

The production of asiaticoside from *Centella asiatica* hairy roots overexpressing the foreign FPS gene

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Objectives

We generated *C. asiatica* hairy root lines overexpressing ginseng farnesyl diphosphate synthase (FPS) gene via a stable genetic transformation system. In tomato, FPS enzyme has been proposed to play essential role in early development of plant organs when cell division and growth occur. By overexpression of a chimeric FPS gene in transgenic plants, Chen et al. (2000, Plant Sci 155:179-185) have demonstrated that FPS performs a regulatory function in sesquiterpene biosynthesis. To investigate asiaticoside accumulation in hairy roots overexpressing ginseng FPS, methyl jasmonate as an elicitor was added to culture medium. In this study, triterpene production from *C. asiatica* hairy roots overexpressing FPS gene was analyzed.

Materials and Methods

○ Plant Materials

Four node segments per petri dish (90×20 mm) were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar at 23±2°C under light conditions.

○ Vector construction

The PCR product containing a PgFPS cDNA from *Panax ginseng* was digested with *Bam*HI and *Sac*I to obtain a 1026 bp fragment containing the complete open reading frame. This fragment was then placed between a CaMV 35S promoter and a NOS terminator to form an intermediate vector.

○ Plant transformation

Formation of *Centella* hairy roots was performed as described by Kim et al. (2007, Plant Cell Rep 26:1941-1949).

Results and Discussion

Transformed root ("hairy roots") cultures have been shown to be a good model for the study of many secondary metabolites. In order to determine whether or not the overexpression of FPS contributes into triterpene biosynthesis, the first step *Centella*

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asiatica (L.) Urban hairy roots overexpressing (HR-FPS) the foreign FPS gene was produced by using the *Agrobacterium rhizogenes* strain R1000, which harbors pCAMBIA1302 including ginseng FPS. Triterpene contents including madecassoside, asiaticoside, madecassic acid and asiatic acid were analyzed to compare with the control and HR-FPS24 for 4 weeks of MJ treatment. As shown in Fig. 1, asiaticoside production in HR-FPS24 line increased 1.78-time than that of the control. However, no differences were observed in any content of madecassoside and asiatic acid between HR-FPS24 and control.

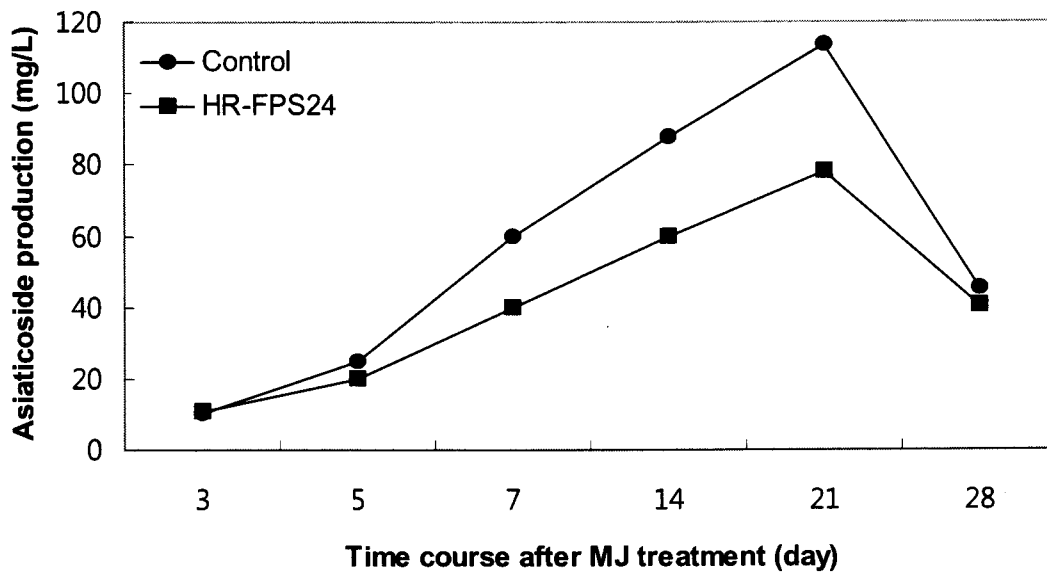


Fig. 1. Time course of asiaticoside production of *C. asiatica* hairy root (HR-FPS24) overexpressing ginseng FPS after MJ treatment.