Preliminary

Characterization of Stilbene Synthase Genes in Japanese Red Pine *(Pinus densiflora)*[*1]

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Genus *Pinus* produces stilbenoid phytoalexins usually in the heartwood. The secondary metabolites are composed of a C_6-C_2-C_6 skeleton, and biosynthesized via phenylpropanoid pathway. Common stilbenoids from pine trees are pinosylvin, pinosylvin monomethyl ether and pinosylvin dimethyl ether. Pinosylvin is an effective fungicide while its monomethyl ether is a strong nematicide.

Stilbene synthase (STS) catalyzes the addition of three malonyl-CoA to a starter CoA ester (cinnamoyl-CoA), producing stilbenoids. They are involved directly in the defense mechanism. Thus, STS gene is an appropriate target for molecular and genetic engineering to achieve wood durability and diseases resistance. In this study, we characterized STS genes in Japanese red pine *(Pinus densiflora)*, which will promise a control of the stilbenoid biosynthesis.

RNA extraction for the pine seedlings was developed by a modified guanidium method[1]. By using this method, high quality intact RNA was prepared from the roots. The cDNA synthesized successively was fractionated (0.3–1.0 kb, 1.0–4.0 kb), and they were introduced into plasmid vector, respectively. Thus each size fractions of the cDNA library were successfully constructed[2]. The library (0.3–1.0 kb) was used as a template for amplificatoin of STS genes (3′-end fragments). After the subcloning, 21 STS cDNA clones were obtained. We classified them into a molecular phylogenetic tree by neighbor-joining method. Three prime untranslated region (UTR) diversity in the STS genes made it clear that STS formed a multigene family. At least, 13 molecular species of STS genes were expressed simultaneously in the roots.

Three STS cDNA clones that carry full coding sequence (ca. 1.4 kb) were isolated from the library (1.0–4.0 kb) by colony hybridization. They showed quite similar sequences (95%) to that from Scots pine *(Pinus sylvestris)*. They had an in-frame stop codon and a few possible polyadenylation signal (AATAAA). The homology in the coding regions of each STS cDNA clones was about 95%. They had a conserved cysteine residue, located in the central section of these proteins (amino acid position 167), which is essential for the catalytic activity of both STS and CHS[3] and probably represents the binding site for cinnamoyl-CoA[4,5]. The amino acid residue around this active site was well conserved. Thus, the cDNAs (*sts* I, *sts* II, *sts* III) have a potential to make functional STS in Japanese red pine *(Pinus sylvestris)*.

Cinnamoyl-CoA synthesized[6] was pure and was enough amounts for recombinant STS assay. The three recombinant STS encoded by STS cDNAs (*STS*-I, II, III) were successfully expressed at high level in *Escherichia coli* cells. The enzyme activity was confirmed in the STS I and II.

By this molecular biological study on the STS, we are now able to prospect manipulation of the pinosylvin biosynthesis.

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