Preliminary

cDNA Analysis of the Genes Increasing Their Expression on Tension wood Formation in *Eucalyptus camaldulensis* L.*[1]

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Introduction

Tension wood is an abnormal wood formed in an inclined tree stem. The change of gravity direction is thought as the principle to form tension wood. From biochemical aspect, several hormones have been examined, and some of them affected to form tension wood. However, these studies have not elucidated the mechanism sufficiently so far. On the other hand, molecular biological technique has advanced extremely in last a couple of decades, and the physiological phenomena of plants have been shown at molecular levels in detail. Recently, we have reported that several genes were expressed in the tissue forming a distinctive feature of tension wood in two weeks after inclination of the trees, and their cDNAs were cloned[1]. In this paper, some of their nucleotide sequences revealed their putative function deduced from similarity search of the sequences.

Eucalyptus camaldulensis L. was used in this study because it grows fast and is useful for a material of paper industry. Tension wood has high cellulose and low lignin contents, so molecular genetics involving tension wood is expected to supply more suitable cultivate.

Materials and Methods

Thirteen cDNA clones, used in this study, were obtained from a cDNA library constructed from mRNA of differentiating xylem of tension wood in *Eucalyptus camaldulensis* L.[1]. Three of them inserted in λ gt11 phage were amplified by polymerase chain reaction (PCR), and their PCR products were sequenced directly. The other ten cDNAs were subcloned from λ gt11 phage to pBluescript SK II plasmid. Then, they were subjected to DNA sequencing by dideoxy chain termination method. After the nucleotide sequences were determined, they were queried to BLAST Search of NCBI to search the similarity to the genes registered in GenBank.

Results and Discussion

Five of thirteen cDNA clones were completely identical in their nucleotide sequences. Thus the clones that we obtained were nine species. Two of nine showed high similarity to registered genes in GenBank.

One of the nucleotide sequence showed high similarity to Glycine max cationic peroxidase 2 (Prx2) mRNA and cotton *(Gossypium hirsutum)* peroxidase mRNA. These peroxidases were closely similar to each other, but they didn't hit to another peroxidases by similality search and the cotton peroxidase was expressed in the cotyledon and extremely induced at only two stages: embryo development and germination[2]. These peroxidases with the tension-wood-specific peroxidase never showed similarity with xylem-specific peroxidase. These findings indicaded that this cDNA clone may not take a role in lignification but a specific role that assumes to concern with forming tension wood.

The other cDNA clone showed high similarity to a part of 3′-terminal side within the open reading frame of cobalamin-independent methionin synthase mRNA obtained from *Coleus blumei*, *Arabidopsis thaliana*, *Catharanthus roseus* and *Mesembryanthemum crystallium*. Methionine synthase transfers methyl-group from methylenetetrahydrofolic acid to homocysteine, a precursor of methionine at the last step of methionine biosynthesis. The methionine further changes to S-adenosylmethionine and works as a donor of methyl-group in methylation. In fact, a cDNA clone that showed high similarity to S-adenosylmethionine synthase was obtained at one hour after inclination of *E. camaldurensis*[3]. Methionine metabolism would be activated in inclined stem. S-adenosylmethionine metabolism seems somehow implicated in plant growth with plant-growth hormones and in plant pathogen interactions[4]. Methionine synthase-like expressed sequence tag (EST) was found many in bent stem of pine, and there are many examples which express methionine synthases because of stress responses[5]. The methionine synthase seems to be expressed response to the stress of inclined stimulus.

Another cDNA clone hit an EST of Eucalyptus globulus bicostata symbiont mRNA in lower similarity with three regions, which were not long and interrupted. The cDNA clone would have another function. The other cDNA

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clones sequenced in this study showed no significant similarity to the genes registered in GenBank. They should be cDNA clones derived from unknown genes.

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