

## Increasing Flavone Levels through Overexpression of Chalcone Isomerase in Hairy Root Cultures of *Scutellaria baicalensis*

*Department of Crop Science, Chungnam National University,  
Nam Il Park, Hui Xu, Xiaohua Li and Sang Un Park\**

### Objectives

we isolated the cDNA encoding chalcone isomerase (EC 5.5.1.6) from *S. baicalensis* and investigated the production of flavones in different organs of *S. baicalensis*. In addition, we analyzed the gene expression level of *SbCHI* in *S. baicalensis* suspension cells under biotic or abiotic stresses. The *S. baicalensis* chalcone isomerase gene was used to increase flavone production in hairy root cultures.

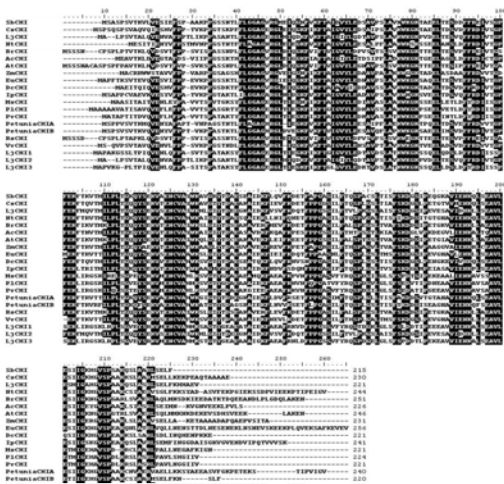
### Materials and Methods

- Methyl Jasmonate Treatment and Wounding of Cell Suspension Cultures
- RNA Extraction and Quantitative Real-time Polymerase Chain Reaction
- Isolation of Chalcone Isomerase cDNA
- Construction of Plasmids for Transformation of *S. baicalensis* Hairy Roots
- Hairy Root Cultures
- High Performance Liquid Chromatography Analysis

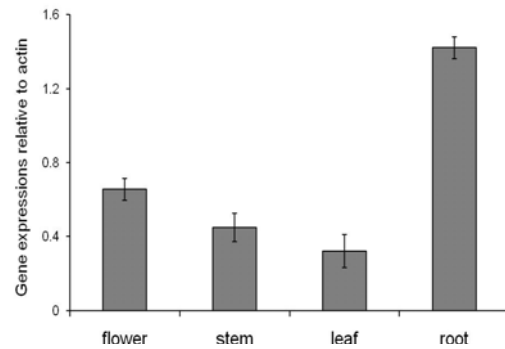
### Results

A cDNA encoding *Scutellaria baicalensis* chalcone isomerase (*SbCHI*) was isolated using rapid amplification of cDNA ends polymerase chain reaction (RACE-PCR). The effects of wounding and methyl jasmonate on the expression of *SbCHI* were determined in *S. baicalensis* cell suspensions. In addition, since the highest expression level of *SbCHI* occurred in the roots of *S. baicalensis*, transgenic hairy root lines were established by using *Agrobacterium rhizogenes*-mediated transformation to overexpress *SbCHI* and increase the production of flavones, such as baicalin, baicalein, and wogonin. These transgenic hairy root lines enhanced *SbCHI* gene expression, elevated CHI activity, and produced more baicalin, baicalein, and wogonin than the control hairy root line. The amount of wogonin in all hairy root cultures was 1.84 - 8.82 times higher than that in wild-type roots. This study showed the importance of CHI in flavone biosynthesis and the efficiency of metabolic engineering in *S. baicalensis*.

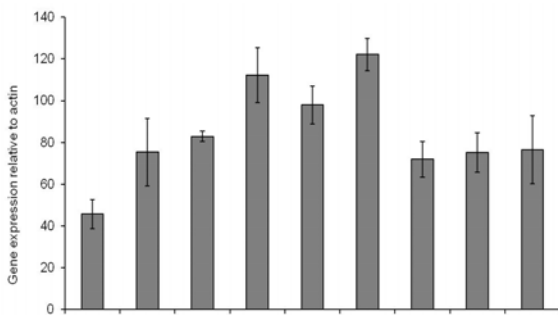
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\* corresponding author: Tel. 042-821-5730, E-mail: [supark@cun.ac.kr](mailto:supark@cun.ac.kr)



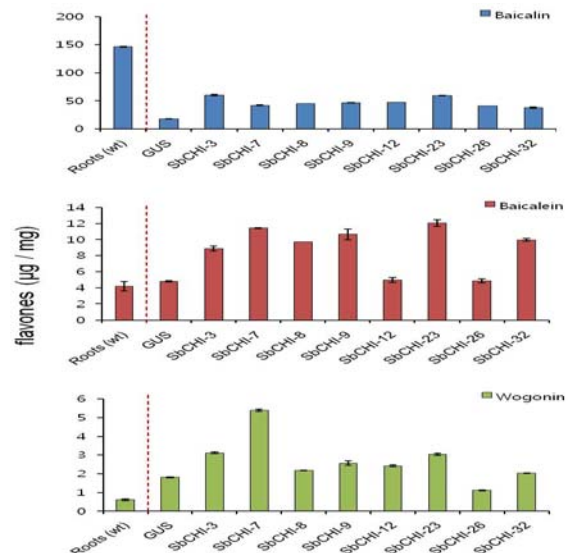
**Figure 1.** Multiple sequence alignment of the amino acid sequences of chalcone isomerase (CHI) from *S. baicalensis* and its orthologs.



**Figure 2.** Expression levels of *CHI* relative to actin in different organs of *S. baicalensis*.



**Figure 3.** Analysis of the expression level of *SbCHI* relative to actin in *SbCHI*-transformed hairy root lines of *S. baicalensis*. GUS; a control hairy root line transformed with the  $\beta$ -glucuronidase (GUS) overexpression vector pGUS, *SbCHI*-n; transgenic hairy root lines ("n" indicates the line number) transformed with the CHI overexpression vector pCHI.



**Figure 4.** Production of baicalin, baicalein, and wogonin by *SbCHI*-transformed hairy root lines. The height of the bars and the error bars indicate the mean and standard deviation ( $n=3$ ), respectively.

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