Chromosome 11: TG523, 29.00cM

Christopher Martin and Douglas Maxwell
University of Wisconsin – Madison: June 9, 2006

PCR primer pairs were designed for RFLP probe: TG523 (Fig. 1).

Fig 1: RFLP map of the bottom 2/3 of Chr. 11 (Adapted from Pan et. al., 1999).

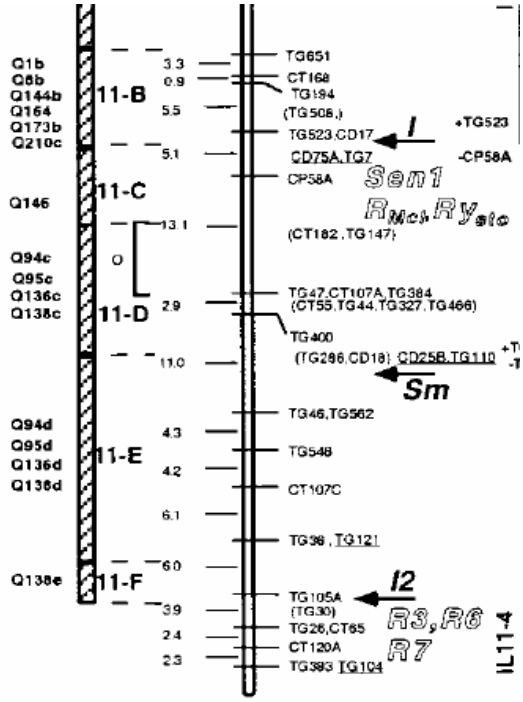

Table 1: Primers from the TG523 probe on Chr. 11.

TG523	Primer Sequence (5' to 3')
GROUP 1	
PTG523F1	GGAAGAGGAGGATTTCAGTCCTGTAG
PTG523F3	CCTGGATTCGTTTTCTTCTCAAGATGG
PTG523R1	GATGTTCTAAGTCAAAAAGTCACAACC
PTG523R4	CCTACTACTTCACTTCCTGGTCATG
GROUP 2	
PTG523R2	GACCACATTCACAAAACTCACTTTTAACC
PTG523R3	CATGAAGGAAATACTAACCAGGAACC
PTG523F2	CCAGTAAGGAGCTTCATTCAATCTATG
PTG523F4	GTTGTTTTGTTCTCTCTCTCTTTCTATTCG

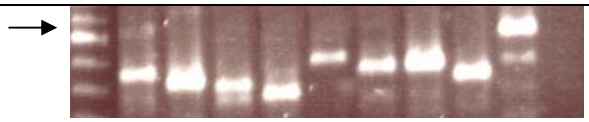

Fig. 2: Agarose gel of the eight TG523 primers and Heinz 1706 DNA. lane 1, 100-bp ladder; lane 2, TG523F1/PTG523R1; lane 3, PTG523F1/PTG523R4; lane 4, PTG523F3/PTG523R1; lane 5, PTG523F3PTG523R4; lane 6, PTG523F2/PTG523R2; lane 7, PTG523F2/PTG523R3; lane 8, PTG523F4/PTG523R2; lane 9, PTG523F4/PTG523R3; lane 10, control, primer pair PTG301F3/PTG301R2 with Heinz 1706 DNA; lane 11, water. Two bands appear in lane 10, our positive control. The band at 700 bp was unexpected and likely indicates contamination. However, since this band did not appear in any of the other lanes and our water was clean, we determined that the contamination was limited to that lane only and that the other data was reliable. Subsequent reactions supported this conclusion (data not shown). The TG523 F2/TG523R2 and TG523F3/TG523R4 primer pairs were chose for use with additional genotypes. Arrow marks the 600-bp fragment.

TG523 RFLP Probe RGH and RGHH Results: Two groups of primers, with four primers in each group were designed from the TG523 probe: GROUP 1- PTG523F1, PTG523F3, PTG523R1, PTG523R4, GROUP 2- PTG523R2, PTG523R3, PTG523F2, and PTG523F4 (Table 1). Primers from the different groups cannot be used with each other due to the place on the RFLP probe from which they were designed. The PTG523F3PTG523R4, PTG523F2/PTG523R2, PTG523F2/PTG523R3, and PTG523F4/PTG523R2 primer pairs each gave single bands with Heinz 1706 DNA with sizes ranging from 400 bp to 600 bp (Fig. 2). The other primer combinations produced multiple bands or streaky bands. One primer pair was chosen from each of the two groups; TG523 F2/TG523R2 and TG523F3/TG523R4 produced the largest and most intense single bands and were thus used with additional genotypes. Heniz1706, Gc9, Gc173, Gh13, Sheriff, LA1777, and LA1968 each produced a strong band at 500 bp with the TG523F2/TG523R2 primer pair. LA1777 and LA1968 were not run with the TG523F3/TG523R4

primer pair, but the remaining samples were and each produced a strong band at 450 bp. These PCR fragments were directly sequenced with both the forward and reverse primers. Sequence was obtained for both the forward and reverse TG523F2/TG523R2 primer pair with Heinz 1706 (acc. no.DQ097530), Gh13 (acc. no. DQ097531), Gc9 (acc. no. DQ097529), Sheriff (acc. no. DQ097532), and Gc173 (acc. no. DQ097533), and LA1968. Sequence was obtained for LA1777 with only the forward TG523F2 primer. Sequence was also obtained for both the forward and reverse TG523F3/TG523R4 primer pair with Heinz 1706 (acc. no. DQ097537), Gc9 (acc. no. DQ097534), Gc173 (acc. no. DQ097535), Gh13 (acc. no. DQ097536), and Sheriff (acc. no. DQ097538). When these sequences were aligned, there were no SNPs or INDELS detected that distinguished the begomovirus-resistant genotypes, Gc9, Gh13, and Gc173, from the susceptible genotype, Heinz 1706. When Heinz 1706 was compared to the LA1777 and LA1968 species there were 6 SNPs between Heinz and LA1777, and 7 SNPs and 2 INDEL between Heinz and LA1968 (Table 2). Thus, there is no evidence that supports an introgression of a begomovirus resistance gene at the *TG523* locus.

Table 2: Sequence differences found at the TG523F2/TG523R2 locus. In instances where an SNP or INDEL was found but not all of the lines gave sequence in that area, the absence of sequence for that line is indicated by N/A. All tested lines not listed matched exactly with Heinz 1706. The nt position is relative to Heinz 1706.

Line	**INDEL**	**INDEL**	**SNP 2**	**SNP 4**	**SNP 5**	**SNP 6**
Heinz 1706	GTT	T	T	T	C	G
LA1777	N/A	N/A	N/A	C	T	G
LA1968	…	.	G	C	C	A
nt Position	76-78 bp	93 bp	104 bp	152 bp	163 bp	202 bp
Line	**SNP 7**	**SNP 8**	**SNP 9**	**SNP 10**	**SNP 11**	
Heinz 1706	C	A	G	A	A	
LA1777	C	T	T	T	G	
LA1968	T	T	T	A	G	
nt Position	277 bp	304 bp	316 bp	344 bp	408 bp	

TG523 RFLP RIL Results: Good sequence was obtained with both of the TG523F2/TG523R2 primers for Heinz 1706 (acc. no. DQ097530), LA3959, LA3966, and LA1968. Good sequence was obtained for LA1777 (acc. no. DQ437771), and LA3967 with only the TG523F2 primer. Upon alignment the three RIL genotypes matched with the Heinz 1706 sequence exactly. When compared to the LA1777 sequence, the RIL differed by 6 SNP's, and from LA1968 by 11 SNP's out of 340 bp (the entire sequence was 455 bp but LA1777 only gave sequence with the forward primer thus limiting the size of overlap). Therefore, there is strong evidence that the RIL genome does not contain an introgression for LA1777 in the TG523F2/TG523R2 region.

In addition, Heinz 1706 (acc. no. DQ097537) and LA3959 gave sequence with both of the TG523F3/TG523R4 primers. The other tested samples did not produce usable sequence data with these primers. Upon alignment, Heinz 1706 and LA3959 were identical. Evidence from other LA1777 sequence indicates that it is quite different from Heinz in this region. Therefore, because the RIL matched with Heinz it is likely that no introgression from LA1777 is present in the TG523F3/TG523R4 region. Overall, the sequence from these two primer pairs provide strong evidence against an introgression from LA1777 at the TG523 locus.

Table 3: Sequence differences found at the TG523F2/TG523R2 locus. In instances where an SNP or INDEL was found but not all of the lines gave sequence in that area, the absence of sequence for that line is indicated by N/A. The nt position is relative to Heinz 1706.

Line	**INDEL**	**INDEL**	**SNP 2**	**SNP 4**	**SNP 5**	**SNP 6**
LA3966	GTT	T	T	T	C	G
LA3967	GTT	T	N/A	T	C	G
Heinz 1706	GTT	T	T	T	C	G
LA1777	N/A	N/A	N/A	C	T	G
LA1968	…	.	G	C	C	A
nt Position	76-78 bp	93 bp	104 bp	152 bp	163 bp	202 bp
Line	**SNP 7**	**SNP 8**	**SNP 9**	**SNP 10**	**SNP 11**	
LA3966	C	A	G	A	A	
LA3967	C	A	G	A	A	
Heinz 1706	C	A	G	A	A	
LA1777	C	T	T	T	G	
LA1968	T	T	T	A	G	
nt Position	277 bp	304 bp	316 bp	344 bp	408 bp	

References

Pan, Q., Liu, Y., Budai-Hadrian, O., Sela, M., Carmel-Goren, L., Zamir, D., and Fluhr, R. 1999. Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: tomato and arabidopsis. *Genetics Society of America* 88:309-322.