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Gene expression associated with enhanced cellular cytokinin level in hybrid poplar

Young Im Choi^{1*}, Eun Woon Noh¹, Jae Soon Lee¹, Hyo shin Lee¹, and Kwan-Sam Choi²

¹Biotechnol. Division, Korea Forest Research Institute, Suwon, 441-350, Rep. of Korea

² Dept. Applied Biology, ChungNam Natl. Univ., Daejeon, 305-762, Rep. of Korea

Objectives

To screen genes that are specifically expressed by the elevated level of cellular cytokinin

Materials and Methods

1. Material

Cell suspensions of transgenic poplars (*Populus alba* x *P. tremula* var. *glandulosa*) carrying a chimeric p35S-*tzs* gene (trans-zeatin secreting locus)

2. Methods

Cell suspensions were prepared from two lines of transgenic poplars that show different degree of the transgene expression (one moderate level, the other high level, determined by RT-PCR and Western blotting). Subtractive hybridization was performed with cDNAs prepared from the cells against nontransformed control cells. The functional classification of the ESTs was done by an EST clustering and analysis system (Insilicogen, Korea).

Results and Discussion

Among the total of 487 cDNA clones from a subtractive cDNA library prepared from cytokinin overexpressing line, 68 contigs consisting of at least 2 or more reads were identified. The remaining 102 clones were singlets. In the case of moderately expressing lines, out of 450 cDNA clones analyzed, 46 contigs and 82 singlets were identified. Most abundant transcripts in over expressed line included metallothionein, peroxidase, extensin like protein, imbibition protein, and lipid transfer protein. As expected, cytokinin binding protein and auxin regulated protein were also present as separate contigs. In contrast, most abundant transcripts in moderately expressed lines were senescence related protein that represented at least 70 times in the library, cytochrome P-450, extensin like protein, and peroxidase. At present, full sequences are being prepared from the ESTs to test their function by over expressing or blocking the genes in transgenic plants.

* Corresponding author : Young Im Choi, TEL: 031-290-1173, E-mail: yichoi99@foa.go.kr