

Chromosome 1: TG301, 7.00cM

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PCR primer pairs were designed for RFLP probe: TG301 (Fig. 1).

Fig. 1: RFLP map of the top of Chr. 1 (Adapted from Pan et. al., 1999).

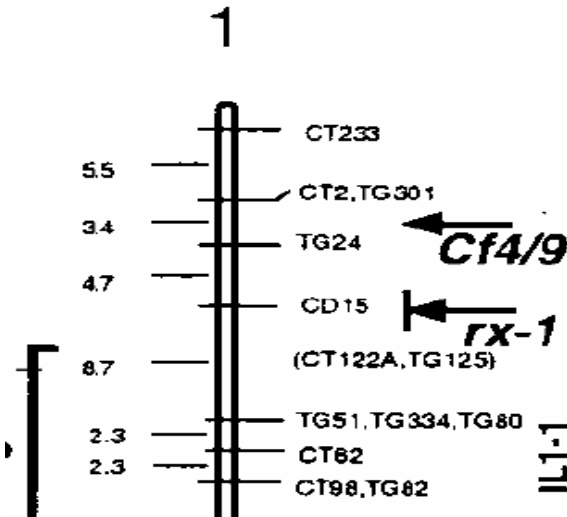


Table 1: Primers from the TG301 probe on Chr 1.

TG301	Primer Sequence (5' to 3')
P301F1	GTGGGAGTTCTTGTCTGAATAAGC
P301F2	GATGACAAGACATGTGAAGAGCG
P301F3	GGAAATTGAAGCACAGTGG
P301R1	CCACAGTGACAATCTTGATCTGCACC
P301R2	GTTTAGGTCTGATTCCCAGC

TG301 RFLP Probe RGH and RGHH Results: Five primers were designed from the TG301 RFLP probe: P301F1, P301F2, P301F3, P301R1, and P301R2 (Table 1). All primer combinations were tested with Heinz 1706 DNA (Fig. 2). Primer pairs P301F2/P301R1, P301F2/P301R2, P301F3/P301R1, P301F3/P301R2, and P301F3/P301R2 gave a single band. Their sizes varied from 550 bp to 650 bp. The primer pair P301F3/P301R2 gave the most intense band of the largest size, so this primer pair was used with the additional genotypes. Heinz 1706, Gc173, Gh13, and Gc9 produced strong bands of 580 bp. These PCR fragments were sequenced with both the forward and reverse primers. The sequence from Gh13 (acc. no. DQ066448), Gc9 (acc. no. DQ066449), and Heinz 1706 (acc. no. DQ66450) was submitted to GenBank. When the sequences were aligned one INDEL was found at nt number 416 (Table 2). Heinz 1706 had a single thiamine nucleotide that none of the other tested sequences had. As a result, 11 additional samples (Gh11, Gc16, Gc173-2, Gc16, Gc173-2-1, H7996, Dominique, Don Raul, Gc173-2-a, M82, M82-2, Silverado) were sequenced with these primers. All of the other samples had the same sequence as Gc9, Gc173, and Gh13. Thus, it was concluded that the Heinz 1706 sequence was unique, and that the INDEL was not associated with begomovirus resistance. Subsequently, *Solanum habrochaites* accessions LA1777 (acc. no. DQ222939) and LA0386 (acc. no. DQ437767) were tested with the P301F3/P301R2 primer pair. These species differed from each other at five SNP and one INDEL and from the breeding lines at seven SNP and two INDEL (Table 2). It is interesting to note that the LA0386 and LA1777 sequences had been identical when tested at other locations. The source of these differences may warrant additional investigation. However, there was no correlation

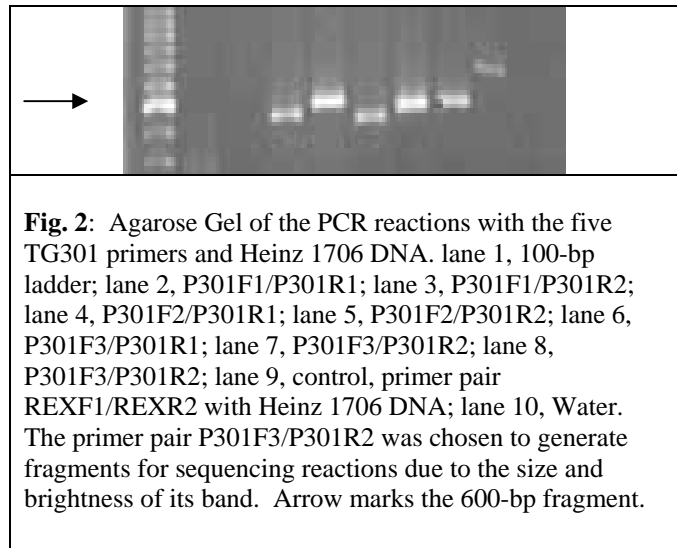


Fig. 2: Agarose Gel of the PCR reactions with the five TG301 primers and Heinz 1706 DNA. lane 1, 100-bp ladder; lane 2, P301F1/P301R1; lane 3, P301F1/P301R2; lane 4, P301F2/P301R1; lane 5, P301F2/P301R2; lane 6, P301F3/P301R1; lane 7, P301F3/P301R2; lane 8, P301F3/P301R2; lane 9, control, primer pair REXF1/REXR2 with Heinz 1706 DNA; lane 10, Water. The primer pair P301F3/P301R2 was chosen to generate fragments for sequencing reactions due to the size and brightness of its band. Arrow marks the 600-bp fragment.

between the species sequences and any of the breeding lines. Therefore, there is no evidence that supports an introgression of a begomovirus-resistance gene from *S. habrochaites* at the *TG301* locus.

Table 2: Sequence differences found at the *TG301* locus. All tested samples not listed matched LA3918 exactly. The sequences of the lines listed were identical in between the listed SNP and INDEL. The nt position is relative to Gh13.

Line	SNP 1	SNP 2	SNP 3	INDEL	SNP 4	SNP 5	INDEL	SNP 6	INDEL	SNP 7
LA1777	A	G	A	.	T	A	.	G	.	A
LA0386	C	G	A	.	C	G	.	C	C	G
Heinz 1706	C	A	T	T	T	G	T	C	.	A
Gh13	C	A	T	T	T	G	.	C	.	A
nt Position	186 bp	291 bp	349 bp	353 bp	366 bp	387 bp	416 bp	417 bp	418 bp	435 bp

TG301 RFLP Probe RIL Results: Sequence for all tested lines (Table 3) was obtained with both primers. When these sequences were aligned, the LA3918 (DQ222938) sequence matched exactly with Gc9 (acc. no. DQ066449), Gh13 (acc. no. DQ066448), and Gc173. Heinz 1706 (acc. no. DQ66450) differed from the RIL by one INDEL. The LA1777(DQ222939) and LA0386 (acc. no. DQ437767) differed from the LA3918 sequence by 5 SNP and 1 INDEL, and 4 SNP and 2 INDEL respectively out of 521 bp. Therefore, the LA3918 sequence does not provide evidence for an introgression from LA1777 near the *TG301* RFLP probe. It is of note that the LA0386 and LA1777 sequences did not match, since at other tested loci they have matched exactly. These sequence differences may warrant additional investigation.

Table 3: Sequence differences found at the *TG301* locus. All tested samples not listed matched LA3918 exactly. The sequences of the lines listed were identical in between the listed SNP and INDEL. The nt position is relative to LA3918.

Line	SNP 1	SNP 2	SNP 3	INDEL	SNP 4	SNP 5	INDEL	SNP 6	INDEL	SNP 7
LA1777	A	G	A	.	T	A	.	G	.	A
LA0386	C	G	A	.	C	G	.	C	C	G
Heinz 1706	C	A	T	T	T	G	T	C	.	A
LA3918	C	A	T	T	T	G	.	C	.	A
nt Position	186 bp	291 bp	349 bp	353 bp	366 bp	387 bp	416 bp	417 bp	418 bp	435 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.