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Research Article

Transcriptome-based identification of MYB transcription factors associated with flavonoid biosynthesis under LED light in *Astragalus membranaceus* (Fisch.) Bunge

Ji W. SEO[1], Hong J. CHOI[1], Jiu PARK[1], Won H. CHOI[1], Eun S. SEONG[2*]

[1]*Kangwon National University, Interdisciplinary Program in Smart Science, Chuncheon, 24341, Republic of Korea; seojiwon789@kangwon.ac.kr; chj001210@kangwon.ac.kr; parkjiu@kangwon.ac.kr; h8168084@kangwon.ac.kr*
[2]*Kangwon National University, Department pf Applied of Plant Sciences, Division of Bioresource Sciences, Chuncheon, 24341, Republic of Korea; esseong@kangwon.ac.kr (*corresponding author)*

Abstract

Astragalus membranaceus, valued for its health benefits due to flavonoids, saponins, and polysaccharides, has been studied mainly for its roots. However, the regulatory role of MYB transcription factors (TFs) in flavonoid biosynthesis under different light conditions remains largely unexplored. In this study, sterile plants were cultured for six weeks under white, red, and blue light. Transcriptome analysis identified MYB genes, which were further characterized by sequence alignment, conserved domain searches, and phylogenetic analysis with *AtMYB* and *GmMYB176*. Gene structures, conserved motifs, and cis-regulatory elements were examined using bioinformatics tools. Expression levels of selected genes were validated by real-time PCR using the $2^{-\Delta\Delta Ct}$ method. Two candidate genes, one *R2R3-MYB* and one *R1-MYB*, were found to be involved in flavonoid biosynthesis. Both showed high expression under white light, with *AmMYB12* exhibiting nearly 20-fold higher expression compared with other conditions. Motif and promoter analyses indicated the presence of multiple MYB-binding sites, suggesting strong regulatory potential. Together, these results indicate that *AmMYB12* may play a critical role in light-regulated flavonoid biosynthesis. This study provides essential data for functional analysis of MYB TFs and enhances understanding of molecular mechanisms underlying flavonoid accumulation in *A. membranaceus* in response to artificial light.

Keywords: *Astragalus membranaceus*; flavonoid biosynthesis; LED light; MYB transcription factors; transcriptome data

Introduction

Astragalus membranaceus B., commonly known as Huangqi, comprises the dried roots of *A. membranaceus* (Fisch.) Bge. or *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao, which are

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predominantly found in northern China, Mongolia, and Korea (Chen *et al.,* 2023). *Astragalus membranaceus* is a perennial herb, an annual stem herb, and a small shrub that can grow to a height of 150–200 cm. Moreover, the flowers of this genus belong to the Fabaceae family, and their fruits contain kidney-shaped seeds (Salehi *et al.,* 2021).

Cytogenetic analyses have shown that *A. membranaceus* is diploid with 2n = 16 (x = 8), consistent with other members of the genus (Xing and Yin, 1984; Konichenko *et al.*, 2014; Dong *et al*., 2024). While artificial tetraploids (2n = 32) can be induced in vitro by colchicine or oryzalin (Chen *et al.,* 2007; Zhang *et al*., 2024), natural and cultivated populations remain diploid. In terms of reproduction, *A. membranaceus* is predominantly insect-mediated and cross-pollinated, although limited self-pollination may occur under restricted pollinator conditions (Qiu *et al.,* 2001; DeBonis and Crone, 2011).

The dried root of *A. membranaceus* (Astragali Radix) is rich in various bioactive compounds, including flavonoids, saponins, and polysaccharides, and has been consumed for more than 2,000 years owing to its health-promoting effects (Durazzo *et al.,* 2021; Cui *et al.,* 2022). The primary bioactive isoflavones extracted from *A. membranaceus*, namely formononetin and calycosin, contribute to its therapeutic efficacy and pharmacological activity (Hu *et al.,* 2023). Traditionally, *A. membranaceus* has been used to treat chronic fatigue, wounds, anemia, loss of appetite, debility, fever, multiple allergies, uterine bleeding, and uterine prolapse (Li *et al.,* 2020; Lee *et al.,* 2021; Gong *et al.,* 2021; Qader *et al.,* 2021; Akbari *et al.,* 2022; Du *et al.,* 2022; Ghasemian *et al.,* 2022; D'Avino *et al.,* 2023; Ullah *et al.,* 2023; Wei *et al.,* 2023). Moreover, contemporary pharmacological studies have indicated that *A. membranaceus* and its active constituents have strong antitumor activity and enhance host immune functions (Li *et al.,* 2020).

Light plays a crucial role in plant physiology, functioning as an energy source for carbon fixation during photosynthesis and as a signal that activates and regulates many key processes related to plant growth and development (Paradiso and Proietti 2022). The important components of light conditions include the light quality (wavelength), light quantity (intensity), photoperiod (duration), and direction (Zhang *et al.,* 2020). Light also serves as a key environmental signal that regulates gene expression through photoreceptor-mediated signaling pathways. For instance, light perception by phytochromes and cryptochromes triggers transcriptional reprogramming of genes associated with growth, stress responses, and secondary metabolism (Jiao *et al.,* 2007). Light-emitting diodes (LEDs), with narrow emission spectra available in various colors, enable the creation of almost any spectrum required for research (Kochetova *et al.,* 2023). Further, they represent a more promising lighting technology than traditional lighting for plant photobiology research (Wu *et al.,* 2020). Specific LED lights significantly affect plant morphogenesis, and different LED lights affect the yields of primary and species-specific secondary metabolites in plants (Xie *et al.,* 2020). For example, in *Arabidopsis thaliana*, red light increases the leaf area growth, biomass, and net photosynthetic rate, whereas blue light enhances the leaf area growth and carotenoid and anthocyanin contents (Yavari *et al.,* 2021). Blue light also increases secondary metabolite accumulation (Xue *et al.,* 2021). Meanwhile, combined red and blue light influence the antioxidant capacity and phenol and flavonoid contents in lettuce, depending on the light intensity, and increase the anthocyanin content and productivity in *Eruca sativa* (Mill) Thell (Pennisi *et al.,* 2020; Veremeichik *et al.,* 2023). In addition, yellow light positively affects flavonoid concentrations in *Epimedium pseudowushanense* (Yang *et al.,* 2019). Moreover, blue light most effectively promotes the accumulation of phytochemicals, including isoflavonoids such as calycosin and formononetin, as well as astragaloside IV and astragaloside I, in the roots of *A. membranaceus* (Fisch.) Bunge (Gai *et al.,* 2023).

MYB transcription factors (TFs) play crucial roles in plant growth, development, metabolism, and responses to environmental stresses and hormone signaling (Li *et al.,* 2023). These include a group of paneukaryotic TFs characterized by a highly conserved N-terminal DNA-binding domain repeat (R) and a variable C-terminal regulatory region (Wu *et al.,* 2022). Tryptophan residues in the DNA-binding domain

promote stability and form a helix-turn-helix structure (Thakur and Vasudev, 2022). Based on the number of domain repeats, MYB TFs are classified into 1R-MYB (with one or two separate repeats), 2R-MYB (R2R3-MYB with two adjacent repeats), 3R-MYB (with three adjacent repeats), and 4R-MYB (with four adjacent repeats) types (Song *et al.,* 2024). The R2R3 type is the most abundant and is thought to have evolved from the 3R type (R1R2R3) through the loss of the R1 repeat (Abubakar *et al.,* 2022).

The R2R3-MYB TFs are essential regulators of flavonoid biosynthesis in all angiosperm species (Albert *et al.,* 2018). Flavonoid biosynthesis has been studied in many plant species, with various TFs, including MYB, basic helix-loop-helix (bHLH), basic leucine zipper (bZIP), WD40, and zinc finger proteins, involved in the process (Zhao *et al.,* 2022). In *A. thaliana*, *AtMYB11*, *AtMYB12*, and *AtMYB111* are classified into subgroup 7 (S7), which is related to flavonol biosynthesis, with *AtMYB12* functioning primarily in the roots and *AtMYB111* in the cotyledons (Stracke *et al.,* 2007; Dong *et al.,* 2024). *MrMYB12* overexpression in *Morella rubra* significantly increases the flavonol content and induces the expression of *NtCHS*, *NtF3H*, and *NtFLS* in transgenic tobacco leaves and flowers (Cao *et al.,* 2021). Moreover, transient *DzMYB1* expression in *Durio zibethinus* increases the flavonoid content in Micro-Tom fruits, regulating flavonoid biosynthesis through interactions with the promoters of various flavonoid biosynthetic genes and their transcriptional activation (Weerawanich *et al.,* 2024). In *Ziziphus* Mill, *MYB44* is involved in the flavonoid biosynthetic pathway during fruit coloration (Muhammad *et al.,* 2023). Further, in *A. thaliana*, *MYB21* and its homologs, *MYB24* and *MYB57*, belonging to subgroup 19, regulate *FLS1* gene expression and promote flavonol biosynthesis (Zhang *et al.,* 2021). In *Glycine max*, the R1-MYB protein encoded by *GmMYB176* regulates expression of the *CHS8* gene and participates in isoflavonoid biosynthesis (Yi *et al.,* 2010).

Even though there have been various studies on *A. membranaceus*, most have focused on the underground parts, and no studies have reported the involvement of MYB TFs in flavonoid biosynthesis in this species under different light conditions. In our previous study, we performed transcriptome analysis using the whole plants of *A. membranaceus* treated with artificial light and registered the data in NCBI (*https://www.ncbi.nlm.nih.gov/sra?linkname=bioproject_sra_all&from_uid=865476*). In this study, we selected one candidate gene each from R2R3-MYB and R1-MYB TFs using the transcriptome data. We performed exon-intron, conserved motif, and *cis*-element analyses using *A. thaliana* and *G. max* as model plants, and compared the gene expression levels of candidate MYB TFs. This study provides fundamental data for the functional analysis of MYB TFs related to flavonoid biosynthesis in whole plants of *A. membranaceus* under different light conditions.

Materials and Methods

Sample preparation and LED treatment

The *A. membranaceus* seeds used in this study were purchased from KS Seed Co. (Incheon, Korea). To obtain sterile *A. membranaceus* plants, seeds were treated with 70% ethanol for 1 min, followed by shaking for 5 min in 3% NaClO. The seeds were then washed three times with sterile distilled water and germinated on 1MS medium for 6 weeks (Figure 1a). The LED treatments used included white light (continuous spectrum, 0.56 μmol/m²/s), red light (631 nm, 2.00 μmol/m²/s), and blue light (464 nm, 1.32 μmol/m²/s) (Figure 1b). The light source was positioned 45 cm from the top of the culture vessel, and the area of the culture vessel was 11 cm. The light cycle was set to 16 h of light and 8 h of darkness. Wavelength measurements were performed for each light source using a PG200N light meter (United Power Research Technology Co., Zhunan Township, Taiwan). After cultivation, the plants were immediately placed in liquid nitrogen, transferred to −80 °C, and stored for subsequent experiments.

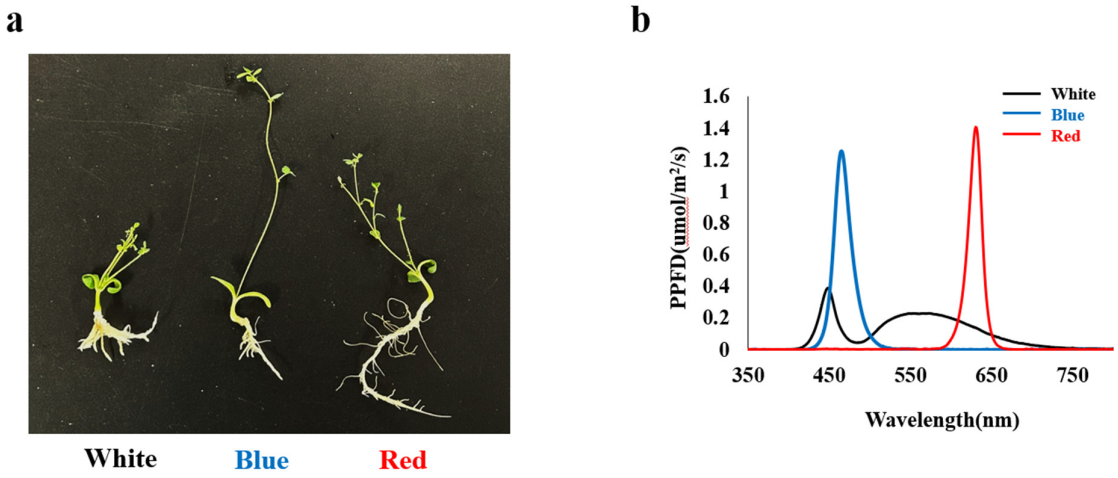


Figure 1. Morphological characteristics of *A. membranaceus* seedlings under different types of LED light. (a); The whole plant morphology. (b); Wavelength of white, blue, red LED lights

Identification of AmMYB genes

The transcriptome data of *A. membranaceus* treated with artificial light sources were retrieved from files previously deposited in NCBI (*https://www.ncbi.nlm.nih.gov/sra?linkname=bioproject_sra_all&from_uid=865476*). Sequences for the *AtMYB* genes from *A. thaliana* and *GmMYB176* from *G. max* were downloaded from PlantTFDB (*https://planttfdb.gao-lab.org/family.php?sp=Ath&fam=MYB*) and NCBI (*https://www.ncbi.nlm.nih.gov/protein/NP_001236048.2*), respectively. To identify the *R2R3-MYB* and *R1-MYB* domains of *MYB* genes, all *MYB* genes were analyzed using the PlantTFDB TF prediction program (*https://planttfdb.gao-lab.org/prediction.php*), the NCBI Conserved Domain Database (*https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi*), and the SMART database (*http://smart.embl-heidelberg.de/*).

Multiple sequence alignment and classification of AmMYB genes through phylogenetic analysis

Phylogenetic trees were constructed using ClustalW default settings for alignment and the general reversible chloroplast model (cpREV) using the maximum likelihood method in MEGA11 (Tamura *et al.,* 2021). Phylogenetic trees were generated for 36 R2R3-MYB and 30 R1-MYB proteins using *AtMYB* and *GmMYB176* as references. Tree visualization was performed using the Interactive Tree of Life tool.

AmMYB gene structure and cis-acting element analysis

The intron structures of the candidate *AmMYB* genes were constructed using the Gene Structure Display Server (*https://gsds.gao-lab.org/*). Conserved motifs in *AmMYB* genes were predicted using the multiple EM for motif elicitation (MEME) program with up to 10 motifs (*https://meme-suite.org/meme/tools/meme*). *Cis*-acting elements in the promoters of *AmMYB* and flavonoid biosynthesis genes were predicted using PlantCARE (*https://bioinformatics.psb.ugent.be/webtools/plantcare/html/)*, and both results were visualized using TBtools (Chen *et al.,* 2020).

Real-time PCR analysis of candidate genes

Total RNA was extracted from in vitro *A. membranaceus* samples using TRIzol reagent (Invitrogen Scientific, Inc., USA). The RNA purity was determined using a Microvolume Spectrophotometer (Keen

Innovative Solutions). cDNA synthesis was conducted using PrimeScript™ RT Master Mix (Perfect Real Time; Takara Korea Biomedical Inc., Seoul, Republic of Korea). For real-time PCR analysis, a 25 μL mixture was prepared using TB Green® Premix Ex Taq™ II (Tli RNaseH Plus; Takara Korea Biomedical Inc., Seoul, Republic of Korea), and analysis was conducted with the CronoSTAR™ 96 Real-Time PCR System (Takara Korea Biomedical Inc., Seoul, Republic of Korea). The PCR conditions were set as follows: initial denaturation at 95 °C for 30 s, followed by two-step amplification (denaturation at 95 °C for 5 s and annealing at 60 °C for 30 s) for 40 cycles. A melting step for the melting curve was performed at 95 °C for 1 min, 60 °C for 15 s, and 98 °C for 5 s. Primers for the reference genes were designed using Primer3Plus (*https://www.primer3plus.com/*) (Table 1). Gene expression levels were determined using Ct values and calculated based on the $2^{-\Delta\Delta Ct}$ method.

Table 1 Reference gene primers used in real-time PCR

Number	Name	Sequence (5' to 3')	Gene ID (Tm, band size)
1	AmMYB12-F	ggtgggtgttggtgaggaaa	Gene_138420T (60.1 °C, 186bp)
2	AmMYB12-R	acactctcccagtcccagtt	
3	AmMYBS3-F	ctgaagccaatcccggtcat	Gene_032600T (60 °C, 154bp)
4	AmMYBS3-R	atgctgactgtcttgagggc	

Statistical analysis

All experiments were performed in triplicate, and significance testing was conducted using IBM SPSS Statistics 26 (SPSS, Armonk, NY, USA). Mean comparison was achieved using Duncan's Multiple Range Test ($P < 0.05$).

Results

Identification and analysis of MYB TFs in A. membranaceus

To identify the R2R3-MYB and R1-MYB genes in *A. membranaceus* treated with different light sources, we used the PlantTFDB TF prediction program, NCBI CDD program, and SMART database to analyze the transcriptome data of *A. membranaceus* registered in NCBI. In total, 36 R2R3-MYB and 30 R1-MYB genes were identified (Table 2 and 3).

Table 2. Information of R2R3-MYB transcription factors in *A. membranaceus*

Reference ID	Description	DESeq Normalization			Blue vs White	Blue vs Red
		Blue	Red	White	log_2FoldChange	log_2FoldChange
Gene_008390T	Transcription factor MYB44	107.66	109.23	112.24	0.06	0.02
Gene_019830T	transcription factor MYB61	71.13	131.26	148.11	1.06	0.88
Gene_025890T	Transcription factor MYB86	73.05	108.35	101.83	0.48	0.57
Gene_032420T	Transcription factor MYB86	64.40	174.42	108.77	0.76	1.44
Gene_033330T	transcription factor MYB102-like	39.41	29.07	56.70	0.52	-0.44
Gene_057070T	transcription factor MYB106-like	0.96	51.97	18.51	4.27	5.76
Gene_061100T	transcription factor MYB83-like	73.05	132.14	81.00	0.15	0.86
Gene_064490T	transcription factor MYB61	49.98	52.85	33.56	-0.57	0.08
Gene_083640T	transcription factor MYB1	75.94	75.76	54.38	-0.48	0.00
Gene_096120T	Transcription factor MYB44	4438.85	4474.15	4317.24	-0.04	0.01
Gene_121270T	transcription factor MYB108-like	148.99	84.57	70.58	-1.08	-0.82
Gene_121530T	Transcription factor MYB36	24.99	23.78	30.09	0.27	-0.07
Gene_138420T	Transcription factor MYB12	76.90	121.57	112.24	0.55	0.66
Gene_153990T	transcription factor MYB78-like	27.87	17.62	15.04	-0.89	-0.66
Gene_193810T	transcription factor MYB33	5.77	7.05	5.79	0.00	0.29
Gene_197070T	transcription factor MYB14-like	56.71	44.05	52.07	-0.12	-0.36
Gene_197720T	Transcription factor MYB44	23.07	24.67	8.10	-1.51	0.10
Gene_199710T	transcription factor MYB88	154.75	161.21	121.50	-0.35	0.06
Gene_212260T	transcription factor MYB52	21.15	30.83	37.03	0.81	0.54

Gene_229540T	transcription factor MYB41-like	25.95	29.95	38.19	0.56	0.21
Gene_231550T	transcription factor MYB4-like	1.92	0.00	3.47	0.85	-Inf
Gene_250860T	transcription factor MYB16-like	24.99	37.00	6.94	-1.85	0.57
Gene_273310T	transcription factor MYB46-like	62.48	59.02	35.87	-0.80	-0.08
Gene_273400T	transcription factor MYB62-like	14.42	1.76	11.57	-0.32	-3.03
Gene_284900T	transcription factor MYB16-like	84.59	137.42	96.04	0.18	0.70
Gene_293270T	transcription factor MYB61	25.95	17.62	2.31	-3.49	-0.56
Gene_295700T	transcription factor MYB44-like	543.08	341.79	436.24	-0.32	-0.67
Gene_301440T	transcription factor MYB20-like	28.84	21.14	17.36	-0.73	-0.45
Gene_337370T	transcription factor MYB111-like	45.18	50.21	33.56	-0.43	0.15
Gene_337580T	transcription factor MYB33	43.25	34.36	45.13	0.06	-0.33
Gene_360960T	transcription factor MYB41	8.65	2.64	26.61	1.62	-1.71
Gene_376100T	transcription factor MYB86	3.84	7.93	11.57	1.59	1.04
Gene_401650T	transcription factor MYB16-like	68.25	81.04	60.17	-0.18	0.25
Gene_425760T	transcription factor MYB41-like	9.61	7.05	5.79	-0.73	-0.45
Gene_431760T	transcription factor MYB86	25.95	32.59	28.93	0.16	0.33
Gene_443460T	transcription factor MYB1-like	0.00	0.00	6.94	Inf	NA

Table 3. Information of R1-MYB transcription factors in *A. membranaceus*

Reference ID	Description	DESeq Normalization			Blue vs White	Blue vs Red
		Blue	Red	White	log2FoldChange	log2FoldChange
Gene_022080T	myb family transcription factor PHL7-like	263.37	214.94	179.35	-0.29	-0.55
Gene_032600T	transcription factor MYBS3	0.96	5.29	4.63	2.46	2.27
Gene_040380T	putative Myb family transcription factor	29.80	14.98	34.71	-0.99	0.22
Gene_050840T	myb family transcription factor PHL5-like	45.18	8.81	8.10	-2.36	-2.48
Gene_064910T	myb family transcription factor EFM-like	189.36	229.92	109.93	0.28	-0.78
Gene_083770T	transcription factor MYB1R1-like	35.56	14.09	8.10	-1.34	-2.13
Gene_093240T	myb family transcription factor PHL5-like	86.51	47.57	65.96	-0.86	-0.39
Gene_098550T	putative Myb family transcription factor	47.10	38.76	50.91	-0.28	0.11
Gene_103670T	transcription factor MYBS1	200.89	208.78	231.43	0.06	0.20
Gene_105350T	myb family transcription factor EFM-like	186.47	161.21	150.43	-0.21	-0.31
Gene_106630T	myb family transcription factor PHL7-like	18.26	31.71	9.26	0.80	-0.98
Gene_109740T	transcription factor MYBS3-like	63.44	79.28	48.60	0.32	-0.38
Gene_110870T	myb family transcription factor PHL8-like	73.05	13.21	53.23	-2.47	-0.46
Gene_114080T	Myb family transcription factor EFM	100.93	148.87	76.37	0.56	-0.40
Gene_157170T	putative Myb family transcription factor	35.56	73.12	31.24	1.04	-0.19
Gene_174490T	myb family transcription factor PHL7-like	27.87	29.95	39.34	0.10	0.50
Gene_200770T	transcription factor MYBC1-like	979.47	1272.04	1203.41	0.38	0.30
Gene_231630T	myb family transcription factor PHL7	1693.65	1562.74	1495.01	-0.12	-0.18
Gene_246100T	Myb family transcription factor EFM	5.77	3.52	0.00	-0.71	-Inf
Gene_260130T	myb family transcription factor PHL8-like	3.84	0.00	2.31	-Inf	-0.73
Gene_276850T	transcription factor MYBS3	70.17	41.40	46.29	-0.76	-0.60
Gene_280130T	myb family transcription factor APL	17.30	29.07	24.30	0.75	0.49
Gene_287830T	myb family transcription factor EFM-like	13.46	7.05	3.47	-1.95	-0.93
Gene_352120T	transcription factor MYB1R1-like	864.12	1082.64	741.72	-0.22	0.33
Gene_361540T	myb-like transcription factor family protein	680.53	662.45	705.85	0.05	-0.04
Gene_365200T	myb family transcription factor EFM-like	129.76	119.80	75.21	-0.79	-0.12
Gene_369550T	putative Myb family transcription factor	24.99	20.26	30.09	0.27	-0.30
Gene_372520T	putative Myb family transcription factor	19.22	10.57	25.46	0.41	-0.86
Gene_407870T	Myb family transcription factor PHL5	0.96	14.98	9.26	3.96	3.27

Phylogenic trees and analysis of candidate AmMYB genes

Based on the 36 R2R3-MYB and 30 R1-MYB genes, phylogenetic trees were constructed using *AtMYB* genes and *GmMYB176*. *Arabidopsis thaliana* R2R3-MYB genes were divided into 32 subgroups, among which *A. membranaceus* R2R3-MYB genes were determined to belong to 16 subgroups, with five genes (Gene_231500T, Gene_229540T, Gene_360960T, Gene_425760T, and Gene_273400T) that did not belong to any group (Figure 2a). Many studies have shown that R2R3-MYB genes are involved in flavonoid biosynthesis in *A. thaliana*, with genes belonging to subgroups S4, S5, S6, and S7. Among the constructed phylogenetic trees, no *A. membranaceus* R2R3-MYB genes clustered with the S4 and S5 subgroups, whereas

one gene clustered with the S6 subgroup, which is known to be involved in anthocyanin biosynthesis, which is not a main substance in *A. membranaceus*; therefore, it was excludedFinally, to select the MYB gene involved in flavonoid biosynthesis in *A. membranaceus* treated with an artificial light source, Gene_138420T was selected as the final gene by considering the DESeq normalization value and log_2fold change value among the genes clustered with *AtMYB11, AtMYB12*, and *AtMYB111* of S7, which are known to be involved in flavonol biosynthesis in *A. thaliana* (Table 2). Additionally, because the major marker compounds of *A. membranaceus*, calycosin and formononetin, belong to the isoflavonoid family, we constructed a second phylogenetic tree using the R1-MYB gene *GmMYB176*, known to be involved in isoflavonoid biosynthesis in *G. max*, and the R1-MYB gene of *A. membranaceus* (Figure 3a). As a result, a total of three genes (Gene_032600T, Gene_109740T, Gene_276850T) were confirmed to be clustered with *GmMYB176*, and Gene_032600T was selected as the final gene considering the DESeq normalization value and log_2foldchange value (Table 3). Finally, two candidate *AmMYB* genes were selected, one R2R3-MYB gene and one R1-MYB gene, which are expected to be involved in flavonoid biosynthesis in *A. membranaceus* treated with an artificial light source.

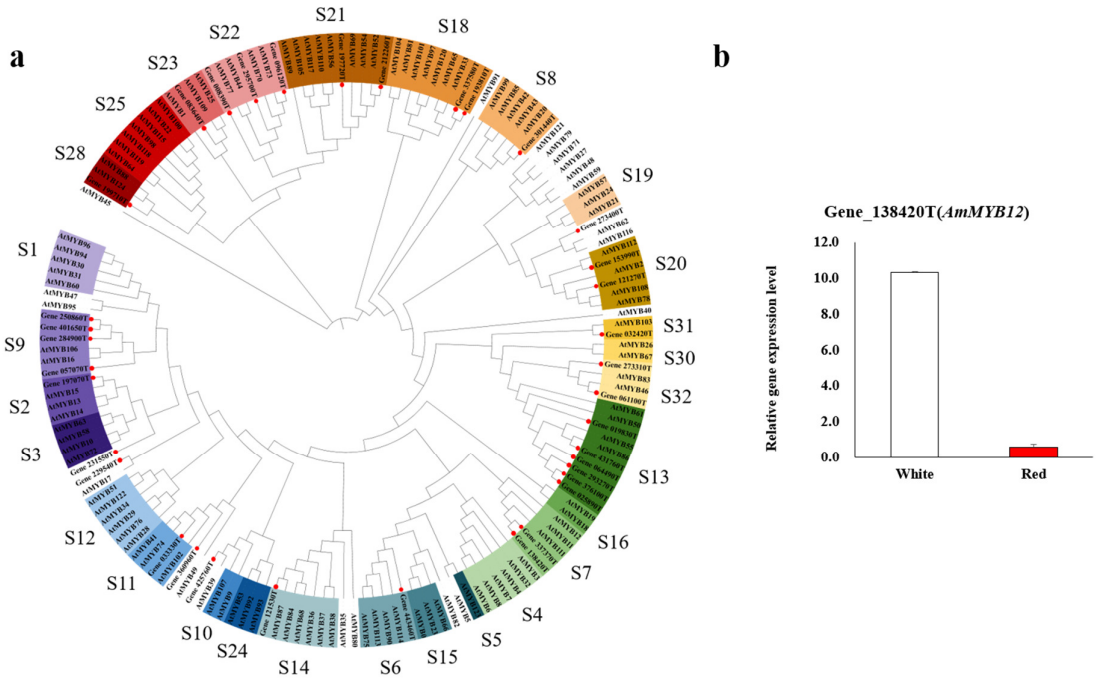


Figure 2. Phylogenic analysis and relative gene expression of R2R3-MYB genes. (a) Phylogenic tree of R2R3-MYB genes in *A. membranaceus* along with R2R3-MYB genes from *A. thaliana*; (b) Relative gene expression level of the candidate R2R3-MYB gene expected to be involved in flavonoid biosynthesis in *A. membranaceus*. Phylogenic tree was created using general reversible chloroplast model (cpREV) option

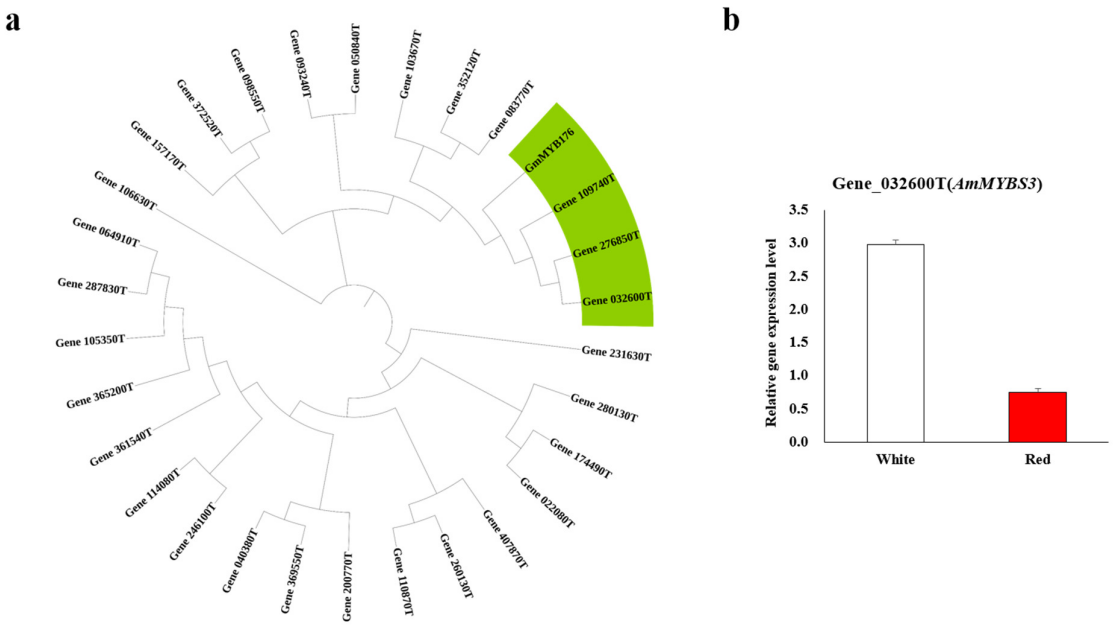


Figure 3. Phylogenic analysis and relative gene expression of R1-MYB genes. (a) Phylogenic tree of R1-MYB genes in *A. membranaceus* along with *GmMYB176* which is R1-MYB protein in *G. max*; (b) Relative gene expression level of the candidate R1-MYB gene expected to be involved in flavonoid biosynthesis in *A. membranaceus*. Phylogenic tree was created using general reversible chloroplast model (cpREV) option

Analysis of AmMYB structures and encoded protein motifs

To gain a comprehensive understanding of the conserved domains in *AmMYB* genes, we first used the gene structure display server to examine the coding sequence (CDS) regions based on the model plants and candidate *AmMYB* genes (Figure 4a). The results confirmed that the CDS regions of the R2R3-MYB candidate genes of *A. membranaceus*, Gene_138420T, *AtMYB12*, *AtMYB11*, and *AtMYB111*, are distributed between approximately 113 and 1365 bp. In addition, the CDS regions of the *A. membranaceus* R1-MYB candidate genes, Gene_032600T and the *GmMYB176* gene of *G. max* were analyzed and found to be located between 134 and 1226 bp; however, intron regions were not identified for either the R2R3-MYB or R1-MYB genes. To identify conserved domains within the CDS regions, we conducted an analysis using the MEME program. E-values greater than 0.05 were excluded to select motifs with statistically significant differences, resulting in the identification of five conserved motifs in both R2R3-MYB and R1-MYB genes. As shown in Figure 4b, the R2R3-MYB genes from *A. membranaceus* and *A. thaliana*, except for *AtMYB111*, contained five motifs, with motifs 1, 2, and 3 encoding the MYB DNA-binding domain, whereas motifs 4 and 5 were determined to be nonfunctional (Table 4). An analysis of the conserved motifs in *GmMYB176* from *G. max* and the R1-MYB candidate genes from *A. membranaceus* revealed that they shared motifs 1, 4, and 5, with *GmMYB176* lacking motifs 2 and 3 (Figure 5b). Motif 1 encodes the MYB DNA-binding domain, whereas the other motifs were determined to be nonfunctional (Table 4). A comparison of these results with the CDS region analysis showed that the MYB DNA-binding domain was located outside the CDS region. To further confirm the motif composition of the conserved domains in the genes, we analyzed them using WebLogo. The results revealed the presence of conserved amino acids, with the R2 domain of R2R3-MYB genes consisting of motifs 1 and 3 and the R3 domain consisting of motif 2 (Figure 4c). The R2 domain was found to contain six conserved glycine (G) residues, whereas the R3 domain was determined to contain four conserved glycine (G) and lysine (K) residues. For the *R1-MYB* genes, the R1 domain only consisted of motif 1, and the conserved

amino acids serine (S), histidine (H), alanine (A), glutamine (Q), lysine (K), tyrosine (Y), and phenylalanine (F) were identified as the conserved residues (Figure 5c).

Table 4. Detail characteristics of reference MYB proteins in *A. membranaceus*

Type	Motif	Domain Sequence	Length	Function
R2R3	1	MGRAPCCEKVGJKKGRWTAEEDEILSKYIQSNGEGSWRS LPKNAGLLRCG	50	MYB DNA-binding
	2	KRGNITPEEEDIIVKLHSTLGNRWSLIASHLPGRTDNEIKN YWNSHLSRK	50	MYB DNA-binding
	3	KSCRLRWINYLRSDL	15	MYB DNA-binding
	4	PPKRKGGRTSRSAMKKNK	18	None
	5	FGEPLDPDEZNALVAWF	17	None
R1	1	KKGVPWTEEEHRRFLIGLQKLGKGDWRGIARNYVVTRT PTQVASHAQKYF	50	MYB DNA-binding
	2	MTRRCSHCSNNGHNSRTCP	23	None
	3	MGNLTLSSVHNHSSSLLAPSSANPSSPCCETPHEPEGYLSD DLAHASTFA	20	None
	4	IRQSNATRRKRRSSLFDMAPDM	41	None
	5	GGVKLFGVRLTDGSIIK	15	None

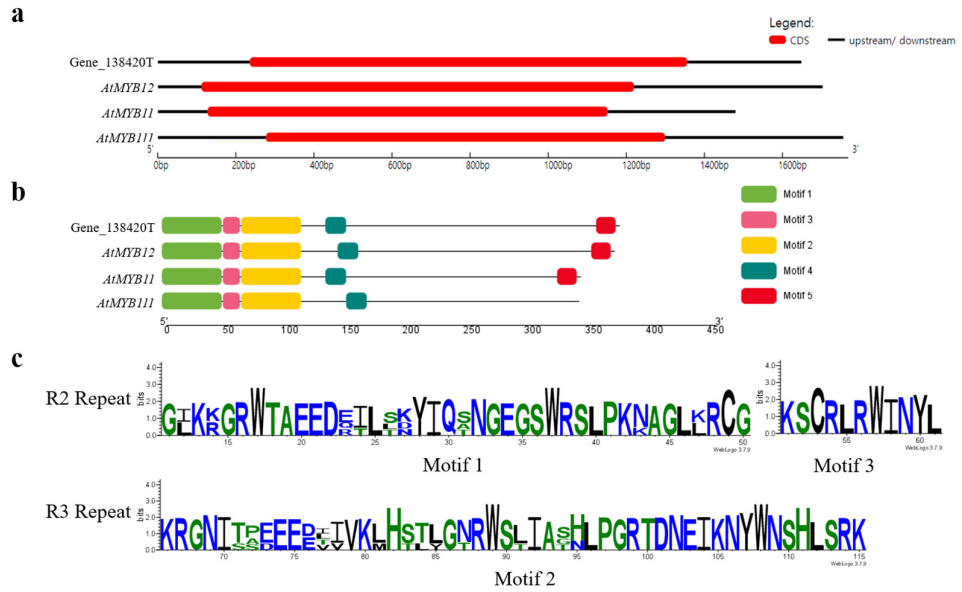
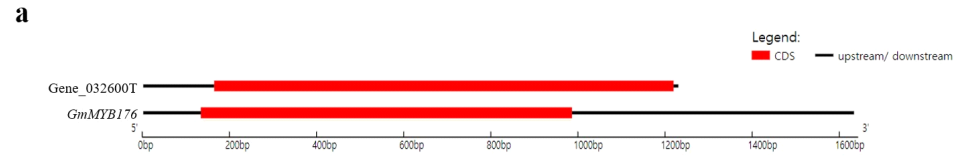


Figure 4. Conserved motif and structure analysis of the R2R3-MYB genes in *A. membranaceus* and *A. thaliana*. (a) CDS regions are shown in red line and up/downstream region in black line, respectively. (b) Groups of R2R3-MYB proteins are highlighted with different colors, and every motif was identified by MEME programThe different colors represent different motifs and position. (c) R2 and R3 repeats of the R2R3-MYB proteins in *A. membranaceus* and *A. thaliana*. English letters indicate amino acid residues and each stack showed the conservation of the sequence



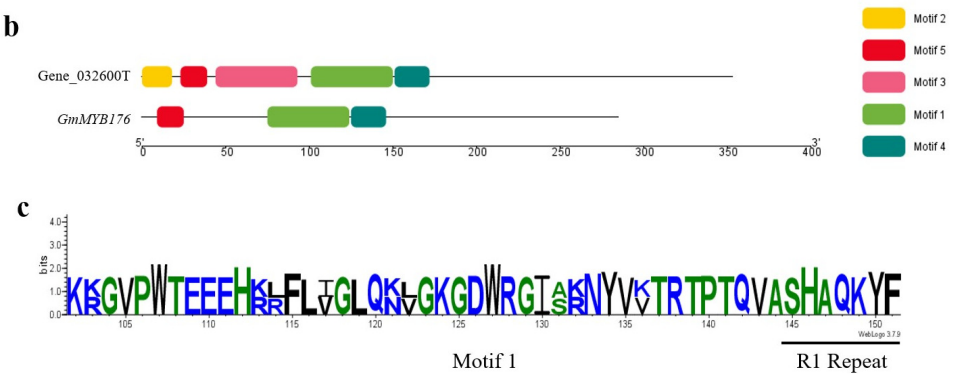


Figure 5. Conserved motif and structure analysis of the R1-MYB genes in *A. membranaceus* and *GmMYB176*. (a) CDS regions are shown in red line and up/downstream region in black line, respectively. (b) Groups of R2R3-MYB proteins are highlighted with different colors, and every motif was identified by MEME program. The different colors represent different motifs and position. (c) R1 repeat of the R1-MYB proteins in *A. membranaceus* and *GmMYB176*. English letters indicate amino acid residues and each stack showed the conservation of the sequence

Cis-acting element analysis of AmMYB and flavonoid biosynthesis genes

Cis-acting elements in gene promoter regions serve as crucial references for predicting the roles of genes in specific biological regulatory processes. Thus, we analyzed *cis*-acting elements in the promoters of R2R3-MYB and R1-MYB in *A. membranaceus* using PlantCARE (Figure 6). We first analyzed the MYB-binding-related *cis*-acting elements that might be involved in flavonoid biosynthesis in the promoters of *A. membranaceus*. This analysis revealed the presence of MBS, MYB-binding sites, a CCAAT-box, and *cis*-acting elements. The presence of these MYB-binding domains in the promoter suggests a potential role in regulating the expression of flavonoid biosynthesis-related genes, such as *AmPAL*, *AmC4H*, *AmCHI*, *Am4CL*, *AmI3'H*, and *AmCHS*. Additionally, since *A. membranaceus* was treated with artificial light, we identified *AmMYB* genes containing light-related *cis*-acting elements, such as the G-box (light response *cis*-acting element), with Gene_032600T (R1-MYB) found to contain these elements. Various other *cis*-acting elements related to hormone responses were also identified, including the abscisic acid-responsive element (ABRE), MeJA-responsive elements (TGACG-motif and CGTCA-motif), salicylic acid-responsive elements (SARE and TCA-element), and the gibberellin-responsive element (TATC-box). These findings suggested that *A. membranaceus* regulates and responds to various hormones. Additionally, *cis*-acting elements involved in anaerobic induction (ARE), and zein metabolism regulation (O2-site) were also present in the *AmMYB* genes.

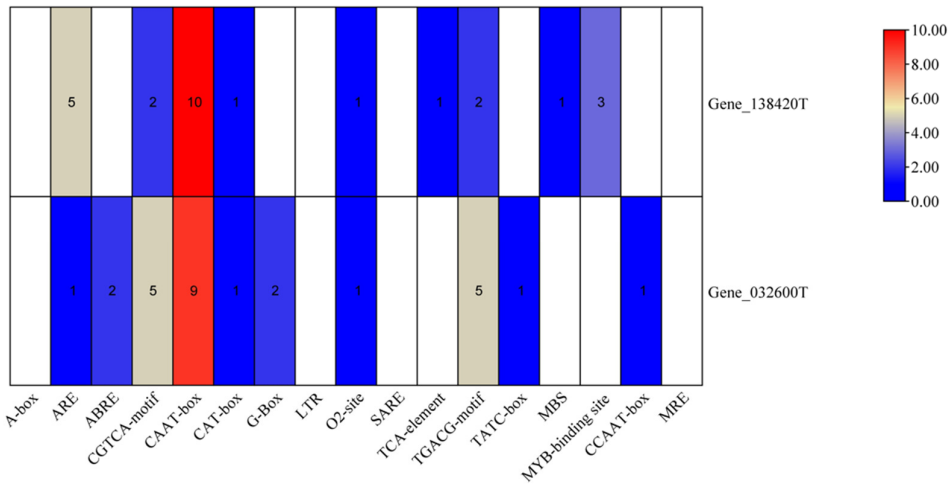


Figure 6. *Cis*-acting elements analysis of *AmMYB* reference genes

Validation of AmMYB and flavonoid biosynthesis gene expression using real-time PCR

The expression levels of the selected candidate MYB genes were confirmed through real-time PCR, and all of them showed high expression levels under white light. In particular, the *AmMYB12* gene showed an mRNA expression level 18 times higher than that under red light (Figure 2b). This suggests that the gene is likely to be involved in formononetin biosynthesis in *A. membranaceus* treated with white light, and this may be related to the result that the content was high under white light as revealed in a previous study.

Discussion

In this study, we aimed to identify MYB TFs related to flavonoid biosynthesis in *A. membranaceus* using transcriptome data from whole plants treated with different artificial light sources. Traditionally, *A. membranaceus* underground parts are used in traditional medicine, whereas the aerial parts are often discarded. Moreover, research on the aerial parts has primarily focused on biological activity or compound contents (Qader *et al.,* 2021; Samuel *et al.,* 2021; Guo *et al.,* 2022). Our study is the first to identify MYB TFs associated with flavonoid biosynthesis in *A. membranaceus* using transcriptome data from the whole plant subjected to different light treatments.

In our previous study, we compared the physiological activity and the content of formononetin (an isoflavone with antioxidant and anti-inflammatory properties), an indicator substance, of *A. membranaceus* treated with white, red, and blue LEDs. When treated with white light, the antioxidant activity and total phenolic content were the highest, and the content of formononetin also showed the maximum value under white light conditions (Seo *et al.,* 2022; Seo *et al.,* 2024). These findings contrast those of studies in which blue light was found to increase the contents of key compounds in the hairy roots of *A. membranaceus*, suggesting that the amount of accumulated substances could vary depending on the plant part treated with different light sources (Gai *et al.,* 2023). Similar variations in secondary metabolite contents and growth patterns have been reported in various plants based on the light wavelength and intensity (Pant *et al.,* 2021; Del-Castillo-Alonso *et al.,* 2021; Le *et al.,* 2022; da Cristina Bungala *et al.,* 2024).

A transcriptome analysis of *A. membranaceus* identified 36 R2R3-MYB and 30 R1-MYB genes (Tables 1 and 2), which are relatively low numbers compared to the numbers of *MYB* genes found in other plant whole-genome analyses. For example, dicotyledons typically have 70-200 R2R3-MYB gene families (Du *et al.,* 2015),

with examples including *A. thaliana* (126), *Oryza sativa* (102), *Zea mays* (157), *Populus trichocarpa* (192), *Camellia sinensis* (122), and *Vitis vinifera* (134) (Stracke *et al.,* 2001; Yanhui *et al.,* 2006; Chai *et al.,* 2014; Wong *et al.,* 2016). Moreover, a recent genome assembly study of *A. membranaceus* var. *mongholicus* might have revealed more *MYB* genes using whole-genome sequencing data (Chen *et al.,* 2023).

We also analyzed the conserved motifs and structures of *MYB* genes in model plants and selected candidate genes. Here, the CDS regions of the candidate genes and those of the model plants were located at similar positions. Both the R2R3-MYB and R1-MYB genes contained five conserved motifs, and although the number of motifs varied among the genes, they were generally uniformly distributed. This indicates that evolutionarily related genes share relatively consistent conserved motifs and perform similar functions, such as MYB-DNA binding.

Several plant *MYB* genes are involved in flavonoid biosynthesis. For example, *FaMYB5* in cultivated strawberries (*Fragaria* × *Ananassa*) increases the expression of genes involved in flavonoid, phenylpropanoid, and lignin biosynthesis (Weerawanich *et al.,* 2024). Further, in chrysanthemum, the overexpression of *CmMYB11* increases flavonol and anthocyanin contents in tobacco (Wang *et al.,* 2023), whereas the overexpression of *CmMYB8* inhibits lignin and flavonoid synthesis (Zhu *et al.,* 2020). *AtMYB11*, *AtMYB12*, and *AtMYB111* from *A. thaliana* and *R2R3-MYB* genes are involved in flavonol synthesis (Stracke *et al.,* 2007), and *GmMYB176* from *G. max* is involved in isoflavonoid synthesis (Yi *et al.,* 2010); therefore, we constructed phylogenetic trees using the R2R3-MYB and R1-MYB genes from *A. membranaceus* transcriptome data (Figure 2a and 3a). We identified two R2R3-MYB genes that clustered with *A. thaliana* S7 and three *R1-MYB* genes that clustered with *GmMYB176*. Promoter analysis of the five *MYB* genes revealed four types of MYB-binding elements, with Gene_138420T (*AmMYB12*) having the most.

Our real-time PCR results showed that *AmMYB12* and *AmMYBS3* exhibit consistent expression patterns, with *AmMYB12* expression being significantly upregulated (Figure 2b). Combining promoter analysis and real-time PCR data, *AmMYB12*, which have the most MYB-binding regions, showed higher expression levels under white light. This suggests that this gene might affect flavonoid biosynthesis in *A. membranaceus* under white light condition.

Light quality plays a critical role in regulating plant secondary metabolism by activating specific signaling pathways. Several studies have identified phytochromes (PHY), cryptochromes (CRY), and HY5 (ELONGATED HYPOCOTYL 5) as key regulators of light-responsive transcriptional networks. Notably, HY5 is a well-established regulator that mediates light responses by directly binding to G-box elements in gene promoters (Gangappa and Botto, 2016). In *Arabidopsis thaliana*, for example, Gangappa and Botto (2016) demonstrated that HY5 coordinates both light and dark responses through regulation of downstream transcription factors. Furthermore, subsequent studies by Gangappa and co-workers have reported its pivotal role in integrating light quality perception with the transcriptional control of secondary metabolism.

Our cis-regulatory element analysis revealed that the promoters of AmMYB12 and AmMYBS3 contain multiple G-box elements, which suggests that these genes may be regulated by light-induced transcription factors such as HY5. Furthermore, previous studies have demonstrated that MYB transcription factors involved in flavonoid biosynthesis (e.g., AtMYB12 in Arabidopsis) are transcriptionally regulated by light signaling components (Stracke *et al*., 2007). These findings suggest a potential regulatory relationship between light quality perception and MYB-mediated control of flavonoid biosynthesis in A. membranaceus.

Although our study does not directly investigate the involvement of PHY, CRY, or HY5 in regulating AmMYB genes, our findings suggest a possible interaction between light quality signaling pathways and MYB transcription factors. Future studies employing ChIP-qPCR to test HY5 binding to the AmMYB12 and AmMYBS3 promoters, as well as functional validation in transgenic systems, could provide further insights into this regulatory mechanism.

Additionally, while we successfully identified candidate MYB transcription factors involved in flavonoid biosynthesis under different light conditions, we did not establish a direct correlation between MYB gene expression and flavonoid accumulation. Future research will focus on quantifying flavonoid content in corresponding plant tissues under identical experimental conditions, thereby validating the functional roles of these MYB genes in flavonoid biosynthesis.

Conclusions

We compared the growth characteristics of *A. membranaceus* treated with artificial light, identified 36 *R2R3-MYB* and 30 *R1-MYB* genes based on transcriptome data. Then we selected candidate genes through phylogenetic tree analysis, DESeq normalization values and log_2foldchange values of MYB genes. The gene structure and conserved motifs of the candidate *AmMYB* genes were confirmed, with both R2R3-MYB and R1-MYB genes containing five motifs. Finally, as a result of measuring the mRNA expression level based on the light source using real-time PCR analysis, the *AmMYB12* gene showed the highest expression level under white light compared to that under red light. This suggests that *AmMYB12* might affect flavonoid biosynthesis in *A. membranaceus* exposed to white light. For more accurate results, future studies should use the recently available whole-genome data of *A. membranaceus* to identify additional *MYB* genes and further investigate their roles. Importantly, our results highlight that AmMYB12 is strongly induced under white light, suggesting its central role in regulating flavonoid biosynthesis in *A. membranaceus*.

Authors' Contributions

Conceptualization: ESS; Data curation: JWS; Formal analysis: JWS, HJC, JP, WHC; Investigation: ESS; Methodology: JWS, HJC, JP, WHC; Project administration: ESS; Resources: ESS; Software: JWS; Supervision: ESS; Validation: JWS; Visualization: JWS; Roles/Writing - original draft: JWS; and Writing - review & editing: ESS.

All authors read and approved the final manuscript.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abubakar AS, Feng X, Gao G, Yu C, Chen J, Chen K, ... Cehn P (2022). Genome wide characterization of R2R3 MYB transcription factor from *Apocynum venetum* revealed potential stress tolerance and flavonoid biosynthesis genes. Genomics 114:110275. *https://doi.org/10.1016/j.ygeno.2022.110275*

Albert NW, Thrimawithana AH, McGhie TK, Clayton WA, Deroles SC, Schwinn KE, ... Davies KM (2018). Genetic analysis of the liverwort *Marchantia polymorpha* reveals that R2R3 MYB activation of flavonoid production in response to abiotic stress is an ancient character in land plants. New Phytologist 218:554-566. *https://doi.org/10.1111/nph.15002*

Akbari F, Azadbakht M, Bagheri A, Vahedi L (2022). In vitro and in vivo wound healing activity of *Astragalus Floccosus Boiss.* (Fabaceae). Advances in Pharmacological and Pharmaceutical Sciences 2022:7865015. *https://doi.org/10.1155/2022/7865015*

Cao Y, Jia H, Xing M, Jin R, Grierson D, Gao Z, ... Li X (2021). Genome-wide analysis of MYB gene family in Chinese bayberry (*Morella rubra*) and identification of members regulating flavonoid biosynthesis. Frontiers in Plant Science 12:691384. *https://doi.org/10.3389/fpls.2021.691384*

Chai G, Wang Z, Tang X, Yu L, Qi G, Wang D, ... Zhou G (2014). R2R3-MYB gene pairs in Populus: evolution and contribution to secondary wall formation and flowering time. Journal of Experimental Botany 65:4255-4269. *https://doi.org/10.1093/jxb/eru196*

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. Molecular Plant 13:1194-1202. *https://doi.org/10.1016/j.molp.2020.06.009*

Chen F, Luo X, Gao W, Liu X, Zheng Y (2007). Induction and characterization of tetraploid *Astragalus membranaceus* plants. Plant Cell Reports 26:641-648.

Chen G, Jiang N, Zheng J, Hu H, Yang H, Lin A, ... Liu H (2023). Structural characterization and anti-inflammatory activity of polysaccharides from *Astragalus membranaceus*. International Journal of Biological Macromolecules 241:124386. *https://doi.org/10.1016/j.ijbiomac.2023.124386*

Chen X, Wang P, Gu M, Lin X, Hou B, Zheng Y, ... Ye N (2021). R2R3-MYB transcription factor family in tea plant (*Camellia sinensis*): Genome-wide characterization, phylogeny, chromosome location, structure and expression patterns. Genomics 113:1565-1578. *https://doi.org/10.1016/j.ygeno.2021.03.033*

Chen Y, Fang T, Su H, Duan S, Ma R, Wang P, ... Zhao M (2023). A reference-grade genome assembly for *Astragalus mongholicus* and insights into the biosynthesis and high accumulation of triterpenoids and flavonoids in its roots. Plant Communications 4:100469. *https://doi.org/10.1016/j.xplc.2022.100469*

Cui L, Ma Z, Wang D, Niu Y (2022). Ultrasound-assisted extraction, optimization, isolation, and antioxidant activity analysis of flavonoids from *Astragalus membranaceus* stems and leaves. Ultrasonics Sonochemistry 90:106190. *https://doi.org/10.1016/j.ultsonch.2022.106190*

da Cristina Bungala LT, Park SU, Van Nguyen B, Lim J, Kim K, Kim JK, ... Yeo HJ (2024). Effect of LED Lights on secondary metabolites and antioxidant activities in red pakchoi baby leaves. ACS omega 9:23420. *https://doi.org/10.1021/acsomega.3c10261*

D'Avino D, Cerqua I, Ullah H, Spinelli M, Di Matteo R, Granato E, ... Daglia M (2023). Beneficial effects of *Astragalus membranaceus* (Fisch.) bunge extract in controlling inflammatory response and preventing asthma features. International Journal of Molecular Sciences 24:10954. *https://doi.org/10.3390/ijms241310954*

Del-Castillo-Alonso MÁ, Monforte L, Tomás-Las-Heras R, Ranieri A, Castagna A, Martínez-Abaigar J, Núñez-Olivera E (2021). Secondary metabolites and related genes in *Vitis vinifera* L. cv. Tempranillo grapes as influenced by ultraviolet radiation and berry development. Physiologia Plantarum 173:709-724. *https://doi.org/10.1111/ppl.13483*

DeBonis A, Crone E (2011). Pollination ecology of *Astragalus filipes* (Fabaceae). Plant Species Biology 26:215-224.

Dong X, Liu X, Cheng L, Li R, Ge S, Wang S, ... Jiang CZ (2024). SlBEL11 regulates flavonoid biosynthesis, thus fine-tuning auxin efflux to prevent premature fruit drop in tomato. Journal of Integrative Plant Biology 66:749-770. *https://doi.org/10.1111/jipb.13627*

Dong J, Wang L, Zhang Y, Liu X, Chen M, Zhao H, Li Q (2024). Advances in cytogenetic and genomic resources of *Astragalus membranaceus*. Frontiers in Plant Science 15:1420512.

Du H, Liang Z, Zhao S, Nan M-G, Tran L-SP, Lu K, ... Li JN (2015). The evolutionary history of R2R3-MYB proteins across 50 eukaryotes: new insights into subfamily classification and expansion. Scientific Reports 5:11037. *https://doi.org/10.1038/srep11037*

Durazzo A, Nazhand A, Lucarini M, Silva AM, Souto SB, Guerra F, ... Santini A (2021). Astragalus (*Astragalus membranaceus* Bunge): botanical, geographical, and historical aspects to pharmaceutical components and beneficial role. Rendiconti Lincei. Scienze Fisiche e Naturali 32:625-642. *https://doi.org/10.1007/s12210-021-01003-2*

Du Y, Wan H, Huang P, Yang J, He Y (2022). A critical review of Astragalus polysaccharides: from therapeutic mechanisms to pharmaceutics. Biomedicine and Pharmacotherapy 147:112654. *https://doi.org/10.1016/j.biopha.2022.112654*

Gai Q-Y, Feng X, Jiao J, Xu X-J, Fu J-X, He X-J, Fu YJ (2023). Blue LED light promoting the growth, accumulation of high-value isoflavonoids and astragalosides, antioxidant response, and biosynthesis gene expression in *Astragalus membranaceus* (Fisch.) Bunge hairy root cultures. Plant Cell Tissue and Organ Culture 153:511-523. *https://doi.org/10.1007/s11240-023-02486-7*

Gangappa SN, Botto JF (2016). The multifaceted toles of HY5 in plant growth and development. Molecular Plant 9:1353-1365. *http://dx.doi.org/10.1016/j.molp.2016.07.002*

Ghasemian Yadegari J, Khudair Khalaf A, Darabi R (2022). Antiparasitic effects and cellular mechanism of *Astragalus maximus* chloroform extract against clinical isolates of *Giardia lamblia*. Research Journal of Pharmacognosy 9:5-13. *https://www.rjpharmacognosy.ir/article_148769.html*

Gong G, Yu H, Zheng Y, Qi B, He H, Yin T, ... Tsim KWK (2021). Astragaloside IV, a saponin from *Astragalus membranaceus* var. *mongholicus*, induces expressions of heme recycle proteins via signaling of Nrf2/ARE in cultured macrophages. Journal of Ethnopharmacology 265:113389. *https://doi.org/10.1016/j.jep.2020.113389*

Guo L, Sun Y, Ping X, Liu J, Wang X, Qin N (2022). Chemical composition and antibacterial activity of ethyl acetate extract of *Astragalus membranaceus* aerial parts. Jounal of Food Safety 42:e12947. *https://doi.org/10.1111/jfs.12947*

Hu Y, Zhai W, Tan D, Chen H, Zhang G, Tan X, ... Wu J (2023) Uncovering the effects and molecular mechanism of *Astragalus membranaceus* (Fisch.) Bunge and its bioactive ingredients formononetin and calycosin against colon cancer: An integrated approach based on network pharmacology analysis coupled with experimental validation and molecular docking. Frontiers in Pharmacology 14:1111912. *https://doi.org/10.3389/fphar.2023.1111912*

Jiao Y, Lau OS, Deng XW (2007). Light-regulated transcriptional networks in higher plants. Nature Reviews Genetics 8:217-230. *https://doi.org/10.1038/nrg2049*

Kochetova GV, Avercheva OV, Bassarskaya EM, Kushunina MA, Zhigalova TV (2023). Effects of red and blue LED light on the growth and photosynthesis of barley (*Hordeum vulgare* L.) seedlings. Journal of Plant Growth Regulation 42:1804-1820. *https://doi.org/10.1007/s00344-022-10661-x*

Konichenko EA, Selyutina IY, Selyutina GG (2014). Chromosome numbers of Fabaceae in Eurasia. Caryologia 67:1-11.

Le AT, Yu JK, Han GD, Do TK, Chung YS (2022). Potential use of colored LED lights to increase the production of bioactive metabolites *Hedyotis corymbosa* (L.) Lam. Plants 11:225. *https://doi.org/10.3390/plants11020225*

Lee SB, Lee JS, Moon SO, Lee HD, Yoon YS, Son CG (2021). A standardized herbal combination of *Astragalus membranaceus* and *Paeonia japonica*, protects against muscle atrophy in a C26 colon cancer cachexia mouse model. Journal of Ethnopharmacology 267:113470. *https://doi.org/10.1016/j.jep.2020.113470*

Li J, Xu S, Mei Y, Gu Y, Sun M, Zhang W, Wang J (2023). Genomic-wide identification and expression analysis of R2R3-MYB transcription factors related to flavonol biosynthesis in *Morinda officinalis*. BMC Plant Biology 23:381. *https://doi.org/10.1186/s12870-023-04394-6*

Li S, Sun Y, Huang J, Wang B, Gong Y, Fang Y, ... Wang H (2020). Anti-tumor effects and mechanisms of *Astragalus membranaceus* (AM) and its specific immunopotentiation: Status and prospect. Journal of Ethnopharmacology 258:112797. *https://doi.org/10.1016/j.jep.2020.112797*

Muhammad N, Luo Z, Zhao X, Yang M, Liu Z, Liu M (2023). Transcriptome-wide expression analysis of MYB gene family leads to functional characterization of flavonoid biosynthesis in fruit coloration of *Ziziphus* Mill. Frontiers in Plant Science 14:1171288. *https://doi.org/10.3389/fpls.2023.1171288*

Pant P, Pandey S, Dall'Acqua S (2021). The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. Chemistry & Biodiversity 18:e2100345. *https://doi.org/10.1002/cbdv.202100345*

Paradiso R, Proietti S (2022). Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: The state of the art and the opportunities of modern LED systems. Journal of Plant Growth Regulation 41:742-780. *https://doi.org/10.1007/s00344-021-10337-y*

Pennisi G, Pistillo A, Orsini F, Cellini A, Spinelli F, Nicola S, ... Marcelis LFM (2020). Optimal light intensity for sustainable water and energy use in indoor cultivation of lettuce and basil under red and blue LEDs. Scientia Horticulturae 272:109508. *https://doi.org/10.1016/j.scienta.2020.109508*

Qader M, Xu J, Yang Y, Wu X, Liu Y, Cao S (2021). Chemistry behind the immunomodulatory activity of *Astragalus membranaceus*. Chinese Medicine and Culture 4:201-210. *https://doi.org/10.4103/cmac.cmac_40_21*

Qiu YX, Chen XL, Fu CX (2001). Reproductive biology of *Astragalus membranaceus* var. mongholicus. Acta Botanica Boreali-Occidentalia Sinica 21:493-498.

Salehi B, Carneiro JNP, Rocha JE, Coutinho HDM, Morais Braga MFB, Sharifi-Rad J, ... de Zarate Machado V (2021). Astragalus species: Insights on its chemical composition toward pharmacological applications. Phytotherapy Research 35:2445-2476. *https://doi.org/10.1002/ptr.6974*

Samuel AO, Huang BT, Chen Y, Guo FX, Yang DD, Jin JQ (2021). Antioxidant and antibacterial insights into the leaves, leaf tea and medicinal roots from *Astragalus membranaceus* (Fisch.) Bge. Scientific Reports 11:19625. *https://doi.org/10.1038/s41598-021-97109-6*

Seo JW, Ham DY, Lee JG, Kim MJ, Yu CY, Seong ES (2024). The effect of different LED wavelengths on the components and biosynthesis of isoflavonoid in sprout *Astragalus membranaceus*. Protoplasma 261:103-110. *https://doi.org/10.1007/s00709-023-01883-1*

Seo JW, Lee JG, Yoo JH, Lim JD, Kim MJ, Seong ES (2022). Growth characteristics and biological activities in *Astragalus membranaceus* seedlings of exposed to different types of artificial light. Korean Journal of Medicinal Crop Science 30:339-346. *https://doi.org/10.7783/kjmcs.2022.30.5.339*

Song G, Yan Y, Guo C, Chen J, Wang Y, Wang Y, ... Pia X (2024). Identification and expression analysis of R2R3-MYB transcription factors associated with flavonoid biosynthesis in *Panax quinquefolius*. International Journal of Molecular Sciences 25:3709. *https://doi.org/10.3390/ijms25073709*

Stracke R, Ishihara H, Huep G, Barsch A, Mehrtens F, Niehaus K, Weisshaar B (2007). Differential regulation of closely related R2R3-MYB transcription factors controls flavonol accumulation in different parts of the *Arabidopsis thaliana* seedling. The Plant Journal 50:660-677. *https://doi.org/10.1111/j.1365-313X.2007.03078.x*

Stracke R, Werber M, Weisshaar B (2001). The R2R3-MYB gene family in *Arabidopsis thaliana*. Current Opinion in Plant Biology 4:447-456. *https://doi.org/10.1016/S1369-5266(00)00199-0*

Tamura K, Stecher G, Kumar S (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38:3022-3027. *https://doi.org/10.1093/molbev/msab120*

Thakur S, Vasudev PG (2022). MYB transcription factors and their role in Medicinal plants. Molecular Biology Reports 49:10995-11008. *https://doi.org/10.1007/s11033-022-07825-z*

Ullah MA, Hassan A, Hamza A (2023). Clinical treatments of various diseases with the root of *Astragalus Membranaceus*. Journal of Clinical Research and Reports 13:299. *https://doi.org/10.31579/2690-1919/299*

Veremeichik G, Grigorchuk V, Makhazen D, Subbotin E, Kholin A, Subbotina N, ... Bulgakov VP (2023). High production of flavonols and anthocyanins in Eruca sativa (Mill) Thell plants at high artificial LED light intensities. Food Chemistry 408:135216. *https://doi.org/10.1016/j.foodchem.2022.135216*

Wang Y, Zhou L-J, Song A, Wang Y, Geng Z, Zhao K, ... Chen F (2023). Comparative transcriptome analysis and flavonoid profiling of floral mutants reveals CmMYB11 regulating flavonoid biosynthesis in chrysanthemum. Plant Science 336:111837. *https://doi.org/10.1016/j.plantsci.2023.111837*

Weerawanich K, Halbwirth H, Sirikantaramas S (2024). A novel MYB transcription factor from durian (*Durio zibethinus*), DzMYB1, regulates flavonoid biosynthesis in fruit pulp. Scientia Horticulturae 333:113246. *https://doi.org/10.1016/j.scienta.2024.113246*

Wei X, Xin J, Chen W, Wang J, Lv Y, Wei Y, ... Xu X (2023). Astragalus polysaccharide ameliorated complex factor-induced chronic fatigue syndrome by modulating the gut microbiota and metabolites in mice. Biomedicine & Pharmacotherapy 163:114862. *https://doi.org/10.1016/j.biopha.2023.114862*

Wong DCJ, Schlechter R, Vannozzi A, Höll J, Hmmam I, Bogs J, ... Matus JT (2016). A systems-oriented analysis of the grapevine R2R3-MYB transcription factor family uncovers new insights into the regulation of stilbene accumulation. DNA Research 23:451-466. *https://doi.org/10.1093/dnares/dsw028*

Wu BS, Hitti Y, MacPherson S, Orsat V, Lefsrud MG (2020). Comparison and perspective of conventional and LED lighting for photobiology and industry applications. Environmental and Experimental Botany 171:103953. *https://doi.org/10.1016/j.envexpbot.2019.103953*

Wu Y, Wen J, Xia Y, Zhang L, Du H (2022). Evolution and functional diversification of R2R3-MYB transcription factors in plants. Horticulture Research 9:uhac058. *https://doi.org/10.1093/hr/uhac058*

Xie D, Chen L, Zhou C, Tarin MWK, Yang D, Ren K, ... Zheng Y (2020). Transcriptomic and metabolomic profiling reveals the effect of LED light quality on morphological traits, and phenylpropanoid-derived compounds

accumulation in *Sarcandra glabra* seedlings. BMC Plant Biology 20:1-18. *https://doi.org/10.1186/s12870-020-02685-w*

Xing H, Yin Z (1984). Karyomorphological studies on medicinal plants in China (III). Cytologia 49:701-707.

Xue A, Liang W, Wen S, Gao Y, Huang X, Tong Y, ... Luo L (2021). Metabolomic analysis based on EESI-MS indicate blue LED light promotes aliphatic-glucosinolates biosynthesis in broccoli sprouts. Journal of Food Composition and Analysis 97:103777. *https://doi.org/10.1016/j.jfca.2020.103777*

Yang Q, Pan J, Shen G, Guo B (2019). Yellow light promotes the growth and accumulation of bioactive flavonoids in *Epimedium pseudowushanense*. Journal of Photochemistry and Photobiology B: Biology 197:111550. *https://doi.org/10.1016/j.jphotobiol.2019.111550*

Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G, ... Xiaoming Q (2006). The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant molecular biology 60:107-124. *https://doi.org/10.1007/s11103-005-2910-y*

Yavari N, Tripathi R, Wu B-S, MacPherson S, Singh J, Lefsrud M (2021). The effect of light quality on plant physiology, photosynthetic, and stress response in *Arabidopsis thaliana* leaves. PloS One 16:e0247380. *https://doi.org/10.1371/journal.pone.0247380*

Yi J, Derynck MR, Li X, Telmer P, Marsolais F, Dhaubhadel S (2010). A single-repeat MYB transcription factor, GmMYB176, regulates CHS8 gene expression and affects isoflavonoid biosynthesis in soybean. The Plant Journal 62:1019-1034. *https://doi.org/10.1111/j.1365-313X.2010.04214.x*

Zhang H, Li X, Wang J, Sun R, Zhao L (2024). Genome-wide identification of secondary metabolite genes in tetraploid *Astragalus membranaceus*. Plants 13:1123.

Zhang X, Bian Z, Yuan X, Chen X, Lu C (2020). A review on the effects of light-emitting diode (LED) light on the nutrients of sprouts and microgreens. Trends in Food Science & Technology 99:203-216. *https://doi.org/10.1016/j.tifs.2020.02.031*

Zhang X, He Y, Li L, Liu H, Hong G (2021). Involvement of the R2R3-MYB transcription factor MYB21 and its homologs in regulating flavonol accumulation in Arabidopsis stamen. Journal of Experimental Botany 72:4319-4332. *https://doi.org/10.1093/jxb/erab156*

Zhao Y, Zhang G, Tang Q, Song W, Gao Q, Xiang G, ... Li X (2022). EbMYBP1, a R2R3-MYB transcription factor, promotes flavonoid biosynthesis in *Erigeron breviscapus*. Frontiers in Plant Science 13:946827. *https://doi.org/10.3389/fpls.2022.946827*

Zhu L, Guan Y, Zhang Z, Song A, Chen S, Jiang J, Chen F (2020). CmMYB8 encodes an R2R3 MYB transcription factor which represses lignin and flavonoid synthesis in chrysanthemum. Plant Physiology and Biochemistry 149:217-224. *https://doi.org/10.1016/j.plaphy.2020.02.010*

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