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Overexpression of cinnamate 4-Hydroxylase gene enhances biosynthesis of decursinol angelate in *Angelica gigas h*airy roots

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Objectives

Although there have only been a few reports of metabolic engineering of coumarin pathways in plants, recent advances have made it feasible in *A. gigas*. In this study, the *AgPAL* and *AgC4H* genes were overexpressed in hairy roots of *A. gigas* and those transgenic hairy roots were compared the production of Decursinol Angelate with that in control hairy roots.

Materials and Methods

- Total RNA Isolation and cDNA Synthesis
- Plasmid Construction for Transformation of *A. gigas* Hairy Roots
- Preparation of *Agrobacterium rhizogenes* and hairy Root Cultures
- Quantitative real-time PCR
- GUS Staining Analysis
- HPLC Analysis of Decursin and Decursinol Angelate

Results

Angelica gigas is a medicinal plant that produces pyranocoumarins, including decursin (**D**) and decursinol angelate (**DA**), which have neuroprotective, anticancer, and antiandrogenic effects. In this study, the coumarin biosynthetic pathway was engineered to increase the production of **DA**. Specifically, a vector was constructed which contained the *A. gigas* phenylalanine ammonia lyase (*AgPAL*) and cinnamate 4-hydroxylase (*AgC4H*) genes that were driven by the cauliflower mosaicvirus (CaMV) 35S promoter. Transgenic hairy roots that overexpressed *AgPAL* or *AgC4H* genes were obtained by using an *Agrobacterium rhizogenes*-mediated transformation system. Among them, only AgC4H-transgenic hairy root lines produced more **DA** than control transgenic hairy root lines. The enhanced gene expression corresponded to elevated C4H activities. This study showed the importance of C4H in the production of **DA** in *A. gigas* hairy root culture.

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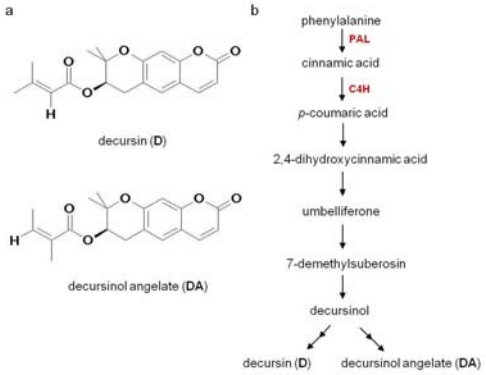

Fig. 1. Chemical structure of decusin (**D**)and decursinol angelate (**DA**), and proposed biosynthetic pathway. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase.

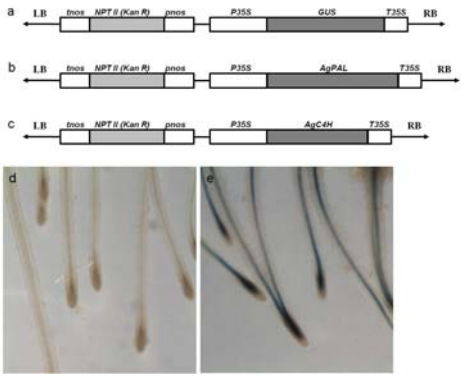

Fig. 2. The plasmid vectors used in transformations and GUS histochemical analysis of transgenic hairy roots. Schematic representation of the (A) pGUS, (B) pAgPAL, and (C) pAgC4H constructs. (D) Hairy roots transformed with *Agrobacterium rhizogenes* R1601 only and (E) hairy roots transformed with *P*35S::GUS.

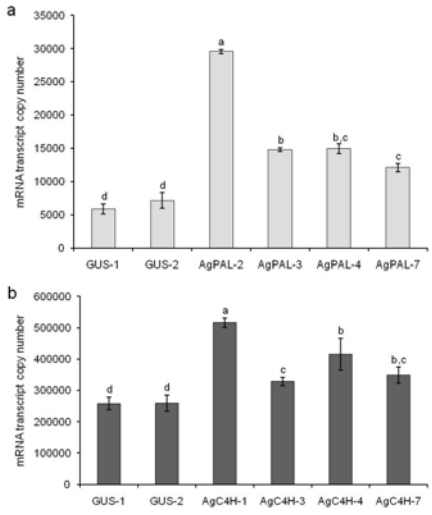

Fig. 3. Analyses of *AgPAL* and *AgC4H* gene expression. (A)Quantitative real-time PCR (qRT-PCR) analysis of *AgPAL* in transgenic hairy root lines; (B) qRT-PCR analysis of *AgC4H* in transgenic hairy root lines. Statistical significance of the differences between treatments was determined using ANOVA followed by paired-group comparisons. Different letters indicate significance at $P<0.05$.

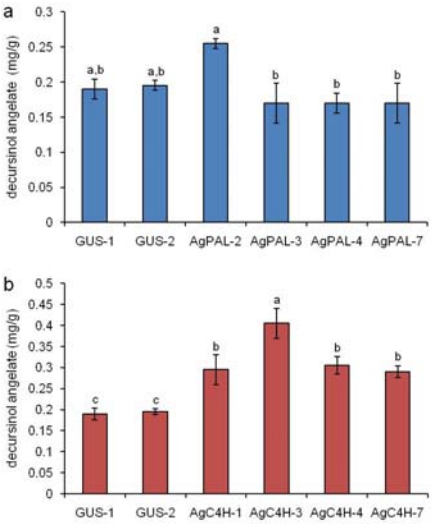

Fig. 4. Decursinol angelate (**DA**) production by *AgPAL*-(A) and *AgC4H*-transformed hairy root lines (B). The height of the bars corresponds to the mean and the error bars indicate standard deviation (n=3). Statistical significance of the differences between treatments was determined using ANOVA followed by paired-group comparisons. Different letters indicate significance at $P<0.05$.