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Detection of Differentially Expressed Genes by cDNA-AFLP in Symbiotic Supernodulating Soybean Mutant

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Objectives

This study was conducted to determine the optimum condition of cDNA-AFLP and to identify differentially expressed genes in symbiotic relationship between supernodulating soybean mutant and *Rhizobium*.

Materials and Methods

- Soybean genotypes: SS2-2 (supernodulating soybean), Sinpaldalkong 2 (wild type of SS2-2) and Jangyupkong (control genotype).
- Bacteria: *Bradyrhizobium japonicum* USDA 110.
- Leaf harvest: 0, 1, 2, 3 weeks after *Rhizobium* inoculation.
- cDNA synthesis: cDNA Synthesis Kit (Roche Applied Science, Manheim, Germany).
- Amplification of selected Transcript-Derived Fragments (TDFs): pGEM-T vector.
- Homology search: BLAST program against EST database.

Results and Discussion

TDFs showed polymorphic banding patterns indenpendently by TAI (time after inoculation) among SS2-2, Sinpaldalkong 2 and Jangyupkong. Using 22 primer combinations of *EcoR*I and *Mse*I, 46 polymorphic bands out of 397 bands were observed on the first week after inoculation (Table 1). The polymorphism level of the TDFs among three genotypes was found to be the highest on one week after inoculation, determining the best time for isolating differentially expressed genes (Fig. 1A). Out of 400 amplicons, 60 differentially expressed cDNA fragments were obtained between SS2-2 and Sinpaldalkong 2. Several TDF shown high homology to ion transporters, translational termination factors, cellular organization proteins were detected only in SS2-2, maybe contributing in controlling symbiotical growth of soybean with *Rhizobium*. Therefore, pathway for regulating symbiosis and their signal transduction between Sinpaldalkong 2 and SS2-2 might be different because different genes involved in metabolism, transcription and electron transport chain were detected (Fig.1B, 1C & Table 2).

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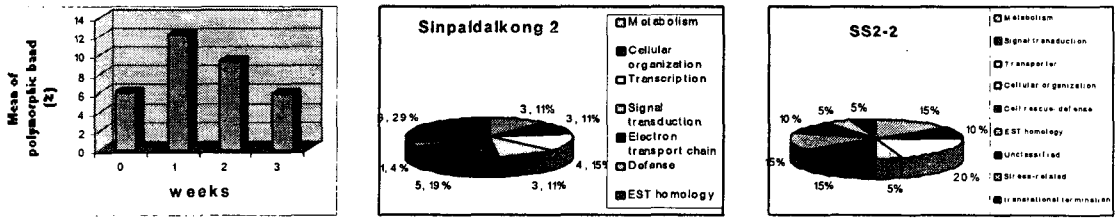
Table 1. Number of polymorphic cDNA-AFLP products generated with 22 different primer combinations of *EcoRI* and *MseI*.

No. total bands	*EcoRI*	*MseI*	Polymorphic bands (week after inoculation)			
			0	1	2	3
19	ACC	CGG	2	3	2	0
18		CGA	2	2	2	2
15		CTA	0	2	3	1
14		CAC	1	1	1	1
14		CTG	2	1	2	2
16		CGC	1	2	1	1
11	AAG	CGG	0	2	1	1
17		CGA	2	0	2	0
12		CAC	0	0	0	0
10		CTG	1	1	0	1
13		CGT	0	2	1	0
18		CGC	1	1	1	0
21	A	CA	2	2	3	1
33		CAT	1	3	2	2
25		CGG	2	3	3	2
23		CGA	1	3	2	2
27		CAC	2	4	3	2
20		CGC	1	2	3	2
12	AC	CA	1	3	1	1
20		CG	2	5	4	1
13	A	CA	0	1	1	1
16		CG	1	3	1	1
397			25	**46**	39	23



A B C

Figure 1. A: Mean of polymorphic cDNA-AFLP, B: Specific genes in Sinpaldalkong 2, C: Specific genes in supernodulating mutant SS2-2.

Table 2. Number of genes showing homology to TDFs grouped by function in supernodulating mutant and its wild type.

Sinpaldalkong 2 (wild type)		SS2-2 (supernodulating soybean)	
Gene function	No.of gene	Gene function	No.of gene
Metabolism	3	Metabolism	3
Cellular organization	3	Cellular organization	1
Transcription	4	Transporter	4
Signal transduction	3	Signal transduction	2
Electron transport chain	5	Translational termination	1
Defense	1	Cell rescue/defense	3
EST homology	8	EST homology	3
		Stress-related	1
		Unclassified	2