INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 26, No. 4, p. 79-86, 2025

RESEARCH PAPER OPEN ACCESS

Cloning and sequence analysis of *GbCHS6* gene from *Ginkgo biloba*

Hualin Zou, Weiwei Zhang*

College of Horticulture and Gardening, Yangtze University, Jingzhou, China

Key words: *Ginkgo biloba*, Chalcone synthase, Molecular cloning, Sequence analysis

http://dx.doi.org/10.12692/ijb/26.4.79-86 Article published on April 04, 2025

Abstract

Chalcone synthase (CHS) is a key enzyme in the flavonoids biosynthetic pathway. In this study, chalcone synthase gene from *Ginkgo biloba* (*GbCHS6*) was cloned. The cDNA fragment of *GbCHS6* was 1191 bp, which encodes 396-amino-acid proteins with a calculated molecular weight of 43.29 kDa and isoelectric point of 6.47. Multiple alignments showed the amino acid sequence of GbCHS6 have extensive homology with those of CHS proteins from other plant. Phylogenetic tree analysis revealed that GbCHS6 had closer relationship with CHSs from Pinaceae and Cupressaceae plants than other plants. Molecular cloning and sequence analysis of GbCHS6 were conducted to further investigate the molecular mechanisms of biosynthese of flavonoids.

* **Corresponding Author:** Weiwei Zhang ✉ wwzhangchn@163.com

Introduction

Ginkgo biloba is native to China, which is the only existing species of Ginkgo in Ginkgoaceae (Gong *et al.*, 2008). It is a deciduous tree with a tree height of 30-40 meters and a conical or broad ovate crown. *G. biloba* leaves are fan-shaped, golden yellow in autumn, and have high ornamental value. So it can be used as a street tree, park tree, and courtyard tree, enhancing the landscape aesthetics of urban environments. *G. biloba* is not only an ornamental tree but also an important medicinal plant. The chemical constituents of *G. biloba* leaves: the terpene trilactones (ginkgolides A, B, C, J, P, and Q, and bilobalides) many flavonol glycosides, proanthocyanidins, alkylphenols, simple phenolic acids, 6-hydroxykynurenic acid, 4-O-methylpyridoxine, and polyphenols. *G. biloba* leaves extract is used in medicine due to its therapeutic actions in regulating cerebral blood flow, protection against free radicals, and delaying the progress of dementia and diabetes (Piazza *et al.*, 2019; Weinmann *et al.*, 2010; Zhou *et al.*, 2010). In the future, *G. biloba* research will make more breakthroughs in the development of medicinal components and make greater contributions to human health and ecological protection with the development of biotechnology.

The biosynthetic pathway of flavonoids has been widely studied at home and abroad, and the main steps of its synthetic pathway have been basically proved (Tohge *et al.*, 2017). Flavonoids are synthesized by the combination of the phenylpropanoid and polyketide pathways. Chalcone synthase (CHS) catalyzes the formation of a molecule of p-coumaroyl-CoA with three molecules of malonyl-CoA to form naringenin chalcone (4, 2, 4, 6-tetrahydroxychalcone), which is the first step in the core flavonoid synthesis pathway (Wang *et al.*, 2021). CHSs are widely found in higher plants and have been cloned in many plants, such as *Carthamus tinctorius* (Tang *et al.*, 2023), *Dysoxylum gotadhora* (Mahajan *et al.*, 2023), *Poncirus trifoliata* (Liu *et al.*, 2022), *Rhododendron delavayi* (Huang *et al.*, 2024), *Vaccinium corymbosum* (Zhang *et al.*, 2023), *Rhododendron* × *hybridum* Hort (Jia *et al.*, 2023), and its function has been clarified.

In this study, the *GbCHS6* gene was cloned and its amino acid sequence was analyzed, providing a foundation for further investigation into the biosynthesis and regulatory mechanisms of flavonoids in *G. biloba*, as well as the evolutionary dynamics of the *CHS* gene family.

Material and methods

Plant material and reagents

G. biloba leaves were collected at the *Ginkgo biloba* Science Research Garden of Yangtze University, Jingzhou, Hubei, China, and immediately placed in liquid nitrogen and stored in a refrigerator at -80℃ for subsequent RNA extraction. Primers synthesis and DNA sequencing were carried out by Sangon biotechnology company (Shanghai, China). Taq DNA polymerase, pMD19-T vector kit and MiniBEST Plant RNA Extraction kit were from Takara Company (Dalian, China). HiScript® Reverse Transcriptase was purchased from Vazyme Company (Nanjin, China). EasyPure PCR Purification Kit was from TransGen Biotech Company (Beijing, China).

RNA isolation and cDNA synthesis

Total RNA was isolated from frozen *G. biloba* leaves by using the MiniBEST Plant RNA Extraction kit, and using 1% agarose gel electrophoresis detection the RNA. Finally, cDNA was synthesized by using HiScript® Reverse Transcriptase.

Full-length cDNA clone of GbCHS6 gene

The specific primers GbCHS6-F (ATGCCTGCAGGAGCGAT) and GbCHS6-R (TTAATTGTTGCAGGGAACGCTTC) were designed by Premier 6.0 software. The PCR was performed under the following reaction system: ddH_2O 9.5 μl, Taq polymerase 12.5 μl, Primer-F 1μl, Primer-R 1μl, cDNA 1μl, and the reaction conditions : 95℃ 3 min initial denaturation; 95℃ 15 s, 56.5℃ 15 s, 72℃ 15 s, 32 cycles, followed by an extension for 5 min at 72℃. The PCR products were separated on a 1% agarose gel

electrophoresis. PCR products were purified and sent to Sangon biotechnology company for sequencing.

Bioinformatics analysis

The amino acid sequences of CHS homologous to GbCHS6 were searched in NCBI (https://www.ncbi.nlm.nih.gov/) database. The ExPASy online website (http://web.expasy.org/protparam/) was utilized to predict the physicochemical properties of the obtained sequence. DNAman 9.0 software was employed to analyze *GbCHS6* gene sequence and amino acid composition. The software MEGA 10.0 was used to construct the evolution tree. Additionally, TMHMM 2.0 online website (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) was used to predict the transmembrane domain of the protein online.

Results

Cloning of the cDNA of GbCHS6

The *GbCHS6* gene was amplified by PCR using the cDNA of *G. biloba* leaves as template. A band of about 1191 bp was detected by agarose gel electrophoresis (Fig. 1), and the band was recovered and sequenced. The sequencing results were consistent with the transcriptome sequence. According to the transcriptome data of *G. biloba*, the ORF length of *GbCHS6* gene is 1191 bp, encoding 396 amino acids (Fig. 2).

Table 1. Composition of GbCHS6 amino acids.

Amino acid name	Number	Frequency	Amino acid name	Number	Frequency
Leu(L)	39	9.80%	Gly(G)	34	8.60%
Ala(A)	33	8.30%	Val(V)	30	7.60%
Lys(K)	27	6.80%	Glu(E)	24	6.10%
Ser(S)	24	6.10%	Asp(D)	22	5.60%
Thr(T)	22	5.60%	Pro(P)	21	5.30%
Ile(I)	20	5.10%	Arg(R)	17	4.30%
Phe(F)	15	3.80%	Asn(N)	14	3.50%
Gln(Q)	12	3.00%	Met(M)	12	3.00%
Tyr(Y)	9	2.30%	Cys(C)	8	2.00%
His(H)	8	2.00%	Trp(W)	5	1.30%

Characterization of the GbCHS6 protein

The physical and chemical properties of GbCHS6 protein are analyzed by ExPasy website (Table 1). Leucine accounted for the largest proportion of amino acid composition, 9.8%, and the molecular weight is 43.29 kDa. The isoelectric point is 6.47, which is an acidic protein. The instability index of GbCHS6 is 39.21, and the grand average of hydropathicity is -0.124, indicating that it was an unstable hydrophilic protein. The total number of negatively charged residues (Asp + Glu) carried by the protein was 46, and the total number of positively charged residues (Arg + Lys) was 44. The prediction results of transmembrane structure (Fig. 3) showed that the probability of each amino acid of GbCHS6 protein located outside the cell membrane was higher than that in the cell membrane and the presence of transmembrane structure. This indicates that the GbCHS6 protein does not contain a transmembrane domain, and the entire polypeptide chain is outside the cell membrane.

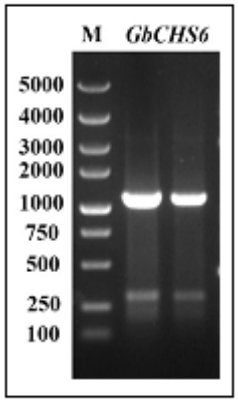

Fig. 1. PCR amplification result of *GbCHS6* gene.
M: DL5000 DNA Marker; *GbChs6*: PCR product of *GbCHS6* gene.

Molecular evolution analysis

The amino acid sequence of GbCHS6 was compared with the CHS amino acid sequence of *Larix kaempferi*, *Picea sitchensis*, *Abies alba*, *Matricaria chamomilla*, *Zea mays* and *Glycyrrhiza uralensis* by DNAMAN9.0. The result showed that the similarities of GbCHS6 with *Larix kaempferi*, *Picea sitchensis*, *Abies alba*, *Matricaria chamomilla*, *Zea mays* and *Glycyrrhiza* uralensis were 88.78%, 89.03%, 88.53%, 78.55%, 78.8% and80.3%, respectively.

Like CHS proteins in other plants, GbCHS6 contains highly conserved amino acid residues in the *CHS* gene family, including seven cyclized amino acid residues, three coenzyme A active binding sites, a Cys, His, Asn catalytic triad and two highly conserved amino acid characteristic sequences (RLMMYQQGCFAGGTVLR and GVLFGFGPGL) (Fig. 4).

The phylogenetic tree of GbCHS6 and CHS encoding amino acid sequences of 14 other plants were constructed by MEGA 10.0. The results showed that GbCHS6 was closely related to the evolution of *CHS* mino acid from plants such as *Picea sitchensis*, *Abies alba*, *Larix kaempferi*, *Pinus radiata*, *Pinus pinaster*, *Cryptomeria japonica*, but far from the evolution of *CHS* genes from *Arabidopsis thaliana*, *Zea mays*, *Matricaria chamomilla* (Fig. 5).

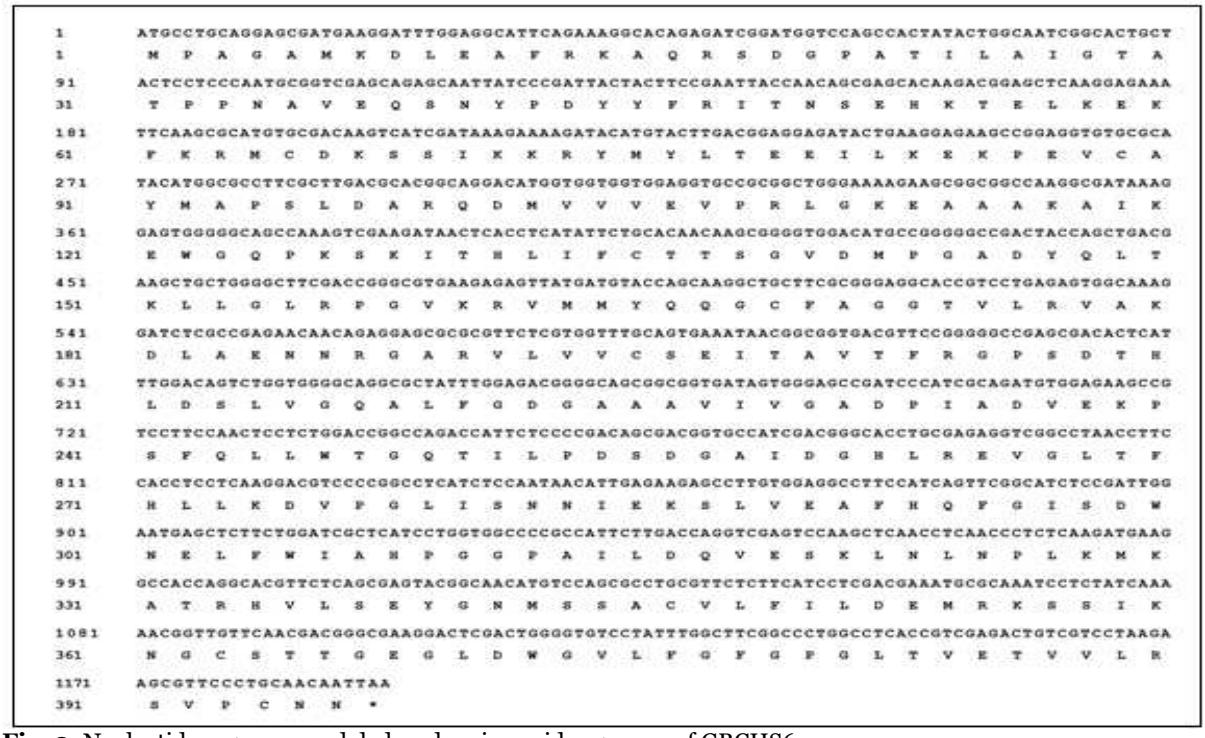

Fig. 2. Nucleotide sequence and deduced amino acid sequence of GBCHS6.

Discussion

Numerous studies have demonstrated that CHS is a critical enzyme in the synthesis of flavonoids, the flavonoids content of the rice protoplasts that overexpressed *CHS* genes was higher (Zhang *et al.*, 2022). Conversely, suppressing the expression of the *CitCHS* genes in plants led to a 41.11% reduction in total flavonoid production compared to non-transgenic control (Wang *et al.*, 2018). Furthermore, research on *G. biloba* chalcone synthase revealed a significant correlation between the expression levels of *CHS* genes and flavonoid content throughout all developmental stages of *G. biloba* leaves (Xu *et al.*, 2007). In this study, *GbCHS6* was isolated from *G. biloba* cDNA. Sequence analysis showed that GbCHS6 encodes a protein of 396 amino acids. The amino acid sequence analysis revealed a similarity of 89.03% between GbCHS protein and *Picea sitchensis* CHS protein. Moreover, the protein encoded by the *GbCHS6* gene contains the typical conserved domains and active site characteristics of CHS.

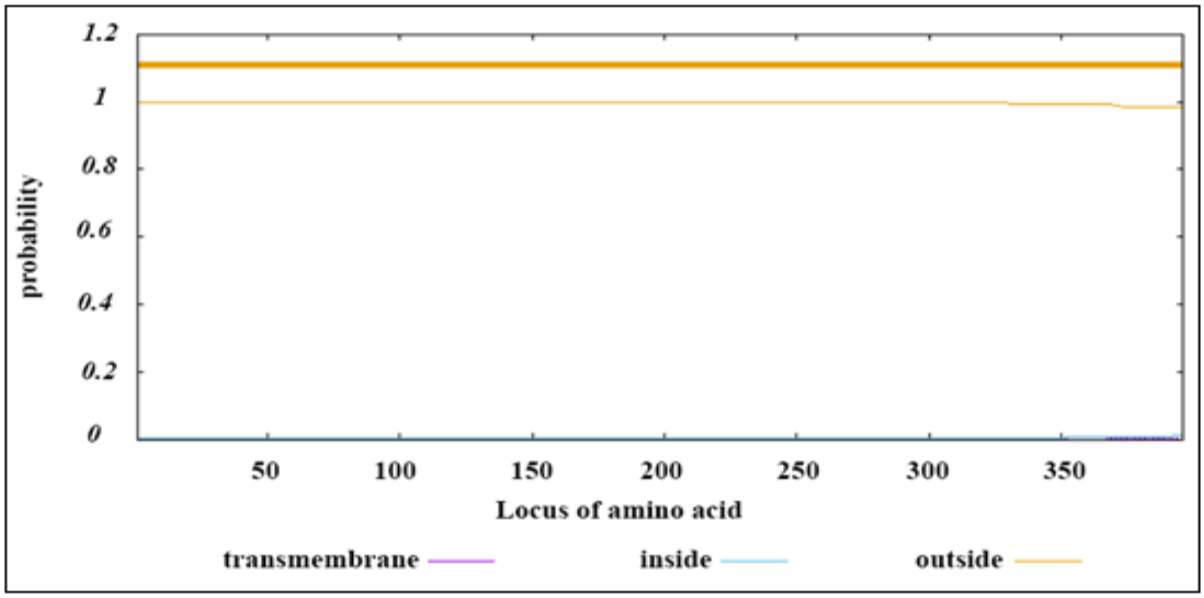

Fig. 3. Transmembrane structure prediction of GbCHS protein.

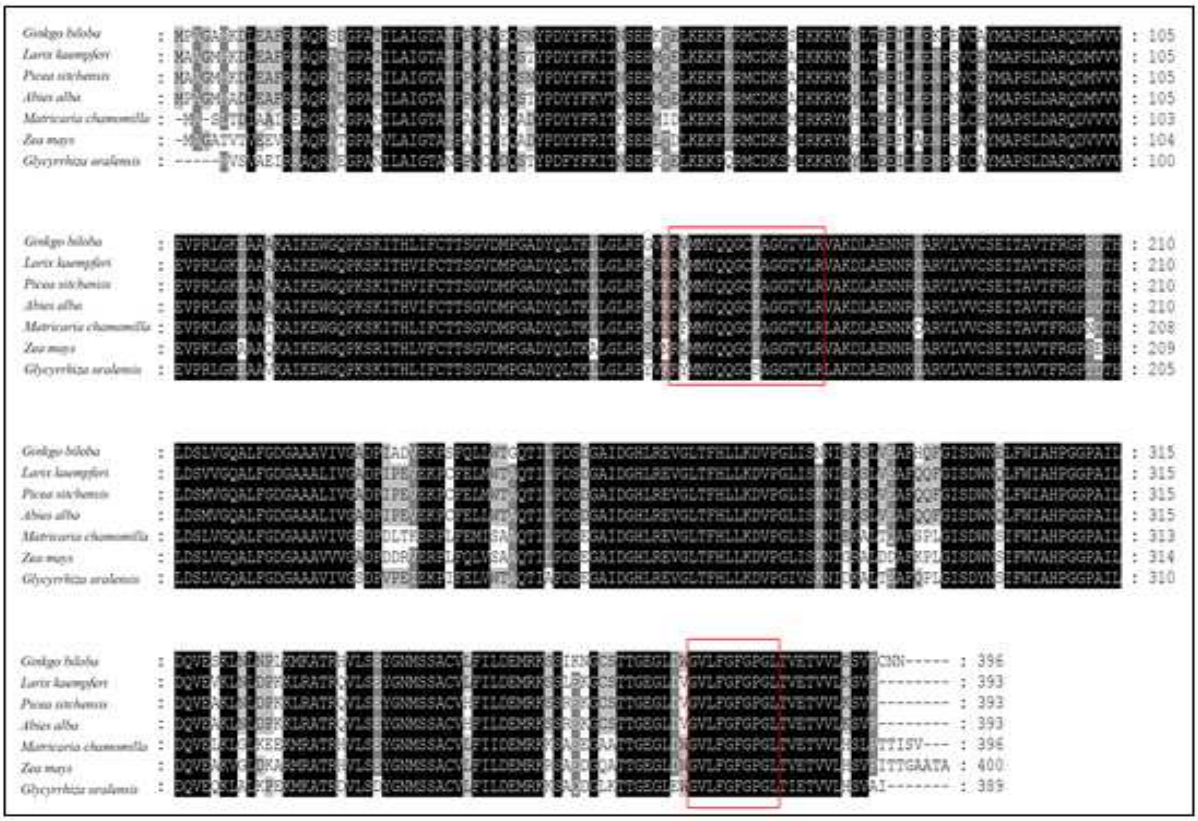

Fig. 4. Sequence multi-alignment of the deduced GbCHS6 protein with other CHS proteins. The species and GenBank accession number are as following: *Larix kaempferi* (QDF21381.1), *Picea sitchensis* (ABK21072.1), *Abies alba* (ABD24230.1), *Matricaria chamomilla* (WOR09430.1), *Zea mays* (NP_001142246.1), *Glycyrrhiza uralensis* (ABM66532.1).

In this study, the bioinformatics analysis of GbCHS protein sequence was carried out to explore its protein characteristics, which laid a good foundation for the functional identification and application of *GbCHS* gene and its encoded protein. In addition, GbCHS protein does not contain signal peptide and transit peptide structure. When expressed in prokaryotes such as *E. coli*, the coding region

sequence can be directly cloned into the expression vector and transformed into the host bacteria, without the need for enzyme digestion or specific PCR primer amplification to remove this part of the structure (Jiang *et al*., 2024; De Marco, 2025). These protein characteristics can provide theoretical guidance for subsequent experiments such as purification, functional identification and expression vector construction of GbCHS fusion protein.

And research shows that the higher production of naringenin was reported in a strain modified both to improve malonyl-CoA and l-tyrosine availability (Gomes *et al*., 2024).

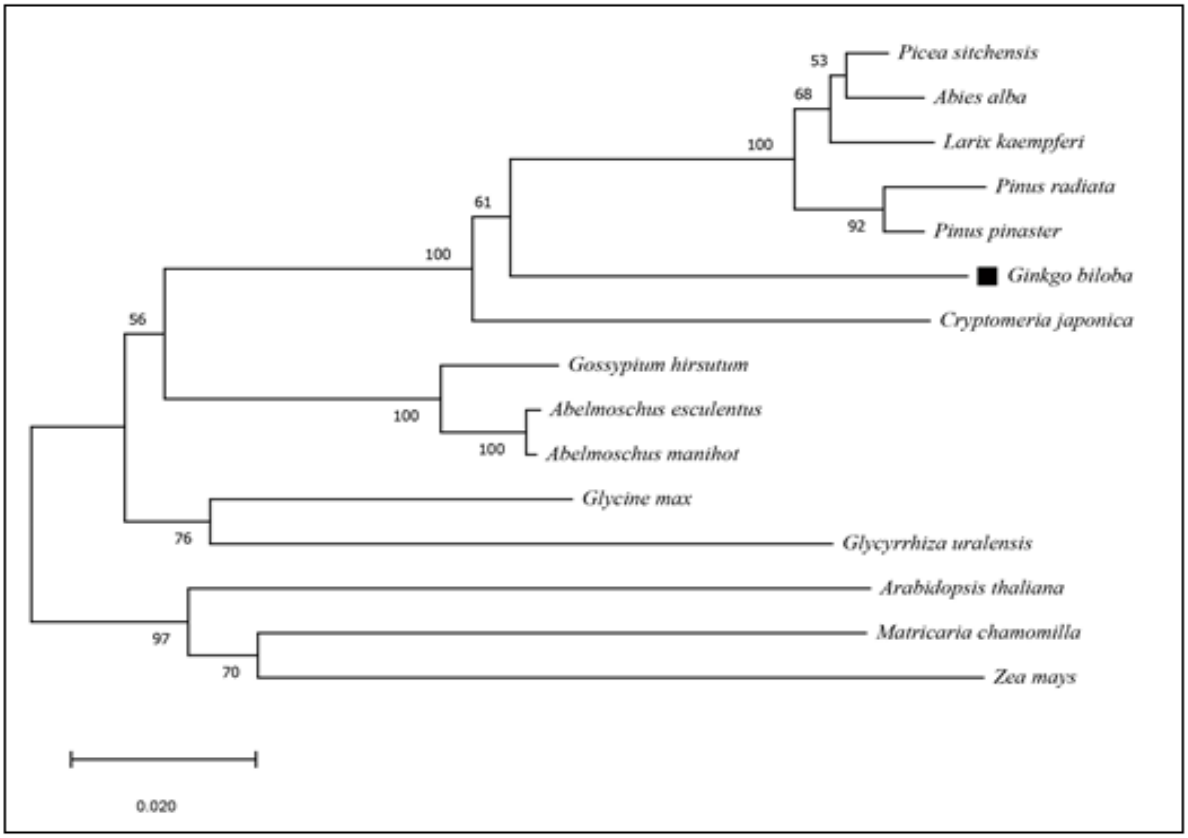

Fig. 5. The phylogenetic tree of CHS including GbCHS6. Phylogenetic analysis of *GbCHS6* mino acid sequence with other plants. Bootstrap values are expressed in percentages and placed at the nodes in the tree. The GenBank accession numbers of the *CHS* mino acid sequences and plant species are as following: P*icea sitchensis* (ABK21072.1), *Larix kaempferi* (QDF21381.1), *Pinus radiata* (AGY80771.1), *Pinus pinaster* (AAP85249.1), *Abies alba* (ABD24230.1), *Cryptomeria japonica* (XP_057812903.1), *Abelmoschus esculentus* (AGW22222.1), *Abelmoschus manihot* (ACE60221.1), *Gossypium hirsutum* (NP_001314206.1), *Glycine max* (NP_001358306.1), *Matricaria chamomilla* (WOR09430.1), *Zea mays* (NP_001142246.1), *Glycyrrhiza uralensis* (ABM66532.1), *Arabidopsis thaliana* (NP_196897.1).

If the subsequent conditions permit, the prokaryotic expression experiment of *GbCHS6* gene can be carried out to construct a heterologous protein expression system.

It provides a theoretical basis and trial direction for the future use of genetic engineering to transform *CHS* gene and produce flavonoids.

Conclusions

In this study, the *GbCHS6* gene from *G. biloba* was successfully cloned and characterized, revealing that it encodes a 396-amino-acid protein with typical conserved domains and active sites of chalcone synthase. Sequence alignment and phylogenetic analysis demonstrated high similarity between GbCHS6 and CHS proteins from gymnosperms such

as *Picea sitchensis* and *Larix kaempferi*, indicating a close evolutionary relationship. Bioinformatics analyses showed that the GbCHS6 protein is an acidic, hydrophilic, and non-transmembrane protein, with properties conducive to heterologous expression. These findings provide a molecular basis for further research into the biosynthesis and regulation of flavonoids in *G. biloba*. The identification of GbCHS6 lays a strong foundation for future functional studies, genetic engineering applications, and the potential development of biotechnological strategies to enhance flavonoid production for medicinal and industrial use.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

De Marco A. 2025. Recent advances in recombinant production of soluble proteins in *E. coli*. Microbial cell factories **24(1),** 1-12. https://doi.org/10.1186/s12934-025-02646-8

Gong W, Chen C, Dobeš C, Fu C, Koch MA. 2008. Phylogeography of a living fossil: pleistocene glaciations forced *Ginkgo biloba* L. (Ginkgoaceae) into two refuge areas in China with limited subsequent postglacial expansion. Molecular phylogenetics and evolution **48(3),** 1094-1105. https://doi.org/10.1016/j.ympev.2008.05.003

Gomes D, Rodrigues JL, Rodrigues LR. 2024. Step-by-step optimization of a heterologous pathway for de novo naringenin production in *Escherichia coli*. Applied Microbiology and Biotechnology **108**(1), 435. https://doi.org/10.1007/s00253-024-13271-7

Huang J, Zhao X, Zhang Y, Chen Y, Zhang X, Yi Y, Ju Z, Sun W. 2024. Chalcone-synthase-encoding *RdCHS1* is involved in flavonoid biosynthesis in *Rhododendron delavayi*. Molecules **29(8),** 1822. https://doi.org/10.3390/molecules29081822

Jiang R, Yuan S, Zhou Y, Wei Y, Li F, Wang M, Chen B, Yu H. 2024. Strategies to overcome the challenges of low or no expression of heterologous proteins in Escherichia coli. Biotechnology advances **75,** 108417. https://doi.org/10.1016/j.biotechadv.2024.108417

Jia YH, He F, Shen ZL, Xie XH, Lv SJ, Jiang BX, Yang GX, Yan YC, Wang DD, Li P, Chen QY, Chen XY, Yan ZW, Wang MY, Mao YB. 2021. Differential contributions of MYCs to insect defense reveals flavonoids alleviating growth inhibition caused by wounding in *Arabidopsis*. Frontiers in plant science **12,** 700555. https://doi.org/10.3389/fpls.2021.700555

Mahajan V, Chouhan R, Jamwal VL, Kapoor N, Gandhi SG. 2023. A wound inducible chalcone synthase gene from *Dysoxylum gotadhora* (*DbCHS*) regulates flavonoid biosynthesis. Physiology and Molecular Biology of Plants **29(7),** 959-969. https://doi.org/10.1007/s12298-023-01344-2

Piazza S, Pacchetti B, Fumagalli M, Bonacina F, Dell'Agli M, Sangiovanni E. 2019. Comparison of two *Ginkgo biloba* L. extracts on oxidative stress and inflammation markers in human endothelial cells. Mediators of inflammation **1**, 6173893. https://doi.org/10.1155/2019/6173893

Tang X, Ren C, Hu J, Chen J, Wang J, Wang R, Wu Q, Liao W, Pei J. 2023. Cloning, expression and activity analysises of chalcone synthase genes in *Carthamus tinctorius*. Chinese herbal medicines **15(2),** 291-297. https://doi.org/10.1016/j.chmed.2022.12.005

Tohge T, de Souza LP, Fernie AR. 2017. Current understanding of the pathways of flavonoid biosynthesis in model and crop plants. Journal of experimental botany **68(15),** 4013-4028. https://doi.org/10.1093/jxb/erx177

Weinmann S, Roll S, Schwarzbach CJ, Vauth C, Willich SN. 2010. Effects of *Ginkgo biloba* in dementia: systematic review and meta-analysis. BMC Geriatrics **10,** 1-12.
https://doi.org/10.1186/1471-2318-10-14

Wang Z, Yu Q, Shen W, El Mohtar CA, Zhao X, Gmitter FG. 2018. Functional study of *CHS* gene family members in citrus revealed a novel *CHS* gene affecting the production of flavonoids. BMC plant biology **18(1),** 1-13.
https://doi.org/10.1186/s12870-018-1418-y

Wu Z, Wu Y. 2023. Molecular characterization of a chalcone synthase gene *RhCHS* from *Rhododendron* × *hybridum* Hort. Gene **857,** 147176.
https://doi.org/10.1016/j.gene.2023.147176

Xu F, Cheng SY, Cheng SH, Wang Y, Du HW. 2007. Time course of expression of chalcone synthase gene in *Ginkgo biloba*. Journal of plant physiology and molecular biology **33(4),** 309-317.

Liu Z, Cheng S, Liu XQ, Kuča K, Hashem A, Al-Arjani AF, Almutairi KF, Abd Allah EF, Wu QS, Zou YN. 2022. Cloning of a *CHS* gene of *Poncirus trifoliata* and its expression in response to soil water deficit and arbuscular mycorrhizal fungi. Frontiers in plant science **13,** 1101212.
https://doi.org/10.3389/fpls.2022.1101212

Zhang Q, Li T, Gao M, Ye M, Lin M, Wu D, Guo J, Guan W, Wang J, Yang K, Zhu L, Cheng Y, Du B, He G. 2022. Transcriptome and metabolome profiling reveal the resistance mechanisms of rice against brown planthopper. International journal of molecular sciences **23(8),** 4083.
https://doi.org/10.3390/ijms23084083

Zhang Z, Qu P, Hao S, Li R, Zhang Y, Zhao Q, Wen P, Cheng C. 2023. Characterization and functional analysis of chalcone synthase genes in Highbush Blueberry (*Vaccinium corymbosum*). International journal of molecular sciences **24(18),** 13882.
https://doi.org/10.3390/ijms241813882

Zhou L, Meng Q, Qian T, Yang Z. 2010. *Ginkgo biloba* extract enhances glucose tolerance in hyperinsulinism-induced hepatic cells. Journal of Natural Medicines **65,** 50-56.
https://doi.org/10.1007/s11418-010-0456-z