGTTTTACCT CTGGTNTTTA TTCATATACA
901 TCAGAATTTT AGTCATTNAT ATGTTTCAAT CTGTATTTTC CNNNCAAGGG GCATCTGTTT
961 TGCTAATTCT GAAACCTTTT TTTTCAAGCAAG AACCTGGGAA CATCTTTGGG ATGATTTGGG
1021 GAAAATCAAN AGANGGGGGA GGATGAGATA GAGGTGGGAN CAAGTTGATT GTGGTTCTGA
1081 TAATNCCTAG TGNACCAACG TT

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