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Overexpression of *Angelica gigas* Phenylalanine Ammonia-Lyase and Cinnamate 4-Hydroxylase Genes Enhances Biosynthesis of Decursinol Angelate in *A. gigas* Hairy Root Cultures

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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 (**1**) and decursinol angelate (**2**),whichhave neuroprotective, anticancer, and antiandrogenic effects. In this study, the coumarin biosynthetic pathway was engineered to increase the production of **2**. Specifically, a vector was constructed which it contained the *A. gigas* phenylalanine ammonia lyase (*AgPAL*) and cinnamate 4-hydroxylase (*AgC4H*) genes that were driven by the cauliflower mosaic virus (CaMV) 35S promoter. Then transgenic hairy root lines were established thatheverexpressed *PAL* or *C4H* genes by using an *Agrobacterium rhizogenes*-mediated transformation system. These transgenic hairy root lines produced more decursinol angelate (**2**) than control hairy root lines. Furthermore, overexpression of *C4H* was more effective than that of *PAL* on the production of **2**. The enhanced gene expression corresponded to elevated PAL and C4H activities. This study showed the importance of PAL and C4H in the production of **2** in *A. gigas* hairy root culture.

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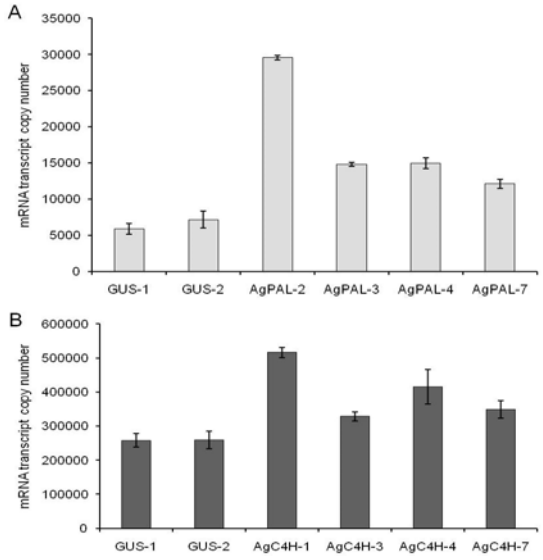

Figure 1. Analyses of *AgPAL* and *AgC4H* gene expression. (A) Quantitative real-time PCR (qRT-PCR) analysis of *AgPAL* in transgenic hairy root lines; (B) qRT-PCR analysis of *AgC4H* in transgenic hairy root lines. GUS-n, hairy root induced by GUS; AgPAL-n, transgenic hairy root lines induced by AgPAL; AgC4H-n, transgenic hairy root lines induced by AgC4H ("n" indicates the hairy root line number). The height of the bars corresponds to the mean and the error bars indicate standard deviation (n=3).

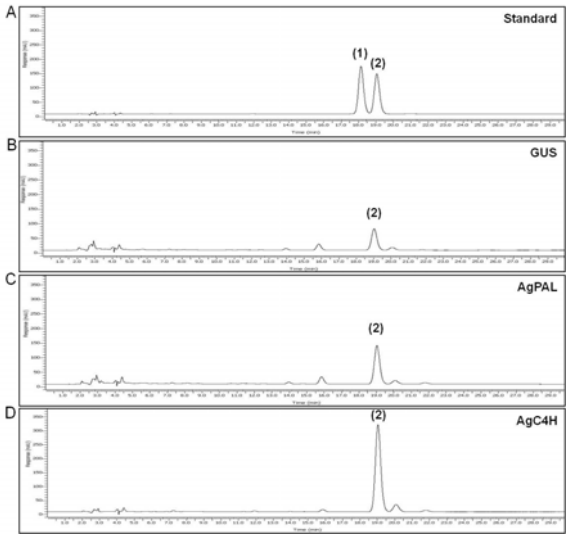

Figure 2. HPLC chromatograms of decursin (**1**) and decursinol angelate (**2**). (A) Standard samples of decursin and decursinol angelate; (B) *GUS*-transformed hairy root line; (C) *AgPAL*-transformed hairy root line; (D) *AgC4H*-transformed hairy root line of *A. gigas*.

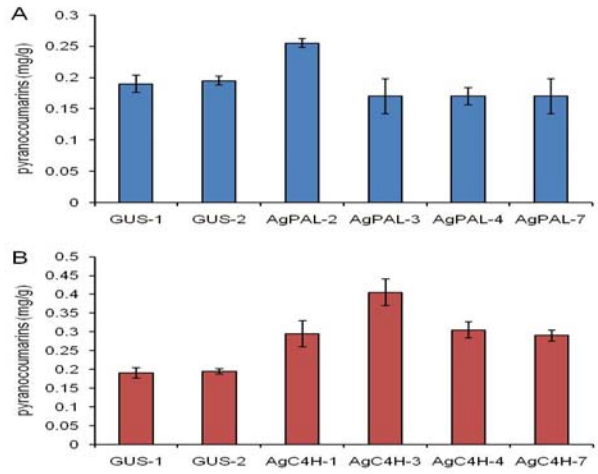

Figure 3. Decursinol angelate (2) production by *AgPAL*- and *AgC4H*-transformed hairy root lines. The height of the bars corresponds to the mean and the error bars indicate standard deviation (n=3).

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