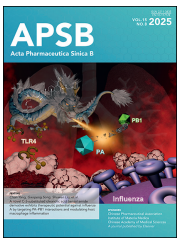
Acta Pharmaceutica Sinica B 2025;15(8):4300–4305

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



SHORT COMMUNICATION

A simple widely applicable hairy root transformation method for gene function studies in medicinal plants

Xue Cao[a,b,†], Zhenfen Qin[a,b,†], Panhui Fan[a,d,†], Sifan Wang[a,b], Xiangxiao Meng[a,b], Huihua Wan[a,b], Wei Yang[a,b], Shilin Chen[c], Hui Yao[d,*], Weiqiang Chen[a,b,*], Wei Sun[a,b,*]

[a]*State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[b]*Key Laboratory of Beijing for Identification and Safety Evaluation of Chinese Medicine, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*
[c]*Innovative Institute of Chinese Medicine and Pharmacy, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan 611137, China*
[d]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100193, China*

Received 27 January 2025; received in revised form 9 March 2025; accepted 14 March 2025

KEY WORDS

Medicinal plants;
Hairy root transformation;
Liquiritin;
GuUGT1;
GuUGT10

Abstract Genetic transformation is a fundamental tool in molecular biology research of medicinal plants. Tailoring transgenic technologies to each distinct medicinal plant would necessitate a substantial investment of time and effort. Here, we present a simple hairy root transformation method that does not require sterile conditions, utilizing *Agrobacterium rhizogenes* strain K599 and the visible RUBY reporter system. Transgenic hairy roots were obtained for six tested medicinal plant species, roots or rhizomes of which have recognized medicinal value, spanning four botanical families and six genera (*Platycodon grandiflorus*, *Atractylodes macrocephala*, *Scutellaria baicalensis*, *Codonopsis pilosula*, *Astragalus membranaceus*, and *Glycyrrhiza uralensis*). Furthermore, two previously identified *Glycyrrhiza uralensis* UGTs that convert liquiritigenin into liquiritin in heterologous systems were studied *in planta* using the method. Our results indicate that overexpression of *GuUGT1* but not *GuUGT10* and Cas9-

*Corresponding authors.
E-mail addresses: wsun@icmm.ac.cn (Wei Sun), wqchen@icmm.ac.cn (Weiqiang Chen), hyao@implad.ac.cn (Hui Yao).
†These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.03.038

mediated knockout of *GuUGT1* profoundly influenced the accumulation of liquiritin and isoliquiritin in licorice roots. Therefore, the method described here represents a simple, rapid and widely applicable hairy root transformation method that enables fast gene functional study in medicinal plants.

1. Introduction

Advances in high-throughput sequencing technologies, especially the release of 1K Medicinal Plant Genomes Project, have generated comprehensive genomic resources for medicinal plants[1]. Concurrently, the refinement of *in vitro* and heterologous expression systems has facilitated functional characterization of numerous biosynthetic genes, significantly accelerated the elucidation of metabolic pathways of valuable phytochemicals[2]. However, the *in planta* biological roles of these identified genes remain poorly resolved, primarily due to the absence of robust, widely applicable genetic transformation protocols in medicinal plants. The deficiency in understanding the *in planta* biological roles of those biosynthetic genes would impede downstream molecular breeding.

Over 540 traditional Chinese medicines listed in Pharmacopoeia of the People's Republic of China are derived from plants[3]. However, developing species-specific genetic transformation protocols using conventional tissue culture for each of the medicinal plant species would be impractical, as traditional tissue culture dependent genetic transformation methods are highly species and genotype-specific[3,4]. Therefore, there is an urgent need to develop widely applicable transformation methods for medicinal plants. Recent breakthroughs in plant genetic transformation have been made, such as *in planta* or *in vitro* plant transformation methods, Cut-Dip-Budding (CDB) gene delivery system, and regenerative activity-dependent *in planta* injection delivery (RAPID)[5-9]. *In planta* or *in vitro* transformation method is based on plant active regeneration capacity with or without the aid of expressing plant development regulators, while CDB system uses *Agrobacterium rhizogenes* (*A. rhizogenes*) to induce hairy root growth and shoot growth from hairy roots. Unlike conventional methods, the RAPID method utilizes *Agrobacterium tumefaciens* introduced *via* meristem injection to produce transformed meristem-derived tissues. *In planta* or *in vitro* transformation works well for tobacco (*Nicotiana benthamiana*), snapdragons (*Antirrhinum majus*), tomato (*Solanum lycopersicum*), Brassica cabbage (*Brassica rapa*) and potato (*Solanum tuberosum*)[7-9]. Currently, the CDB system has been effectively utilized to transform a variety of medicinal plants, including *Taraxacum mongolicum*, *Rehmannia glutinosa*, *Salvia miltiorrhiza*, *Cannabis sativa* and *Polygala tenuifolia*[5,6,10].

In this study, we developed a simple hairy root transformation method to a wider range of root and rhizome medicinal plants. Using licorice as a model system, we demonstrated that this method can be effectively used to study gene function *in planta via* overexpression and gene editing. Therefore, this hairy root transformation method represents a simple, rapid, and widely applicable transformation technology that significantly accelerates the gene function validation in medicinal plants.

2. Results and discussion

We first selected a model herb licorice (*Glycyrrhiza uralensis*) for application and subsequent evaluation of the transformation method. Licorice has substantial market demand, well-defined metabolic and biosynthetic pathways, and the accessibility of comprehensive genomic data. A binary vector containing *RUBY* reporter gene, confers bright red color to transgenic tissue due to synthesis of betalain, was used to test the transformation[11,12]. Two hundred and eleven three weeks old licorice seedlings were cut at the base of the stem and the shoots were inoculated with suspension of *A. rhizogenes* K599, Ar. Qual and C58C1 for 16 h. Explants were subsequently transferred to vermiculite moisture-retaining darkness for 24 h, and then cultivated in moisture-retaining photoperiodic conditions for 3 days (Fig. 1A). After about 40 days of cultivation under photoperiod of 16 h light/8 h dark, red hairy roots were observed for 66 of the 211 inoculated plants (Fig. 1B, Supporting Information Table S1). The strain of *A. rhizogenes* K599 showed the highest transformation efficiency compared with Ar. Qual and C58C1 (Table S1). Interestingly, 2−3 months later, three plants regenerated from a single plant with red hairy roots showed red stem (Supporting Information Fig. S1A and S1B).

Been able to produce red hairy roots, we next tested the *in planta* functions of two previously characterized genes that were

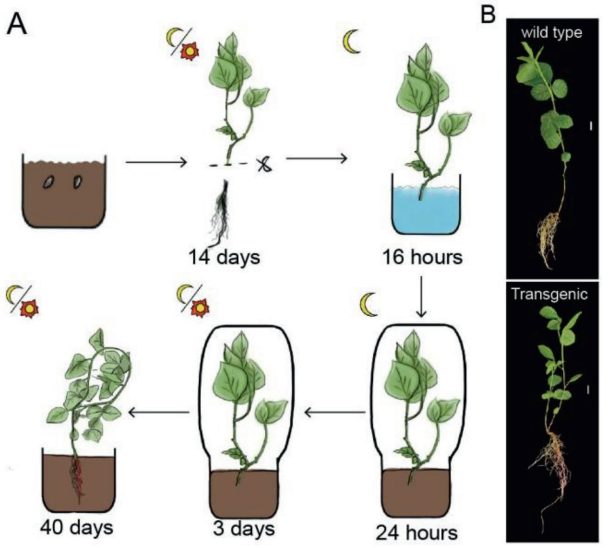


Figure 1 The hairy root transformation method for licorice. (A) The workflow chart of hairy root transformation method. (B) The comparison of wild-type and red hairy roots of licorice. Scale bar = 1 cm.

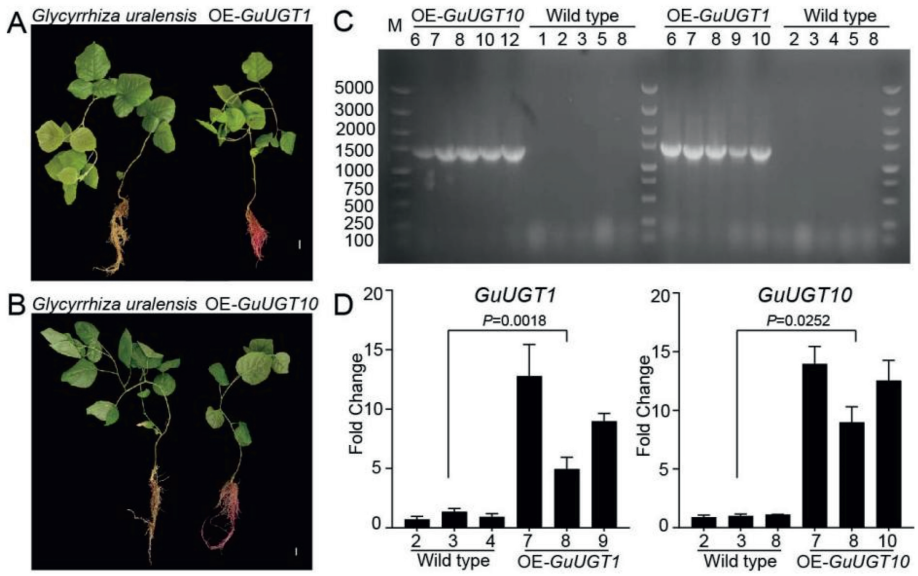


Figure 2 Overexpression of *GuUGT1* and *GuUGT10* in licorice. (A, B) The representative transgenic licorice overexpressing *GuUTG1* or *GuUGT10*. Scale bar = 1 cm. (C) Genotype analysis of the transgenic and WT plants; (D) qRT-PCR analysis of representative transgenic licorice overexpressing *GuUGT1* and *GuUGT10*. Data are presented as mean ± SD ($n = 3$).

shown to convert liquiritigenin into liquiritin in heterologous expressing systems[12-14]. Liquiritin, derived from glycosylation of liquiritigenin at C4′-*O*, is one of the key chemical compounds used to evaluate the quality of licorice. To investigate which *UGTs* are responsible for the liquiritin biosynthesis in licorice, we cloned the *GuUGT1*[14] and *GuUGT10*[12] from the root cDNA of *Glycyrrhiza uralensis*. The amino acid sequences of the cloned UGTs are consistent with the published ones. Moreover, the UGTs proteins purified from *Escherichia coli* extracts were also capable of catalyzing the glycosylation of liquiritigenin at the C4′-*O* position (Supporting Information Fig. S2). Hence, the *GuUGT1* and *GuUGT10* genes were separately cloned into the pCAMBIA1305-RUBY vector and transferred into the *G. uralensis* using the method (Fig. 2A and B). Among the inoculated plants, 15 out of 38 (39%) transformed with *GuUGT1* and 12 out of 36 (33%) transformed with *GuUGT10* developed red hairy roots, showing an average hairy root induction rate of 36% that is consistent with our previous data (Table S1). Representative plants exhibiting abundant red hairy roots for each gene were selected for further analysis.

To further confirm transgenesis of red hairy roots, PCR amplification using primer sets targeting the *GuUGTs-RUBY* junctions detected the ∼1.5 kb segment in all 10 tested plants (Fig. 2C, Supporting Information Table S2). Furthermore, quantitative real-time PCR (qRT-PCR) analysis revealed that expression levels of *GuUGT1* and *GuUGT10* in the red roots were more than five times higher than those in the wild type. These results indicate that *GuUGT1* and *GuUGT10* were over-expressed in red hairy roots (Fig. 2D).

To quantify the concentrations of liquiritigenin, isoliquiritigenin, liquiritin, and isoliquiritin in transgenic licorice root. We first developed a quantitative detection method using the MRM mode on a UPLC−QQQ−MS/MS system. Three representative transgenic plants for each gene were selected for analysis. Overexpression of *GuUGT1* significantly increased liquiritin (48.1840 ± 9.8072 μg/g FW) and isoliquiritin (4.6542 ± 1.4041 μg/g FW) compared to the wild-type plants (7.4506 ± 2.0061 μg/g FW and 0.6795 ± 0.1304 μg/g FW, respectively). In contrast, overexpression of *GuUGT10* did not result in significant changes in the concentrations of liquiritin and isoliquiritin (Fig. 3, Supporting Information Tables S4 and S5). These findings indicate that *GuUGT1*[14] rather than *GuUGT10*[12], plays a pivotal role in catalyzing the formation of liquiritin and its isomer isoliquiritin in licorice plants.

To further elucidate the functional role of *GuUGT1*, we constructed a dual-target knockout vector (CR-UGT1) to disrupt the *GuUGT1* gene by CRISPR/Cas9. PCR screening identified 29 independent transgenic lines containing the *Cas9* gene. Among these, two lines harbored definitive knockout mutations, corresponding to an overall editing efficiency of 6.9% (2/29) at the plant level. Sequencing of the PCR products revealed both of the knockout lines (CR-UGT1-1 and CR-UGT1-2) harbored heterozygous mutations. Specifically, these mutations consisted of the

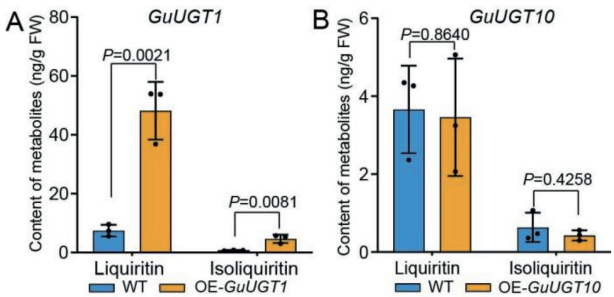


Figure 3 Liquiritin and isoliquiritin concentration in wild-type (WT) and transgenic hairy roots. (A) Analysis of liquiritin and isoliquiritin content in *GuUGT1* overexpressing roots (OE-*GuUGT1*) and WT; (B) Analysis of liquiritin and isoliquiritin content in *GuUGT10* overexpressing roots (OE-*GuUGT10*) and WT. Data are presented as mean ± SD ($n = 3$). $P < 0.05$ indicates a statistically significant difference in liquiritin and isoliquiritin concentration in WT and transgenic plants.

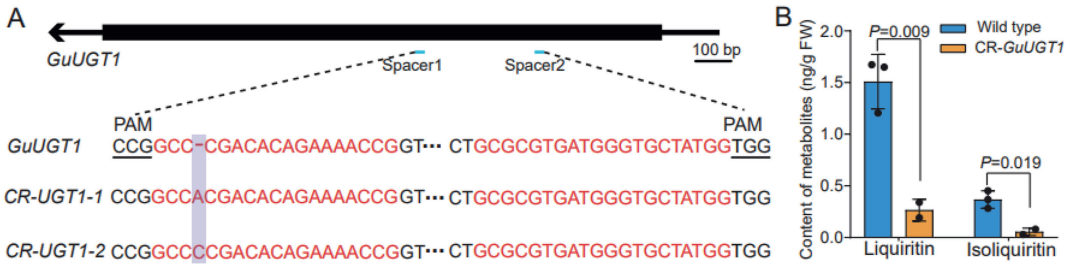


Figure 4 *GuUGT1* mutations generated by CRISPR/Cas9 and phenotyping analysis. (A) Depiction of *GuUGT1* mutations generated by CRISPR/Cas9. Blue lines indicate the targeted sites of the guide RNAs. Black lines indicate the proto-spacer adjacent motif (PAM). The sequences of the two mutants *CR-UGT1-1* and *CR-UGT1-2* are shown. (B) Liquiritin and isoliquiritin contents in wild type *Glycyrrhiza uralensis* and *UGT1* mutants. $P < 0.05$ indicates a statistically significant difference in liquiritin and isoliquiritin concentration in WT and transgenic plants.

insertion of thymine (T) or guanine (G) after the 733rd nucleotide of open reading frame, yielding stop codons that would produce truncated GuUGT1 proteins (Fig. 4A, Supporting Information Fig. S3).

To assess the impact of *GuUGT1* disruption on metabolite change, we analyzed the contents of liquiritin and isoliquiritin in the two knockout lines using UPLC−QQQ−MS/MS system. The results show a significant reduction in the levels of liquiritin and isoliquiritin in the knockout lines compared to the wild type (Fig. 4B, Supporting Information Table S6). The decrease of liquiritin and isoliquiritin supports that *GuUGT1* is a pivotal gene in the conversion of liquiritigenin and isoliquiritigenin into liquiritin and isoliquiritin in licorice.

Been able to perform overexpression and gene-editing in licorice, we further evaluated whether we could transform other medicinal plant species whose root/rhizome of pharmaceutical importance. Five plant species including *Platycodon grandiflorus* (Campanulaceae), *Atractylodes macrocephala* (Asteraceae), *Scutellaria baicalensis* (Lamiaceae), *Codonopsis pilosula* (Campanulaceae), and *Astragalus membranaceus* (Fabaceae) that spanning four botanical families and six genera were tested. Remarkably, four of the five species exhibited efficient transformation rates averaging 30%, with the exception that *Codonopsis pilosula* showed a comparatively lower success rate of 12.5% (Fig. 5, Supporting Information Table S7). This demonstrates the broad utility of our method across diverse medicinal plant taxa.

3. Conclusions

In summary, we reported a simple, rapid and wildly applicable hairy root transformation method that does not need sterile conditions and specialized equipment. The method enables efficient gene transfer and gene-editing in medicinal plants within two months. All six tested root/rhizome used medicinal plants were successfully transformed. Moreover, Functional characterization of *GuUGT1* in *Glycyrrhiza uralensis* by overexpression and CRISPR/Cas9 mediated gene-editing confirmed its *in planta* role in regulating liquiritin biosynthesis, highlighting the method's utility for uncovering biosynthetic pathways and regulatory

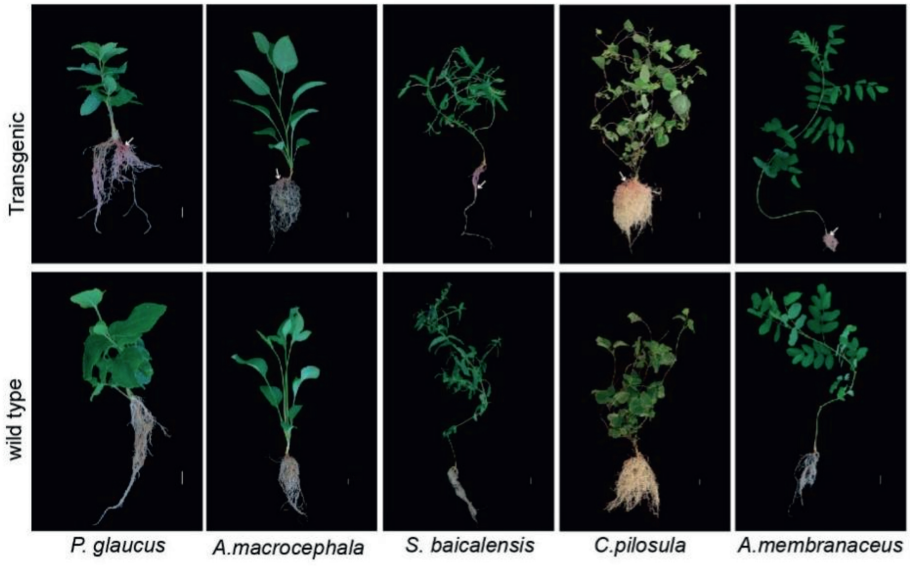


Figure 5 The application of hairy root transformation method to root/rhizome used medicinal plants. Transgenic (upper row) and wild type (lower row) of Jiegeng (*Platycodon glaucus*), Baizhu (*Atractylodes macrocephala*), Huangqin (*Scutellaria baicalensis*), Dangshen (*Codonopsis pilosula*) and Huangqi (*Astragalus membranaceus*) plants are shown, with white arrows indicate the transgenic root with red color. Scale bar = 1 cm.

mechanisms of bioactive compounds. Unlike the CDB and RAPID systems, which aim to generate intact transgenic plants through root suckering and strong plant regenerative capacity, respectively, we focus on expanding the range of medicinal plants that can be rapidly transformed, enabling the investigation of *in planta* gene function. Therefore, the method described here represents a versatile platform for rapid gene functional study in non-model medicinal plants and genetic tools for fast optimization of metabolite production in medicinal plants whose roots or rhizome synthesized valuable phytochemicals.

4. Experimental

4.1. Plant materials, growth condition and sample preparation

Seeds of *Glycyrrhiza uralensis* were obtained from Gansu province, while seeds of Baizhu, Huangqi, Huangqin, Jiegeng and Dangshen seeds were procured from Anguo Hebei province. Plants were grown in artificial climate chamber at 25 °C with a photoperiod of 16 h light and 8 h dark. After 60 days, roots of *G. uralensis* were harvested, frozen in liquid nitrogen, and stored at −80 °C before RNA and metabolites extraction.

4.2. Plant transformation

A. rhizogenes K599, Ar.Qual and C58C1 stocks with desired constructs were first inoculated into 1 mL liquid Luria-Bertaniin (LB) medium and cultured 6−8 h at 28 °C and 200 rpm. One hundred μL liquid *A. rhizogenes* was spread on LB agar plate and kept at 28 °C overnight. Cells were collected and resuspended in transformation solution [10 mmol/L $MgCl_2$, 10 mmol/L 2-(*N*-morpholino) ethanesulfonic acid (MES) pH 5.6, 100 mmol/L acetosyringone (AS)] to OD_{600} = 0.5−0.6. Three weeks old plants were cut at the base of stems, and the stems were immediately inoculated with *A. rhizogenes* and then kept in the darkness for 16 h. Following this, the explants were transferred into vermiculite and kept in high humidity in darkness for 24 h, then exposed to a photoperiod of 16 h light/8 h darkness cycles for three days. After removing the covers, the plants were cultivated in a standard growth chamber for approximately 40 days, after which the emergence of red hairy roots was observed.

4.3. Total RNA isolation, cDNA synthesis and gene cloning

Total RNA was extracted from the root of *G. uralensis* using the Quick RNA isolation Kit according to the manufacturer's protocol (ZH120; Huayueyang, China). 1 μg of RNA was reverse transcribed using the StarLighter Script RT All-in-One Mix (catalog number FS-P1001, Foreverstar, China), following the manufacturer's protocol to synthesize the first-strand cDNA. The full-length sequences of *GuUGT1* and *GuUGT10* genes were amplified with KOD One™ PCR master Mix (KMM-101, TOYOBO, Japan) from the cDNA using specific primers (Table S1).

4.4. Vector construction and A. rhizogenes preparation

The over-expression binary vector for *GuUGT1* and *GuUGT10* were constructed using a modified pCAMBIA1305, with the incorporation *RUBY* as the reporter gene that was driven by the cauliflower mosaic virus (CaMV) 35S promoter. The *GuUGT1*-targeting CRISPR/Cas9 binary vector was constructed using the modular plasmid pKSE401[15] with *Cas9* expression cassette, sgRNA scaffold, promoter *U6-26* (*AtU6-26p*), and reporter system. The Cas9 expression cassette: A plant codon-optimized *Streptococcus pyogenes* Cas9 nuclease is driven by the cauliflower mosaic virus (CaMV) 35S promoter. sgRNA scaffold: Dual sgRNA expression units under the control of the *Arabidopsis thaliana*. *AtU6-26p* each followed by a ribozyme (HDV and HH) for precise sgRNA processing. An eGFP reporter gene under the *Arabidopsis thaliana UBQ10* promoter for visual screening of transformed tissues. Two 20-bp protospacer sequences adjacent to the NGG proto-spacer adjacent motif (PAM) were selected from exon regions of *GuUGT1* using the tool of https://crispor.gi.ucsc.edu. The vector construction was carried out using the Golden Gate method. All of the plasmids were introduced into *A. rhizogenes* K599 cells through the heat-shock transformation method according to the manufacturer (AC1080; WEIDI, China). All of the primers are listed in Table S2.

4.5. Genotyping analysis

DNA was isolated from leaves, stems, and roots using the CTAB method[16]. The PCR reaction was as follows: 1 μL of diluted genomic DNA (100 ng/μL), 0.2 μL of each forward and reverse primer, 5 μL 2 × Rapid Taq Master Mix (P222-01, Vazyme, China), finally added ddH_2O to 10 μL. The PCR conditions were as follows: 94 °C for 3 min; followed by 30 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 50 s; 72 °C for 5 min; and final temperature of 16 °C. The products were separated on a 1.5% agarose gel. The amplified DNA fragments were sequenced at the Beijing Genomics Institute, Beijing, China. All of the primers were listed in Table S2.

4.6. qRT-PCR analysis

The qRT-PCR was performed using the AceQ qPCR SYBR Green Master Mix (Q111-02; Vazyme, China) and Rotor-GeneQ Real-time PCR Detection System (QIAGEN, Rotor-Gene Q MDx 5plex HRM (CA), USA). The reaction of qRT-PCR was 15 μL, made up of 3 μL diluted cDNA template, 0.3 μL of the forward primer, 0.3 μL of the reversed primer, 7.5 μL of the AceQ qPCR SYBR Green Master Mix, and 3.9 μL ddH_2O. These reaction conditions were as follows: 95 °C for 2 min, followed by 45 cycles at 95 °C for 10 s, 60 °C for 15 s and 72 °C for 15 s. The β-Tubulin was used as an internal reference. The three biological duplications in the qRT-PCR were used to compute the final relative expression levels of target genes using the fold change = $2^{-\Delta\Delta Ct}$ method. All of the primers are listed in Table S2.

4.7. Content analysis of liquiritigenin, isoliquiritigenin, liquiritin, and isoliquiritin

The fresh roots were ground into a fine powder in liquid nitrogen. A hundred mg ground powder was suspended in 1 mL 75% methanol in 2 mL tube. The samples were ultrasonically treated for 45 min at room temperature. Following this, samples were kept at 4 °C overnight to remove the impurity. After centrifugation (GS-X1, Gene, China) at 12,000 rpm for 10 min, the supernatant

was filtered with a 0.22 μm Nylon ultra-filtration membrane (Nylon 66; Tianjin Jin Teng, China). 100 μL extracts were transferred into screw top vials and 3 μL extracts were measured by an Agilent UPLC-MS instrument (1290II-G6400, Agilent, USA) equipped with the Agilent InfinityLab poroshell 120 EC-C18 (2.1 mm × 150 mm, 2.7 μm) (693775-902T; Agilent, USA) under positive modes employed the multiple reaction monitoring (MRM) mode for metabolites analysis (Supporting Information Table S3). Column temperature was 35 °C. Solvents for the mobile phase were 0.1% formic acid in water (A) and acetonitrile (B). The gradient elution program was as follows: 0−8 min, 19% B; 8−20 min, 19%−50% B; 20−25 min, 50%−100% B; 25−28 min, 100% B; 28−29 min, 100%−19% B; 29−32 min, 19% B. The flow rate was 0.3 mL/min. Details of the ion pair information can be found in Table S3.

Acknowledgments

The Fundamental Research Funds for the Central public welfare research institutes (No. ZZ15-YQ-043, China); Scientific and technological innovation project of China Academy of Chinese Medical Sciences (CI2023E002); National Key R&D Program of China (2023YFC3504104); National Natural Science Foundation of China (32070368). We are grateful for the instrument support provided by the large-scale instrument sharing platform of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

Author contributions

Wei Sun, Weiqiang Chen, Hui Yao and Xue Cao designed the experiments. Xue Cao, Zhenfen Qin, Panhui Fan, Sifan Wang, Huihua Wan, Xiangxiao Meng, Hui Yao and Wei Yang performed the experiments. Zhenfen Qin, Huihua Wan, and Wei Yang performed the metabolites analysis. Sifan Wang, Xiangxiao Meng and Weiqiang Chen performed the genetic analysis. Xue Cao and Zhenfen Qin wrote the original draft article; Shilin Chen, Weiqiang Chen and Wei Sun wrote the final article. All authors discussed the results and commented on the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2025.03.038.

References

1. Su XJ, Yang LL, Wang DL, Shu ZQ, Yang YC, Chen SL, et al. 1 K medicinal plant genome database: an integrated database combining genomes and metabolites of medicinal plants. *Hortic Res* 2022;**9**: uhac075.
2. Courdavault V, O'Connor SE, Jensen MK, Papon N. Metabolic engineering for plant natural products biosynthesis: new procedures, concrete achievements and remaining limits. *Nat Prod Rep* 2021;**38**: 2145−53.
3. Committee TP. *Pharmacopoeia of the People's Republic of China 2020 edition*. Beijing: China Medical Science Press; 2020.
4. Huang LQ, Guo LP, Zhang XB, Yu LY, Sun JH. Species of Chinese materia medica resources based on the fourth national survey of Chinese materia medica resources. *China J Chin Mater Med* 2024;**2**: 183−6.
5. Cao XS, Xie HT, Song ML, Lu JH, Ma P, Huang BY, et al. Cut−dip−budding delivery system enables genetic modifications in plants without tissue culture. *Innovation (Camb)* 2023;**4**:100345.
6. Cao XS, Xie HT, Song ML, Zhao LH, Liu HL, Li GF, et al. Simple method for transformation and gene editing in medicinal plants. *J Integr Plant Biol* 2024;**66**:17−9.
7. Lian ZY, Nguyen CD, Liu L, Wang GL, Chen JJ, Wang SH, et al. Application of developmental regulators to improve *in planta* or *in vitro* transformation in plants. *Plant Biotechnol J* 2022;**20**: 1622−35.
8. Maher MF, Nasti RA, Vollbrecht M, Starker CG, Clark MD, Voytas DF. Plant gene editing through *de novo* induction of meristems. *Nat Biotechnol* 2019;**38**:84−9.
9. Mei GG, Chen A, Wang YR, Li SQ, Wu MY, Hu YL, et al. A simple and efficient *in planta* transformation method based on the active regeneration capacity of plants. *Plant Commun* 2024;**5**:100822.
10. Ajdanian L, Niazian M, Torkamaneh D. Optimizing *ex vitro* one-step RUBY-equipped hairy root transformation in drug- and hemp-type Cannabis. *Plant Biotechnol J* 2024;**22**:1957−9.
11. He YB, Zhang T, Sun H, Zhan HD, Zhao YD. A reporter for non-invasively monitoring gene expression and plant transformation. *Hortic Res* 2020;**7**:152.
12. Chen K, Hu ZM, Song W, Wang ZL, He JB, Shi XM, et al. Diversity of *O*-glycosyltransferases contributes to the biosynthesis of flavonoid and triterpenoid glycosides in *Glycyrrhiza uralensis*. *ACS Synth Biol* 2019;**8**:1858−66.
13. Wang HT, Wang ZL, Chen K, Yao MJ, Zhang M, Wang RS, et al. Insights into the missing apiosylation step in flavonoid apiosides biosynthesis of Leguminosae plants. *Nat Commun* 2023;**14**:6658.
14. Yin Y, Li YP, Jiang D, Zhang XN, Gao W, Liu CS. *De novo* biosynthesis of liquiritin in *Saccharomyces cerevisiae*. *Acta Pharm Sin B* 2020;**10**:711−21.
15. Xing HL, Dong L, Wang ZP, Zhang HY, Han CY, Liu B, et al. A CRISPR/Cas9 toolkit for multiplex genome editing in plants. *BMC Plant Biol* 2014;**14**:327.
16. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 1980;**8**:4321−5.