MOLECULAR CLONING AND CHARACTERIZATION OF *CAD* AND *OMT* GENES INVOLVED IN LIGNIN AND LIGNAN BIOSYNTHESIS

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Lignin and lignans are typical phenylpropanoid compounds derived from the cinnamate/monolignol pathway. Lignins confer mechanical strength to plant cell walls and the plant as a whole, while lignans have a wide variety of biological functions and activities, not only for the plant itself, but also for other organisms.

In the author's current research, the author has tried to elucidate genes that are involved in lignin and lignan biosynthesis. To do so, the author has conducted experiments to isolate cDNAs which are possibly involved in lignin/lignan biosynthesis, and characterize their recombinant proteins.

Characterization of *Carthamus tinctorius* cinnamyl alcohol dehydrogenases

Cinnamyl alcohol dehydrogenase (CAD) is an NADPH-dependent enzyme that is known to be responsible for the last reductive step in the monolignol biosynthesis, where coniferaldehyde and sinapaldehyde, are reduced into their corresponding monolignols, coniferyl alcohol and sinapyl alcohol. This is thought to be one of the factors in determining the lignin content in wood. In the current research, the author carried out the characterization of three cDNAs encoding cinnamyl alohol dehydrogenase (CAD) in *Carthamus tinctorius* (safflower), which were named *CtCAD1*, *CtCAD2*, and *CtCAD3*, respectively. The plant was chosen due to its significant amount of lignin and lignan production in its seed developing stage.

In this research, the author conducted functional characterization of the recombinant CtCADs by use of GCMS-based analysis. In addition, the author also carried out quantification of *CtCAD* expression in different *C. tinctorius* tissues.

Molecular cloning of *Anthriscus sylvestris O*-methyltransferase cDNAs

S-Adenosyl-L-methionine (SAM)-dependent *O*-methyltransferases (OMTs) play important roles in plant secondary metabolite biosynthesis. They are involved in the synthesis of methylated plant phenolics, including lignin, lignan, flavonoids, and other phenylpropanoids. These plant OMTs transfer a methyl group from SAM to the hydroxyl group of the methyl acceptor.

In order to further understand the roles of *O*-methyltransferases (OMTs) in the biosynthesis of an antitumor lignan, podophyllotoxin, via yatein, the author isolated seven *OMT* cDNAs from an *Anthriscus sylvestris* (cow parsley) cDNA library. *A. sylvestris* was chosen because it produces significant amount of yatein. Substrates of the recombinant OMTs will be determined by GCMS-based assay analysis.