COSII Marker C2_At2g14260, 38.30cM

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

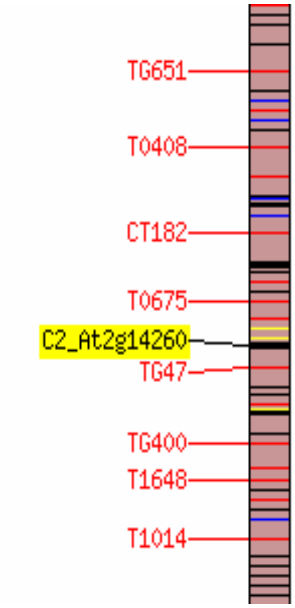

Table 1: Primers from C2_At2g14260 on Chr. 11

Primer Name	Sequence (5' to 3')
DM11 - F6	AGGATCTATACCCCTCTATAGAGCC
DM11 - R6	TTATTGGGTGAAGTCCCACCTCC

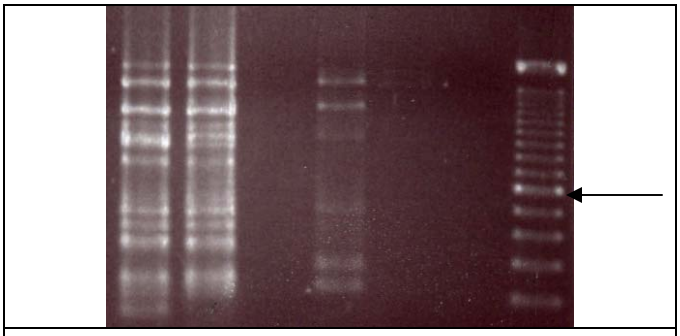

Fig. 2: Agarose gel of the PCR reactions with the DM11F6/DM11R6 primers from Chr. 11. lane 1, Heinz 1706; lane 2, Gh13; lane 3, Gc9; lane 4, LA1777; lane 5, LA2779; lane 6, water; lane 7, 100-bp ladder. Arrow marks the 600-bp fragment.

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.

DM11F6-R6 Results: The DM11F6/DM11R6 primer pair was chosen from the list of COSII primers on the SGN website (Solanaceae Genomics Network, 2006). The primer combination produced three distinct banding patterns (Fig. 2). Heinz 1706 and Gh13 produced multiple bands ranging from 200bp to greater than 1400bp in size. LA1777 had a similar banding pattern, but the bands are weaker and less in number. Gc9 and LA2779 did not produce any bands.

Thus, the *L. chilense* parent and the *L. chilense* breeding line had a distinctly different banding pattern than the other plants. This may be indicative of an *L. chilense* introgression in this area of the genome in Gc9. It is possible that these primers might be used as molecular markers to track introgressions from LA2779 at this locus. This could readily be accomplished with a multiplex PCR so as to eliminate the possibility of bad DNA or human error as an explanation for the lack of bands in Gc9 or LA2779.

Table 2: Results of the PCR with the DM11F6/DM11R6 primer pair.

Plant Line	**Result**
Heinz and Gh13	Many strong bands ranging from 200bp to greater than 1400bp
LA1777	Many weak bands ranging from 200bp to greater than 1400bp
LA2779 and Gc9	No bands

References

2006. "COS II Markers." Solanaceae Genomics Network. Cornell University. <http://www.sgn.cornell.edu/markers/cosii_markers.pl> (July 19, 2006).

Budiman, MA., Mao, L., Wood, TC., and Wing, RA. 2000. A deep-coverage tomato BAC library and prospects toward development of an STC framework for genome sequencing. Genome Res. 10:129-136.

Czosnek, H., Vidavski, F., Mejia, L., Lapidot, M., Maxwell, D., and Havey, M. 2004. "Molecular Marker-Assisted Breeding for Resistance to Whitefly-Transmitted Geminiviruses Infecting Tomato in Guatemala." *Achievements for CDR Grant 2004*. <http://www.plantpath.wisc.edu/InVirLab/CDR-Grant04.htm> (October 5, 2004).

Mejía, L., Teni, R.E., Vidavski, F., Czosnek, H., Lapidot, M., Nakhla, M.K., and Maxwell D.P. 2004. Evaluation of tomato germplasm and selection of breeding lines for resistance to begomoviruses in Guatemala. Acta Hort. (in press).

Nakhla, M., Sorenson, A., Mejía, L., Ramírez, P., Karkashian, J.P., and Maxwell, D., "Molecular Characterization of Tomato-Infecting Begomoviruses in Central America and Development of DNA-Based Detection Methods." *International Plant Virology Laboratory*. <http://www.plantpath.wisc.edu/invirlab/docs/Beg-CA-Final.htm> (October 5, 2004).

Omnikowski, R. 2004. Pedigree of variety Heinz 1706. Report of the Tomato Genetics Cooperative 54: 27.

Scott, J.W., Stevens, M.R., Barten, J.H.M., Thome, C.R., Polston, J.E., Schuster, D.J. and Serra, C.A. 1995. Introgression of resistance to whitefly-transmitted geminiviruses from *Lycopersicon chilense* to tomato. Taxonomy, Biology, Damage Control and Management, Ed. by D. Gerling and R.T. Mayer, Intercept Ltd., Andover, UK. p. 357-367.