BMC Plant Biology

RESEARCH Open Access

Genome-wide identification of the *UGT* genes family in *Acer rubrum* and role of *ArUGT52* in anthocyanin biosynthesis under cold stress

Khan Arif Kamal[1], Faheem Afzal Shah[2], Yue Zhao[1], Zhu Chen[2], Songling Fu[1], Zhiyong Zhu[3], Jie Ren[2*] and Hua Liu[1*]

Abstract

Acer rubrum is a widespread *Acer* species valued for its vibrant autumn foliage. The *UGT* (UDP-glycosyltransferase) gene family is integral to the biosynthesis of anthocyanins, the pigments responsible for leaf coloration. This study aimed to comprehensively identify and characterize the *UGT* gene family in the *A. rubrum* genome. The results of the phylogenetic analysis of 249 *ArUGTs* revealed 18 distinct subgroups. Conserved motif analysis demonstrated structural similarities within subgroups. Gene duplication analysis identified 21 tandem and 66 segmental duplication events across chromosomes. Transcriptomic data from autumn leaves of different colours and under low-temperature stress were analyzed for *ArUGT* expression patterns. Compared to controls, 44 *UGTs* were upregulated and 99 downregulated in yellow leaves, while 59 were upregulated and 84 downregulated in red leaves. Low-temperature treatments showed upregulation of 18 *UGTs* at 10 °C and 40 *UGTs* at 4 °C. Downregulation was observed in 7 *UGTs* at 10 °C and 33 *UGTs* at 4 °C. Among all *UGT* genes, *ArUGT52* was common in highly expressed genes in both red leaf and low-temperature stress. Furthermore, the transient overexpression of *ArUGT52* in tobacco plants demonstrated cytoplasmic localization and a marked increase in anthocyanin levels under cold stress. In vitro, biochemical assay results indicated that the *ArUGT52* was involved in anthocyanin biosynthesis via the glucosylation of anthocyanidins. This study provides insights into the genetic mechanisms of leaf coloration and the potential of *UGT* manipulation for enhancing plant responses to low-temperature stress. These findings have applications in ornamental horticulture and agriculture.

Keywords *Acer rubrum*, UDP-glycosyltransferase (*UGTs*), Anthocyanin, Cold stress, Leaf colour

Introduction

Acer rubrum, also known as red maple, is a deciduous tree species endemic to the eastern United States. It is highly admired for its remarkable ornamental qualities and lumber production [1, 2]. According to Harris et al. [3], red maples are among the *Acer* genus species that are most often planted as ornamental trees. Among the most eye-catching aspects of *A. rubrum* is the incredible seasonal and cultivar-specific variety in leaf color. Flavonoid pigments are mainly responsible for the broad spectrum of colors seen, ranging from verdant green to flaming red [4]. According to Tanaka et al. [5], plant pigmentation is a

*Correspondence:
Jie Ren
renjieaaas@sina.com
Hua Liu
liuhuanmg@126.com
[1]School of Forestry & Landscape Architecture, Anhui Agricultural University, Hefei, Anhui 230036, China
[2]Institute of Agricultural Engineering, Anhui Academy of Agricultural Sciences, Hefei, Anhui 230031, China
[3]Ningbo City College of Vocational Technology, Ningbo 315502, China

crucial characteristic in ornamental horticulture, and flavonoids, betalains, and carotenoids impact it. In order to manipulate plant color, it is essential to understand the intricate gene network that controls the production and accumulation of these pigments [6].

The anthocyanin colors produced by the flavonoid biosynthetic pathway in *A. rubrum* make its fall leaves so eye-catching [7]. Our understanding of the phenylpropanoid metabolic route, a key mechanism in flavonoid production, has been dramatically enhanced by studies conducted on model plants such as maize and *A. thaliana* [8–10]. According to many studies [6, 11–13], anthocyanins, which may be found in a variety of plant parts, are the ones that give rise to a wide range of colors. The UFGT enzyme plays a critical role in their synthesis, acylation, and glycosylation, as well as adding sugars [14].

Flavonoids are among the many plant metabolites that are glycosylated by the vast and varied family of enzymes known as UDP-glycosyltransferases (*UGTs)* [15]. According to [16], flavonoids' stability, solubility, and appearance are all greatly affected by glycosylation. The anthocyanin-forming process is facilitated by the UDP-glucose flavonoid 3-O-glycosyltransferase (UFGT) enzyme, which is one of the *UGTs* [17].

The formation of various anthocyanin colors is made possible by UFGT-encoding genes found in many plants and display substrate selectivity [18]. Enzymes like anthocyanidin 3-O-glycosyltransferase and anthocyanidin 5, 3-O-glucosyltransferase enable one set of enzymes to facilitate the first glycosylation of anthocyanin precursors [18–21]. A further set of UFGTs changes the anthocyanin structure by catalyzing glycosylation processes that follow. Some examples of such enzymes are anthocyanidin 3-O-glucoside-6-O-rhamnosyltransferase [22], anthocyanidin 3-O-glucoside 6-O-rhamnosyltransferase [23], and anthocyanidin 3-O-glucoside-2′-O-xylosyltransferase [24].

The conserved PSPG box motif, which consists of 44 amino acids in the C-terminal region, is a unique feature of the UGT protein family [16, 25–27]. The core regions and folding patterns of plant UGT proteins are often conserved. According to Nair et al. [28], the receptors are recognized mainly by their C-terminal domains, whereas specific glycosyl donors are recognized by their N-terminal domains.

UGT gene family seems to have a common evolutionary ancestor [29]. According to Rehman et al. [30], several *UGT* genes have been found in different plants by the use of the PSPG motif. Among these genes, 122 have been found in *A. thaliana*, 148 in maize, 184 in grape, and 128 in soybean. High conservation at 17 amino acid positions was found in sequence analysis of five terpenoid-related *UGT* genes in *Panax notoginseng*, indicating functional importance [31]. Numerous studies have elucidated *UGTs* in significant plant species such as *A. thaliana* [15], *Glycine max* [32], *Malus domestica* [33], and *Vitis vinifera* [34]. Genome-wide investigations of *UGT* genes in *Populus trichocarpa* shed light on their expression in specific tissues and reactions to abiotic stress [35].

Despite the significance of the *UGT* gene family in different plant species, the identification and functional analysis of the *UGT* gene family in *A. rubrum* was elusive. Therefore, the current study aims to discover and conduct a complete analysis of the *UGT* gene family in the *A. rubrum* genome. We analyzed motif composition, gene structure, and duplication events in order to gain insight into the evolutionary connections of *ArUGTs*. A gene expression study was conducted using transcriptomic data and qPCR analysis on red-colored fall leaves of *A. rubrum*, as well as leaves subjected to low-temperature stress. The gene "*ArUGT52*" with a high level of expression was defined functionally by temporarily expressing it in tobacco leaves. An in vitro biochemical investigation was conducted to examine the process of anthocyanin production by *ArUGT52*. This study holds potential for elucidating the genetic mechanisms governing leaf color variation in *A. rubrum*, with potential applications for manipulating ornamental traits and deepening our understanding of plant responses to environmental research.

Materials and methods

Identification of *UGTs*

We employed a comprehensive two-pronged approach to identify UDP-glycosyltransferase (*UGT*) genes within the *A. rubrum* genome. First, we used 122 established UGT protein sequences from *A. thaliana* (TAIR database v10.0) as queries in a BLASTP similarity search against the *A. rubrum* protein database. Second, we used a Hidden Markov Model (HMM) with the canonical *UGT* domain (Pfam00201) to probe against the same *A. rubrum* database. This dual strategy ensured redundancy and increased the likelihood of identifying all potential UGTs. All sequences identified by either the BLASTP or HMM search was retained. To verify the presence of the *UGT* domain in candidate sequences and minimize false positives, we conducted analyses using the SMART, PFAM, and CDD databases. This rigorous validation process ensured the inclusion of only genuine *UGT* genes in our dataset.

Phylogenetic analysis, gene structure, and conserved motif determination

To investigate the evolutionary links between the discovered *A. rubrum UGTs* (*ArUGTs*) and known *A. thaliana UGTs*, we generated a phylogenetic tree using MEGA11. The JTT substitution model and 1500 bootstrap iterations were used with the Neighbor-joining (NJ) technique. The

web program iTOL was used to visualize the resultant unrooted tree. Understanding gene structure is crucial for insights into gene function and evolution. We analyzed the structure of *ArUGTs* using TBtools, focusing on the distribution of exons and introns. Additionally, we used the MEME Suite 5.1.1 to identify conserved protein motifs within the *ArUGT* sequences. These motifs often represent functionally essential regions and can aid in classifying *UGTs* based on potential substrate specificity.

Chromosomal location, gene duplication events

First, we prepared the required data, such as gene annotation files (GFF) and a file containing gene IDs and chromosomal locations, in order to use TBtools to identify gene duplication occurrences and determine chromosomal locations. To gain insights into the genomic distribution of *UGT* genes, we mapped the *ArUGTs* onto *A. rubrum* chromosomes using the MG2C online tool. We prepared the reference genome sequence file in FASTA format and gene sequences or IDs for gene duplication occurrences. We used tools like MCScanX Wrapper and One Step MCScanX in the Gene Duplication section of TBtools-II. In order to find duplicate genes (segmental and tandem), we loaded the reference genome and gene sequence files, set up the analysis settings, and then performed the analysis. The outcomes were utilized to display duplication events within the *ArUGT* family.

Collinearity analysis of UGTs

To understand the evolutionary history of *ArUGTs* in broader plant lineages, we conducted comparative genomic analyses using MCScanX (within TBtools). Specifically, we examined syntenic relationships between *ArUGTs* and *UGT* gene families in *A. yangbiense*, *Populus trichocarpa*, *Gossypium hirsutum*, *Vitis vinifera*, and *A. thaliana*. Synteny refers to the conserved order of genes on chromosomes across different species and provides clues about shared ancestry.

Plant materials collection and treatments

To investigate the role of *ArUGTs* in leaf color variation and environmental responses, we utilized pre-existing RNA-seq and new experimental data. Leaves color variation from the same *A. rubrum* trees were sampled across different color stages during the autumn season. These samples were immediately flash-frozen in liquid nitrogen for gene expression analysis. Cold stress response of *A. rubrum* seeds was stratified at 4 °C, and germinated seedlings were grown under standard conditions. *A. rubrum* seedlings were produced via tissue culture to avoid genotype-phenotype variation. Eight-month-old seedlings were subjected to either 4–10 °C cold stress for 24 h, after which leaf samples were collected and flash-frozen for subsequent analysis. All experiments included three biological replicates for accurate statistical assessment.

RNA extraction and gene expression analysis

The RNA was extracted from all plant samples using the RNeasy Plant Mini Kit, including on-column DNAse I treatment. RNA quality and quantification were assessed using a NanoDrop 2000c spectrophotometer. First-strand cDNA synthesis was performed using the HiScript II Q RT SuperMix kit. Gene expression patterns of *ArUGTs* were analyzed using quantitative real-time PCR (qPCR) with gene-specific primers (Supplementary Table S1). The $2^{-\Delta\Delta Ct}$ method determined relative gene expression, normalized against the *A. rubrum ACTIN2* gene. Technical and biological replicates were included to ensure reliable and reproducible results.

Statistical analysis

We implemented a comprehensive set of statistical analyses to systematically identify genes with a potential role in leaf color variation and cold stress responses. We then determined *ArUGT* expression levels utilizing quantitative real-time PCR (qPCR). We analyzed our qPCR results using one-way ANOVA followed by Tukey's HSD post-hoc test. ANOVA enables us to examine whether there are significant variations in the overall expression of the *ArUGT* gene across the stress treatments and color groups. By identifying the precise pairings of samples that exhibit statistically significant differences, Tukey's HSD fortifies our inferences regarding the involvement of *ArUGTs* in the processes under investigation.

Sub-cellular localization

Protein regulation mechanisms and possible roles may be better understood by tracing their subcellular distribution. We fused the coding sequence (CDS) of *ArUGT52* with the green fluorescent protein (GFP) gene in the *pCambia1302* vector to find its location. We transiently produced this construct in *Nicotiana benthamiana* leaves after introducing it into *Agrobacterium.* A Nikon C2-ER confocal laser microscope was used to study the localization of GFP-tagged *ArUGT52.*

Transient expression of *ArUGT52* in tobacco (*Nicotiana Benthamiana*)

We used a transient expression system using tobacco (*Nicotiana benthamiana*) as a model organism to examine the *ArUGT52* gene's function. We used PCR using gene-specific primers to amplify the *ArUGT52* coding sequence from cDNA extracted from *A. rubrum* leaves (Supplementary Table S1). Initial propagation and sequence verification were accomplished by cloning the PCR product into the pEASY®-Blunt Vector. Next, the *ArUGT52* sequence was inserted into the plant

expression vector *pCAMBIA1302* using the NcoI and SpeI restriction enzymes, creating the *pCAMBIA1302-ArUGT52* construct. This construct was introduced into *Agrobacterium rhizogenes* (EHA105), a soil bacterium commonly used for plant transformation. Finally, the transformed *Agrobacterium* introduced the construct into tobacco plants. To ensure the reliability of our results, we generated three independent transgenic lines, each representing a separate transformation event.

Extraction and determination of anthocyanins

We used a well-established procedure to identify and quantify anthocyanins in our plant samples. We accomplished this by immersing samples in a 5 mL HCl-ethanol solution heated to 55 °C in a water bath for two hours. Hydrolysis degrades complex anthocyanin structures into their fundamental parts. After hydrolysis, the liquid extract (supernatant) containing the anthocyanins was centrifuged to separate it from the residual plant material. Second, we quantified anthocyanins using a spectrophotometer, which evaluates a sample's light absorption. The extracted anthocyanins were concentrated and tested for absorbance at specific wavelengths (530 nm, 620 nm, and 650 nm). These wavelengths match the absorption peaks of anthocyanins. The spectrophotometer was calibrated using a blank solution of 0.1 mol/L HCl-ethanol. Using the absorbance values, we estimated the anthocyanin concentration using a formula used in prior work [36]. This method takes into account the absorbance readings, extract volume, and sample weight to calculate the amount of anthocyanin in the original plant tissue.

In vitro biochemical assay

The biochemical test followed the recognized methodology reported by Lee et al. [37]. The *ArUGT52* gene was amplified using the primers given in (Supplementary Table S1), and then inserted into the *pGEX4T-1* vector. GST-ArUGT52 fusion proteins were extracted from Escherichia coli strain BL21 using glutathione-sepharose 4B beads from Pharmacia Biotech in the United States. A 200 µL reaction mixture was prepared using 2 µg of recombinant GST-ArUGT52 protein and a reaction solution of 50 mM Tris-HCl (pH 7.0), 14 mM 2-mercaptoethanol, 5 mM UDP-glucose, and 1 mM substrates. After 30 min of incubation at 30 °C, the mixture was quenched with 50 µL of methanol containing 1% hydrochloric acid for cyanidin and pelargonidin and 20 µL of trichloroacetic acid (240 mg/mL) for quercetin and kaempferol. These reaction mixtures were immediately frozen in liquid nitrogen and kept at -20 °C before further analysis using high-performance liquid chromatography (HPLC). Each sample was injected onto a reverse-phase C18 column (WATERS ACQUITY UPLC HSS T3 1.8 µm 2.1 × 100 mm) with a gradient that was linear of 10 to 50% acetonitrile in water containing 0.5% trifluoroacetic acid with a flow rate of 0.5 mL/min. Detection was carried out using a High-performance liquid chromatography (Agilent 1290, Agilent, USA) system equipped with a Liquid mass spectrometry instrument (SCIEX Triple Quad™ 6500 + LC-MS/MS, AB SCIEX, USA). This allowed for both UV-Vis and MS/MS analysis of the reaction products. Flavanols were initially detected at a wavelength of 340 nm and anthocyanidins/anthocyanins at 520 nm. Further confirmation of compound identity was achieved using MS/MS by analyzing the fragmentation patterns of the detected compounds. Flavonoid substrates and standards were obtained from Sigma-Aldrich (St. Louis, Missouri, United States). Absorbance spectra and mass spectra of the detected compounds are presented in Supplementary Fig. S2A and B.

Results

ArUGTs gene discovery in the genome of the *Acer rubrum* and protein characteristics

A comprehensive genome-wide analysis was conducted to identify UDP-glycosyltransferase (*UGT*) genes in the *A. rubrum* genome. Using a combination of domain analysis and Hidden Markov Model (HMM) searches, we discovered 249 *ArUGT* genes. Detailed information on these *ArUGT* genes is provided in (Supplementary Table S2). Our analysis revealed significant variation in the characteristics of ArUGT proteins. Protein sequence lengths ranged from 88 amino acids (ArUGT6) to 908 amino acids (ArUGT241), with the majority (73.38%) falling between 400 and 499 amino acids (Supplementary Fig. S1A and B). Similarly, calculated molecular weights ranged from 9.87 kDa to 99.78 kDa (Supplementary Fig. S1C). Most ArUGT proteins (80.24%) had molecular weights between 40 and 59.9 kDa (Supplementary Fig. S1D). Notably, ArUGT6 displayed both the shortest sequence length (88 AA) and the lightest molecular weight (9.87 kDa), while ArUGT241 exhibited the most lengthy sequence (908 AA) and the heaviest molecular weight (99.78 kDa).

ArUGTs gene chromosomal locations and duplication events

The distribution of the 249 identified *ArUGT* genes was investigated across the 30 chromosomes of the *A. rubrum* genome (Fig. 1). The number of *ArUGT* genes per chromosome varied, with chromosome LG 24 containing the most (24) and chromosomes LG 13, LG 20, and LG 36 containing the fewest (1). This uneven distribution highlights the potential for differential regulation and function of *ArUGTs* on different chromosomes. Gene duplication is a major evolutionary force driving gene family expansion and diversification. We identified 21 tandem duplication events, where pairs of closely related *ArUGTs* occur adjacent to each other on the same

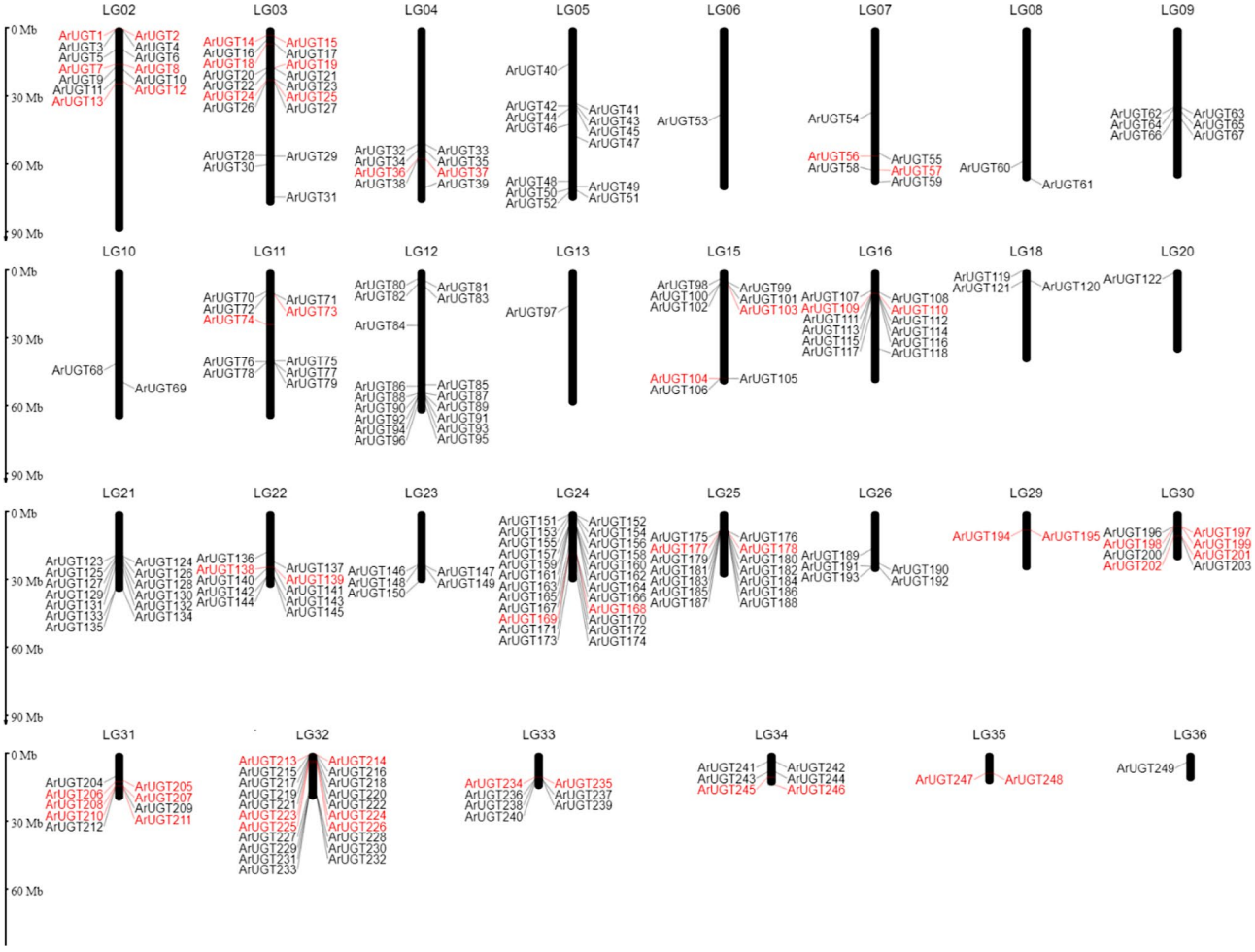

Fig. 1 *ArUGTs* family Gene distribution across *A. rubrum* chromosomes and Gene duplication events. Distribution of *ArUGTs* transcription factor across *A. rubrum* chromosome, and tandem duplication, red colour labeled genes are tandem duplicates. To understand the genomic distribution of *UGT* genes, we used the MG2C online tool to map the *ArUGTs* onto *A. rubrum* chromosomes. We prepared a reference genome sequence file in FASTA format along with gene sequences or IDs to identify gene duplication events. For this purpose, we employed tools such as MCScanX Wrapper and One Step MCScanX in the Gene Duplication section of TBtools-II. To find duplicate genes (both segmental and tandem), we loaded the reference genome and gene sequence files, configured the analysis settings, and performed the analysis. The results were then used to illustrate duplication events within the *ArUGT* family

chromosome (Fig. 1). Additionally, 66 segmental duplication events were identified (Supplementary Fig. S2A and B). Segmental duplications involve duplicating the chromosomal regions, potentially leading to dispersing *ArUGT* copies across different chromosomes. Further analysis revealed 882 paralogous gene pairs within the *ArUGT* family (Supplementary Table S3). Paralogs are genes that arise from duplication events within a single genome. Out of the 249 *ArUGTs*, 36 were found to be singletons (lacking paralogs). Interestingly, a significant portion of *ArUGTs* possessed either one (47 genes), two (45 genes), or three (51 genes) paralogs (Supplementary Fig. S3). These findings suggest that *ArUGT* gene duplication, both tandem and segmental, has played a significant role in the expansion and evolution of the *UGT* gene family within *A. rubrum*.

Motif analysis of the identified ArUGTs proteins

We performed a motif analysis using the MEME tool to gain insights into potential functional domains within ArUGT proteins. This analysis revealed ten conserved motifs (Motif1-Motif10) across the 249 ArUGT proteins (Supplementary Fig. S4, Supplementary Table S4). Motif lengths ranged from 15 amino acids (Motif10) to 34 amino acids (Motif8). These conserved motifs likely represent functionally essential regions of the proteins. Interestingly, while some motifs were common to many ArUGT proteins, others were found only in specific subsets. This variation in motif distribution suggests potential functional diversification within the ArUGT family. Understanding the specific roles of these motifs could provide clues about how different ArUGTs contribute to diverse processes in *A. rubrum*.

Phylogenetic analysis of the ArUGTs gene family

To study the evolutionary links between ArUGT proteins, we generated a phylogenetic tree using MEGA11. The study contained 249 UGT protein sequences from *A. rubrum* and 122 established UGTs from *A. thaliana* [29], which allowed us to categorize ArUGTs based on common ancestry with known *A. thaliana* subfamilies [38]. Our findings showed that ArUGT proteins were divided into 18 unique subgroups (Fig. 2), showing high diversity within the UGT gene family in *A. rubrum*. The prevalence of *ArUGTs* was unequal across these groupings. Group (E) was the biggest, with 38 *ArUGTs* members, while Group (F) had just 6 *ArUGTs*. Notably, the *A. thaliana* protein *AtUGT78D2*, recognized for its function in anthocyanin biosynthesis, is closely related to numerous *ArUGTs* (*ArUGT52*, *ArUGT11*, *ArUGT41*, *ArUGT50*, *ArUGT6*, and *ArUGT40*). This evolutionary link implies that these particular *ArUGTs* may also play a role in anthocyanin synthesis in *A. rubrum.*

Duplication and synteny analysis of ArUGTs gene

To gain insights into the evolutionary history of the *UGT* gene family in *A. rubrum*, we conducted both gene duplication analysis and comparative genomics to understand how *ArUGTs* relate to *UGTs* in other species. We constructed syntenic maps comparing *A. rubrum* to five other plants (*P. trichocarpa*, *A. thaliana*, *Vitis vinifera*, *Gossypium hirsutum*, and *A. yangbiense*). The analysis showed that 96 *UGT* gene pairs are in the same line between *A. rubrum* and *P. trichocarpa*. Additionally, 35 *UGT* gene pairs are in the same line between *A. rubrum* and *A. thaliana*. Furthermore, 126 genes have a similar function between *A. rubrum* and *A. yangbiense*, and 100 genes have a similar function between *A. rubrum* and *Gossypium hirsutum.* Lastly, there are 67 genes with a similar function between *A. rubrum* and *Vitis vinifera* (Fig. 3 and Supplementary Table S5).

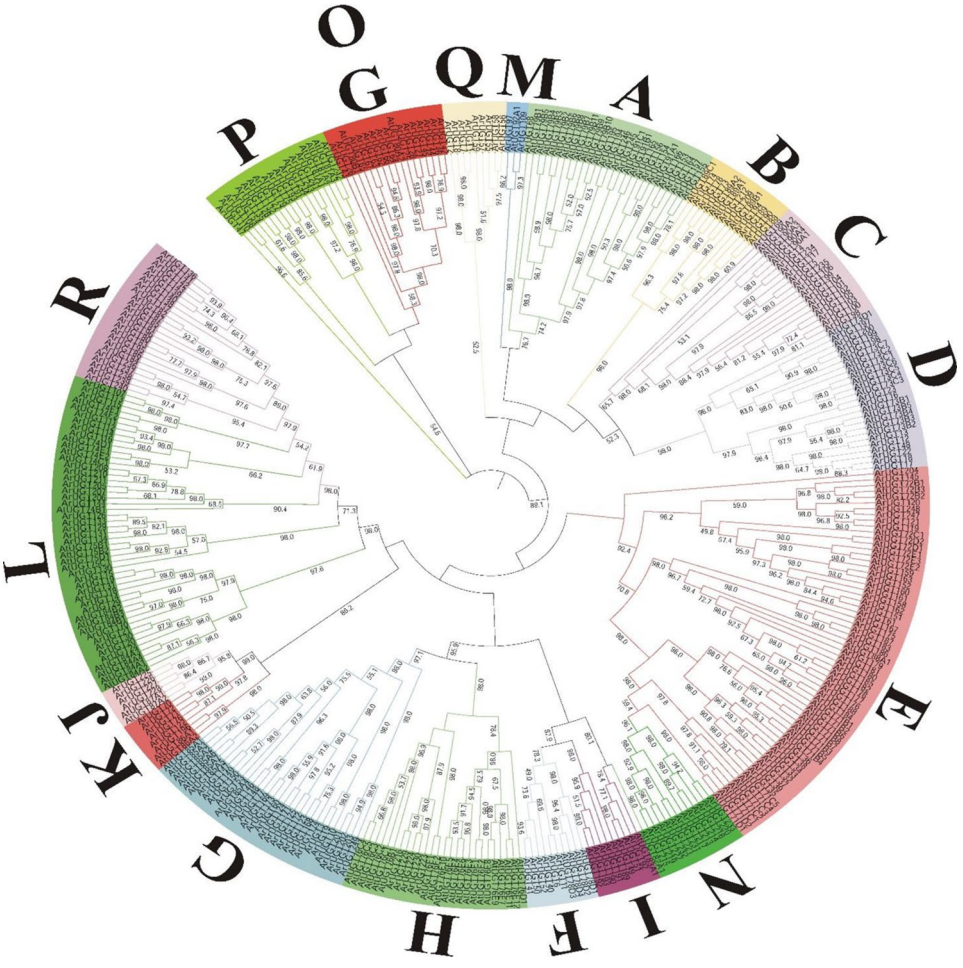

Fig. 2 Phylogenetic tree of identified *ArUGT* proteins. A phylogenetic analysis of *A. rubrum ArUGT* family genes. The MUSCLE and MEGA 11.0 software was used for the sequence alignment and construction of the phylogenetic tree (parameters: p-distance, complete deletion, bootstrap: 1500 replicates) using the complete length sequences of 249 *A. rubrum ArUGTs* and 122 *A. thaliana* AtUGTs

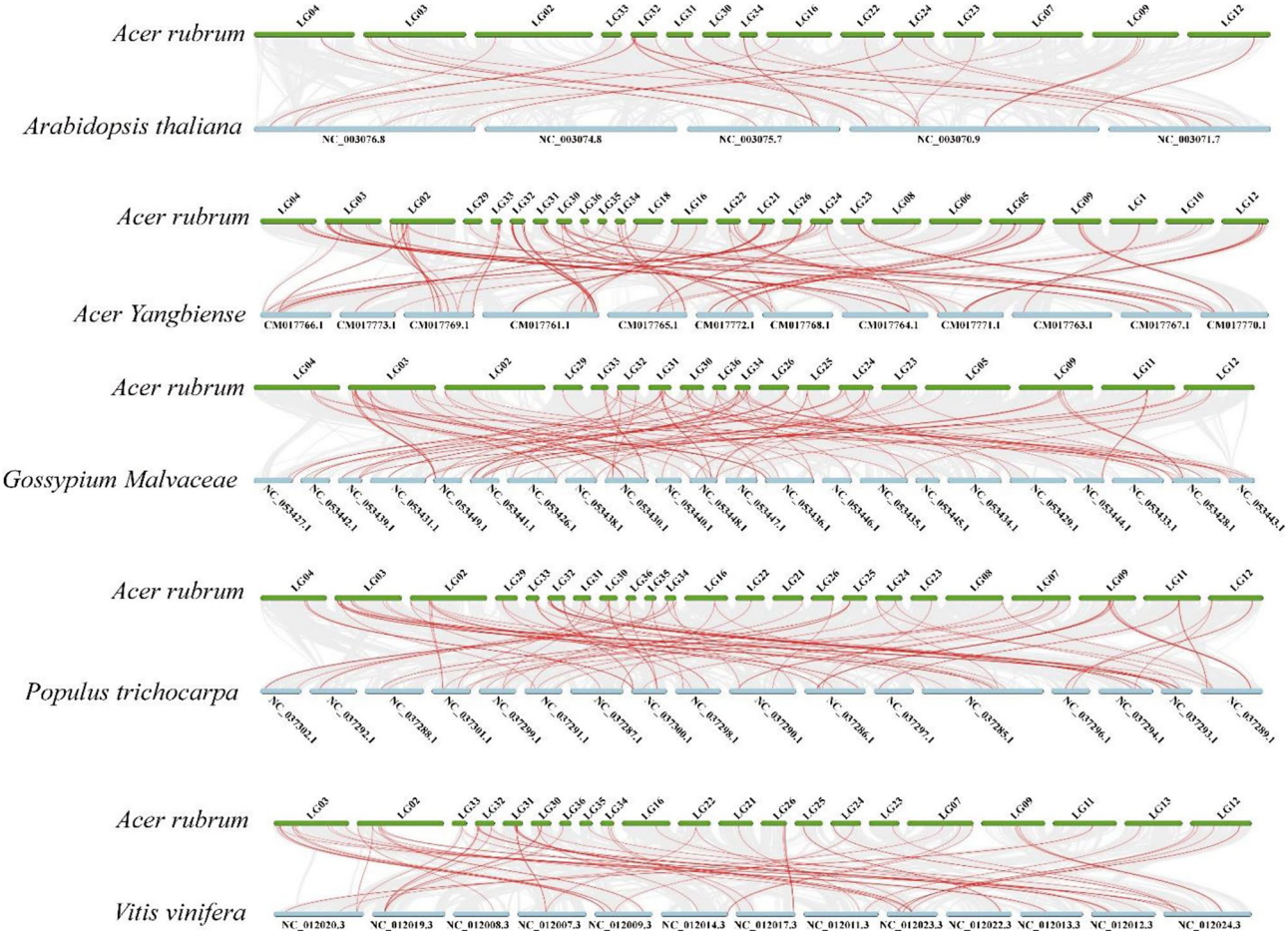

Fig. 3 Synteny analyses of the *ArUGT* family gene with the *UGT* family of other species. Synteny analysis between the *ArUGT* genes and other plant species. The synteny analyses were performed using TBtool's "Dual Synteny Plotter" option. The genome information files (GFF files) and genomic sequences (fasta files) of *P. trichocarpa*, *A. thaliana*, *Vitis vinifera*, *Gossypium hirsutum*, and *A. yangbensi* were downloaded from NCBI. Grey lines represent the collinear relationship between *A. rubrum* and other plant species' genomes. In contrast, highlighted red lines represent the collinear relationship of *ArUGT* genes with other plant species' *UGT* genes. Chromosomes of different plant species are shown in different colours

Expression analysis of *ArUGT* genes in different colors of leaves of *Acer rubrum*

To investigate the role of *ArUGT* genes in the striking color variation of *A. rubrum* leaves, we performed expression profiling of *ArUGTs* in leaves exhibiting distinct colors (green, yellow, and red) (Fig. 4A). Samples were collected from *A. rubrum* trees at the Anhui Academy of Agricultural Sciences. The transcriptomic analysis revealed differential expression patterns of *ArUGT* genes across the three-color groups (Fig. 4B). Several genes, including *ArUGT52*, *ArUGT102*, *ArUGT107*, *ArUGT218*, *ArUGT126*, *ArUGT103*, *ArUGT197*, and *ArUGT138* were upregulated in red leaves compared to green leaves (Fig. 4B). To validate our findings, we performed qRT-PCR analysis, which confirmed the trends observed in our transcriptomic data (Fig. 4C). These results strongly suggest that differential regulation of specific *ArUGT* genes plays a crucial role in the diverse leaf coloration of *A. rubrum*.

Expression analysis of ArUGT genes under low-temperature stress

To investigate the possible significance of *ArUGTs* in *A. rubrum's* cold stress response, we evaluated their expression at two low temperatures (10 °C and 4 °C) (Fig. 5A). Our findings demonstrated that multiple *ArUGT* genes were significantly upregulated at both temperatures. At 4 °C, the *ArUGT52*, *ArUGT24*, *ArUGT21*, *ArUGT46*, *ArUGT20*, *ArUGT148*, *ArUGT41* and *ArUGT50* had higher level of expression (Fig. 5B). We corroborated these transcriptome results using qRT-PCR (Fig. 5C), which confirmed the increase of *ArUGTs* during cold stress. These findings indicate that *ArUGTs* play a role in the cold-stress response in *A. rubrum*.

Identification of cis-acting element in the promoter region of ArUGTs

To further understand how *ArUGT* gene expression is regulated, we investigated cis-acting elements in their

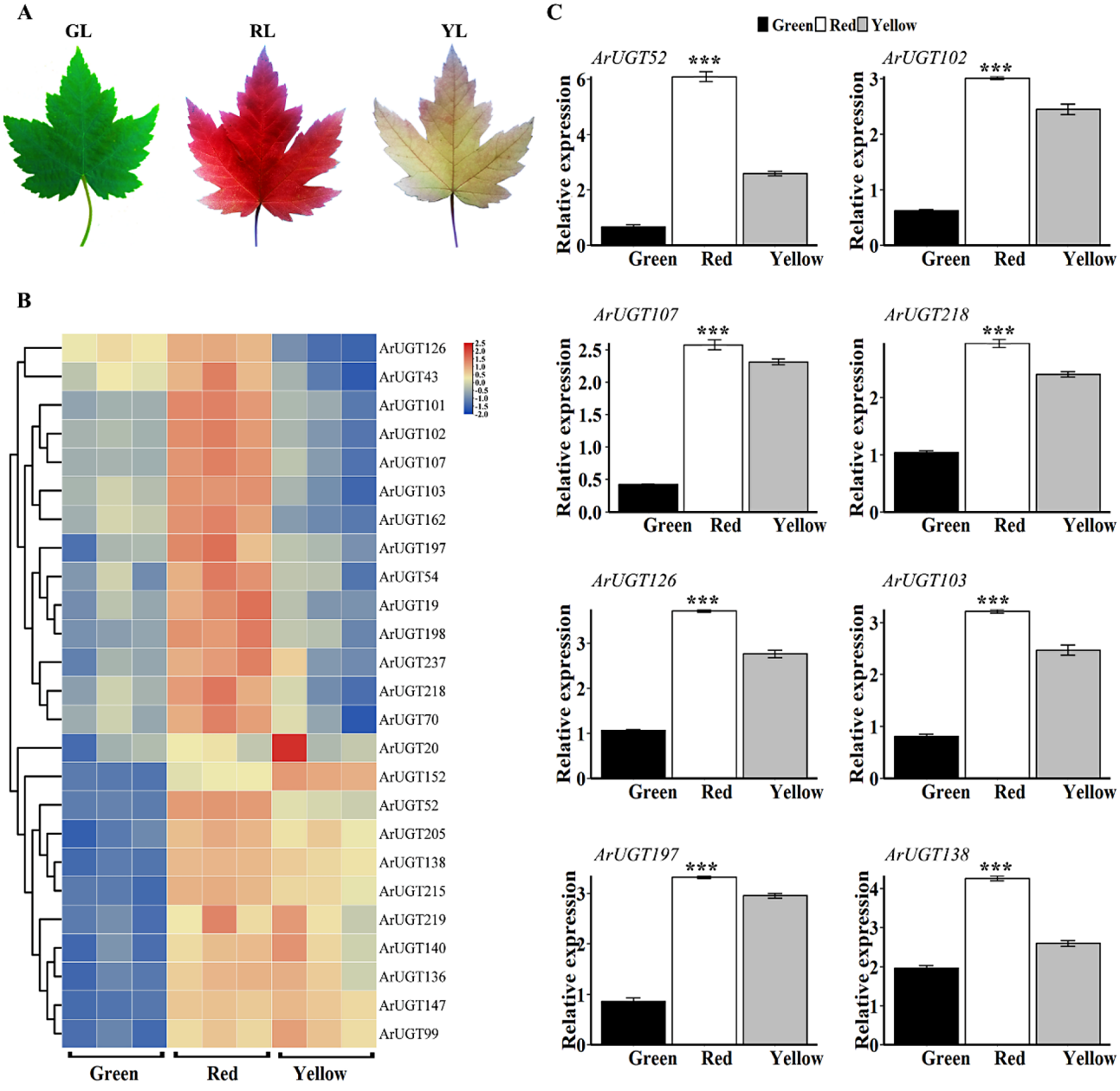

Fig. 4 Heatmap of the transcriptomic expression levels of *ArUGT* genes in green, red, and yellow leaves of *A. rubrum*.**A** leaves samples were collected in mid-to-late November. **B** The figure data represents the log scale of transcription reads (FPKM values) of *ArUGTs* in green, red, and yellow leaves of *A. rubrum*. The figure was illustrated using the heatmap function of TBtools-II software v2.030. In this figure, GL represents the green leaf, RL is the red leaf, and YL means the yellow leaf of *A. rubrum*.**C** The relative expression level of *ArUGTs* was analyzed using the $2^{-\Delta\Delta Ct}$ method. *ArACTIN2* was used as a reference gene. Data are means of three biological replicates. The qPCR data was statistically analyzed via One-way Analysis of variance, and multiple comparisons were made with HSD Tuckey's test at $p < 0.001$ significant level ($n = 3$). Asterisks (***) show a significant difference at $p < 0.001$. In this figure, GL represents the green leaf, RL is the red leaf, and YL means the yellow leaf of *A. rubrum*

promoter regions. These short DNA sequences serve as binding sites for transcription factors, which influence gene activity. Using the PlantCARE database, we analyzed the promoter regions of all identified *ArUGTs*, including a focused analysis of *ArUGT52*. Across the *ArUGT* promoters, we found four main types of cis-elements: those associated with light responsiveness, circadian clock control, environmental stress responses (including cold), and hormone signaling pathways (Supplementary Fig. S5). Importantly, many *ArUGT* promoters, including *ArUGT52*, contained binding sites for transcription factors known to regulate anthocyanin production. Specifically, the *ArUGT52* promoter contained MYB binding sites. This suggests that *ArUGT52* expression is likely influenced by a combination of factors, including light, circadian rhythms, and stress conditions. This complex

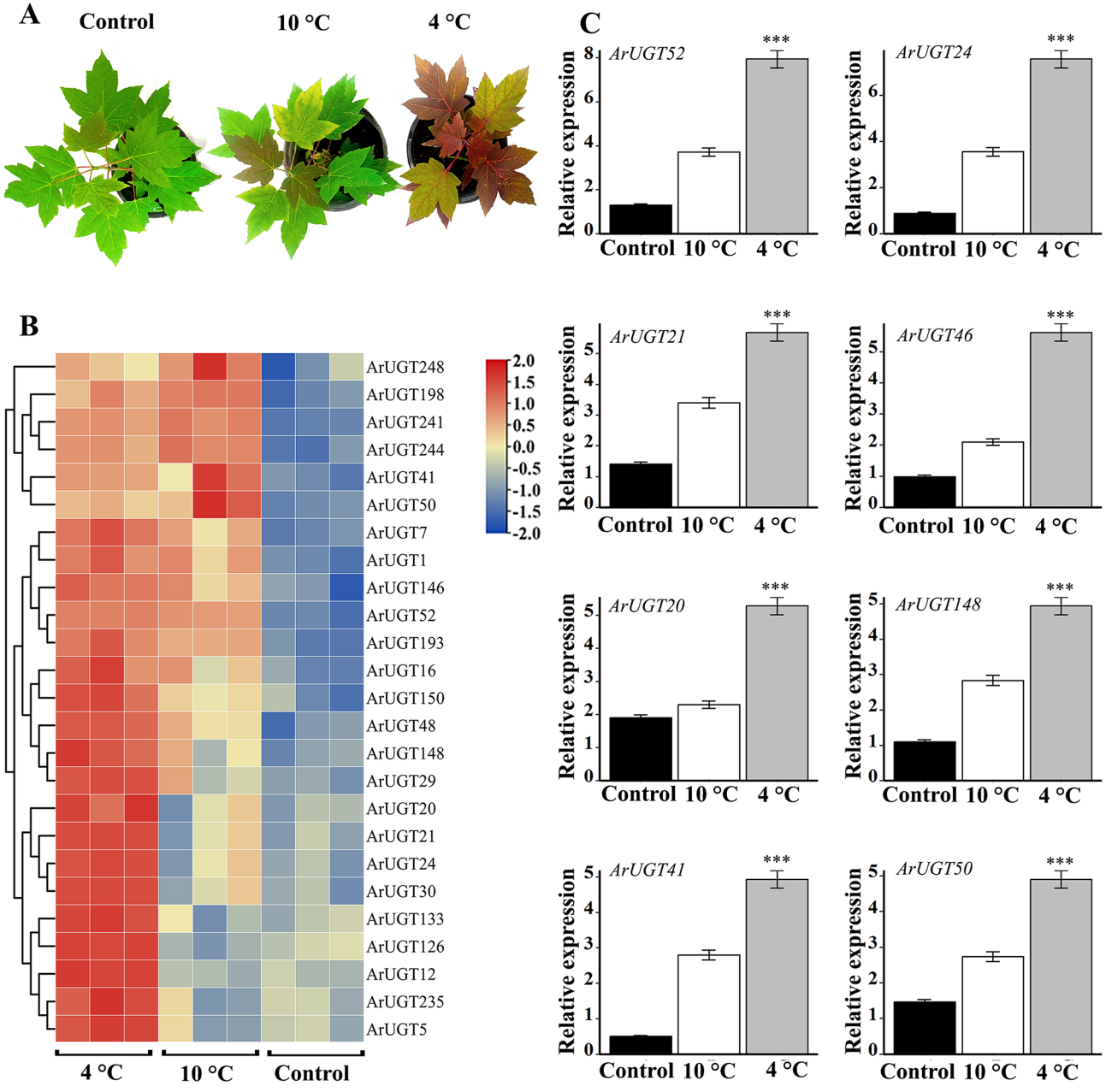

Fig. 5 Heatmap of the transcriptomic expression levels of *ArUGT* genes under cold stress. **A** Leaves samples were collected from Eight-month-old seedlings in control (22 °C) and cold stress (10 °C and 4 °C) for 24 h. **B** The figure data was extracted from the transcriptomic data representing the log scale of transcription reads (FPKM values) of *ArUGTs*. Data are means of three biological replicates. The figure was illustrated using the heat map function of TBtools-II software v2.030. **C** The relative expression level of *ArUGTs* was analyzed using the $2^{-\Delta\Delta Ct}$ method. *ArACTIN2* was used as a reference gene. Data are means of three biological replicates. The qPCR data was statistically analyzed via One-way Analysis of variance, and multiple comparisons were made with HSD Tuckey's test at $p < 0.001$ significant level ($n = 3$). Asterisks (***) show a significant difference at $p < 0.001$. In this figure, GL represents the green leaf, RL is the red leaf, and YL means the yellow leaf of *A. rubrum*

regulatory network may fine-tune *ArUGT52* expression to control anthocyanin biosynthesis in response to various internal and external cues.

Subcellular localization of the ArUGT52

To determine the subcellular localization of *ArUGT52*, expression in *Nicotiana benthamiana* leaf cells was performed using Agrobacterium rhizogenes-mediated transformation. We constructed a fusion protein of *ArUGT52* and the green fluorescent protein (GFP) tag, expressed from the *pCambia1302-ArUGT52*-GFP plasmid. A control construct (*pCambia1302*-GFP) expressing GFP alone was used for comparison. Microscopic examination revealed that the *ArUGT52*-GFP fusion protein

localized predominantly within the cytoplasm (Fig. 6A). In contrast, the GFP alone control showed a more diffuse pattern throughout the cell. This finding suggests that *ArUGT52* may function primarily within the cytoplasm, where its potential interaction partners or substrates are likely located.

Transient overexpression of ArUGT52 in tobacco (Nicotiana Benthamiana)

To study the function of *ArUGT52*, we used transient overexpression in tobacco leaves. we infiltrated leaves with *Agrobacterium* rhizogenes expressing either the *pCambia1302-ArUGT52* construct or an empty *pCambia1302* vector as a control. We found a considerable increase in anthocyanin concentration in leaves overexpressing *ArUGT52* compared to controls (Fig. 6B and D). This study implies that *ArUGT52* contributes to anthocyanin accumulation in *Nicotiana benthamiana*. Furthermore, when *ArUGT52*-overexpressing plants were exposed to cold stress, their anthocyanin levels rose even more (Fig. 6C and E). This finding suggests that *ArUGT52* may contribute to controlling anthocyanin production in *A. rubrum* in response to cold temperatures.

ArUGT52 glycosylates flavonols and anthocyanins in vitro

It has been found that AtUGT78D2, an UDP-flavonoid glucosyl transferase (UGT), may glucosylate flavonols [39]. Building on this information, we tested if *ArUGT52*, a homologous gene from a different plant species, could

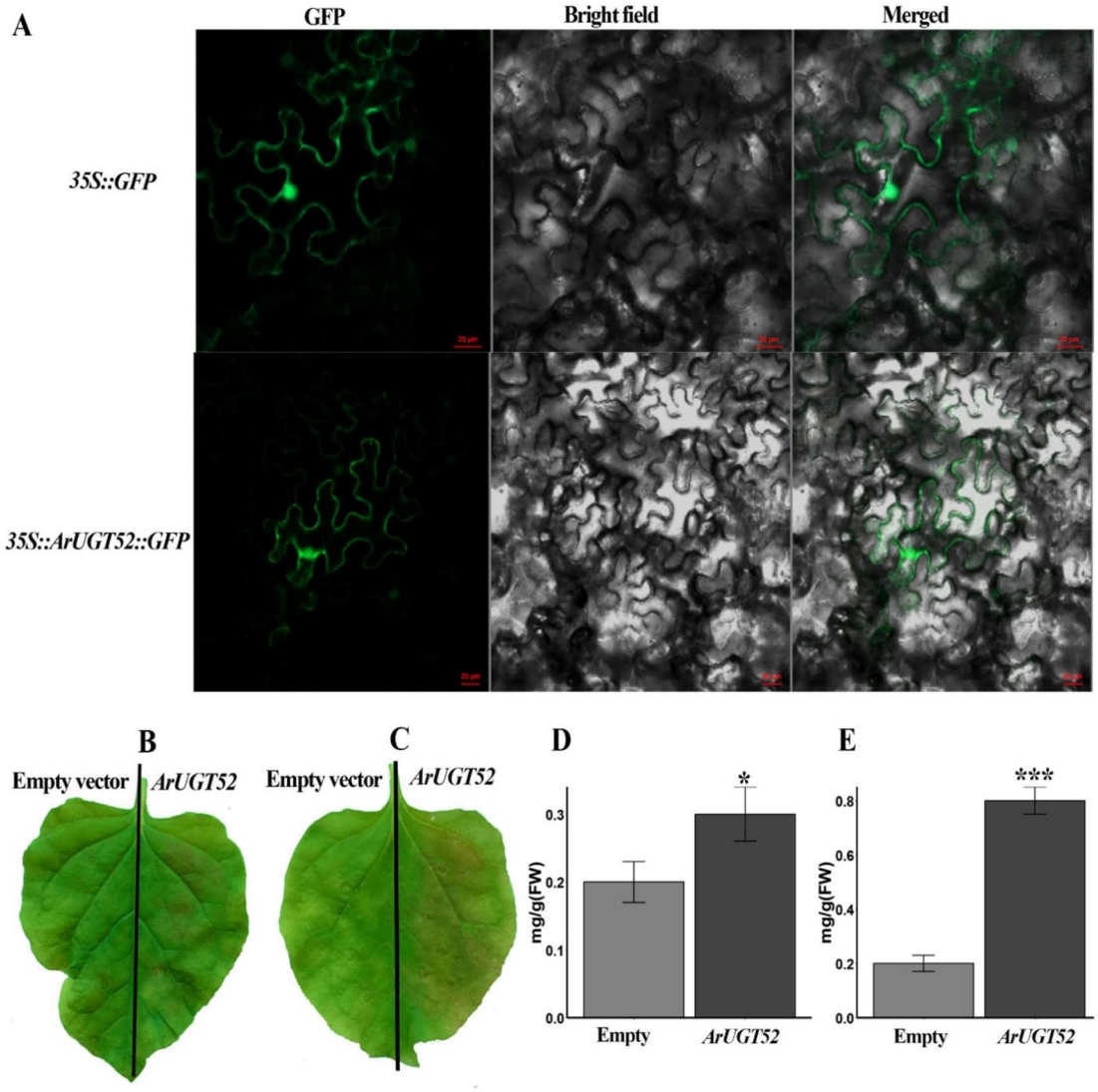

Fig. 6 Transient expression of *ArUGT52*.**A** Sub-cellular localization of *ArUGT52*. **B** Transient expression of *ArUGT52* in tobacco leaves under control condition. **C** Low-temperature treated *ArUGT52* transient expressed tobacco seedlings. **D** Anthocyanin levels in transient expressed *ArUGT52* in tobacco leaves under control condition. **E** Anthocyanin levels in transient expressed *ArUGT52* in tobacco leaves under cold condition

also glucosylate flavonols and anthocyanidins. We generated and purified recombinant *ArUGT52*, a fusion protein with GST (glutathione S-transferase) to test. Biochemical reactions were then conducted using various flavonol and anthocyanidin substrates, and the products were analyzed using HPLC. The results indicated that the GST enzyme alone did not catalyze the glucosylation of flavonol substrates. However, when GST was fused with *ArUGT52*, the glucosylation of flavonols, specifically quercetin and kaempferol, was observed. Furthermore, this fusion protein could also glycosylate anthocyanidin substrates, including pelargonidin and cyaniding (Fig. 7). These findings suggest that *ArUGT52* exhibits biochemical activity similar to its *A. thaliana* orthologue, *AtUGT78D2*, in glucosylating flavonols and anthocyanidins, which is expanding our understanding of the functional conservation of *UGT* enzymes across plant species and provides insights into the biosynthesis of flavonoid glycosides in *A. rubrum*.

Discussion

This study identifies and characterizes the *UGT* family in *A. rubrum*, an important ornamental tree. We conducted a comprehensive analysis encompassing motif composition, gene structure, phylogenetic relationships, tandem duplication events, gene replication events, and collinear relationships with other species. Furthermore, we investigated the expression profiles of *ArUGT* genes in red leaves across changing seasons and under low-temperature conditions. Additionally, a co-expression analysis of *ArUGTs* with various transcription factors was performed to predict their potential roles in regulating secondary metabolites like anthocyanin biosynthesis. After finding a common highly expressed gene, *ArUGT52*, in both transcriptomic data of red leaves and under low temperature, we genetically transformed *ArUGT52* in *Nicotiana benthamiana* and confirmed its involvement in anthocyanin biosynthesis under both control and low-temperature.

Our analysis revealed that *ArUGT* genes associated with anthocyanin metabolism exhibit conserved sequence motifs and gene architectures, consistent with previous research on *UGT* genes in upland cotton [29], pomelo [40]. and *A. thaliana* [29]. It is worth mentioning that 80.7% (200 out of 249) of these genes had 1–2 exons. In upland cotton, 17 out of 36 UGT members contained one intron, while 19 possessed two introns. Around 50% of *UGT* genes in *A. thaliana* and pomelo do not have introns [40]. The *UGTs* in *Gossypium hirsutum* primarily contain one or two introns, with only a few exhibiting multiple introns [40]. This suggests a degree of conservation in *UGT* gene structure across diverse plant species, although variations exist. The high proportion of *ArUGTs* with 1–2 exons might reflect specific evolutionary pressures or functional adaptations in *A. rubrum*. Notably, motif arrangements within evolutionarily related *ArUGT* groups displayed significant similarity, suggesting that motif positioning and gene structure within these subfamilies support the phylogenetic classification of *ArUGTs*.

Gene duplication events are believed to impact the variability in the number of *UGT* family members [41]. In this study, a total of 21 tandem duplications were found on various chromosomes. Additionally, 66 segmental duplications were found on different chromosomes. The genome of *A. rubrum* has experienced tandem and segmental duplications, resulting in increased gene copies, which could contribute to new gene functions and the expansion of gene families. Interestingly, *A. rubrum* has a significantly higher number of segmental duplication genes compared to *Nelumbo nucifera* [42], *Manihot esculenta Crantz* [43], and *Broussonetia papyrifera* [44], which have 7, 24, and 28 segmental duplications, respectively. This suggests that segmental duplication may have played a more prominent role in the evolution of the *UGT* gene family in *A. rubrum*. The results of this study indicate that the duplication of *ArUGTs* in *A. rubrum* depends upon both tandem and segmental duplication events.

After comparing the *UGT* gene families in *A. thaliana*, the 249 *UGT* genes were classified into 18 evolutionary groupings. However, as noted by the reviewer, this may not represent the full diversity of *UGT* groups across the plant kingdom. 38 genes were discovered in group E of *A. rubrum* and have been reported to facilitate the conversion of terpenes and flavonoids. Additionally, 10 genes were found in Group F, which are known to be involved in anthocyanin biosynthesis [45]. For example, *ArUGT52*, found in group E, forms a cluster with *A. thaliana UGT78D2*. *UGT82A1* catalyzes the addition of glucose molecules to flavonols and anthocyanins at the 3-OH position and leads to the accumulation of anthocyanin [37, 46].

Previous research has shown the importance of plant secondary product glycosyltransferases (PSPGs) in regulating secondary plant metabolite production [31, 47]. Interestingly, analysis of the 249 ArUGT proteins from all 18 evolutionary groups revealed the consistent presence of a conserved PSPG box in each group, implying that ArUGT proteins may be involved in the glycosylation of secondary metabolites across diverse evolutionary lineages in *A. rubrum*. Furthermore, observed differences in glycosylation patterns between evolutionary groups, together with specificities found in PSPG motif analysis, provide further support for the probable role of these 249 *UGT* genes in secondary metabolite synthesis.

The cis-elements found in the promoter of *ArUGTs* were divided into four major groups: (1) light

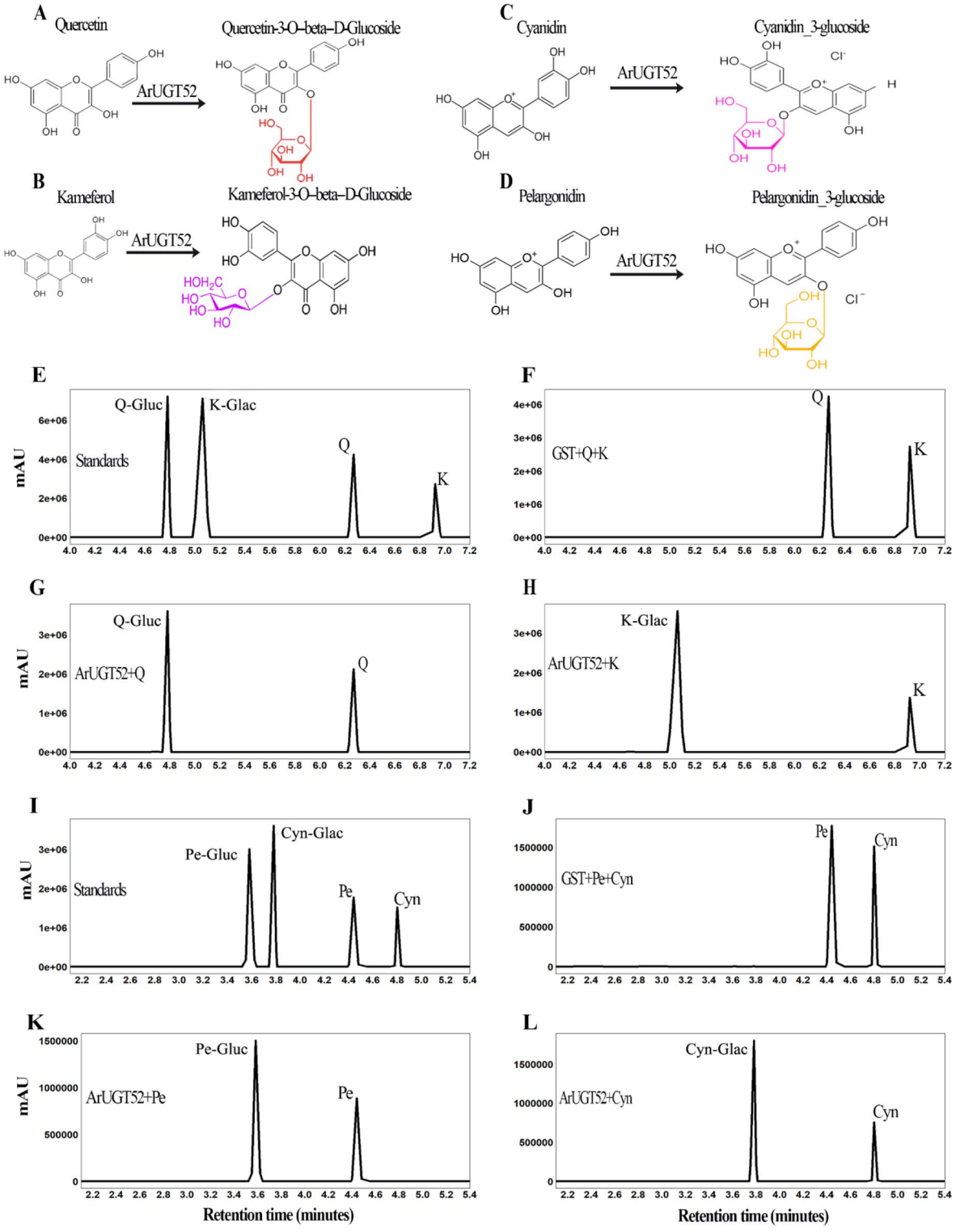

Fig. 7 ArUGT52 regulated the glucosylation of anthocyanidins and flavonols. **A** representation of ArUGT52 enzyme glucosylation of quercetin (Q) into quercetin 3-O-glucoside (Q-Gluc). **B** ArUGT52 enzyme glucosylation of kaempferol (K) into kaempferol 3-O-glucoside (K-Glac). **C** ArUGT52 enzyme glucosylation of cyanidin (Cyn) into cyanidin 3-O-glucosides (Cyn-Gluc). **D** ArUGT52 enzyme glucosylation of pelargonidin (Pe) into pelargonidin 3-O-glucosides (Pe-Glac). **E** standards mean HPLC profile of flavonols and flavonol 3-O-glucosides. **F** GST + Q + K, GST did not glucosylate the Q and K. **G** ArUGT52 + Q represented the conversion of quercetin to quercetin 3-O-glucoside by ArUGT52 enzyme. **H** ArUGT52 + K represented the conversion of kaempferol to kaempferol 3-O-glucoside by ArUGT52 enzyme. **I** standards mean HPLC profile of pelargonidin and pelargonidin 3-O-glucosides. **J** GST + Pe + Cyn, GST did not glucosylate the Pe and Cyn. **K** ArUGT52 + Pe represented the conversion of pelargonidin to pelargonidin 3-O-glucoside by the ArUGT52 enzyme. **L** ArUGT52 + Cyn represented the HPLC profile of cyanidin and cyanidin 3-O-glucosides

responsiveness, (2) circadian rhythm, (3) environmental adaptation (including responses to low temperatures, defense, and stress), and (4) hormonal control (particularly, responses to ABA and Jasmonic acid). Light, the circadian clock, low temperatures, and phytohormones have a significant influence on anthocyanin production and stability. Light quality, intensity, duration, and the circadian clock may all impact anthocyanin accumulation in plants [48–51].

According to recent research, low temperatures alter both anthocyanin production and stability [52]. Plant hormones also influence the production of anthocyanins. Several studies have demonstrated that auxin and gibberellins diminish anthocyanin accumulation by blocking the transcription of anthocyanin-producing genes [53–55]. Various studies suggest that cytokinins, abscisic acid (ABA), jasmonic acid, ethylene, and brassinosteroids enhance anthocyanin production by activating genes involved in its biosynthesis [55–57].

The findings showed that the ArUGTs promoter region has a binding site for all anthocyanin-producing components. As a result, the data suggest that numerous factors may influence anthocyanin production in *ArUGTs*. Several *UGTs* have been shown to have elevated relative expression levels in red-colored leaves. This research found that numerous genes were elevated in yellow and red leaves, consistent with prior results. Previous research has shown that *UGT79B1* is highly elevated in the red autumn leaves of *Fraxinus angustifolia* [58]. Another study found that various UDP-glucuronosyltransferases (*UGTs*) have unique expression patterns throughout fall. According to [59], there was a substantial association between anthocyanin levels and gene expression. When anthocyanin levels were high, expression levels were much more remarkable. These data imply that *UGTs* play an essential role in the coloration of *A. rubrum* leaves.

The identification of *ArUGT52* as a key regulator of anthocyanin biosynthesis in *A. rubrum* has important implications for breeding programs. By targeting *ArUGT52* expression or manipulating its regulatory network, breeders could potentially enhance the intensity and stability of red leaf coloration in cultivated varieties. This could lead to the development of new *A. rubrum* cultivars with improved aesthetic qualities, increasing their value in the horticultural market.

Cold response to *UGTs* and accumulation of anthocyanin under cold stress [60–62]. Furthermore, one research found that aberrant expression of the *UGT79B2/B3* genes significantly increased the accumulation of anthocyanin pigments in plants subjected to low temperatures. Plants harboring mutations in the *UGT79B2* and *UGT79B3* genes have lower amounts of anthocyanin pigments. CBF1 (CRT/DRE-binding factor 1, also known as DREB1B) regulates the expression of *UGT79B2* and *UGT79B3* genes in response to low temperatures [60]. Several *ArUGTs* were shown to be upregulated in *A. rubrum* leaves when exposed to various low temperatures. These findings indicate that certain *ArUGTs* are quite sensitive to low temperatures. Another report stated that *AtUGT78D2* played a role in anthocyanin biosynthesis, and the phylogenetic tree results of this study showed that *ArUGT52* grouped with *AtUGT78D2* and that its expression levels were much more significant in red and low-temperature treated leaves of *A. rubrum*, showing that *ArUGT52* likely retains its conserved roles in regulating anthocyanin biosynthesis in *A. rubrum* in response to low temperatures.

In this work, the results of phylogenetic tree group F revealed that *ArUGT52* was related with *AtUGT78D2*, which has been linked to anthocyanin biosynthesis [37]. Notably, transcriptome data analysis in red-colored leaves exposed to cold stress indicated a considerable elevation of *ArUGT52* expression. This finding encouraged additional investigation into *ArUGT52*. As a result, we performed an overexpression investigation in *Nicotiana benthamiana* leaves, subjecting the transgenic plants to cold stress. The findings showed that overexpressing *ArUGT52* increased anthocyanin content, demonstrating *ArUGT52's* ability to increase anthocyanin concentrations in *Nicotiana benthamiana* leaves. Cold stress increased anthocyanin levels in *ArUGT52*-overexpressed *Nicotiana benthamiana* leaves. These results are consistent with prior research that found *UGT78* enhances anthocyanin production in tea plants under cold stress [63]. The finding that *ArUGT52* also enhances anthocyanin production under cold stress suggests its potential role in improving cold tolerance in *A. rubrum*. Further research is needed to explore this possibility and investigate whether manipulating *ArUGT52* expression can enhance cold tolerance in *A. rubrum* cultivars.

The findings of the in vitro biochemical experiment revealed that *ArUGT52* was engaged in flavonol glucosylation as well as anthocyanin production by glucosylating anthocyanidin substrates such as pelargonidin and cyanidin. These findings were consistent with previous studies showing that the biosynthesis of anthocyanin is orchestrated by specific genes in *A. thaliana*, namely *UGT75C1* and *UGT78D2*, which encode anthocyanin 5-O-glucosyltransferase and flavonoid 3-O-glucosyltransferase enzymes, respectively [37, 64]. Petunia hybrida flowers accumulated blue and purple anthocyanins due to the overexpression of anthocyanin 3′-O-glucosyltransferase (3'GT) from Gentiana triflora [65].

While our study provides valuable insights into the *UGT* gene family in *A. rubrum*, it is important to acknowledge some limitations. Our analysis focused primarily on gene expression in leaves, and further research

is needed to investigate the roles of *ArUGTs* in other tissues and developmental stages. Additionally, functional characterization of other *ArUGT* genes, especially those highly expressed in red leaves or under cold stress, would provide a more complete understanding of their roles in anthocyanin biosynthesis and stress response. Despite these limitations, our findings provide a foundation for future research and have significant implications for *A. rubrum* breeding programs.

Conclusion

This study provides a comprehensive analysis of the *UGT* gene family in *A. rubrum*, revealing its role in leaf coloration and response to low-temperature stress. The identification of 249 *ArUGT* genes, their phylogenetic relationships, expression patterns, and the functional characterization of *ArUGT52* significantly enhance our understanding of the genetic mechanisms underlying leaf color diversity in this species.

Our findings have important implications for both ornamental horticulture and ecological research. The potential for manipulating *UGT* gene expression to enhance autumn leaf color in *A. rubrum* cultivars holds promise for the development of new varieties with improved aesthetic qualities. Moreover, understanding the role of *ArUGTs* in response to low-temperature stress can shed light on the adaptive mechanisms of this species in a changing climate. Future research could explore the specific functions of other highly expressed *ArUGT* genes, investigate their interactions with other regulatory pathways, and assess their potential for improving cold tolerance in other plant species.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-06043-y.

Supplementary Material 1
Supplementary Material 2
Supplementary Material 3
Supplementary Material 4
Supplementary Material 5
Supplementary Material 6
Supplementary Material 7
Supplementary Material 8
Supplementary Material 9
Supplementary Material 10

Acknowledgements
The results shown here are in part based upon data generated by the repository: https://www.ncbi.nlm.nih.gov/.

Author contributions
K. A. K, S. F, L.H, and J.R created the concept and formulated the method. F.A.S, Y.Z and Z. C participated in collecting plant samples and conducting gene expression analysis using qPCR. Khan A.K and F. A. S conducted bioinformatics and statistical analysis and wrote the manuscript. The manuscript was revised by S.F, L.H, and J.R.

Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 26 September 2024 / Accepted: 31 December 2024
Published online: 05 March 2025

References

1. Bissiwu P, Kulkarni K, Melmaiee K, Elavarthi S. Physiological and molecular responses of red maple (Acer rubrum l.) cultivars to drought stress. Plant Breed Biotechnol. 2022;10(1):62–74.
2. Li C, Seeram NP. Ultra-fast liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry for the rapid phenolic profiling of red maple (Acer rubrum) leaves. J Sep Sci. 2018;41(11):2331–46.
3. Harris A, Chen Y, Olsen RT, Lutz S, Wen J. On merging Acer sections Rubra and Hyptiocarpa: Molecular and morphological evidence. PhytoKeys 2017;(86):9.
4. Chen Z, Lu X, Xuan Y, Tang F, Wang J, Shi D, Fu S, Ren J. Transcriptome analysis based on a combination of sequencing platforms provides insights into leaf pigmentation in *Acer rubrum*. BMC Plant Biol. 2019;19(1):240–240.
5. Tanaka Y, Ohmiya A. Seeing is believing: engineering anthocyanin and carotenoid biosynthetic pathways. Curr Opin Biotechnol. 2008;19(2):190–7.
6. Han M, Yang C, Zhou J, Zhu J, Meng J, Shen T, Xin Z, Li H. Analysis of flavonoids and anthocyanin biosynthesis-related genes expression reveals the mechanism of petal color fading of Malus hupehensis (Rosaceae). Brazilian J Bot. 2020;43(1):81–9.
7. Lu Z, Wen J, Ma Q, Yan K, Du Y, Chen Z, Lu X, Ren J, Wang Y, Li S. Transcriptome profiling provides insights into leaf color changes in two Acer palmatum genotypes. BMC Plant Biol. 2022;22(1):1–14.
8. Hongxia W, Fan W, Li H, Yang J, Huang J, Zhang P. Functional characterization of Dihydroflavonol-4-reductase in anthocyanin biosynthesis of purple sweet potato underlies the direct evidence of anthocyanins function against abiotic stresses. PLoS ONE. 2013;8(11):e78484–78484.
9. Panfeng Y, Deng R, Huang Y, Stael S, Shi J, Shi G, Lv B, Li Q, Dong Q, Wu Q, et al. Diverse biological effects of glycosyltransferase genes from Tartary buckwheat. BMC Plant Biol. 2019;19(1):339–339.
10. Shoib Ahmad B, Mohiuddin T, Basu S, Swarnkar MK, Malik AH, Wani ZA, Abbas N, Singh AK, Ashraf N. Comprehensive transcriptome analysis of Crocus sativus for discovery and expression of genes involved in apocarotenoid biosynthesis. BMC Genomics. 2015;16(1):698–698.
11. Hugo KD, Nelson OE. Genetic control of UDPglucose:flavonol 3-O-glucosyltransferase in the endosperm of maize. Biochem Genet. 1977;15(5–6):509–19.
12. Jing L, Xiao, Zhang JQ, Wu ZC, Lai B, Huang XM, Qin YH, Wang HC, Hu GB. Functional characterization of a glucosyltransferase gene, LcUFGT1, involved in the formation of cyanidin glucoside in the pericarp of Litchi chinensis. Physiol Plant. 2016;156(2):139–49.
13. Shozo K, Ishimaru M, Ding CK, Yakushiji H, Goto N. Comparison of UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (Vitis vinifera) and their sports with red skin. Plant Sci. 2001;160(3):543–50.
14. Yasumasa M, Ishiguro K, Tanaka Y, Iida S, Hoshino A. Spontaneous mutations of the UDP-glucose:flavonoid 3-O-glucosyltransferase gene confers pale- and

dull-colored flowers in the Japanese and common morning glories. Planta. 2015;242(3):575–87.
15. Li J, Liu X, Gao Y, Zong G, Wang D, Liu M, Fei S, Wei Y, Yin Z, Chen J. Identification of a UDP-Glucosyltransferase favouring substrate-and regio-specific biosynthesis of flavonoid glucosides in Cyclocarya paliurus. Phytochemistry. 2019;163:75–88.
16. Chen K, Hu Z-m, Song W, Wang Z-l, He J-b, Shi X-m, Cui Q-h, Qiao X, Ye M. Diversity of Glycosyltransferases contributes to the biosynthesis of Flavonoid and Triterpenoid glycosides in *Glycyrrhiza uralensis*. ACS Synth Biol. 2019;8(8):1858–66.
17. Wei S, Liang L, Meng X, Li Y, Gao F, Liu X, Wang S, Gao X, Wang L. Biochemical and molecular characterization of a flavonoid 3-O-glycosyltransferase responsible for anthocyanins and flavonols biosynthesis in Freesia Hybrida. Front Plant Sci. 2016;7:410.
18. Jun C, Wei G, Zhou H, Gu C, Vimolmangkang S, Liao L, Han Y. Unraveling the mechanism underlying the glycosylation and methylation of anthocyanins in peach. Plant Physiol. 2014;166(2):1044–58.
19. Nina VF, Furtek DB, Nelson OE. Cloning of the bronze locus in maize by a simple and generalizable procedure using the transposable controlling element activator (ac). Proc Natl Acad Sci U S A. 1984;81(12):3825–9.
20. Takayuki T, Nishiyama Y, Hirai MY, Yano M, Nakajima Ji, Awazuhara M, Inoue E, Takahashi H, Goodenowe DB, Kitayama M, et al. Functional genomics by integrated analysis of metabolome and transcriptome of Arabidopsis plants over-expressing an MYB transcription factor. Plant J. 2005;42(2):218–35.
21. Jun O, Kanno Y, Itoh Y, Tsugawa H, Suzuki M. Anthocyanin biosynthesis in roses. Nature. 2005;435(7043):757–8.
22. Keiko Y-S, Fukushima A, Nakabayashi R, Hanada K, Matsuda F, Sugawara S, Inoue E, Kuromori T, Ito T, Shinozaki K, et al. Two glycosyltransferases involved in anthocyanin modification delineated by transcriptome independent component analysis in Arabidopsis thaliana. Plant Journal: Cell Mol Biology. 2012;69(1):154–67.
23. Johan K, Souer E, de Graaff A, Xue Y, Mol J, Koes R. Cloning and structural analysis of the anthocyanin pigmentation locus Rt of Petunia hybrida: characterization of insertion sequences in two mutant alleles. Plant J. 1994;5(1):69–80.
24. Shin'ya S, Suzuki H, Ichimaida F, Yamaguchi M-a, Iwashita T, Fukui Y, Hemmi H, Nishino T, Nakayama T. UDP-glucuronic Acid:anthocyanin glucuronosyltransferase from Red Daisy (Bellis perennis) flowers. J Biol Chem. 2005;280(2):899–906.
25. Gachon CM, Langlois-Meurinne M, Saindrenan P. Plant secondary metabolism glycosyltransferases: the emerging functional analysis. Trends Plant Sci. 2005;10(11):542–9.
26. Khorolragchaa A, Kim Y-J, Rahimi S, Sukweenadhi J, Jang M-G, Yang D-C. Grouping and characterization of putative glycosyltransferase genes from Panax ginseng Meyer. Gene. 2014;536(1):186–92.
27. Lorenzo C, Malnoy M, Goremykin V, Nikiforova S, Martens S. A genome-wide phylogenetic reconstruction of family 1 UDP-glycosyltransferases revealed the expansion of the family during the adaptation of plants to life on land. Plant J. 2012;69(6):1030–42.
28. Nair PC, Chau N, McKinnon RA, Miners JO. Arginine-259 of UGT2B7 confers UDP-sugar selectivity. Mol Pharmacol. 2020;98(6):710–8.
29. Xiao X, Lu Q, Liu R, Gong J, Gong W, Liu A, Ge Q, Li J, Shang H, Li P, et al. Genome-wide characterization of the UDP-glycosyltransferase gene family in upland cotton. 3 Biotech. 2019;9(12):453–453.
30. Rehman HM, Nawaz MA, Shah ZH, Ludwig-Müller J, Chung G, Ahmad MQ, Yang SH, Lee SI. Comparative genomic and transcriptomic analyses of Family-1 UDP glycosyltransferase in three Brassica species and Arabidopsis indicates stress-responsive regulation. Sci Rep. 2018;8(1):1875.
31. Wang D, Wang J, Shi Y, Li R, Fan F, Huang Y, Li W, Chen N, Huang L, Dai Z. Elucidation of the complete biosynthetic pathway of the main triterpene glycosylation products of Panax notoginseng using a synthetic biology platform. Metab Eng. 2020;61:131–40.
32. Liu Y, Tikunov Y, Schouten RE, Marcelis LFM, Visser RGF, Bovy A. Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: a review. Front Chem. 2018;6:52–52.
33. Carolina E-P, Billet K, Lanoue A, De Craene J-O, Glévarec G, Pichon O, Clastre M, Courdavault V, St-Pierre B et al. Giglioli-Guivarc'h N : Genome-wide identification and biochemical characterization of the UGT88F subfamily in Malus x domestica Borkh. Phytochemistry 2019, 157:135–144.
34. Jochen B, Jaffé FW, Takos AM, Walker AR, Robinson SP. The grapevine transcription factor VvMYBPA1 regulates proanthocyanidin synthesis during fruit development. Plant Physiol. 2007;143(3):1347–61.
35. Rehman HM, Khan UM, Nawaz S, Saleem F, Ahmed N, Rana IA, Atif RM, Shaheen N, Seo H. Genome Wide Analysis of Family-1 UDP Glycosyltransferases in Populus trichocarpa Specifies Abiotic Stress Responsive Glycosylation Mechanisms. Genes 2022, 13(9).
36. Chen Z, Lu X, Li Q, Li T, Zhu L, Ma Q, Wang J, Lan W, Ren J. Systematic analysis of MYB gene family in Acer rubrum and functional characterization of ArMYB89 in regulating anthocyanin biosynthesis. J Exp Bot. 2021;72(18):6319–35.
37. Lee Y, Yoon HR, Paik YS, Liu JR, Chung W-i, Choi G. Reciprocal regulation ofArabidopsis UGT78D2 and BANYULS is critical for regulation of the metabolic flux of anthocyanidins to condensed tannins in developing seed coats. J Plant Biology. 2005;48(4):356–70.
38. Tamura K, Stecher G, Kumar S. Evolution: MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol. 2021;38(7):3022–7.
39. Lim EK, Ashford DA, Hou B, Jackson RG, Bowles DJ. Arabidopsis glycosyltransferases as biocatalysts in fermentation for regioselective synthesis of diverse quercetin glucosides. Biotechnol Bioeng. 2004;87(5):623–31.
40. Wu B, Liu X, Xu K, Zhang B. Genome-wide characterization, evolution and expression profiling of UDP-glycosyltransferase family in pomelo (Citrus grandis) fruit. BMC Plant Biol. 2020;20:1–12.
41. Kondo M, Ikenaka Y, Nakayama SM, Kawai YK, Mizukawa H, Mitani Y, Nomiyama K, Tanabe S, Ishizuka M. Uridine diphosphate-glucuronosyltransferase (UGT) 2B subfamily interspecies differences in carnivores. Toxicol Sci. 2017;158(1):90–100.
42. Li Y, Zhu F-L, Zheng X-W, Hu M-L, Dong C, Diao Y, Wang Y-W, Xie K-Q, Hu Z-L. Comparative population genomics reveals genetic divergence and selection in lotus, Nelumbo nucifera. BMC Genomics. 2020;21:1–13.
43. Wu C, Dai J, Chen Z, Tie W, Yan Y, Yang H, Zeng J, Hu W. Comprehensive analysis and expression profiles of cassava UDP-glycosyltransferases (UGT) family reveal their involvement in development and stress responses in cassava. Genomics. 2021;113(5):3415–29.
44. Wang F, Su Y, Chen N, Shen S. Genome-wide analysis of the UGT gene family and identification of flavonoids in Broussonetia papyrifera. Molecules. 2021;26(11):3449.
45. Song Z, Niu L, Yang Q, Dong B, Wang L, Dong M, Fan X, Jian Y, Meng D, Fu Y. Genome-wide identification and characterization of UGT family in pigeonpea (Cajanus cajan) and expression analysis in abiotic stress. Trees. 2019;33:987–1002.
46. Speeckaert N, El Jaziri M, Baucher M, Behr M. UGT72, a major glycosyltransferase family for flavonoid and monolignol homeostasis in plants. Biology. 2022;11(3):441.
47. Zhang Z, Zhuo X, Yan X, Zhang Q. Comparative genomic and transcriptomic analyses of family-1 UDP glycosyltransferase in Prunus mume. Int J Mol Sci. 2018;19(11):3382.
48. Gao C, Sun Y, Li J, Zhou Z, Deng X, Wang Z, Wu S, Lin L, Huang Y, Zeng W. High light intensity triggered Abscisic Acid Biosynthesis mediates anthocyanin Accumulation in Young leaves of Tea Plant (Camellia sinensis). Antioxidants. 2023;12(2):392.
49. Harmer SL, Hogenesch JB, Straume M, Chang H-S, Han B, Zhu T, Wang X, Kreps JA, Kay SA. Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. Science. 2000;290(5499):2110–3.
50. Tao R, Bai S, Ni J, Yang Q, Zhao Y, Teng Y. The blue light signal transduction pathway is involved in anthocyanin accumulation in 'Red Zaosu'pear. Planta. 2018;248:37–48.
51. Zhang Q, Yang W, Liu J, Liu H, Lv Z, Zhang C, Chen D, Jiao Z. Postharvest UV-C irradiation increased the flavonoids and anthocyanins accumulation, phenylpropanoid pathway gene expression, and antioxidant activity in sweet cherries (Prunus avium L). Postharvest Biology Technol. 2021;175:111490.
52. Wang L, Sang W, Xu R, Cao J. Alteration of flesh color and enhancement of bioactive substances via the stimulation of anthocyanin biosynthesis in 'Friar'plum fruit by low temperature and the removal. Food Chem. 2020;310:125862.
53. Ji X-H, Zhang R, Wang N, Yang L, Chen X-S. Transcriptome profiling reveals auxin suppressed anthocyanin biosynthesis in red-fleshed apple callus (Malus sieversii f. Niedzwetzkyana). Plant Cell Tissue Organ Cult. 2015;123:389–404.
54. Liu Z, Shi M-Z, Xie D-Y. Regulation of anthocyanin biosynthesis in Arabidopsis thaliana red pap1-D cells metabolically programmed by auxins. Planta. 2014;239:765–81.
55. Loreti E, Povero G, Novi G, Solfanelli C, Alpi A, Perata P. Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in Arabidopsis. New Phytol. 2008;179(4):1004–16.

56. El-Kereamy A, Chervin C, Roustan JP, Cheynier V, Souquet JM, Moutounet M, Raynal J, Ford C, Latché A, Pech JC. Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. Physiol Plant. 2003;119(2):175–82.
57. Shan X, Zhang Y, Peng W, Wang Z, Xie D. Molecular mechanism for jasmonate-induction of anthocyanin accumulation in Arabidopsis. J Exp Bot. 2009;60(13):3849–60.
58. Wang Y, Zhen J, Che X, Zhang K, Zhang G, Yang H, Wen J, Wang J, Wang J, He B. Transcriptomic and metabolomic analysis of autumn leaf color change in Fraxinus Angustifolia. PeerJ. 2023;11:e15319.
59. Wen C-H, Lin S-S, Chu F-H. Transcriptome analysis of a subtropical deciduous tree: autumn leaf senescence gene expression profile of formosan gum. Plant Cell Physiol. 2015;56(1):163–74.
60. Li P, Li YJ, Zhang FJ, Zhang GZ, Jiang XY, Yu HM, Hou BK. The Arabidopsis UDP-glycosyltransferases UGT79B2 and UGT79B3, contribute to cold, salt and drought stress tolerance via modulating anthocyanin accumulation. Plant J. 2017;89(1):85–103.
61. Schulz E, Tohge T, Zuther E, Fernie AR, Hincha DK. Flavonoids are determinants of freezing tolerance and cold acclimation in Arabidopsis thaliana. Sci Rep. 2016;6:34027.
62. Yang Q, Zhang Y, Qu X, Wu F, Li X, Ren M, Tong Y, Wu X, Yang A, Chen Y, et al. Genome-wide analysis of UDP-glycosyltransferases family and identification of UGT genes involved in abiotic stress and flavonol biosynthesis in Nicotiana tabacum. BMC Plant Biol. 2023;23(1):204.
63. Zhao M, Jin J, Gao T, Zhang N, Jing T, Wang J, Ban Q, Schwab W, Song C. Glucosyltransferase CsUGT78A14 regulates flavonols accumulation and reactive oxygen species scavenging in response to cold stress in Camellia sinensis. Front Plant Sci. 2019;10:1675.
64. Shi MZ, Xie DY. Biosynthesis and metabolic engineering of anthocyanins in Arabidopsis thaliana. Recent Patents Biotechnol. 2014;8(1):47–60.
65. Fukuchi-Mizutani M, Okuhara H, Fukui Y, Nakao M, Katsumoto Y, Yonekura-Sakakibara K, Kusumi T, Hase T, Tanaka Y. Biochemical and molecular characterization of a novel UDP-glucose: anthocyanin 3′-O-glucosyltransferase, a key enzyme for blue anthocyanin biosynthesis, from Gentian. Plant Physiol. 2003;132(3):1652–63.

Publisher's note