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

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence (5' to 3')</th>
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<tr>
<td>DM11 - F11</td>
<td>ATGGTTTGTCAAATTTTGTGTTCC</td>
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<tr>
<td>DM11 - R11</td>
<td>AAGAGTTTGAATGTAGGGTATGAATG</td>
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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, Ge9; 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 Ge9 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*. Ge9 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 Ge9 (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 Ge9 were supplied by Dr. L. Mejia, Universidad de San Carlos, Guatemala City. The susceptible line, Heinz 1706, was supplied by Dr. R. Ozminkowski, 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 MgCl₂, 0.2-µl Taq DNA polymerase, 5-µl each forward and reverse sense primer at 10µM, 5-7 µl of DNA extract, and H₂O. 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.

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:

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ORIGIN
1  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
61  GAAGATCGATG AAGAATCTTG ATGGATCAGG TATTTTCTCT TTTCTTCTCT
121  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
181  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
241  GAAGATCGATG AAGAATCTTG ATGGATCAGG TATTTTCTCT TTTCTTCTCT
301  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
361  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
421  GAAGATCGATG AAGAATCTTG ATGGATCAGG TATTTTCTCT TTTCTTCTTCT
481  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
541  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
601  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
661  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
721  GAAGATCGATG AAGAATCTTG ATGGATCAGG TATTTTCTCT TTTCTTCTTCT
781  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
841  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
901  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
961  GGAGGATACCC TCTTCCCTCA ATCTTCTCAAC CTTTCCCTCA
1021 GAAGATCGATG AAGAATCTTG ATGGATCAGG TATTTTCTCT TTTCTTCTTCT
1081  GCCTCTGTTT CTAGGGCTAT TTCTTCTTCT TTTCTTCTTCT
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SEQ LA2779 DM11F11-R11, Genbank Accession DQ855118, 1348 bp:

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References


Nakhla, M., Sorenson, A., Mejia, L., Ramirez, P., Karkashian, J.P., and Maxwell, D., “Molecular Characterization of Tomato-Infecting Begomoviruses in Central America and Development of DNA-