PA7

Differentially Expressed Genes in Soybean treated with O_3 and UV-B

Jung-Hun Moon[1], Jae Eun Lee[2], Yook Han Kim[2], Young-Up Kwon[2], Woncheol Yim[1], Sung Don Lim[1], Sang-Kyu Lee[1], Kyung-Hee Kim[1], Si-Nae Han[1], Poo-Reum Oh[1], Sun Hee Woo[3], and Byung-Moo Lee[1]*

[1] Department of Plant Biotechnology, Dongguk University, Seoul 100-715, Korea
[2] National Institute of Crop Science, RDA, Suwon 441-857, Korea
[3] Department of Crop Science, Chungbuk National University, Cheongju 361-763, Korea

Objective

The objective of this study was used a new differential display method, termed an Annealing Control Primer (ACP) system, to analyze differentially expressed genes (DEGs) in Soybean.

Materials and Methods

▷ Plant materials : O_3 (200ppb) treated Dawonkong and Jinpumkong, and UV-B (10 kJ/㎡ /day) treated Cheongjakong and Jinpumkong for 5 days of each 4 h period.
▷ RNA isolation : Total RNA was isolated from leaves using TRIZOL reagent (invitrogen)
▷ cDNA synthesis and PCR primers : GeneFishing™ DEG kit and 120 arbitrary ACPs (Seegene, Korea).
▷ Cloning : T&A Cloning Vector kit (Real Biotech Corporation, Taiwan).
▷ BLAST search : NCBI (http://www.ncbi.nlm.nih.gov)

Results

On the basis of differential expression levels of mRNA fragments observed on agarose gels, DEGs bands were detected at control and O_3 or UV-B treated soybeans using 120 arbitrary ACPs. DEGs bands discovered 408 bands of O_3 treated soybeans (Cheongjakong and Jinpumkong) and 421 bands of UV-B treated soybeans (Dawonkong and Jinpumkong). Differential banding patterns observed 153 bands of up regulated and 255 bands of down regulated in O_3 treated soybeans and 207 bands of up regulated and 214 bands of down regulated in UV-B treated soybeans (Fig. 1. and Table 1).

These 100 PCR products of DEGs bands in O_3 treated soybeans were cloned and their DNA sequences were analyzed using BLAST. The sequence characterization of differentially expressed transcripts are summarized in Table 2.

Corresponding author : Byung-Moo Lee Tel : 02-2260-3307 E-mail : bmlee@dongguk.edu

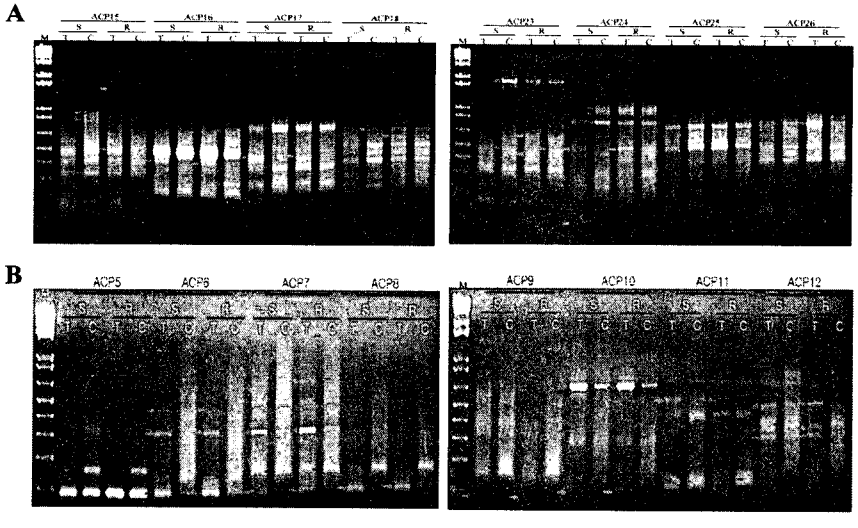

Fig. 1. Ethidium bromide stained 2% agarose gels show differential banding patterns obtained from control and treated (A : O_3, B : UV-B) samples, using a set of arbitrary ACPs (5'-primer) and a dT-ACP2 (3'-primer). Arrow indicated differential cDNA bands (green arrow : up regulated, red arrow : down regulated).

Table 1. Differential banding patterns of up- and down-regulated in O_3 and UV-B treated soybean.

Treatments / Cultivar / Regulated	O_3		UV-B	
	Jinpumkong (sensitive)	Cheongjakong (insensitive)	Dawonkong (sensitive)	Jinpumkong (insensitive)
Up	61	92	116	89
Down	225	30	113	103

Table 2. Sequence characterization of differentially expressed transcripts.

Blast accession no.[a]	Putative identity	Number of clones[b]
AY575953	Glycine max beta-carotene hydroxylase mRNA	75
DQ317523	Glycine max cultivar PI 437654 chloroplast	6
U39567	Glycine max ribulose-1,5-bisphosphate carboxylase small subunit mRNA	7
M64267	Glycine max iron superoxide dismutase (FeSOD) mRNA	4
U39475	Glycine max chlorophyll a/b-binding protein (cab3) mRNA	4
AY563043	Glycine max phosphoenolpyruvate carboxylase (PEPC17) mRNA	5

[a] GenBank accession number of the most similar sequence as identified by BLASTN alignments.

[b] Number of clones assigned to the same GenBank accession.