JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2024 | Volume 12 | Issue 2

Original paper

Ginkgo biloba GbbHLH13 transcription factor regulates flavonoid biosynthesis

Ying Li[1,2*]

[1.] College of Horticulture and Gardening, Yangtze University, Jingzhou 434025, Hubei, China
[2.] Hubei key Laboratory of Spices & Horticultural Plant Germplasm Innovation & Utilization, Yangtze University, Jingzhou 434025, Hubei, China

Abstract: *Ginkgo biloba* is the oldest living plant on Earth and one of the most widely used natural medicines worldwide. Flavonoids extracted from *G. biloba* have been shown to have protective effects against cardiovascular and cerebrovascular diseases. The bHLH transcription factors (TFs) are among the most important families of transcription factors in plants, playing a crucial role in regulating plant growth, development, and secondary metabolism. In this study, *GbbHLH13* was isolated and identified. It encodes a protein consisting of 732 amino acids, and transient expression assays in tobacco indicated that *GbbHLH13* is localized in the nucleus. Exogenous hormone treatments revealed that the expression of *GbbHLH13* is affected by methyl jasmonate (MeJA) and abscisic acid (ABA). Transient overexpression experiments further showed that it upregulates the transcription levels of flavonoid metabolism-related genes *PAL*, *CHI*, and *C4H*, suggesting that *GbbHLH13* may be involved in flavonoid biosynthesis.

Keywords: *Ginkgo biloba*, *GbbHLH13*, flavonoids, subcellular localization, RT-qPCR.

OPEN ACCESS

Edited by
Prof. Suresh Kumar,
Indian Agricultural Research Institute, India

Date
Received: 1 November 2024
Accepted: 6 November 2024
Published: 12 November 2024

Correspondence
Ying Li
liyly1219@163.com

Citation
Li, Y. (2024). *G inkgo biloba GbbHLH13* transcription factor regulates flavonoid biosynthesis. *J Plant Mol Breed 12* (2): 1-12. doi: 10.22058/jpmb.2024.2044701.1310.

Introduction

In our country, *Ginkgo biloba* resources are widely distributed and abundant, accounting for over 75% of the world's *G. biloba* resources (Wang et al., 2013). They possess unique morphological characteristics, systematic evolution, and taxonomic status. All parts of *G. biloba* have different medicinal values, making it one of the world's most widely used natural medicines. It is commonly used to treat asthma, hypertension, atherosclerosis, and to improve cardiovascular and peripheral vascular diseases (Li et al., 2019). *G. biloba* is especially rich in secondary metabolites (Liu et al., 2021), such as flavonoids, terpenoids, polysaccharides, and phenolic acids, with flavonoids and terpenoids being the main active medicinal components (Liu et al., 2022). Ginkgo flavonoids possess antibacterial (Tagousop et al., 2018), anti-inflammatory (Maleki et al., 2019), and antioxidant activities, as well as neuroprotective effects (Singh et al., 2019) and the ability to regulate the functions of key cell enzymes (Heim et al., 2002). They are essential ingredients in various nutritional supplements, pharmaceuticals, and the cosmetics industry.

Transcription factors are a class of proteins that have specific DNA-binding domains and can bind to cis-regulatory elements to regulate gene expression at the transcriptional level (Biłas et al., 2016). bHLH transcription factors are a type of transcription factor that contains a basic helix-loop-helix (bHLH) structural domain, playing important roles in regulating plant growth and development, stress responses, and signal transduction (Hao et al., 2021). In plants, the first protein reported to have a bHLH structural domain is the product of the *R* gene in *Zea mays* L., which is involved in the regulatory synthesis of flavonoids and anthocyanins (Ludwig et al., 1989). In yeast, bHLH proteins participate in chromosome segregation and metabolic regulation (Robinson and Lopes, 2000). In animals, bHLH proteins are involved in sensing environmental signals, regulating the cell cycle and circadian rhythms, as well as modulating various developmental processes (Stevens et al., 2008). In recent years, a large number of *bHLHs* have been identified in plants such as *Arabidopsis thaliana* (L.) Heynh. (Toledo-Ortiz et al., 2003), carrot (Chen et al., 2015), and *Phyllostachys edulis* (Carrière) J. Houzeau (Cheng et al., 2018), with comprehensive genome-wide characterization conducted. To date, over 160 bHLH transcription factors have been isolated and identified, of which approximately 30% have defined functions (Pires and Dolan, 2010). Among the members of the bHLH transcription factor family in different plants, various bHLH proteins have distinct functions and are involved in different regulatory domains such as plant growth and development and morphogenesis. For example, the Arabidopsis thaliana bHLH proteins HEC2 and HEC3 are involved in regulating the development of the gynoecium (Gremski et al., 2007); additionally, *MdbHLH10* in apples can induce anthocyanin accumulation in both homologous and heterologous systems (Espley et al., 2007). Research has shown that bHLH transcription factors mainly regulate gene expression through two mechanisms. One involves forming MBW complexes by binding to MYB or WD40 transcription factors for transcriptional regulation. The second mechanism involves directly binding to the promoters of structural genes to exert regulatory effects. While there are many studies on the regulatory role of MBW complexes, the direct binding of bHLH factors to promoters requires further investigation. For instance, in apples, *MdbHLH3* can directly bind to the promoters of *MdDFR* and *MdUFGT* to activate their expression (Xie et al., 2012). Additionally, the bHLH transcription factor *VvMYC1* interacts with *MYB5a*, *MYB5b*, *MYBA1/A2*, and *MYBPA1* to induce the synthesis of anthocyanins and proanthocyanidins (Hichri et al., 2010). It is known that most bHLH transcription factors involved in flavonoid synthesis in various plants belong to the IIIf subgroup. Members of the bHLH III(d+e) subfamily can regulate jasmonic acid signaling to enhance the plant defense systems in apples and strawberries, promoting the biosynthesis of flavonoids.

So far, there has been little research on bHLH transcription factors related to ginkgo flavonoids, and the molecular mechanisms by which bHLH transcription factors regulate flavonoid synthesis in *G. biloba* remain unclear. In previous studies, the GbbHLH gene family has been identified (Zhou et al., 2020). In this study, *GbbHLH13* was isolated for the first time. Through exogenous hormone treatment and transient overexpression, it was

found that *GbbHLH13* and flavonoid-related structural genes were significantly upregulated, suggesting that *GbbHLH13* may be associated with flavonoid biosynthesis in *G. biloba*.

Materials and Methods

Cloning and identification of GbbHLH13

The nucleic acid sequence of *GbbHLH13* was obtained from genomic and transcriptomic databases based on previous studies. Using ginkgo leaf cDNA as a template, specific primers (*GbbHLH13*-U/D) were designed with Primer 6 software to amplify *GbbHLH13*, which was then cloned into the pMD19-T vector (Supplementary Table S1). The relative molecular weight and isoelectric point of *GbbHLH13* were predicted using the ExPASy platform. Homologous protein sequences of *GbbHLH13* were downloaded from NCBI, and amino acid sequence alignment was performed using DNAMAN 9.0. The upstream 2000 bp of *GbbHLH13* was extracted as the promoter sequence using TBtools, and cis-regulatory elements in the promoter region were predicted online using PlantCARE, visualization analysis of *GbbHLH13* was conducted using TBtools. Phylogenetic analysis of bHLHs involved in flavonoid regulation across different species was conducted using TBtools, and the results were compared with *GbbHLH13*.

Expression levels of GbbHLH13 in different tissues at various developmental stages

The *G. biloba* materials were sourced from a 37-year-old "Buddha's Hand" *G. biloba* in the ginkgo garden at the West Campus of Yangtze University. Samples from different tissues were collected from March to September 2024. Microstrobilus (M) were collected on March 23; ovulate strobilus (OS) were collected on April 10 and April 17; roots (R) were collected on April 10; stems (S) were collected on April 3, April 17, May 7, May 21, June 3, July 1, and July 15; leaves (L) were collected on March 23, April 3, April 17, May 7, May 21, June 3, July 1, July 15, August 13, and August 26; and fruits (F) were collected on May 7, May 21, June 3, July 1, July 15, August 13, and August 26. The samples were quickly frozen in liquid nitrogen for subsequent experiments.

For qRT-PCR validation, total RNA was extracted from *G. biloba* using the Takara MiniBEST Plant RNA Extraction Kit. The first-strand cDNA was synthesized using the HiScript III First Strand cDNA Synthesis Kit from Vazyme, Nanjing. qRT-PCR was performed according to the protocol of ChamQ Universal SYBR qPCR Master Mix. The primers *DGbbHLH13*-U/D were used to detect the expression of GbbHLH13 in *G. biloba* tissues at different developmental stages. *GbGAPDH* was chosen as the reference gene. Statistical analysis was conducted using IBM SPSS Statistics 26, and relative expression levels were calculated using the 2-ΔΔCt method, presented as mean ± standard error (SE). Duncan's multiple range test was employed to assess significant differences between means, with a significance level set at $P < 0.05$.

Exogenous hormone treatment of G. biloba seedlings

When the one-year-old *G. biloba* seedlings reached 8-10 leaves, plants with consistent growth and no visible disease were selected. The seedlings were treated by spraying both sides of the leaves with 1 mM MeJA, 100 μM ABA, and 1% ethanol solution as a control. Each treatment had three replicates, with eight seedlings per replicate. Treatments were applied at 8:00 AM, and leaves were collected at 0, 3, 6, 12, 24, and 48 hours post-treatment. The samples were quickly frozen in liquid nitrogen, and RNA was extracted and reverse transcribed for subsequent RT-qPCR analysis of *GbbHLH13* expression.

Subcellular localization of GbbHLH13

First, the subcellular localization of the GbbHLH13 protein was predicted using the WoLF PSORT online tool. The *GbbHLH13*, with the stop codon removed, was constructed into the pICH86988 and pICSL50008 fusion vectors. The correctly sequenced recombinant plasmid pICH86988-*GbbHLH13*-pICSL50008 was then introduced into Agrobacterium GV3101 via electroporation. Infection solution (10 mM MES, 10 mM $MgCl_2$, 100 μM acetosyringone, pH = 5.6) was injected into 4-5 week-old tobacco leaves for transient expression. Three days later, fluorescence was observed using a laser confocal microscope (Leica TCS SP8) to detect the localization of *GbbHLH13* in the plant.

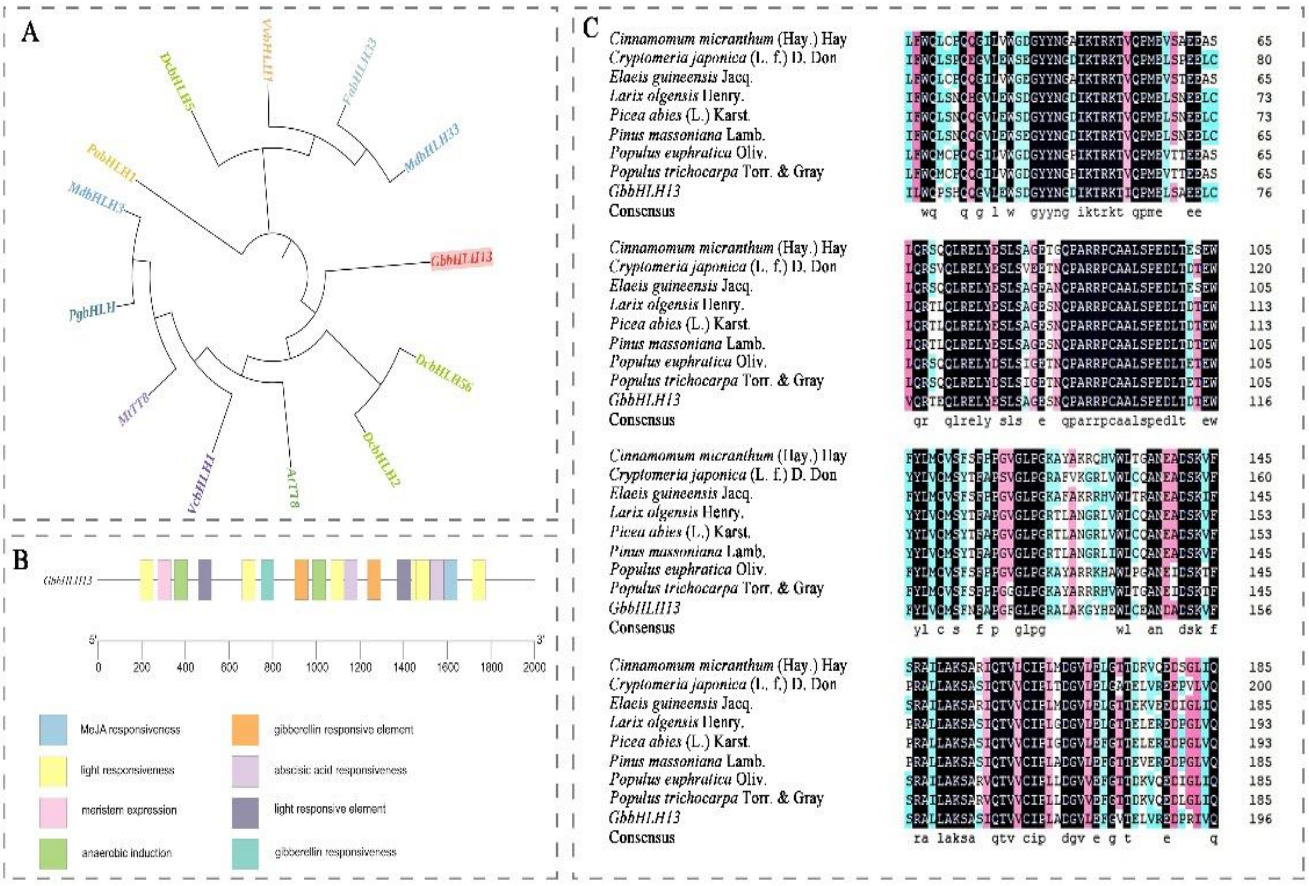

Figure 1. Characterization of *GbbHLH13*. (A) Phylogenetic tree analysis between *GbbHLH13* and verified bHLHs in flavonoid metabolism pathway in other species. Information of gene IDs from other species was shown in Table S1. (B) Promoter analysis for *GbbHLH13*. (C) Multiple sequence alignment for *GbbHLH13* conserved domain and bHLH conserved domains of other species downloaded from NCBI.

Transient overexpression of GbbHLH13 in G. biloba

To investigate the role of *GbbHLH13* in the flavonoid biosynthesis of *G. biloba*, the overexpression vector pCY-H05252 was selected. PCR amplification was conducted using the primers pCY-H05252-*GbbHLH13*-U/D, and the full-length sequence of *GbbHLH13* was subsequently inserted to construct an overexpression recombinant vector. This vector was then transferred into Agrobacterium tumefaciens GV3101 via electroporation and employed for transient overexpression experiments in *G. biloba*. Annual Ginkgo seedlings exhibiting uniform growth and free from disease were selected for these transient expression experiments. The bacterial cultures of pCY-H05252-*GbbHLH13*-GV3101 and pCY-H05252-GV3101 were activated. Once the optical density at 600 nm (OD600) of the bacterial suspension reached approximately 1.0, the culture was centrifuged at 4000 rpm for 5 minutes to collect the bacterial cells, which were then resuspended in an infection solution containing 10 mM MES, 10 mM $MgCl_2$, and 100 μM acetosyringone at pH 5.6. The left leaf of each seedling was injected with the infection solution containing pCY-H05252-*GbbHLH13*, while the right leaf received the control solution. Following a 24-hour incubation period in the dark, the samples

were cultured under normal conditions for one week. Subsequently, samples were rapidly frozen in liquid nitrogen for further experiments.

Results

Isolation and analysis of GbbHLH13

The full length of *GbbHLH13* is 2199 bp, encoding 732 amino acids; the molecular weight is 8.2 kDa, and the isoelectric point is 5.68. Promoter analysis showed that *GbbHLH13* contains a large number of light-responsive elements, gibberellin, methyl jasmonate, and abscisic acid response-related elements; it is also related to anaerobic induction and tissue meristem. (Figure 1B). By comparing bHLH protein sequences with high similarity to *GbbHLH13* using NCBI Blast function, it was found that *GbbHLH13* protein shares a common conserved domain with bHLH proteins of other plants (Fig. 1C). To study the phylogenetic evolution of *GbbHLH13*, this study selected 12 bHLH amino acid sequences involved in flavonoid regulation to construct a phylogenetic tree. The results showed that *GbbHLH13* has a relatively close genetic relationship with dracaena, apple, and grape (Figure 1A).

Expression levels of GbbHLH13 in different tissues at different stages

The expression levels of *GbbHLH13* were analyzed in *G. biloba* microstrobilus (M), ovulate strobilus (OS), roots (R), stems (S), leaves (L), and fruits (F) at different developmental stages. Samples included one period for roots and male flowers, two periods for female flowers, seven periods for stems and fruits, and ten periods for leaves. The results indicated that *GbbHLH13* expression in leaves showed a trend of rising and then falling, peaking at L2 and experiencing a brief increase at L7. The expression was highest in the first period of stems, significantly notable in the first and fourth periods of fruits, and markedly higher in female flowers compared to male flowers and roots (Figure 2). In summary, *GbbHLH13* is expressed in various tissues of *G. biloba* at different stages, with an overall declining trend over time, significant expression in leaves, and almost no expression in roots.

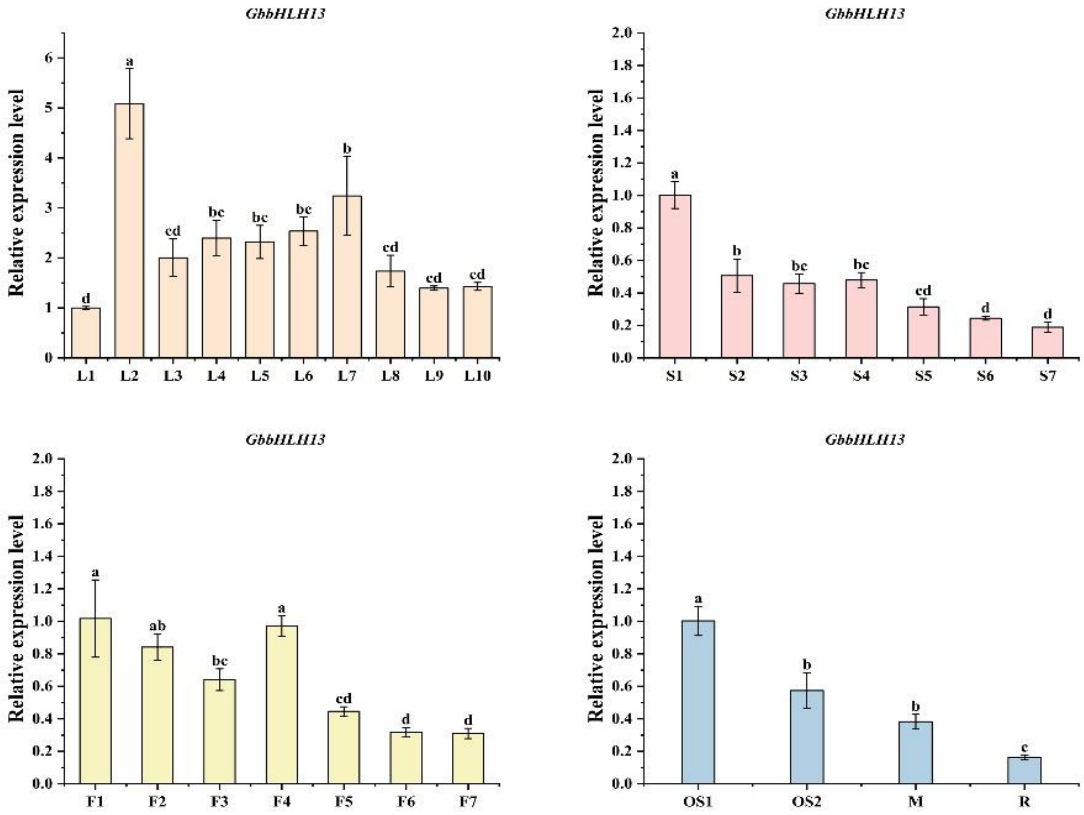

Figure 2. Expression levels of *GbbHLH13* in different tissues at different developmental stages of *Ginkgo biloba*.

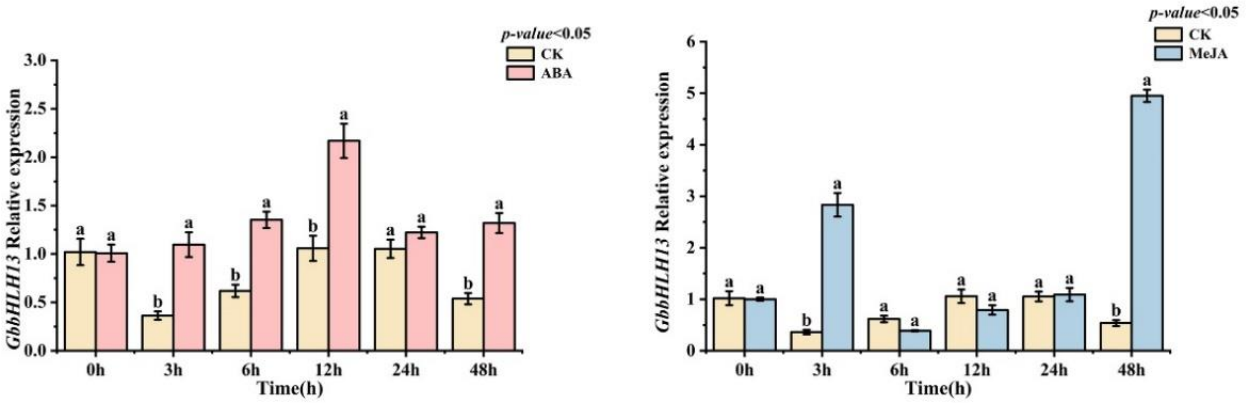

Figure 3. Expression of *GbbHLH13* after exogenous hormone treatment of *Ginkgo biloba.*

Exogenous hormone treatment of G. biloba seedlings

In this study, online prediction of the *GbbHLH13* promoter revealed the presence of response elements for MeJA, ABA, and GA3. Research has shown that treatment with MeJA and ABA can effectively increase flavonoid accumulation. For instance, *MdMYB1* in apple can activate anthocyanin biosynthesis under the influence of ABA, and the isolated *MdbZIP44* from apple can enhance the binding of *MdMYB1* to the promoters of downstream target genes, thus promoting anthocyanin accumulation(An JianPing et al., 2018). Similarly, ABA treatment has been shown to increase the content of flavonoids in pigeon pea (Yang et al., 2021). Additionally, ABA treatment of Fragaria chiloensis can enhance the expression of *FcPAL*, *FcCHS*, and *FcANS*(Mattus-Araya et al., 2022). Studies have found that *Carthamus tinctorius* L. upregulates the expression of *CHSs*, *CHIs*, and *HCTs* while downregulating *ANRs* and *ANSs* through MeJA treatment, thereby promoting flavonoid biosynthesis. In *Camellia vietnamensis* (Chen et al., 2020), MeJA treatment increases the expression of key genes involved in flavonoid synthesis, such as *PAL*, *4CL*, *CHI*, and *FLS*, after 2 hours (Yan et al., 2022). Furthermore, after MeJA treatment, *AmMYB30* interacts with the promoters of *AmCHS*, *AmFLS*, and *AmF3H* to induce flavonoid accumulation (Qi et al., 2024). MeJA treatment also upregulates the expression of key genes like MYC transcription factors in the flavonoid biosynthesis pathway of *Dendrobium officinale* (Jia et al., 2024).

To explore the relationship between *GbbHLH13* and flavonoid content, 1 mM MeJA and 100 μM ABA were applied to the leaves of one-year-old *G. biloba* seedlings. The gene expression patterns of *GbbHLH13* in hormone-treated leaves at different time points were analyzed. The results indicated that after ABA treatment, the expression of *GbbHLH13* first increased and then decreased, peaking at 12 hours. In contrast, after MeJA treatment, *GbbHLH13* expression rose sharply, then fell, and subsequently increased again to reach a peak (Figure 3). These findings suggest that *GbbHLH13* may be involved in the regulation of flavonoids in *G. biloba*.

Subcellular localization of GbbHLH13

Online prediction indicates that *GbbHLH13* is localized in the nucleus. By constructing the fusion expression vector pICH86988-*GbbHLH13*-pICSL50008, using pICH86988-pICSL50008 as a control, we employed laser confocal microscopy to detect the subcellular localization of *GbbHLH13* fused with green fluorescent protein. The experimental results showed that the control group displayed fluorescence at both the cell membrane and nucleus. In contrast, when expressing the pICH86988-*GbbHLH13*-pICSL50008 fusion, strong fluorescence was only observed in the nucleus, which is consistent with the expected results (Figure 4).

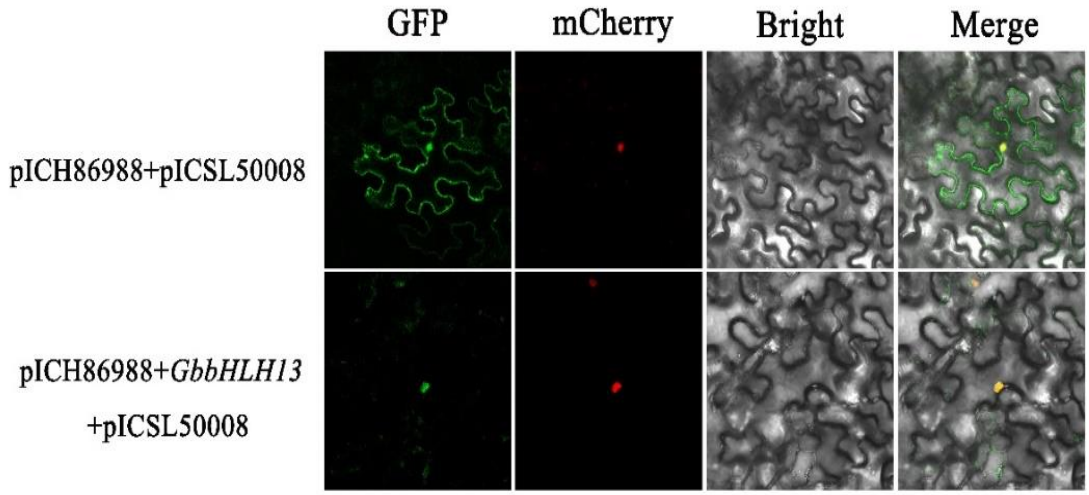

Figure 4. Subcellular localization of *GbbHLH13*.

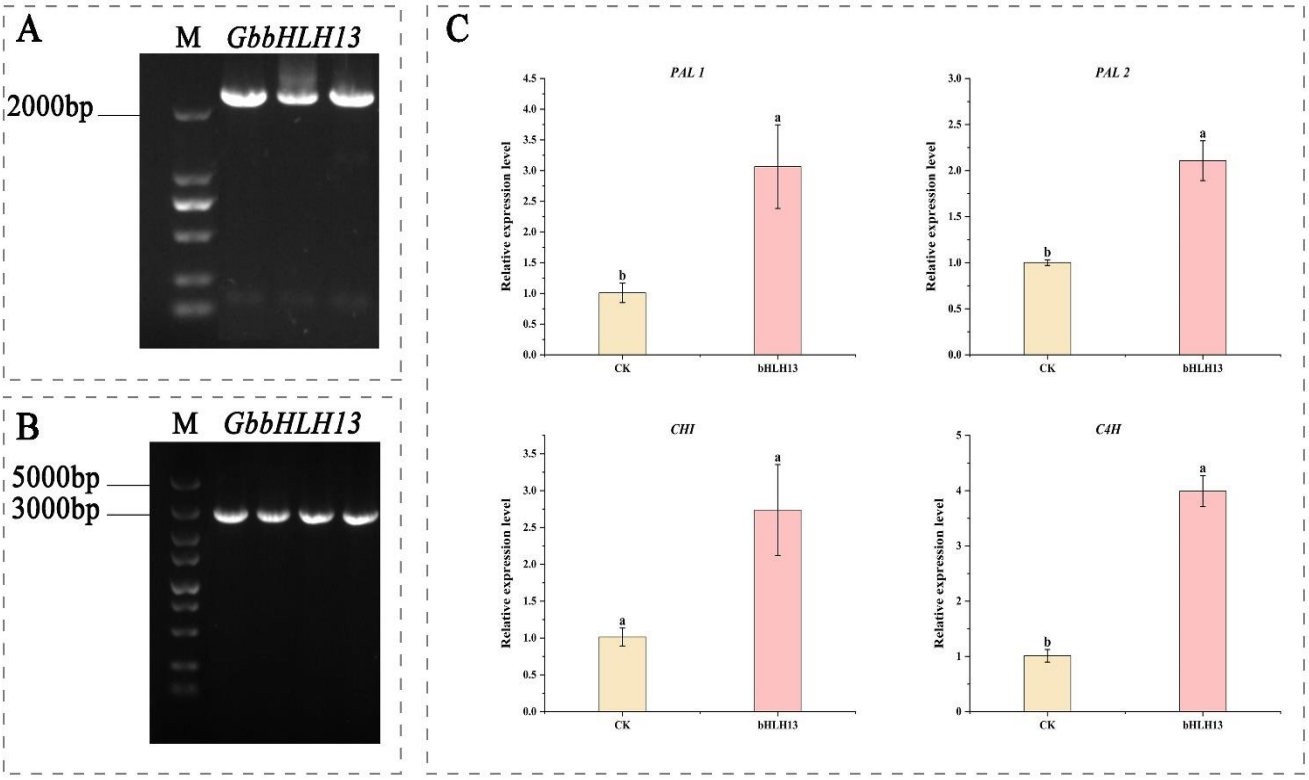

Figure 5. (A) *GbbHLH13* clone product electrophoresis pattern. (B) pCY-H05252-*GbbHLH13* electrophoresis pattern. (C) *GbbHLH13* regulates expression changes of structural genes.

Transient overexpression of GbbHLH13 in G. biloba

Since a stable genetic transformation system has not yet been established for ginkgo, this study chose to use a transient overexpression method by injecting ginkgo leaves to predict the function of *GbbHLH13*. After cloning *GbbHLH13* (Figure 5A), the pCY-H05252-*GbbHLH13* vector was constructed (Figure 5B). The pCY-H05252-*GbbHLH13* vector was used as the experimental group, while pCY-H05252 empty vector served as the control group. Four structural genes involved in the flavonoid biosynthesis pathway in *G. biloba PAL 1*, *PAL 2*, *C4H*, and *CHI* were selected as targets. RT-qPCR experiments were conducted to investigate the expression changes of these four structural genes in the experimental and control groups. The results showed that the expression levels of *PAL 1*, *PAL 2*, and *C4H* significantly increased in a short time (Figure 5C), suggesting that *GbbHLH13* may be involved in the regulation of flavonoids in *G. biloba*.

Discussion

This study provides the first analysis and identification of *GbbHLH13*, which is located in the nucleus. Its nucleotide sequence is 2199 bp, encoding 732 amino acids. It was found that *GbbHLH13* shares common conserved domains with bHLH proteins from various plants, including *Cryptomeria japonica* (Thunb. ex L. f.) D. Don (XP_057844388.1), *Larix gmelinii* (Rupr.) Kuzen. (UNP37115.1), *Pinus massoniana* Lamb. (UGN74583.1), *Picea abies* (L.) H. Karst. (ANB66421.1), *Elaeis guineensis* Jacq. (XP_010906882.1), *Populus trichocarpa* Torr. & Gray (XP_006383662.2), *Cinnamomum micranthum* (Hay.) Hay (RWR95376.1), and *Populus euphratica* Oliv. (XP_011032729.1), (Figure 1C). The phylogenetic tree indicates that *GbbHLH13* is phylogenetically related to the identified bHLH transcription factors involved in flavonoid regulation. Previous studies have shown that *DcbHLH2*, *DcbHLH5*, and *DcbHLH56* regulate flavonoid biosynthesis by binding to promoters(Zhu et al., 2020). Heterologous expression of *PgMYB5*-like and *PgbHLH* in pomegranate induced the accumulation of dihydroflavonols(Arlotta et al., 2020). Research has indicated that the expression of *TT8* is controlled by different combinations of MYB and bHLH transcription factors in plants, specifically inducing the biosynthesis of proanthocyanidins(Baudry et al., 2006). Overexpression of *VvbHLH1* significantly increased the accumulation of flavonoids in transgenic Arabidopsis plants, while enhancing salt and drought tolerance(Wang et al., 2016). *MtTT8*, *MtWD40-1*, and MYB transcription factors interact with *MtPAR* or *MtLAP1* to activate the promoters of flavonoid reductase and anthocyanin synthase, thereby regulating anthocyanin biosynthesis(Li et al., 2016). Differential expression gene (DEG) analysis revealed that the expression pattern of MYB-bHLH-WD40 genes positively correlates with anthocyanin accumulation in blueberry fruits(Zhao et al., 2019). In summary, *GbbHLH13* may play a regulatory role in the flavonoid synthesis pathway. Promoter analysis can help reveal the potential response mechanisms of genes to multiple stresses. Binding sites related to meristem formation, organogenesis, and anaerobic induction were identified in the *GbbHLH13* promoter, suggesting that *GbbHLH13* is closely associated with plant growth and development. When plants are subjected to specific environmental influences, plant hormone signaling pathways are activated to regulate various downstream biological processes. Exogenous hormone treatments showed that the expression of *GbbHLH13* is influenced by MeJA and ABA, both of which have been shown to affect flavonoid synthesis in various plants. Therefore, it can be inferred that *GbbHLH13* may regulate flavonoid synthesis. Additionally, transient overexpression in ginkgo leaves indicated that *GbbHLH13* also upregulated the transcription levels of genes related to flavonoid metabolic pathways, including *PAL*, *CHI*, and *C4H*.

Conclusion

In this study, GbbHLH13, located in the nucleus, was cloned for the first time, and its function was analyzed and identified. It is speculated that GbbHLH13 is involved in the regulation of flavonoid synthesis in Ginkgo through hormone treatment. Furthermore, the transient expression of GbbHLH13 in Ginkgo can upregulate the transcription levels of genes related to the flavonoid metabolism pathway, specifically PAL, C4H, and CHI. Additionally, the transcription factor (TF)

protein that interacts with GbbHLH13 should be further identified and analyzed.

This study suggests that *GbbHLH13* may be involved in the flavonoid metabolic pathway, providing valuable insights for expanding the control network of flavonoid metabolism. Future research is needed to explore the downstream target genes of *GbbHLH13* and how they participate in the regulation of flavonoids in *G. biloba*.

Supplementary Materials

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_717860.html.

Supplementary Table S1. *GbbHLH13* genes and primers information.

Author Contributions

Conceptualization, formal analysis, visualization, writing- original draft, validation, and investigation; Y. L.

Funding

This research received no external funding.

Acknowledgments

Thanks to the author's contribution.

Conflict of Interest Statement

The author declares no conflict of interest.

References

An JianPing, A.J., Yao JiFang, Y.J., Xu RuiRui, X.R., You ChunXiang, Y.C., Wang XiaoFei, W.X., and Hao YuJin, H.Y. (2018). Apple bZIP transcription factor MdbZIP44 regulates abscisic acid-promoted anthocyanin accumulation. *Plant Cell Environ.*41(11): 2515-2714.

Arlotta, C., Puglia, G.D., Genovese, C., Toscano, V., Karlova, R., Beekwilder, J., De Vos, R.C., and Raccuia, S.A. (2020). MYB5-like and bHLH influence flavonoid composition in pomegranate. *Plant Sci.* 298: 110563.

Baudry, A., Caboche, M., and Lepiniec, L. (2006). TT8 controls its own expression in a feedback regulation involving TTG1 and homologous MYB and bHLH factors, allowing a strong and cell - specific accumulation of flavonoids in *Arabidopsis thaliana*. *Plant J.* 46(5): 768-779.

Biłas, R., Szafran, K., Hnatuszko-Konka, K., and Kononowicz, A.K. (2016). Cis-regulatory elements used to control gene expression in plants. *Plant Cell Tissue Organ. Cult.* 127: 269-287.

Chen, J., Wang, J., Wang, R., Xian, B., Ren, C., Liu, Q., Wu, Q., and Pei, J. (2020). Integrated metabolomics and transcriptome analysis on flavonoid biosynthesis in safflower (*Carthamus tinctorius* L.) under MeJA treatment. *BMC Plant Biol.* 20: 1-12.

Chen, Y.-Y., Li, M.-Y., Wu, X.-J., Huang, Y., Ma, J., and Xiong, A.-S. (2015). Genome-wide analysis of basic helix− loop− helix family transcription factors and their role in responses to abiotic stress in carrot. *Mol. Breed.* 35: 1-12.

Cheng, X., Xiong, R., Liu, H., Wu, M., Chen, F., Yan, H., and Xiang, Y. (2018). Basic helix-loop-helix gene family: Genome wide identification, phylogeny, and expression in *Moso bamboo*. *Plant Physiol. Biochem.* 132: 104-119.

Espley, R.V., Hellens, R.P., Putterill, J., Stevenson, D.E., Kutty - Amma, S., and Allan, A.C. (2007). Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. *Plant J.* 49(3): 414-427.

Gremski, K., Ditta, G., and Yanofsky, M.F. (2007). The HECATE genes regulate female reproductive tract development in *Arabidopsis thaliana*. *Dev. J. 134 (20): 3593–3601.*

Hao, Y., Zong, X., Ren, P., Qian, Y., and Fu, A. (2021). Basic helix-loop-helix (bHLH) transcription factors regulate a wide range of functions in *Arabidopsis*. *Int. J. Mol. Sci.* 22(13): 7152.

Heim, K.E., Tagliaferro, A.R., and Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J. Nutr. Biochem.* 13(10): 572-584.

Hichri, I., Heppel, S.C., Pillet, J., Léon, C., Czemmel, S., Delrot, S., Lauvergeat, V., and Bogs, J. (2010). The basic helix-loop-helix transcription factor MYC1 is involved in the regulation of the flavonoid biosynthesis pathway in grapevine. *Mol. Plant* 3(3): 509-523.

Jia, Y., Meng, W., Chen, G., Fan, X., Zhang, Y., Ding, A., Xu, M., Hu, G., Tan, M., and Xiang, Z. (2024). The regulation mechanism of MYC on MeJA-induced flavonoids synthesis in *Dendrobium officinale*. *J Plant Growth Regul.*: 1-16.

Li, M., Li, B., Xia, Z.-M., Tian, Y., Zhang, D., Rui, W.-J., Dong, J.-X., and Xiao, F.-J. (2019). Anticancer effects of five biflavonoids from *Ginkgo biloba* l. Male flowers in vitro. *Molecules* 24(8): 1496.

Li, P., Chen, B., Zhang, G., Chen, L., Dong, Q., Wen, J., Mysore, K.S., and Zhao, J. (2016). Regulation of anthocyanin and proanthocyanidin biosynthesis by *M edicago truncatula* b HLH transcription factor M t TT 8. *New Phytol.* 210(3): 905-921.

Liu, L., Wang, Y., Zhang, J., and Wang, S. (2021). Advances in the chemical constituents and chemical analysis of *Ginkgo biloba* leaf, extract, and phytopharmaceuticals. *J. Pharm. Biomed. Anal.* 193: 113704.

Liu, Y., Xin, H., Zhang, Y., Che, F., Shen, N., and Cui, Y. (2022). Leaves, seeds and exocarp of *Ginkgo biloba* L.(*Ginkgoaceae*): A comprehensive review of traditional uses, phytochemistry, pharmacology, resource utilization and toxicity. *J. Ethnopharmacol.* 298: 115645.

Ludwig, S.R., Habera, L.F., Dellaporta, S.L., and Wessler, S. (1989). Lc, a member of the maize R gene family responsible for tissue-specific anthocyanin production, encodes a protein similar to transcriptional activators and contains the myc-homology region. *Proc. Natl. Acad. Sci. U.S.A.* 86(18): 7092-7096.

Mattus-Araya, E., Guajardo, J., Herrera, R., and Moya-León, M.A. (2022). ABA speeds up the progress of color in developing *F. chiloensis* fruit through the activation of *PAL*, *CHS* and *ANS*, key genes of the phenylpropanoid/flavonoid and anthocyanin pathways. *Int. J. Mol. Sci.* 23(7): 3854.

Pires, N., and Dolan, L. (2010). Origin and diversification of basic-helix-loop-helix proteins in plants. *Mol. Biol. Evol.* 27(4): 862-874.

Qi, L., Ma, Y., Zheng, L., Yuan, Z., Sun, H., Richardson, J.E., Zhou, Y., and Gao, F. (2024). Combined transcriptome and metabolome analysis highlights a central role of *AmMYB30* in *MeJA* induced flavonoid accumulation in *Astragalus membranaceus*. *Food Biosci.* 61: 104565.

Robinson, K.A., and Lopes, J.M. (2000). Survey and summary: Saccharomyces cerevisiae basic helix–loop–helix proteins regulate diverse biological processes. *Nucleic Acids Res.* 28(7): 1499-1505.

Singh, S.K., Srivastav, S., Castellani, R.J., Plascencia-Villa, G., and Perry, G. (2019). Neuroprotective and antioxidant effect of *Ginkgo biloba* extract against AD and other neurological disorders. *Neurotherapeutics* 16(3): 666-674.

Stevens, J.D., Roalson, E.H., and Skinner, M.K. (2008). Phylogenetic and expression analysis of the basic helix-loop-helix transcription factor gene family: genomic approach to cellular differentiation. *Differentiation* 76(9): 1006-1042.

Tagousop, C.N., Tamokou, J.-d.-D., Ekom, S.E., Ngnokam, D., and Voutquenne-Nazabadioko, L. (2018). Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action. *BMC Complement Altern Med* 18: 1-10.

Toledo-Ortiz, G., Huq, E., and Quail, P.H. (2003). The Arabidopsis basic/helix-loop-helix transcription factor family. *The Plant Cell* 15(8): 1749-1770.

Wang, F., Zhu, H., Chen, D., Li, Z., Peng, R., and Yao, Q. (2016). A grape bHLH transcription factor gene, VvbHLH1, increases the accumulation of flavonoids and enhances salt and drought tolerance in transgenic *Arabidopsis thaliana*. *Plant Cell Tiss Organ Cult* 125: 387-398.

Wang, J., Cao, F., Su, E., Wu, C., Zhao, L., and Ying, R. (2013). Improving flavonoid extraction from *Ginkgo biloba* leaves by prefermentation processing. *J. Agric. Food Chem.* 61(24): 5783-5791.

Xie, X.B., Li, S., Zhang, R.F., Zhao, J., Chen, Y.C., Zhao, Q., Yao, Y.X., You, C.X., Zhang, X.S., and Hao, Y.J. (2012). The bHLH transcription factor *MdbHLH3* promotes anthocyanin accumulation and fruit colouration in response to low temperature in apples. *Plant. Cell Environ.* 35(11): 1884-1897.

Yan, H., Zheng, W., Wang, Y., Wu, Y., Yu, J., and Xia, P. (2022). Integrative metabolome and transcriptome analysis reveals the regulatory network of flavonoid biosynthesis in response to MeJA in *Camellia vietnamensis* Huang. *Int. J. Mol. Sci.* 23(16): 9370.

Yang, W., Li, N., Fan, Y., Dong, B., Song, Z., Cao, H., Du, T., Liu, T., Qi, M., and Niu, L. (2021). Transcriptome analysis reveals abscisic acid enhancing drought resistance by regulating genes related to flavonoid metabolism in *Pigeon Pea*. *Environ. Exp. Bot.* 191: 104627.

Zhao, M., Li, J., Zhu, L., Chang, P., Li, L., and Zhang, L. (2019). Identification and characterization of MYB-bHLH-WD40 regulatory complex members controlling anthocyanidin biosynthesis in blueberry fruits development. *Genes* 10(7): 496.

Zhou, X., Liao, Y., Kim, S.-U., Chen, Z., Nie, G., Cheng, S., Ye, J., and Xu, F. (2020). Genome-wide identification and characterization of bHLH family genes from *Ginkgo biloba*. *Sci. Rep.* 10(1): 13723.

Zhu, J.-H., Xia, D.-N., Xu, J., Guo, D., Li, H.-L., Wang, Y., Mei, W.-L., and Peng, S.-Q. (2020). Identification of the bHLH gene family in *Dracaena cambodiana* reveals candidate genes involved in flavonoid biosynthesis. *Ind. Crops Prod.* 150: 112407.

JPMB
Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2024 | Volume 12 | Issue 2

مقاله پژوهشی

نقش فاکتور رونویسی *GbbHLH13* در تنظیم بیوسنتز فلاونوئید در گیاه جینکو بیلوبا

یانگ لی*[۱،۲]

[۱] دانشکده باغبانی و باغداری، دانشگاه یانگ تسه، جینگژو 434025، هوبی، چین
[۲] آزمایشگاه مرکزی نوآوری و استفاده از ژرم پلاسم گیاهان باغبانی و ادویه هوبی، دانشگاه یانگ تسه، جینگژو 434025، هوبی، چین

چکیده: جینکو بیلوبا از قدیمی‌ترین گیاهان زنده روی زمین و یکی از پرمصرف ترین داروهای طبیعی در سراسر جهان می‌باشد. بررسی‌ها نشان داده است که فلاونوئیدهای استخراج شده از *G. biloba*، دارای اثرات محافظتی در برابر بیماری‌های قلبی عروقی و عروقی مغز می‌باشند. فاکتورهای رونویسی bHLH (TFs) یکی از مهم‌ترین خانواده‌های فاکتورهای رونویسی در گیاهان بوده که نقش مهمی در تنظیم رشد، نمو و متابولیسم ثانویه گیاه دارند. در این مطالعه جداسازی و شناسایی ژن *GbbHLH13* مدنظر قرار گرفت. توالی این ژن، پروتئینی متشکل از ۷۳۲ اسید آمینه را کد می‌نماید. سنجش بیان موقت این ژن در تنباکو نشان داد که بیان ژن *GbbHLH13* در هسته می‌باشد. تیمارهای هورمونی اگزوژن نشان داد که بیان *GbbHLH13* تحت تأثیر دو هورمون اسید آبسیزیک (ABA) و متیل جاسمونات (MeJA) قرار دارد. آزمایش‌های بیش‌بیان موقت این ژن نشان داد که سطح رونویسی ژن‌های مرتبط با متابولیسم فلاونوئید شامل *PAL* ، *CHI*، و *C4H* افزایش یافته که احتمالا می‌تواند حاکی از نقش *GbbHLH13* در بیوسنتز فلاونوئید باشد.

کلمات کلیدی: جینکو بیلوبا، GbbHLH13، فلاونوئیدها، مکان‌یابی درون سلولی، RT-qPCR.

ویراستار علمی
دکترسروش کومار،
موسسه تحقیقات کشاورزی هندوسنان، هند

تاریخ
دریافت: ۱۱ آبان ۱۴۰۳
پذیرش: ۱۶ آبان ۱۴۰۳
چاپ: ۲۲ آبان ۱۴۰۳

نویسنده مسئول
یانگ لی
liyly1219@163.com

ارجاع به این مقاله
Li, Y. (2024). *Ginkgo biloba GbbHLH13* transcription factor regulates flavonoid biosynthesis. *J Plant Mol Breed 12* (2): 1-12. doi: 10.22058/jpmb.2024.2044701.1310.