

Development of PCR-Based Markers for Resistance to Sclerotinia Stem Rot (*Sclerotinia sclerotiorum*) in Soybean



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ABSTRACT

Resistance to Sclerotinia stem rot (*S. sclerotiorum*) in soybean has been shown to be quantitatively inherited. Two major QTL associated with disease resistance were mapped to molecular linkage groups (LGs) A2 and B2; however, genetic gaps were found in these QTL regions. The development of cDNA microarray technology presents a means of identifying possible genes involved in quantitative resistance traits. The objectives of the present study were to (i) analyze the profiles of differentially expressed genes in stem tissue of soybean seedlings challenged with the fungal pathogen and (ii) develop PCR-based molecular markers associated with differentially expressed genes to determine if they map to identified QTL. Two soybean genotypes, Williams 82 (S) and PI194639 (R), were grown hydroponically in a growth chamber under controlled light intensity and temperature. Total RNA samples from stems collected at 0, 6, 18, and 48 hours post inoculation (hpi) were fluorescently labeled with Cy3 or Cy5 dyes and hybridized onto soybean microarrays containing over 9,000 gene representatives. Data were normalized using the R/manova package (BioConductor). An ANOVA test was run to determine the significance in the variability of the data. Time point 0 was eliminated to obtain a factorial model balanced for time. Significant differences for 231 genes ($\alpha = 0.01$) were found between treatments (inoculated vs. uninoculated) across the time points, 18 and 48 h, and genotypes. Also, significant differences occurred in 758 genes between treatments across both time points for the resistant genotype (PI194639) ($\alpha = 0.05$) and 363 genes for the susceptible genotype (Williams 82) ($\alpha = 0.05$). Some of these genes have annotations suggestive of potential involvement in the defense response of soybean against fungal infection and/or colonization. Target region amplification polymorphism (TRAP) technique was employed to develop DNA molecular markers using expressed sequence tags (EST) of 24 selected genes from the soybean genome database, consisting of 24 forward and 24 reverse oligos. EST-based oligos were used as fixed primers, and combined with arbitrary primers labeled with fluorescent dyes (6-FAM, HEX, and NED). The markers detected in parental screening were utilized to genotype 155 F4:5 RILs of the Merit x PI194639 population. QTL analysis indicated that although no TRAP markers were mapped to previously identified QTL, several markers were mapped to new genomic regions in LGs L, E and B1. The results showed that the TRAP technique may provide an efficient tool to detect new markers associated with these loci. Additional molecular marker development is in progress.

METHODOLOGY

Table 1. List of selected genes showing significantly ($P < 0.01$) differential expression in PI194639 inoculated with the pathogen *S. sclerotiorum*.

No.	Gene ID	Log2 Ratio	Gene Annotation
1	AW755244	1.04	A12q21410F1K23.17 [<i>Arabidopsis thaliana</i>]
2	AW290429	1.51	EL2K21.24 [<i>Arabidopsis thaliana</i>]
3	AW716059	2.73	putative cytochrome P450 [<i>Glycine max</i>]
4	AW760827	1.01	homeodomain leucine zipper protein HD22 [<i>Phaseolus vulgaris</i>]
5	AW780561	1.22	ADP glucose pyrophosphatase small subunit Cpgs2 [<i>Cicer arietinum</i>]
6	AW831752	1.68	hypothetical protein [<i>Nicotia glauca</i>]
7	BF610459	1.01	glycine-rich protein [<i>Oenothera lutea</i>]
8	BF07307	1.19	auxin diene reductase [<i>Glycine max</i>]
9	BG550623	1.29	unknown protein [<i>Arabidopsis thaliana</i>]
10	BF93976	1.11	PDH-like protein [<i>Arabidopsis thaliana</i>]
11	AW164290	-1.38	putative WD-40 repeat protein [<i>Arabidopsis thaliana</i>]
12	AW201661	-1.23	ubiquitin-protein ligase E3- α protein [<i>Arabidopsis thaliana</i>]
13	AW201677	-1.34	glucanase/transferase-14 [<i>Vigna angularis</i>]
14	AW201760	-1.21	putative protein [<i>Arabidopsis thaliana</i>]
15	AW201886	-1.51	homeodomain leucine zipper protein HD22 [<i>Phaseolus vulgaris</i>]
16	AW705015	-1.43	L-ascorbate oxidase [<i>Medicago truncatula</i>]
17	BF090401	-1.36	hypothetical protein; protein id: A12Q2060.1 [<i>Arabidopsis thaliana</i>]
18	BF090676	-1.36	hypothetical protein [<i>Arabidopsis thaliana</i>]
19	BF069974	-1.35	ABC transporter-like protein [<i>Arabidopsis thaliana</i>]
20	BF027278	-1.23	CaLb protein [<i>Arabidopsis thaliana</i>]
21	BF069094	-1.29	31 kDa protein [<i>Glycine max</i>]
22	BF071554	-1.41	OSINB4073L04.17 [<i>Oryza sativa</i>]
23	BG551049	-1.32	putative UDP-glucose glucosyltransferase [<i>Arabidopsis thaliana</i>]
24	BG790284	-1.41	LRP protein S/D4 [<i>Pennisia x hybrida</i>]
25	BF16529	-1.38	(AL050399) putative protein [<i>Arabidopsis thaliana</i>]
26	BF785941	-1.44	sulfate transporter 2 [<i>Lysoopersicon esculentum</i>]
27	BF786583	-1.33	putative protein [<i>Arabidopsis thaliana</i>]

Table 2. Primer sequences were designed based upon expressed sequence tags (ESTs) of soybean sequence database. These oligos were used as fixed primers.

No.	Primer name	Primer sequence	bp
		F: 5'-----3'	
		R: 5'-----3'	
1	TC186985	AGAGATGGCTCGTGATTCG	20/25
2	TC176524	AAACACAGCTCAGTCTCCAAAT	22/21
3	TC174879	GAACACCATACCCAGGAAGTA	23/19
4	TC178242	TGCTGTCTCCCAAAAAATCTA	23/15
5	TC192832	GAAGAGGGGTAGAGAGCTGCT	23/25
6	TC177882	CCAGAGTAGCCAGCAAAATAGC	24/23
7	TC191301	TTTAGCCGGCGTCTTGAA	19/20
8	TC184669	TTCCGTTCTTGGCATTCG	20/25
9	TC185581	TCGTCTTGATCGTCTGATGAA	25/25
10	TC173366	CGAGGCCACATAGATTTGAAG	23/21
11	TC188296	CAAAATGTGAATAATGAGATCGT	24/26
12	TC190255	TGAGATGATGGTAAATGATGATG	27/29

Fig. 1. Schematic illustration of target region amplification polymorphism (TRAP) technique.

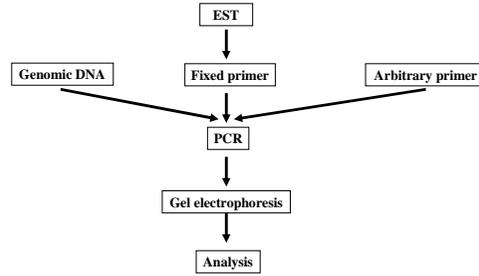


Fig. 2. Gel electrophoresis was performed using ABI Prism 377 DNA sequencer, Illinois Genetic Marker Center, University of Illinois – Urbana.



RESULTS AND DISCUSSION

Fig. 3. TRAP profiles show polymorphisms produced by a number of combinations of fixed and arbitrary primers. Panel A: FAM-labeled primers. Panel B: HEX-labeled primers.

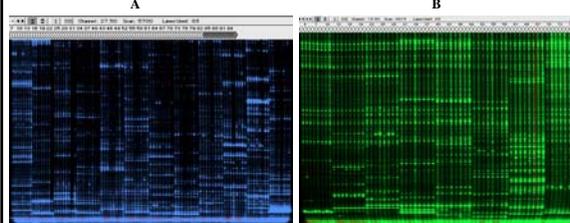


Table 4. Molecular polymorphisms were produced with several primer combinations.

Number	Fixed primer		Arbitrary primer		Polymorphism
	TC code	F/R	Code	Label	
1	TC186985	F/R	TRAP03	FAM	+
2	TC176524	F	TRAP03	FAM	++
3	TC178242	F	TRAP03	FAM	++
4	TC177882	F	TRAP03	FAM	++
5	TC185581	F	TRAP03	FAM	++
6	TC190255	F	TRAP03	FAM	++
7	TC176524	R	TRAP03	FAM	++
8	TC174879	R	TRAP03	FAM	++
9	TC185581	R	TRAP03	FAM	++
10	TC190255	R	TRAP03	FAM	+
11	TC186985	F	TRAP02	FAM	+
12	TC174879	F	TRAP02	FAM	++
13	TC177882	F	TRAP02	FAM	++
14	TC173366	F	TRAP02	FAM	+
15	TC186985	F	TRAP08	HEX	+
16	TC174879	F	TRAP08	HEX	++
17	TC177882	F	TRAP08	HEX	+
18	TC186985	R	TRAP08	HEX	+
19	TC176524	R	TRAP08	HEX	+
20	TC174879	R	TRAP08	HEX	+
21	TC185581	R	TRAP08	HEX	+
22	TC173366	R	TRAP08	HEX	+

Figure 4. Gel images show segregation of TRAP markers used to genotype a RIL population. Panel A: marker TC6F03 Panel B: marker TC6R08.

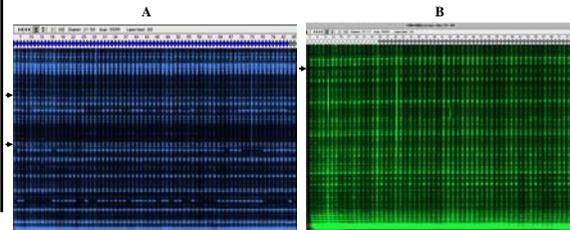


Fig. 5. SSR markers were mapped to LGs K, A2, J, and B2. Among A2 and B2 QTLs were previously reported, several new TRAP markers were mapped to LG A2 and particularly LG K.

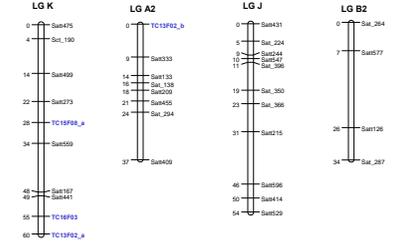
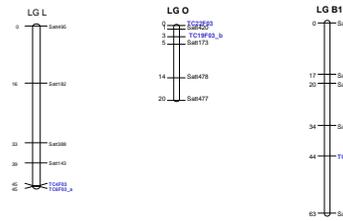


Fig. 6. Several additional TRAP were mapped to LGs L, O, and B1.



Five putative QTL for resistance to Sclerotinia stem rot in soybean were identified in F4:5 recombinant inbred lines (RIL) derived from a Merit (susceptible) x PI194639 (resistant) cross. Of these, two major QTL were mapped to molecular linkage groups (LG) A2 and B2 (Fig. 5). However, large gaps were found in some QTL regions, such as LGs B2 and L (Fig. 5 and 6), suggesting that additional molecular markers associated with these loci need to be developed.

A cDNA microarray study of two soybean genotypes (PI194639 and Williams 82) challenged with the fungal pathogen indicated that hundreds of genes (Table 1) were potentially involved in disease resistance; some genes may have potential to be utilized for the development of PCR-based DNA markers.

Twelve primers were initially designed based on expressed sequence tags (EST) from the soybean sequence database and screened for DNA polymorphisms. No polymorphisms were found among eight soybean genotypes studied, including PI194639 and Merit. It was speculated that intron sequences of gene structures in genomic DNA were problematic. Thus, a preliminary study was conducted using single forward or reverse oligos (Table 2) of the designed primers as fixed primers to combine with arbitrary primers as previously reported using the TRAP technique (Hu and Vick, 2003). Screening for polymorphisms produced a number of DNA polymorphisms among eight soybean genotypes studied (Fig. 3). These markers were subsequently used to genotype a RIL population (Fig. 5).

Genetic linkage maps indicated that several new TRAP markers were mapped to LG A2 and K where putative QTL were previously reported (Vuong et al., 2004). In addition, many TRAP markers were also mapped to LG L, O, and B1. Although no new QTLs were detected in these LG, the association of new TRAP markers and A2 and K QTL indicated the TRAP genotyping technique may provide an efficient tool to detect EST-based molecular markers associated with resistance to Sclerotinia stem rot.

CITED REFERENCES

- Hu, J. and B. Vick. 2003. Target region amplification polymorphism: A novel marker technique for plant genotyping. *Plant Molecular Biology Reporter* 21: 289-294.
- Vuong, T.D., J.J. Zou, G.L. Hartman, and S.J. Clough. 2003. Microarray analysis of defense against Sclerotinia stem rot in soybean. (Abstract) *Plant and Animal Genomics Conference XII*, Jan. 9-14, 2004, San Diego, CA.
- Vuong, T.D., G.L. Hartman, and B. W. Diers. 2004. Identification of QTL for soybean resistance to Sclerotinia stem rot (*S. sclerotiorum*) in the MeritxPI194639 population. (Abstract) *The 10th Biennial Conference of the Cellular and Molecular Biology of the Soybean*, Aug. 8-11, 2004, Columbia, MO.

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