

Metabolic engineering in *Scutellaria baicalensis*

Department of Crop Science, Chungnam National University

Nam Il Park, Hui Xu and Sang Un Park*

Objectives

The phytochemicals, i.e. baicalin, baicalein, and wogonin, are only some of the many compounds produced by *S. baicalensis*; its rich supply of secondary metabolites and its importance as a medicinal plant make it an excellent model system for continued research of useful medicinal compounds. Having determined that several key enzymes in the *S. baicalensis* flavone biosynthetic pathway, we are focused our efforts on producing transgenic hairy roots via the introduction of flavone biosynthetic genes.

Materials and methods

- Callus induction and cell suspension culture
- Methyl jasmonate treatment and wounding of cell suspension cultures
- RNA Extraction and quantitative real-time polymerase chain reaction
- Isolation of cDNA encoding flavone biosynthetic genes
- Plasmid Construction for transformation of *S. baicalensis* hairy roots
- Preparation of *Agrobacterium rhizogenes* and hairy root cultures
- High Performance Liquid Chromatography (HPLC) analysis

Results

The root of *Scutellaria baicalensis* Georgi as a *Lamiaceae* family has been extensively used as a herbal therapy to treat cardiovascular dysfunction. The involvement of genes in flavones biosynthesis was investigated in different organs and suspension cells obtained from *S. baicalensis*. cDNAs encoding phenylalanine ammonia lyase isoforms (*SbPAL1*, *SbPAL2*, and *SbPAL3*) and chalcone isomerase (*SbCHI*) from *S. baicalensis* were isolated using rapid amplification of cDNA ends (RACE)-PCR. These cDNAs were used to show the expression level in different organs of *S. baicalensis*. These genes were upregulated in suspension cells of *S. baicalensis* with biotic/abiotic stress factors.

Due to the highest level of *SbPAL1*, *2*, *3* and *SbCHI* genes in roots among different organs of *S. baicalensis*, the transgenic hairy root lines were established that overexpressed *SbPAL1*, *2*, *3* and *SbCHI* genes by using an *Agrobacterium rhizogenes*-mediated transformation. These transgenic hairy root lines produced more baicalin, baicalein, and wogonin than control

* corresponding author: Tel. 042-821-5730, E-mail: supark@cun.ac.kr

본 연구과제는 한국한의학연구원 “한의생체 에너지 대사기반 노화 조절연구” 사업의 지원에 의하여 이루어진 연구결과임

hairy root line. In particular, wogonin content was 1.84–8.82 times higher increased in all hairy root culture than wild type roots. The flavone biosynthetic pathway was engineered to increase baicalin, baicalein, and wogonin production in hairy root culture. And we also introduce the transcription factors, *leaf color (Lc)* gene and *production of anthocyanin pigments1 (PAP1)* gene, to the hairy root. This study showed the importance of PALs, CHI and transcription factors (Lc and PAP1) in flavone biosynthesis and demonstrates the efficiency of metabolic engineering.

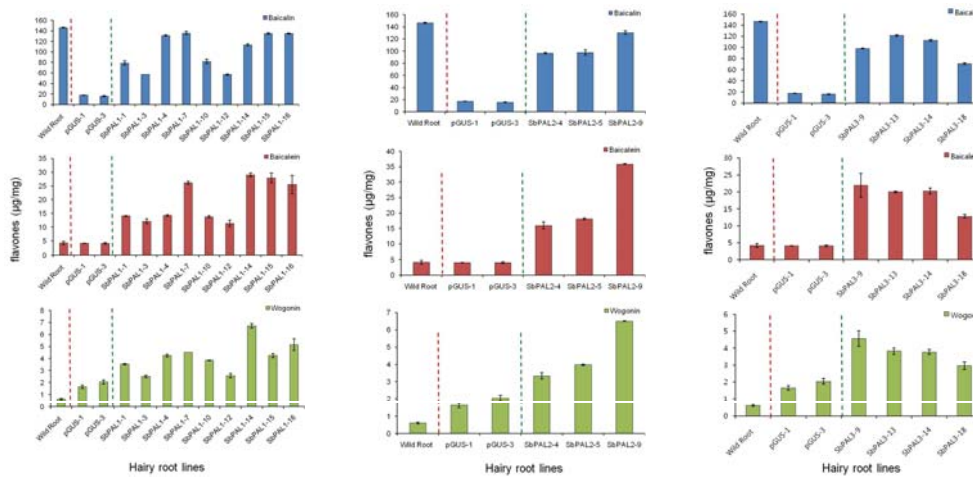


Fig. 1 Analysis of flavones production in *SbPALS*-overexpressed hairy root lines of *S. baicalensis*. Production of baicalin, baicalein, and wogonin by *SbPALS*-overexpressed hairy root lines

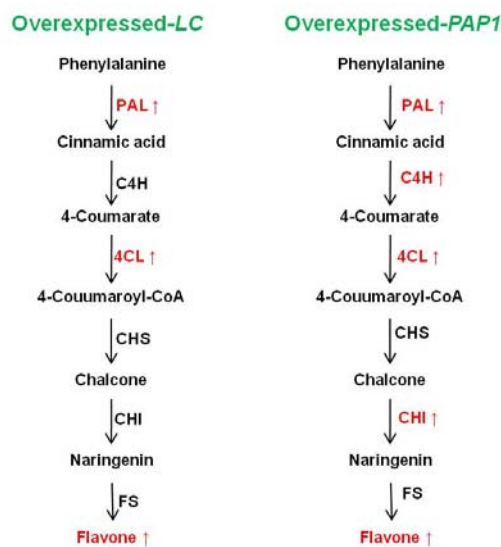


Fig. 2 Upregulation of the flavone biosynthetic genes and flavone compounds by overexpression of the transcription factor LC and PAP1 in *S. baicalensis*