C4. cDNA-AFLP for Discriminating Hypernodulating Soybean Mutant

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Objectives

The cDNA-AFLP was used to identify differentially expressed genes after inoculation of *Bradyrhizobium* by comparing hypernodulating soybean mutant, SS2-2 with its wild type Sinpaldalkong 2.

Materials and Methods

○ ***RNA preparation and cDNA synthesis***

Total RNA was isolated from root of Shinpaldalkong 2 and SS2-2 soybean after inoculation of Bradyrhizobium (USDA110), and was extracted with RNeasy kit (Qiagen). Double stranded cDNA was synthesized using cDNA synthesis system (Roche) with 10㎍ of total RNA.

○ ***Template for analysis of cDNA-AFLP***

A total of 30ng of cDNA were used for cDNA-AFLP analysis. Digestion and ligation reactions were performed in the same tube at one time with restriction enzyme (MseI and EcoRI) and adapter using plant AFLP kit (Applied Biosystem). Preamplication was performed in 20㎕ of areaction mixture that contained 0.2mM dNTP, 3mM $MgCl_2$, 0.3μM of two different primer(Eco+1A, Mse+1C). PCR was performed for 30cycles with following temperature profile: 30 sec at 94℃, 30 sec at 45℃, 30 sec at 72℃. The reaction mixture was then diluted with 135㎕ of TE buffer.

○ ***Reaction for analysis of AFLP***

EcoRI+NNN primers, each with three selective nucleotides at the 3' end, were labelled with fluorescent dye blue, green and yellow. Reactions were performed in 10 ㎕ of a reaction mixture that contained 0.2mM dNTP, 3mM $MgCl_2$, 76 *p*mol of lablled EcoRI(+AGG, ACC, ACT) primer and 125pmol of MseI(+CAA, CAT, CTT, CTG, CAC) primer, and add 2.5㎕ of diluted preamplication mix. The temperature profile for PCR was as follows. The first cycle included 30 sec at 94℃, 30 sec at 65℃, 2min at 72℃. For the next following 32 cycles, the annealing temperature was reduced by -0.7℃ per cycle. The final 23cycles included 30 sec at 94℃, 30 sec at 56℃, 2min at 72℃. After amplication, 2㎕ of the reaction mixture were combined with an equal volume of dye solution. The mixture was heated for 5min at 95℃ and then quickly cooled to 4℃. The sample was loaded on a 7% polyacrylamide/6M UREA gel. Electrophoresis was performed with ABI 377(Perkin-Elmer).

Summary

1. The cDNA-AFLP was thought to be useful to identify genes associated with early nodulation genes.
2. A total of 37 DNA fragments were found to be differentially expressed between two soybean genotypes.
3. DNA fragments will be sequenced and their function will be identified.

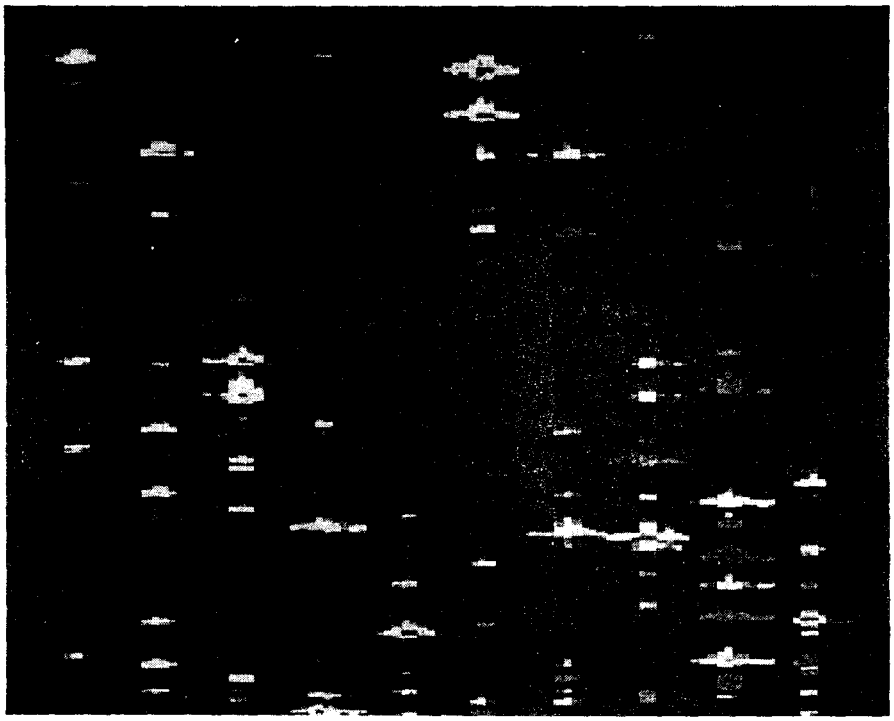

Figure 1. The cDNA-AFLP fingerprinting of Shinpaldalkong 2 and SS2-2 as affected by primer combinations.

Table 1. The cDNA-AFLP band identified as genes expressed differentially between Sinpladlakong 2 and SS2-2.

Primer combinations		Sinpaldalkong 2	SS2-2
EcoRI	MseI	(bp)	(bp)
AAC	CAA	210	19
AAC	CAT	158, 480	200
AAC	CTT	180, 280	61
AAC	CTG	20, 20	108, 158
AAC	CAC	327	76
AAG	CAA	-	51
AAG	CAT	33	-
AAG	CTT	78, 302, 327, 470	136, 313
AAG	CTG	217, 228	91, 118, 197
AAG	CAC	-	39, 100
ACA	CAA	-	233
ACA	CAT	222	116
ACA	CTT	-	114
ACA	CTG	51	125
ACA	CAC	85	90