COSII Marker C2_At4g32930, 88.00 cM

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

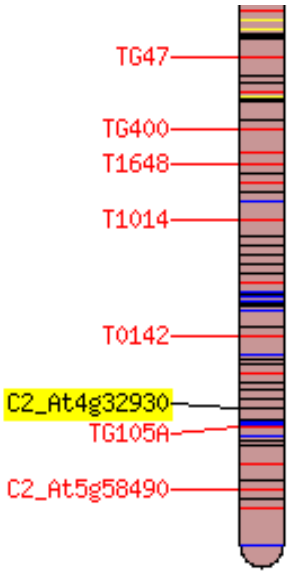

Table 1: Primers from C2_At4g32930 on Chr. 11

Primer Name	Sequence (5' to 3')
DM11 - F12	TCCTCTTCCTATTGGCAAGGGC
DM11 - R12	TGGACACTCCCCCTTTTCATCATAC

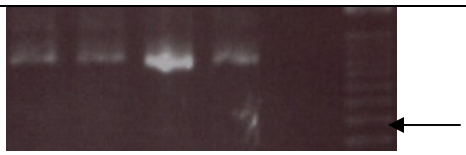

Fig. 2: Agarose gel of the PCR reactions with the DM11F112/DM11R12 primers from Chr. 11. lane 1, Gh13; lane 2, Gc9; lane 3, LA1777; lane 4, LA2779; lane 6, water; lane 7, 100-bp ladder. Arrow marks the 600-bp fragment.

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.

DM11F12-R12 Results: The DM11F12/DM11R12 primer pair was chosen from the list of COSII primers on the SGN website (Solanaceae Genomics Network, 2006). The primer combination produced a strong single band at greater than 1400-bp with all tested samples. The PCR fragments were directly sequenced with both the forward and reverse primers. Gh13 and LA1777 each produced good sequence with the forward primer. No other samples produced any sequence data. Upon alignment, Gh13 differed from LA1777 by 25 SNP and 2 INDEL in roughly 780-bp of overlap. This strongly indicates that Gh13 does not contain an introgression from LA1777 in this region. Thus, it is not likely that a molecular marker for begomovirus resistance can be found at this location. While it is possible that an introgression from L. chilense could have been found in Gc9 at this location, the extensive sequence data from neighboring COSII markers makes this possibility seem highly unlikely.

SEQ LA1777_DM11F12, Genbank Accession DQ855119, 830 bp;

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ORIGIN
1       TGACCCAGAA CTAGTTTACA CTGAATTTAA AATCACCGTG ACAGCCTGGA ATTTGAGGTG
61      AGATACAGAG TTAATTAGGA TATCTTTAAC ATTCAGAATG GGTTAAGCCA TAGTAAATGC
121     CCCTGTTGTT GTTTCTTAGC TCTACATGTT GAATAACTTT TGATATCCTT GGAAGTCTGT
181     TTTGAGCCTT AATTGGTAGC TTCTTTTTTG TTTTTTCTCC TTTGATATCC TATTGCTATT
241     TTACAGCTCT TGATGCTGTA TAGATTTGAT TGATTACTAA GTCTTTTGTG ACTTTTGCTT
301     CCAGTGTAAG TTCTGTGGCA GGGATGGTAC AATAACCATG ATCACTGGCC GCGGCCGTCC
361     TCTGACTCAC GCTGATAGTG AAGCTGGAAA ATCTGCACCT TTGATGCTAT TTGAGTGCAG
421     GGGTTTTGAG CCGCTGGATT ATGTTTTCCA AGGAGAATGG GAAGCTAAAT CTGTAAGCTT
481     CTTCCCTGGC TTTCTGCTTT GGTGTTTCTT AAAATTAAAG AAAAAATTTA TCATAAAAGA
541     AGTTGTCGAA ATATTTAATT CAGGTATGAG ATTTCTTTGC CCTATTAATT TTTCATTTGT
601     TTTGAGCTGT TGTTGGGGTT CTTTGGATAT ATTTCTATTT CTTTAACGAG GTCATGTATC
661     TCCTAGTTAA CTTCTTGGAG AATATGTTCT GCTTACATGT CTAGAGGTGG CAAGATTATC
721     CCGTGAAATC GTAATTTGCC CATGTTTGGG CTGATTATTG GCCCACATTT CTATTAGCTC
781     AGGCCCATCA AAAATTTGGG GCTAATATTT AGCCCAAATT GGNCCCATGA
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SEQ GH13_DM11F12: 809 bp;

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ORIGIN
1       TGGGNGNTCT GAGTGCATAT TTGTTAGNTT TAGATATGAC CCANAACTAG NTTTACACTG
61      AATTTAAAAT CACTGTGACA GCCGGGAATT TGAGGTGAGA TACAGAGTNA ATTAGGANAT
121     CTTTAACATT CAGAATGGGT AAAGCCATAG TAAATGCCCC GGTTGTTGTT TCTTAGCTCA
181     ACANGNTGAA TAATTTTTTA TATCCTTGGA AGTCTGTTTT GAGCCTTAAT TGGTACCNTC
241     TTTTTTGTTT TTTCTCCTTT GATATCCTAT TGCTATTTTG CAGCTCTTGA TGTTGCATAG
301     ATNTGATTGA TTACTAAGTC TTTTGTGACT TTTGCNTCCA GTGTAAGTTC TGTGGCAGGG
361     ATGGNACAAT AACCATGATC ACTGGCCGCG GCCGNCCTCT GACTCACGCT GATAGTGAAG
421     CTGGAAAATC TGCACCTTTG ATGCTATTTG AGTGCAGGGG TTTTGAGCCG CTGGATTATG
481     TTTTCCAAGG AGAATGGGAA GCTAAATCTG TAAGCTTCTT CCCTGGCTTT CTGCTTTGGT
541     GTTTCTTAAA ATTAAAGAAA ATTTTTATCA TAAAAAAAGT TGTCGAAATA TTTAATTCAG
601     GTATGAGATT TCTTTGCCCT ATTAATTTTT CATTTGTTTT GAGCNGTTGT TGGGATTCTT
661     AGGATATATT TCTATNNCTT TAACGAGGTC ATGTATCTCC TAGTTAAACT TCTTGGAGAA
721     TATGTTCTGC TTACATGTCT AGAGGTGGCA AGATTATCCC GTGAAATCGT AATCTGCCCA
781     TGTTTGGGGC TGATNATTGG GCCCCACAC
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