

04-3-19

Characterization of Cinnamyl Alcohol Dehydrogenase (CAD) *AtCAD-H* in *Arabidopsis thaliana* Using Antisense Approach

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Objectives

To isolate and characterize *AtCAD-H* gene from *Arabidopsis thaliana* and to develop the regulator of CAD enzyme through the *in vitro* expression of *AtCAD-H* in *E. coli*.

Materials and Methods

1. Plant materials : *Arabidopsis thaliana* ecotype Columbia
2. Methods: plant transformation using antisense cDNA, RT-PCR, sequence analysis, protein over-expression *in vitro*, SDS-PAGE, lignin staining.

Results and Discussion

The essential gene of living organisms can be a good target for development of growth regulator like herbicide in plants. Antisense approach is very powerful for studying the essential gene because partial disruption of endogenous gene by antisense RNA could reduce the lethality of mutant.

The cDNA of *AtCAD-H* gene was isolated from *Arabidopsis thaliana* through the sequence and phylogenetic analysis of the annotated genes as a CAD homologues. Of the nine putative CADs, three including *AtCAD-H* show a unique group from the other six genes at the phylogenetic tree which is constructed by the nine annotated CAD homologues sequence from Genbank. As a results of RT-PCR analysis, the gene expressed highly at the stem of plant when compared to the other organs whereas consistently at all developmental stage. The mutant plant with antisense construct of *AtCAD-H*, showed growth inhibition when compared to wild type *Arabidopsis*. The lignin composition was also studied by histochemical lignin staining.

The molecular weight of *AtCAD-H* protein expressed by pCALn expression vector for inducible over-expression in *E. coli* was about 36kD in SDS-PAGE. The purified protein had a substrate specificity to coniferyl alcohol and aldehyde. This indicates that *AtCAD-H* is a coniferyl alcohol dehydrogenase.

The further study for other substrate specificity and screening of inhibitor for enzyme activity is in progress. (confer the other poster presentation posted by above authors)

Acknowledgement : This research was partially supported by a grant (CG1312) from Crop Functional Genomics Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of Republic of Korea, and by technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea

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