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Enhancement of flavone levels through overexpression of chalcone isomerase in hairy root cultures of *Scutellaria baicalensis*

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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. baicalenesis* 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. After the treatment of wounding or methyl jasmonate, *SbCHI* transcripts were increased in *S. baicalensis* cell suspensions. Overexpressed- and silenced-*SbCHI* transgenic hairy root lines were established by using *Agrobacterium rhizogenes*-mediated system. Overexpressed-*SbCHI* hairy root lines not only enhanced *SbCHI* gene expression, but also produced more flavones (i.e., baicalin, baicalein, and wogonin) than the control hairy root line. In contrast, silenced-*SbCHI* hairy root lines reduced *SbCHI* transcripts and flavones production compared to those of the control hairy roots. In addition to the amount of wogonin in all hairy root cultures was increased compared to wild-type roots of *S. baicalensis*. Finally, this study showed the importance of CHI in flavone biosynthesis and the efficiency of metabolic engineering in *S. baicalensis* hairy roots.

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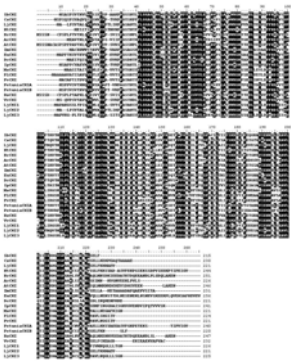

Fig. 1 Multiple sequence alignment of the amino acid sequences of chalcone isomerase (CHI) from *S. baicalensis* and its orthologs.

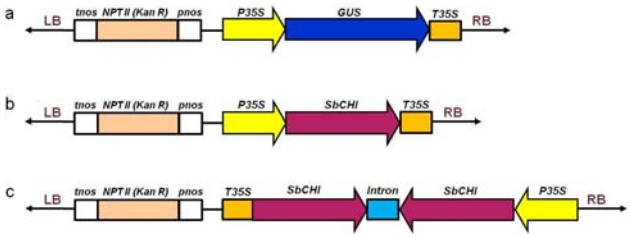

Fig. 2 The plasmid vectors used in transformations. Schematic representation of the (a) pGUS, (b) pSbCHI and (c) pRNAi-SbCHI constructs. LB, left border repeats; RB, right border repeats; P35S, CaMV 35S promoter; T35S, CaMV35S terminator; NPTII, neomycin phosphotransferase II; pnos, nos promoter; tnos, nos terminator; GUS, b-glucuronidase; SbCHI, *S. baicalensis* CHI.

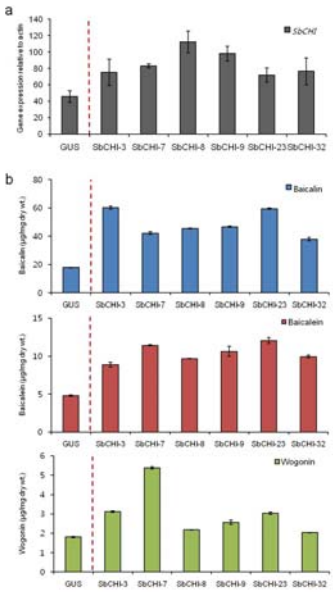

Fig. 3 Analysis of *CHI* expression and flavones production in *SbCHI*-overexpressed hairy root lines of *S. baicalensis*. (a) The expression level of *SbCHI* relative to actin in *SbCHI*-overexpressed hairy root lines of *S. baicalensis*. (b) Production of baicalin, baicalein, and wogonin by *SbCHI*-overexpressed hairy root lines.

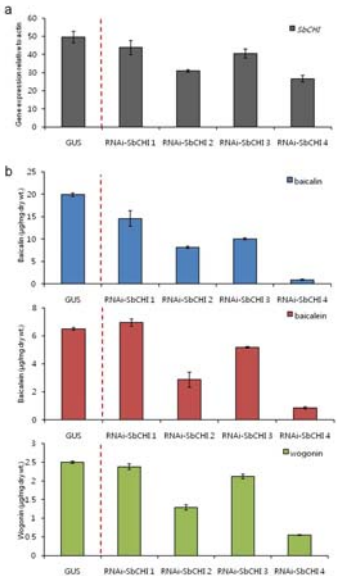

Fig. 4 Analysis of *CHI* expression and flavones production in *SbCHI*-silenced hairy root lines of *S. baicalensis*. (a)The expression level of *SbCHI* relative to actin in *SbCHI*-silenced hairy root lines of *S. baicalensis*. (b)Production of baicalin, baicalein, and wogonin by *SbCHI*-silenced hairy root lines.