COSII Marker C2_At5g51700, 103.00cM

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

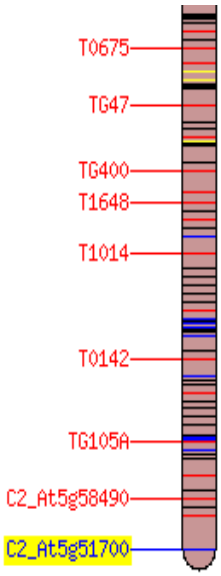

Table 1: Primers from C2_At5g51700 on Chr. 11

Primers	Sequence (5' to 3')
DM11 - F14	AGATGCCACCAGGGATTCTTTTG
DM11 - R14	AGCAGTGTCGTGATTTTCCTTTTC

Fig. 2: Agarose gel of the PCR reactions with the DM11F14/DM11R14 primers from Chr. 11. lane 1, Heinz 1706; lane 2, Gh13; lane 3, Gc9; lane 4, LA1777; lane 5, LA2779; lane 6, water; lane 7, 100-bp ladder. 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; Ozminkowski, 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. Ozminikowski, 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-µl reactions containing: 5-µl 2.5mM deoxynucleotide triphosphates (dNTPs), 5-µl 10X buffer, 5-µl 25 mM $MgCl_2$, 0.2-µl *Taq* DNA polymerase, 5-µl each forward and reverse sense primer at 10µM, 5-7 µl of DNA extract, and H_20. Some PCR reactions were run with 25-µl reactions. When this was the case, the concentrations of all chemicals were exactly half of what appeared in the 50-µl 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.

DM11F14-R14 Results: The DM11F14/DM11R14 primer pair was chosen from the list of COSII primers on the SGN website (Solanaceae Genomics Network, 2006). The primer pair produced a strong single band with all tested samples at roughly 520-bp (Fig. 2). This PCR product was directly sequenced with both the forward and reverse primer pair. Heinz 1706, Gc9, and Gh13 each produced excellent sequence with both primers. LA1777 gave sequence with only the reverse primer and LA2779 gave sequence with only the forward primer. Upon alignment there were no SNP or INDEL that distinguished the begomovirus resistant breeding lines from the susceptible control Heinz 1706. LA1777 differed from the breeding lines by 5 SNP and one INDEL in 200-bp of overlap. LA2779 differed from the breeding lines by 12 SNP and two INDEL in roughly 400-bp of overlap (the area of overlap varies because the sequence quality was variable and only excellent sequence was used in the comparison). Therefore, the breeding lines do not contain an introgression from the parents at this location, and there is no indication that a molecular marker for begomovirus resistance can be found here.

SEQ Heinz_DM11F14-R14, Genbank Accession DQ855121, 506 bp;

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ORIGIN
1       AGATGCCACC AGGGATTCTT TTGTTCTGAT CATGGTAAGT CTTCCATTTT CTAACTTCAT
61      CTAACATTAT ACTTTGGAAG ACTAGGACAA TTCTAGACTT CTAGTTGAGT TTAGCAAAAT
121     TGAAAAATAG CAGGGGAGGC TGTTCATTCT ATAGATGCTG AACTCAACCT TCCCAGTTGT
181     GACTTCCTAG TAGGTTAATA ACGAGGAAAA AGTGTGTTTT CATTTTCACT CTGCCTTTTT
241     CTACTCCCAT TTAGAGACTC TTTTGCTTGT AGAGCCTACT CTGATCAGTT CTTGTGCTAT
301     CATGTTTCAT TTAAGGTTCA CGACCCAGAG AAGCAATTCC AAAAGCATCA AATACAGTAA
361     CATCTCTACC TTCTGAGAGC AATACAGTTG TACAGCAAGA CCATCCAGCT CCAGTGAAAA
421     AGAAAATTGA TATAAATGAG CCCCAAATTT GTAAAAACAA GAGCTGCGGT AAGACCTTTA
481     CAGAAAAGGA AAATCACGAC ACTGCT
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SEQ LA1777_DM11R14: 241 bp;

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ORIGIN
1       CAACCTTCTC AGTTGTGACT TCCTANTAGG TTAATAACGA GGAAAAAGTG TGTTTTCATT
61      TTCACTCTGC CTTTTTCTAC TCCCATTTTA GAGACTCTTT TGCTTGTAGT GCCGACTCTG
121     ATCAGTTCTC GTGCTATCAT GTTTCATTTA AGGTTCACGA CCCAGAGAAG CAATTCCAAA
181     AGCATCAAAT ACAGTAACAT CTCTACCTTC TGAGAGCAAT ACAGTTATAC AGCAAGACCA
241     A
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SEQ LA2779, GenBank Accesión DQ855122, DM11F14: 464 bp;

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ORIGIN
1       AGGAGCTCAT TTCACTTCAT CTACATTATA CTTTGGAAGA CTAGGACAAT TCTAGACCNT
61      TTGTGGAGTT TAGCAAAGCT GAAAGGTAGC AGGGGAGGCT GTTCATTCTA TAGATGCTGA
121     ACTCAACCTT CTCAGTTGTG ACTTCCTAGT AGGTTAATAA CGAGGAAAAA TTGTGTTTTC
181     ATTTTCATTC TGCCTTTCTC TACTCCCATT TTAGACTCTT TTGCTTGTAG TGCCGACTCT
241     GATCAGTTCT CGTGCTATCA TGTTTCATTT AAGGTTCACA ACCCAGAGAA GCAATTCCAA
301     AAGCATCAAA TACAGTNACA TCTCTACCTT CTGAGAGCAA TACAGTTGTA CAGCAAGACC
361     ATCCAGCTCC AGTGAAAAAG AAAATTGATA TAAATGAGCC CCAAATTTGT AAAAACAAGA
421     GCTGCGGTAA GACCTTTACA GAAAAGGAAA ATCACGACAC TGCT
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