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RESEARCH ARTICLE

AcMYB12 and *AcMYB29* promote flavonol biosynthesis through transcriptional regulation in onion (*Allium cepa* L.)

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Highlights

- *AcMYB12* and *AcMYB29* exhibit both functional redundancy and distinct roles in regulating flavonol biosynthesis.
- The differential binding activity of AcMYB12 and AcMYB29 with *cis*-elements results in distinct regulatory functions.

Abstract

Flavonols possess significant medical value and are essential for plant stress resistance. These compounds constitute primary components of the nutritional value in onions, particularly in edible portions. While the flavonol biosynthetic pathway has been extensively studied, its regulatory mechanisms in onions remain incompletely understood. This investigation identified flavonol biosynthesis and regulatory genes through analysis of transcriptome and metabolomics data from different developmental stages of 'SA1'. Two R2R3-MYB transcription factors, AcMYB12 and AcMYB29, were identified as positive regulators of onion flavonol biosynthesis. Transcriptional activation assays demonstrated that both could activate *AcCHS*, *AcF3′H*, and *AcFLS*. Yeast one-hybrid assays confirmed their direct binding to these gene promoters. The expression levels of flavonol pathway genes and flavonol contents in *AcMYB12/AcMYB29*-overexpressing onion calli and Arabidopsis plants were significantly higher than those in the control group. Transient silencing assays revealed partial functional redundancy between these two transcription factors. Notably, their regulatory capabilities exhibited significant differences. AcMYB12 predominantly regulates flavonol accumulation, while AcMYB29 specifically influences quercetin. Further investigation of the molecular mechanisms underlying differential regulation indicated variations in *cis*-elements within flavonol pathway gene promoters and differences in binding activity between transcription factors and *cis*-elements.

Keywords: onion, flavonols, *AcMYB12*, *AcMYB29*, *cis*-element, flavonol pathway genes

1. Introduction

Onions (*Allium cepa* L.) represent one of the most significant horticultural crops globally, distinguished by their unique flavor and nutritional properties (Rodrigues *et al.* 2011; Lee *et al.* 2013; Perez-Gregorio *et al.* 2014). The abundance of secondary metabolites in onions contributes to these characteristics, with flavonoid compounds serving as principal contributors (Slimestad *et al.* 2007). Research has demonstrated that onions contain diverse flavonoid compounds, predominantly flavonol and anthocyanin. The concentration of flavonoids varies among onion varieties. Yellow onions contain substantial amounts of flavonols, while red onions possess both high flavonol content and significant anthocyanin levels. White onions contain minimal detectable flavonoid compounds. Among horticultural crops, onions demonstrate exceptionally high flavonol content, surpassing numerous other plant species (Ko *et al.* 2015).

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Flavonoid compounds represent widely distributed secondary metabolites in plants and serve essential functions in plant growth and development (Stracke *et al.* 2007). These compounds encompass various categories, including flavonols, anthocyanins, isoflavones, and flavones, each serving distinct roles in plants (Shen *et al.* 2022). While anthocyanins, notable for their distinctive colors, have received extensive research attention, flavonols, despite their less visible nature, remain vital for plant function. In onions, quercetin and isorhamnetin constitute the primary flavonol forms, contributing both to onion development and providing significant nutritional and medical benefits (Nile *et al.* 2017).

The biosynthesis of flavonols, which is well characterized in most horticultural crops, initiates with the phenylalanine pathway (Wang *et al.* 2018b). Phenylalanine undergoes catalysis by several enzymes including phenylalanine lyase (PAL), carnosilicate 4-hydroxylase (C4H) (Williams *et al.* 2005; Li *et al.* 2015), chalcone synthetase (CHS), chalcone isomerase (CHI), and flavono-3-hydroxylase (F3H), forming dihydroflavonols. These dihydroflavonols comprise dihydrokaempferol, dihydroquercetin and dihydromyricetin. Dihydrokaempferol transforms to dihydroquercetin through flavonoid 3′-hydroxylase (F3′H) catalysis, and to dihydromyricetin through flavonoid-3′,5′-hydroxylase (F3′5′H) catalysis. flavonol synthetase (FLS) then converts these dihydroflavonols into flavonols (Zhang *et al.* 2017). FLS serves as a key rate-limiting enzyme in flavonol synthesis, while F3′H plays a crucial role in quercetin synthesis (Meng *et al.* 2019). Furthermore, quercetin converts to isorhamnetin through quercetin 3-*O*-methyltransferase (QOMT) catalysis, representing another main subclass of flavonols. However, the complete regulatory mechanisms of flavonol synthesis in plants remain to be fully elucidated.

In *Arabidopsis* (*Arabidopsis thaliana*), research has established that members of the subgroup 7 family of R2R3-MYB transcription factors (SG7-MYB) regulate flavonol biosynthesis (Stracke *et al.* 2007). Similar regulatory patterns by transcription factors in this family appear in other crops, including tomato (*Solanum lycopersicum*), grape (*Vitis vinifera*), and pear (*Pyrus bretschneideri*). *Arabidopsis* contains three SG7-MYB members: AtMYB11, AtMYB12, and AtMYB111. The *myb11myb12myb111* triple mutant exhibits significantly reduced flavonol content compared to the wild type, with flavonols becoming nearly undetectable, demonstrating the essential role of the SG7-MYB family in regulating flavonol biosynthesis in *Arabidopsis* (Stracke *et al.* 2007; Yao *et al.* 2020). In freesia (*Freesia hybrida*), researchers have identified four SG7-MYB family members that modulate flavonol synthesis to varying degrees (Shan *et al.* 2020). These MYB members bind to promoters of flavonol biosynthesis genes, including *CHS*, *F3H*, *F3′H*, and *FLS*, enhancing their transcription and subsequent flavonol accumulation (Shan *et al.* 2020).

While SG7-MYB transcription factor family members demonstrate functional conservation across various plants, distinct functional differences exist among these family members (Stracke *et al.* 2007; Shan *et al.* 2020). For instance, AtMYB111 and AtMYB12 regulate flavonol synthesis in cotyledon and roots, respectively. The specific flavonol composition in different *Arabidopsis* seedling parts depends on differential responsiveness of flavonol glycosidase-encoding genes and other downstream enzymes to SG7-MYB factors (Stracke *et al.* 2007). Freesia's four family members exhibit varying abilities to regulate flavonol pathway genes. FhMYBF2 demonstrates significantly higher activation capacity for *FhCHS* compared to other members, while FhMYBF3 shows higher activation capacity for *FhFLS1*. In pear, which possesses two family members, only PbMYB12b's function in regulating flavonol biosynthesis has been demonstrated, while the other members' function remains undefined (Zhai *et al.* 2019). The mechanisms underlying tissue specificity and functional differences in regulating flavonol biosynthesis among SG7-MYB members in multi-member plants require further investigation.

In the yellow onion 'SA1', renowned for high flavonol content, we have identified two SG7 family members, AcMYB12 and AcMYB29. These members exhibit functional differences in regulating target genes, resulting in varied synthesis of flavonol classes, potentially due to distinct binding activities between transcription factors and *cis*-elements of target gene promoters. These discoveries advance understanding of SG7MYB family members' role in flavonol biosynthesis regulation and establish groundwork for investigating secondary metabolism regulatory mechanisms in onion.

2. Methods and materials

2.1. Plant materials and growth conditions

The onion varieties utilized in this study were 'SA1' for real-time quantitative PCR (RT-qPCR), antisense oligodeoxynucleotide (asODN) and tissue at different developmental stages. The wild type of *Arabidopsis* is Columbia (Col-0). Multiple onion varieties, including yellow onion 'SA1' and 'L19005', red onion 'Meihong' and 'L17106' and white onion 'C1827' and 'C1828' were selected to determine flavonol content and pathway gene expression. The seeds were obtained from the Onion Molecular Breeding Laboratory of Northeast Agricultural University, China.

The growth conditions for the onions utilized in multi-omics analysis and RT-qPCR were described previously (Qin *et al.* 2023). The growth conditions for onion epidermal cells for subcellular localization and *Arabidopsis* were described previously (Jia *et al.* 2024).

2.2. Construction of plasmids

For the yeast one-hybrid assay, pAbAi and pGADT7 plasmids were utilized. The coding sequence (CDS) of *AcMYB12* and *AcMYB29* were amplified using KOD One™ PCR Master mix (Toyobo, Nanjing, China) and inserted into the pGADT7 vector using a BioSharp Seamless Cloning Kit (Anhui, China). The promoters of *AcCHS*, *AcF3′H*, and *AcFLS*, including sequences upstream of ATG at –1,637, –1,738, and –1,987 bp, respectively, were amplified. The corresponding *cis*-element tandem sequences were subsequently amplified and inserted into the pAbAi vector. For the transcriptional activation assay, pGreenII0800 and pGreenII62SK plasmids were selected. The transcription factor was inserted

into pGreenII62SK, and the promoter and its truncated fragment were inserted into pGreenII0800. pSuper1300-eGFP plasmid was selected for genetic transformation and subcellular localization of plants, and the CDS of *AcMYB12* and *AcMYB29* were inserted into it. Appendix A contains the primers used.

2.3. Plant transformation

For overexpression in onion callus, onion seeds were rinsed with flowing water for 4 h and subsequently soaked in warm soup for 1 h. The seed skin from the germination site was excised in a sterile environment and placed uniformly on a solid MS (Murashige and Skoog) medium. When the seeds developed germs of appropriate length, the young germs from the seed skin were excised and cultured on P5 medium (MS medium with picloram) for 7 d. They were then immersed in *Agrobacterium* solution for 10 min, followed by water drainage using filter paper. The callus was cultured in medium containing hygromycin and ampicillin on P5 medium, with medium replacement every 2 weeks. After 40 d, the more prominent callus was selected for subsequent experiments. The overexpression in *Arabidopsis* followed the method previously described (Jia *et al.* 2024).

2.4. Extraction of RNA and RT-qPCR

The extracted samples, stored at –80°C, were pulverized in a mortar with liquid nitrogen. RNA extraction was performed using the Freezol Reagent RNA Extraction Kit (Vazyme, Nanjing, China), following the manufacturer's instructions. The HiScript 1st Strand cDNA Synthesis Kit +gDNA wiper (Vyzyme, Nanjing, China) was employed to obtain cDNA from the extracted RNA, whereas Primer Premier 5 designed quantitative primers were used for the gene of interest, with *Actin* serving as the reference gene. The RT-qPCR reaction procedure and system followed the protocols provided by the QPK-201CH Fluorescence Quantification Kit (Toyobo, Shanghai, China). Quantitative fluorescence assay from Bio-rad (California, USA) was utilized for RT-qPCR. The relative expression levels of genes were calculated using the $2^{-\Delta\Delta ct}$ method. The detailed primers information can be found in Appendix A.

2.5. Determination of onion flavonol content by high performance liquid chromatography (HPLC)

The flavonol content determination in onion followed a previously described method (Ko *et al.* 2015), with modifications. A 0.2 g sample was extracted with 8 ml of 79% (v/v) methanol solution using ultrasonic treatment for 3 min at 60°C. The solution was then adjusted to 10 ml with 16% HCl (to hydrolyze flavonol glycoside to its corresponding flavonol monomer for accurate content determination). Glycosides underwent hydrolysis for 2 h in an 80°C thermostatic water bath. Each sample set included three biological replicates, with a minimum of three separate injections per sample extract. The supernatant was centrifuged through a 0.22 μm filter membrane and transferred to a 1 mL liquid phase bottle for analysis.

The mobile phase of the HPLC comprised mobile phase A (0.2% trifluoroacetic acid solution) and mobile phase B (100% methanol solution). The elution protocol proceeded as follows: 0.0–5.0 min, 5% B isovoltage elution; 5.0–20.0 min, 5–50% B linear gradient elution; 20.0–35.0 min, 50% B isovoltage; 35.0–38.0 min, 100% B isovoltage elution; 38.0–45.0 min, 5% B isovoltage elution with a flow rate of 1.0 mL min^{-1}. UV absorbance measurements were conducted at 370 nm.

Standard curves for each flavonol compound were established using identical methodology, with standards analyzed every seven samples. Serial dilutions from 0.00 to 800.00 μg mL^{-1} were prepared for each standard (400 μg mL^{-1} for isorhamnetin). These standard curves established the range of flavonol concentration (Appendix B).

2.6. Subcellular localization

The plasmid from 1.2 was introduced into *Agrobacterium*. Onion epidermis was prepared according to established methods and infected with *Agrobacterium* (Sun *et al.* 2007). The onion epidermis underwent soaking for approximately 10 min, followed by water absorption from the surface. The tissue was then transferred to MS medium for dark culture for 3 d. Observation was performed using a laser confocal microscope (Olympus FV3000, Tokyo, Japan).

2.7. Yeast one-hybrid assay

The constructed pAbAi plasmids from 1.2 were linearized using BbsI or BstBI and transferred into YIHGold yeast cells along with pGADT7-*AcMYB12* or pGADT7-*AcMYB29*. The pGADT7-AD and PAbAi-promoters co-transferred into yeast served as the control. The interaction between the promoter and transcription factor was verified according to the Y1HGold-pAbAi Yeast One-Hybrid Interaction Proving Kit (Coolaber, Beijing, China) instructions.

2.8. Transcriptional activation assay

The constructed recombinant pGrrenII0800 and pGrrenII62SK plasmids were transferred into *Agrobacterium*. The luciferase (LUC)/REN values were measured according to the Dual-Luciferase Report Assay System (Promega, Wisconsin, USA) instructions, and chemiluminescence imaging analysis was performed to determine the transcriptional activation ability.

2.9. *In situ* staining of diphenylborinic anhydride (DPBA)

The DPBA dye solution was prepared according to the method described by Liang et al (Liang *et al.* 2020). Samples underwent staining in an ethanol solution containing DPBA and Triton X-100 for a minimum of 2 h before observation using a fully automated chemiluminescence image analysis system (Tanon 5200, Guangzhou, China). Fluorescence values were quantified using ImageJ software.

2.10. Instantaneous silencing by asODNs in onion seedlings

The application of asODN for temporary gene silencing in plant species has been extensively documented (Xie *et al.* 2014). The CDS of *AcMYB12* and *AcMYB29* served as the input sequence, and the corresponding asODN was

generated using Sligo software (https://sfold.wadsworth.org/cgi-bin/soligo.pl). Onion seedlings germinated for approximately 15 d were selected. After removing the portion below the cotyledon, the seedlings were immersed in an 80 mmol L^{-1} sucrose solution containing 10 μmol L^{-1} asODN. A control group was treated with the same concentration ODN. Samples were collected at different timepoints for RT-qPCR and flavonol determination.

2.11. Statistical analysis

Data significant differences were determined by the two-tailed Student's *t*-test, with a significance level of $P<0.05$.

3. Results

3.1. Metabolomics of 'SA1' revealed the accumulation pattern of flavonol at different periods

Metabolomic and transcriptomic analyses were previously conducted on onion bulbs during different expansion periods (Qin *et al*. 2023). Metabolomics analysis identified the types of onion flavonoids and their accumulation at various developmental stages. The results indicated that yellow onion 'SA1' primarily contained flavonols and flavanones, with quercetin representing the dominant form of onion flavonols (Fig. 1-A and B; Appendix C). Analysis of flavonols accumulation from period I (early expansion stage of bulb) to period II (rapid expansion stage of bulb) revealed rapid accumulation during this interval. From period II to period III (late expansion stage of bulb), all substances exhibited a decreasing trend except for the fourth and fifth subclasses (Fig. 1-C–E). The venn diagram illustrated that the fourth and fifth subclasses primarily consisted of quercetins and isorhamnetins, while other subclasses predominantly contained kaempferols (Appendices D and E). The HPLC analysis confirmed the reliability of the metabolome data. Furthermore, the HPLC results demonstrated that the total flavonols content remained stable (Appendix C), as quercetins and kaempferols constituted more than 90% of the total flavonols.

3.2. KEGG pathway analysis of transcriptome and metabolome associations obtained flavonol pathway genes

The KEGG pathways associated with temporal differences in the transcriptome and metabolome were annotated as "Flavonoid biosynthesis", "Phenylpropanoid biosynthesis", and "Flavonoid and flavonol biosynthesis" (Fig. 2-A and B). These pathways exhibited significant enrichment from period I to period II (Fig. 2-A and C). The "Phenylpropanoid biosynthesis" pathway demonstrated significant enrichment from period II to period III (Fig. 2-B and D). Analysis of the KEGG pathway results identified 10 genes involved in flavonoid synthesis, comprising two *CHSs*, three *CHIs*, one *F3H*, one *F3′H*, and three *FLSs* as initial candidate genes (Appendices F and G).

3.3. Flavonol pathway genes were identified by RT-qPCR and asODN

The heatmap analysis of FPKM (Fragments Per Kilobase of exon model per Million mapped fragments) values for the flavonol pathway genes in the transcriptome demonstrated a substantial increase in the expression levels of the following genes from period I to period II: *CHS* (*Cluster-29851.120211*, *Cluster-29851.120213)*, *CHI (Cluster-29851.108784*, *Cluster-29851.132018*, *Cluster-29851.114529)*, *F3H (Cluster-29851.111199)*, *F3′H (Cluster-29851.111946)*, *FLS (Cluster-29851.119571*, *Cluster-29851.115498, Cluster-29851.92187)* (Fig. 3-A, Appendix H). This increase correlated strongly with the flavonol content. RT-qPCR analysis of these candidate genes validated the transcriptome accuracy (Fig. 3-B). Furthermore, *Cluster-29851.120213*, *Cluster-29851.108784*, *Cluster-29851.132018*, *Cluster-29851.114529*, *Cluster-29851.111199*, *Cluster-29851.111946*, and *Cluster-29851.119571* exhibited stronger correlation with the flavonol accumulation pattern compared to other family members, warranting further investigation.

To determine these genes' role in regulating flavonol biosynthesis in onions, asODNs were employed to suppress transcript abundance (Zhao *et al.* 2021). The analysis revealed significant reduction in transcript levels of each gene after 12 h of treatment (Fig. 3-C). Examination of flavonol content in treated seedlings (Fig. 3-D) showed significant decrease in flavonol levels of asODN-*AcCHS* (*Cluster-29851.120213*), asODN-*AcCHI* (*Cluster-29851.108784*), asODN-*AcF3H* (*Cluster-29851.111199*), and asODN-*AcFLS* (*Cluster-29851.119571*) compared to the control (Fig. 3-D; Appendix I). Quercetin content decreased in asODN-*AcF3′H* (*Cluster-29851.111946*), confirming *AcF3′H*'s essential role in quercetin biosynthesis (Fig. 3-D). These findings identified these genes as candidates responsible for flavonol biosynthesis.

3.4. Promoter *cis*-elements analysis of flavonol pathway genes

To identify potential transcription factors, the *cis*-elements of the candidate gene promoters underwent analysis. The investigation revealed several types of *cis*-elements, including MYB, MYC, ABRE, G-Box, and ARE (Fig. 3-E). ABRE potentially mediates abscisic acid responsiveness, while ARE associates with anaerobic induction (Zhang *et al.* 2024). G-box and GT1-motif are responsive to light, and these regulatory elements occur frequently in the analyzed promoters (Liu *et al.* 2019). MYB, a crucial *cis*-element regulating secondary metabolic synthesis, demonstrated the highest prevalence among the promoters of these genes. These elements serve as binding sites for MYB transcription factors. The R2R3-MYB family members demonstrate a crucial role in regulating secondary metabolic synthesis (Ma and Constabel 2019). Consequently, this transcription factor family warranted further investigation to identify potential regulators of flavonol biosynthesis.

3.5. Correlation analysis of flavonol pathway genes and R2R3-MYB family revealed potential transcription factors of regulating flavonol biosynthesis

A total of 117 transcripts belonging to the R2R3-MYB family were identified in the transcriptome. Through gene

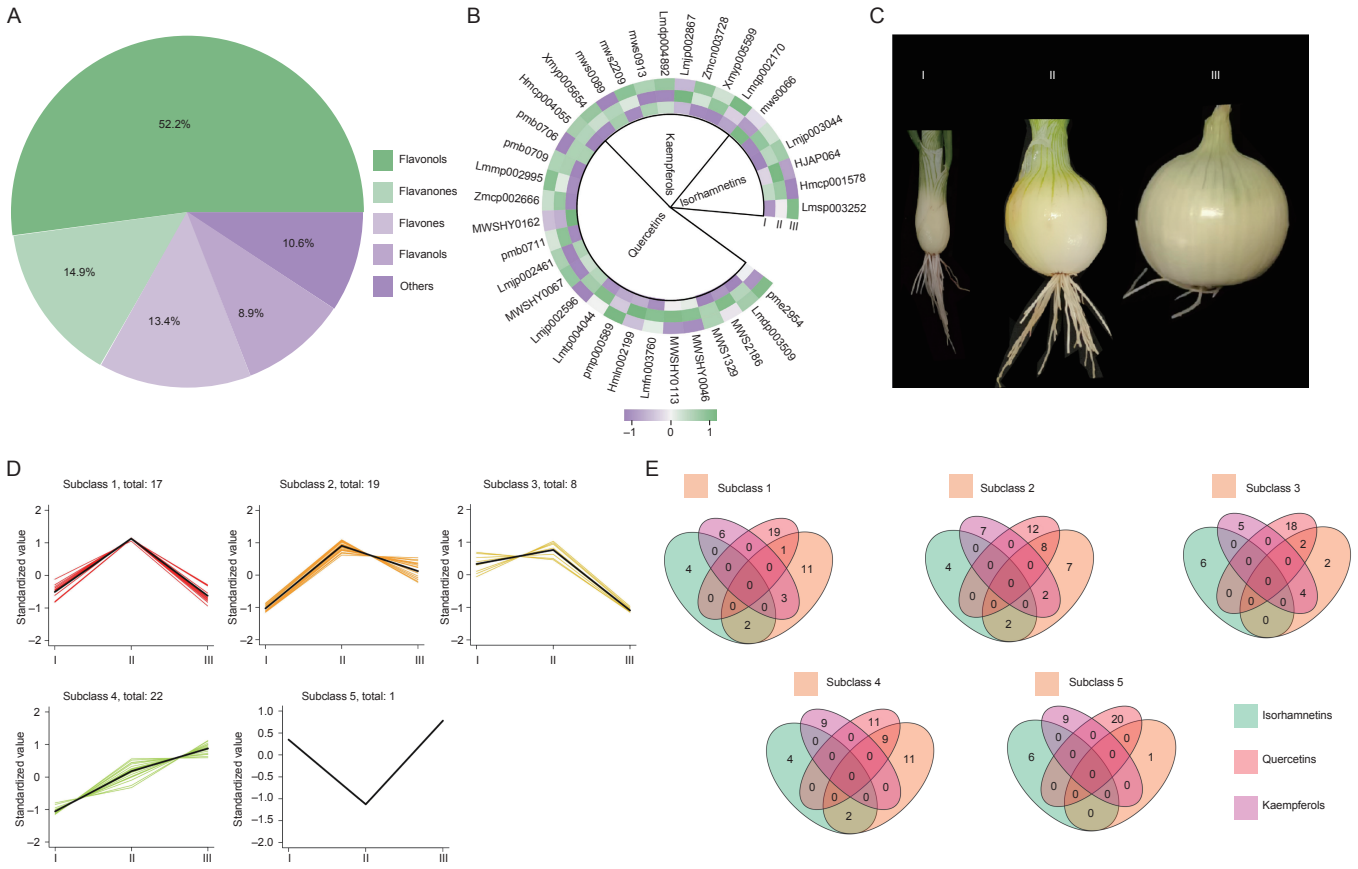


Fig. 1 Transcriptome data analysis of 'SA1'. A, pie chart. Within the metabolome, the categories of flavonoid compounds detected in 'SA1'. B, ring heatmap. In the metabolome, the accumulation of flavonol compounds in different periods of 'SA1'. I, bulb early expension period; II, bulb rapid expension period; III, bulb late expension period. The heat map represents the relative amount of material in the metabolome (logarithmic). C, a schematic representation of the three key developmental periods of the onion bulb (I, II, III). D, K-means cluster analysis. The flavonoid compounds in the metabolome were classified into five categories based on relative content trends. E, Venn graph. Venn diagram illustrating the distribution of the five classes from (D) among three flavonol compounds (quercetin, kaempferol, isorhamnetin).

expression correlation analysis, a co-expression network was established to associate these transcripts with flavonol pathway genes (r>0.7, P<0.1). A total of 57 highly associated genes were identified in leaf, and 31 highly associated genes were detected in bulb (Fig. 4-A and B). Among these, 14 genes demonstrated high association with flavonol biosynthesis genes in both tissues, and four genes exhibited positive correlation, indicating their potential function as positive regulators of flavonol biosynthesis (Fig. 4-C). RT-qPCR validation confirmed the accuracy of the transcriptome data, with results aligning with the transcriptome findings (Fig. 4-D).

3.6. Differential expression of different tissues of bulbs further revealed candidate R2R3-MYB

Previous research has established that the outer layer of bulbs contains substantially higher flavonol concentrations compared to the inner layer (Ko *et al.* 2015). Analysis of the corresponding tissues confirmed that the outer layer contained approximately eight-fold higher flavonol levels than the inner layer, where only trace amounts were detected (Fig. 5-A–C). The expression levels of flavonol pathway genes *AcCHS, AcF3′H,* and *AcFLS* exhibited significantly higher expression in the outer layer relative to the inner layer (Fig. 5-D and E). These tissue-specific expression patterns facilitated further screening to determine the regulatory role of the three transcription factors in flavonol synthesis. The analysis revealed that expression patterns of *Cluster-29851.52146* and *Cluster-29851.85298* strongly correlated with changes in flavonols and related genes, while *Cluster-29851.24248* expression showed no significant difference (Fig. 5-F). Consequently, *Cluster-29851.52146* and *Cluster-29851.85298* were identified as candidate genes.

3.7. Inhibition of *Cluster-29851.52146* transcription significantly reduced the flavonol content in onion seedlings

asODN was employed to suppress the transcription of *Cluster-29851.52146* and *Cluster-29851.85298* (Appendix J). The expression levels exhibited significant inhibition after 12 h (Fig. 5-G). Analysis of flavonol pathway genes revealed significant downregulation in asODN-*Cluster-29851.52146* treated samples, while *Cluster-29851.85298* showed no significant alterations (Fig. 5-H). Flavonol content analysis at 72 h confirmed these findings, demonstrating significantly reduced levels in asODN-*Cluster-29851.52146* compared to control samples (Fig. 5-I). These results establish *Cluster-29851.52146* as a positive regulator

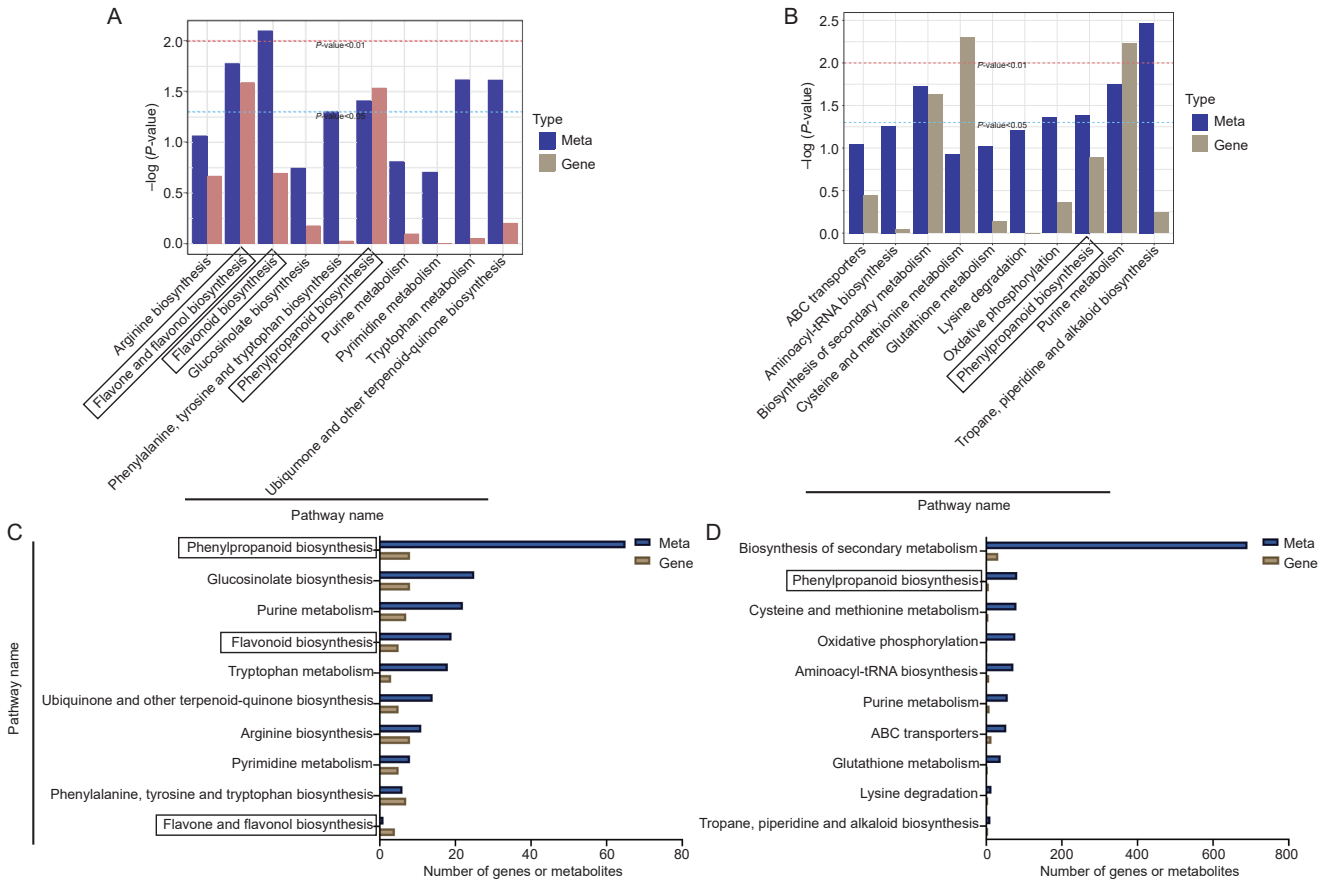


Fig. 2 KEGG pathway enrichment analysis during three developmental periods in bulbs. A and B represent the top 10 biosynthetic pathways significantly enriched by *P*-value (A, period II *vs*. period I; B, period III *vs*. period II). C and D indicate the number of metabolites and genes in the biosynthetic pathways annotated in the metabolome and transcriptome (C, period II *vs*. period I; D, period III *vs*. period II). Meta, metabolites.

of flavonol biosynthesis, while *Cluster-29851.85298* demonstrates no regulatory function in flavonol synthesis.

3.8. AcMYB12 and AcMYB29 are members of SG7-MYB family

Analysis of Cluster-29851.52146 revealed significant homology with *Arabidopsis* and other crop species within the SG7-MYB family, indicating its classification within this family. This gene has been designated as *AcMYB12* (Fig. 6-A). Phylogenetic analysis incorporating R2R3-MYB family members from onion and the SG7-MYB family demonstrated that AcMYB29 also belongs to this family (Fig. 6-A) (Schwinn *et al.* 2016). Multiple-sequence alignment confirmed the presence of characteristic SG7-MYB family functional domains in both AcMYB12 and AcMYB29 (Appendix K). Given the established role of SG7-MYB family members in flavonol regulation, the functional significance of *AcMYB29* warrants further investigation (Stracke *et al.* 2007).

3.9. *AcMYB12* and *AcMYB29* exhibit redundancy and difference in function

Instantaneous silencing experiments in onion seedlings revealed that the expression levels of *AcCHS*, *AcF3′H*, and *AcFLS* in asODN-*AcMYB29* were significantly reduced compared to the control, with *AcF3′H* exhibiting particularly pronounced down regulation (Fig. 6-C and D). The flavonol content demonstrated a significant reduction, particularly in the proportion of quercetin among total flavonols (Fig. 6-E). These observations contrasted with the results obtained for asODN-*AcMYB12*. The findings indicate distinct functional differences in quercetin accumulation regulation. However, in both asODN-*AcMYB12* and asODN-*AcMYB29*, the expression levels of *AcCHS* and *AcFLS* increased after 24 h, suggesting also regulatory redundancy of *CHS* and *FLS* (compensating for each other's regulatory function). To further examine this hypothesis, dual silencing of both *AcMYB12* and *AcMYB29* was conducted. During simultaneous silencing, flavonol pathway gene expression remained unchanged after 24 h, confirming their redundancy in flavonol biosynthesis co-regulation (Fig. 6-D and E). These results demonstrate that *AcMYB12* and *AcMYB29* function as positive regulators in flavonol synthesis with partial functional redundancy and differentiation.

3.10. *AcMYB12* and *AcMYB29* significantly increased the flavonol content in *Arabidopsis* and onion callus

Analysis of the *cis*-elements in the promoter of flavonol pathway genes (*AtCHS*, *AtF3′H*, and *AtFLS*) in *Arabidopsis*

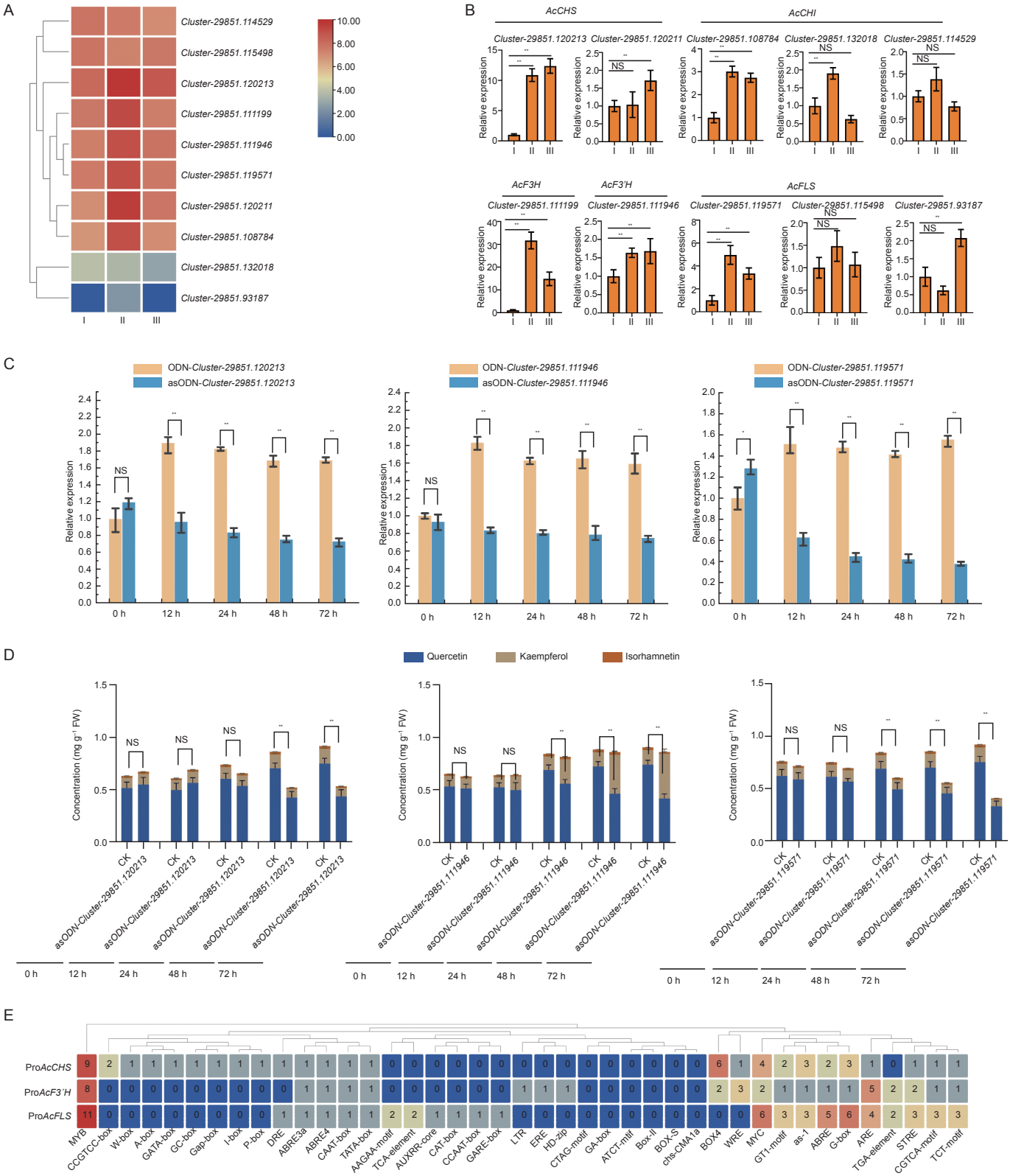


Fig. 3 Transcriptome data analysis of 'SA1', RT-qPCR of candidate genes and prediction of promoter homologous binding sites. A, cluster heatmap of the FPKM values in the transcriptome. B, RT-qPCR of candidate genes involved in flavonol biosynthesis. C, RT-qPCR in onion seedling. Transiently silenced seedlings using the asODN. ODN (oligodeoxynucleotide): control group; asODN: experimental group. Data are means±SD (n=3). D, the content of flavonol in onion seedling by HPLC. Data are means±SD (n=5). For panels B–D statistically significant differences were determined by the two-tailed Student's t-test (*, P<0.05; **, P<0.01). Quercetin+Keamferol+Isorhamnetin=Flavonol. E, heatmap analysis of the *cis*-elements of the promoter. The *cis*-elements of the promoters of *AcCHS*, *AcF3′H* and *AcFLS* were analyzed using PlantCARE and the *cis*-elements were visualized using a heat map.

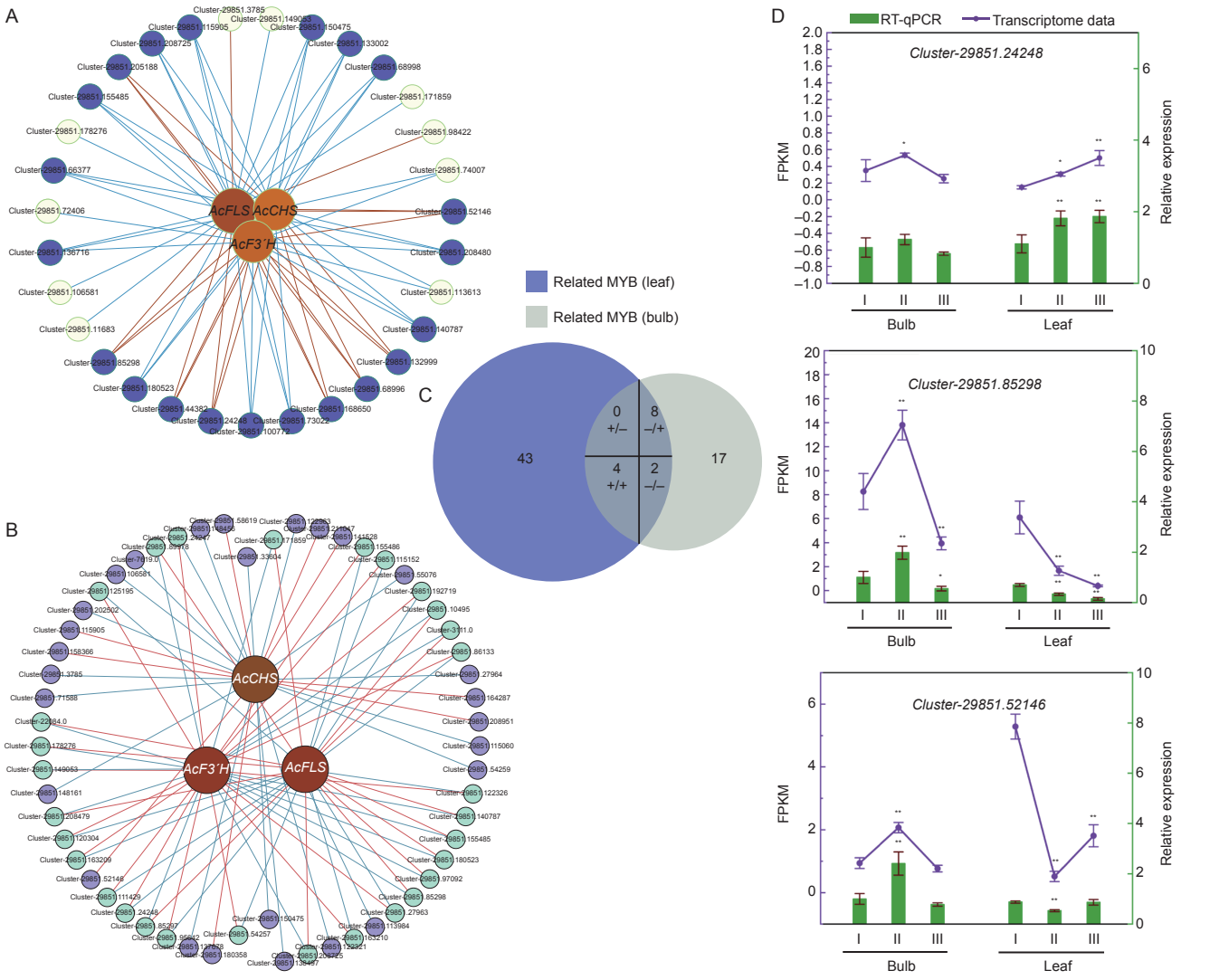


Fig. 4 R2R3-MYB screening through expression association analysis. A and B, expression correlation analysis between three key flavonol pathway genes and R2R3-MYB transcription factor family genes in bulb (A) and leaf (B) (P<0.1, r>0.7). C, Venn diagram analysis comparing candidate MYB family genes in leaves versus bulbs. D, RT-qPCR analysis of candidate MYB expression in leaves and bulbs. Data represent means±SD (n=5). Statistical significance was determined using two-tailed Student's t-test (*, P<0.05; **, P<0.01).

revealed significant enrichment of MYB *cis*-elements (Appendix L). Given that SG7-MYB family members AtMYB11, AtMYB12, AtMYB111 influence flavonol accumulation through regulation of flavonol pathway genes, *AcMYB29* and *AcMYB12* were ectopically overexpressed in *Arabidopsis* to examine their roles in flavonol biosynthesis. The cDNA of *AcMYB29* and *AcMYB12* were incorporated into the genome, with three T_0 plants initially selected. Genomic PCR verification confirmed successful insertion of *AcMYB12* and *AcMYB29* into the *Arabidopsis* genome. Following continuous culture and propagation, the corresponding T3 lines *AcMYB29*-OE1/OE2/OE3 and *AcMYB12*-OE1/OE2/OE3 were established (Fig. 7-A).

Semi-quantitative RT-PCR results demonstrated stable expression of *AcMYB12* and *AcMYB29* in the *AcMYB29*-OE and *AcMYB12*-OE lines (Fig. 7-B). Expression levels of flavonol pathway genes in overexpressed lines increased significantly. DPBA staining revealed markedly higher fluorescence intensity in the overexpressed leaves compared to the control (Fig. 7-C). HPLC analysis confirmed significantly elevated flavonol content in the overexpressed leaves (Fig. 7-D). These findings further indicate that *AcMYB12* and *AcMYB29* regulate flavonol biosynthesis and maintain functional conservation in *Arabidopsis*.

The genes were introduced into the immature embryo of the onion variety 'SA1', generating overexpressed callus (Fig. 7-E). PCR analysis confirmed successful integration of *AcMYB12* and *AcMYB29* into the onion genome (Appendix M). Expression analysis of flavonol pathway genes in *AcMYB29*-OE and *AcMYB12*-OE calluses demonstrated significantly higher levels of *AcCHS* and *AcFLS* compared to the control group , while *AcF3′H* exhibited high expression exclusively in *AcMYB29*-OE callus (Fig. 7-G).

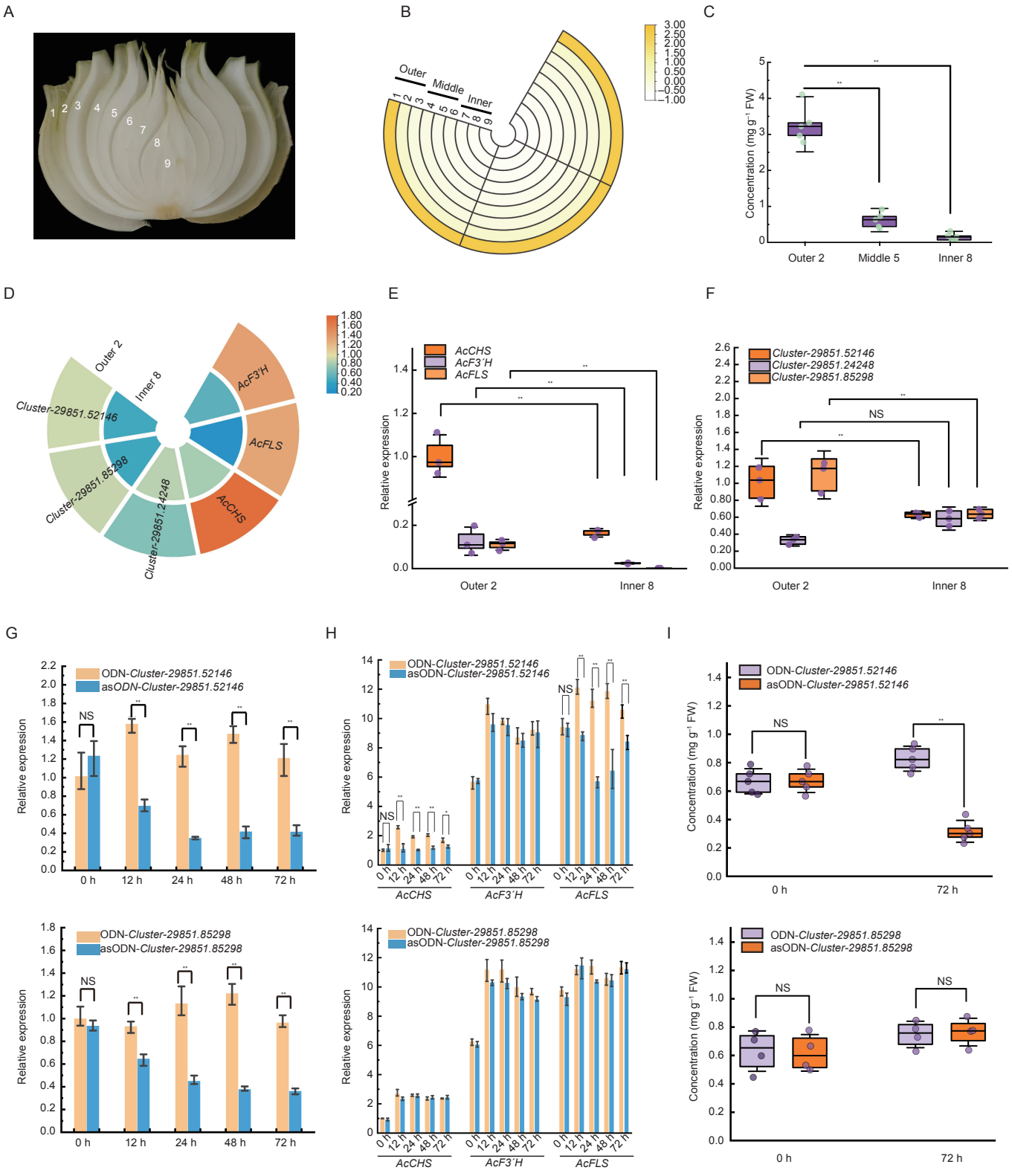


Fig. 5 Tissue-specific expression and transient silencing analysis for identifying R2R3-MYB regulators of flavonol biosynthesis. A, longitudinal section of 'SA1' bulbs. Numbers indicate layer positions (outside to inside) of the bulb in period II. B, flavonol content visualization of corresponding layers in (A) through ring heatmap. C, HPLC analysis of flavonol content in scales at layers 2, 5, and 8. Data represent means±SD (n=5). D, FPKM values (logarithmic) for layers 2 and 8 scales from transcriptomic data, visualized *via* ring heatmap. E and F, RT-qPCR analysis of layers 2 and 8. Data represent means±SD (n=3). G and H, RT-qPCR analysis of asODN-treated onion seedlings. Data represent means±SD (n=3). I, flavonol content analysis in transiently silenced onion seedlings. Data represent means±SD (n=5). For panels C and E–F statistical significance determined by two-tailed Student's t-test (*, P<0.05; **, P<0.01).

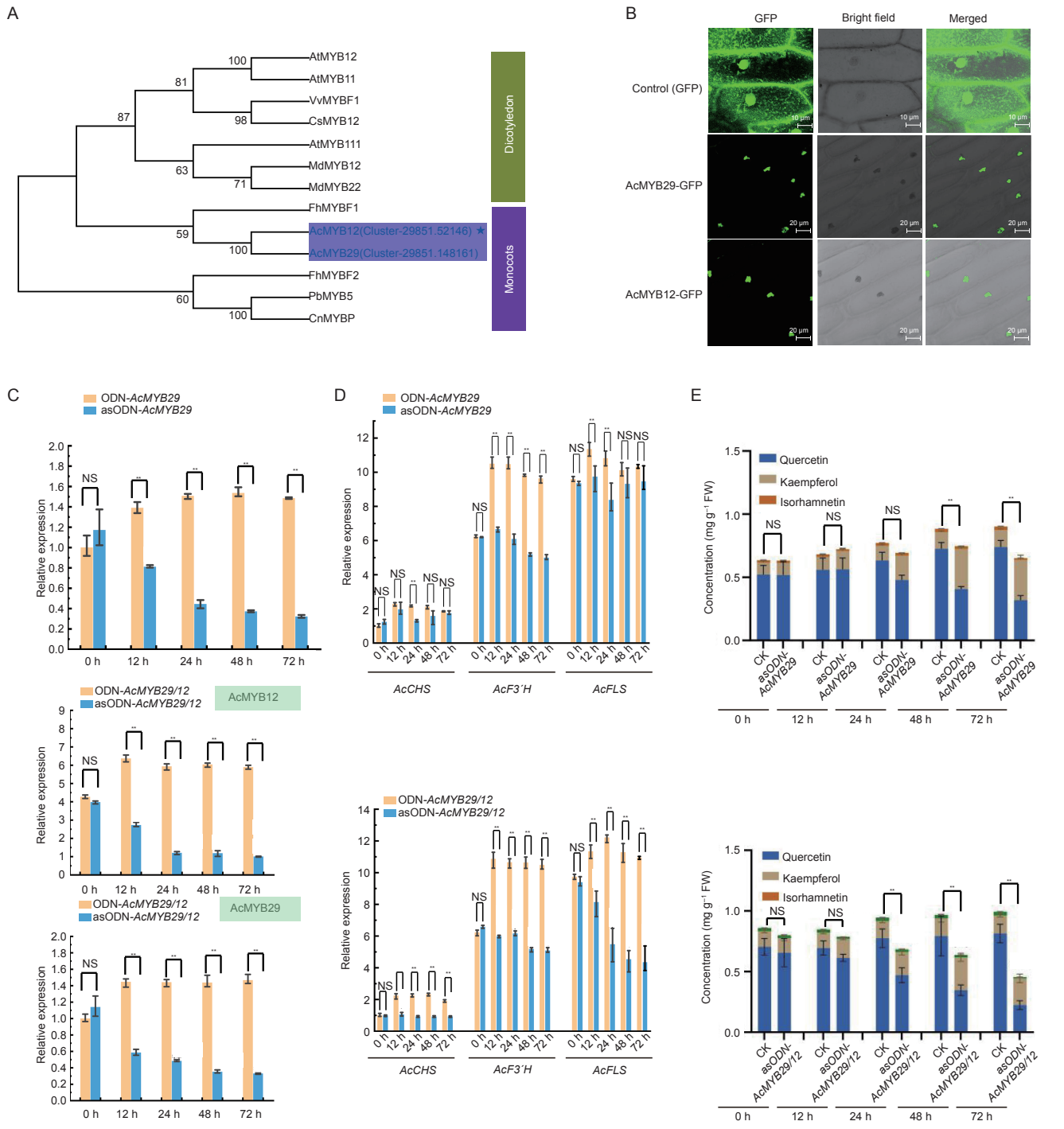


Fig. 6 Homology and functional analysis of AcMYB12 and AcMYB29. A, phylogenetic analysis of the R2R3-MYB. The phylogenetic tree was constructed using MEGA (MEGA 7.0.26), employing the neighbor-joining statistical method and Bootstrap method with 'No. of Bootstrap Replications=1,000' as the test of phylogeny. Plant species analyzed included At (*Arabidopsis thaliana*), Vv (*Vitis vinifera*), Cs (*Camellia sinensis*), Md (*Malus domestica*), Fh (*Freesia hybrida*), Pd (*Pyrus bretschneideri*), Cn (*Cocos nucifera*). B, subcellular localization of MYB12 and MYB29 in the onion epidermis. The first row depicts the localization results of onion epidermal cells imported into the pSuper1300-eGFP empty vector, bar=10 nm. Rows 2, 3 show the localization results of onion epidermal cells with the pSuper1300-eGFP vector connecting AcMYB29, AcMYB12, bar=20 nm. C and D, RT-qPCR in onion seedlings with transient silencing of *AcMYB29* and simultaneous transient silencing of *AcMYB29* and *AcMYB12*. E, flavonol content in seedlings with transient silencing of *AcMYB29* and simultaneous transient silencing of *AcMYB29*, *AcMYB12*. Data are means±SD (n=5). Statistically significant differences were determined by the two-tailed Student's t-test (*, P<0.05; **, P<0.01).

These findings align with instantaneous silencing, further indicating their differential ability to regulate *F3′H*. To explore this further, different classes of flavonols in onion callus were analyzed. The analysis revealed significantly higher flavonol content in the callus compared to the control group (Fig. 7-H and I). Additionally, the

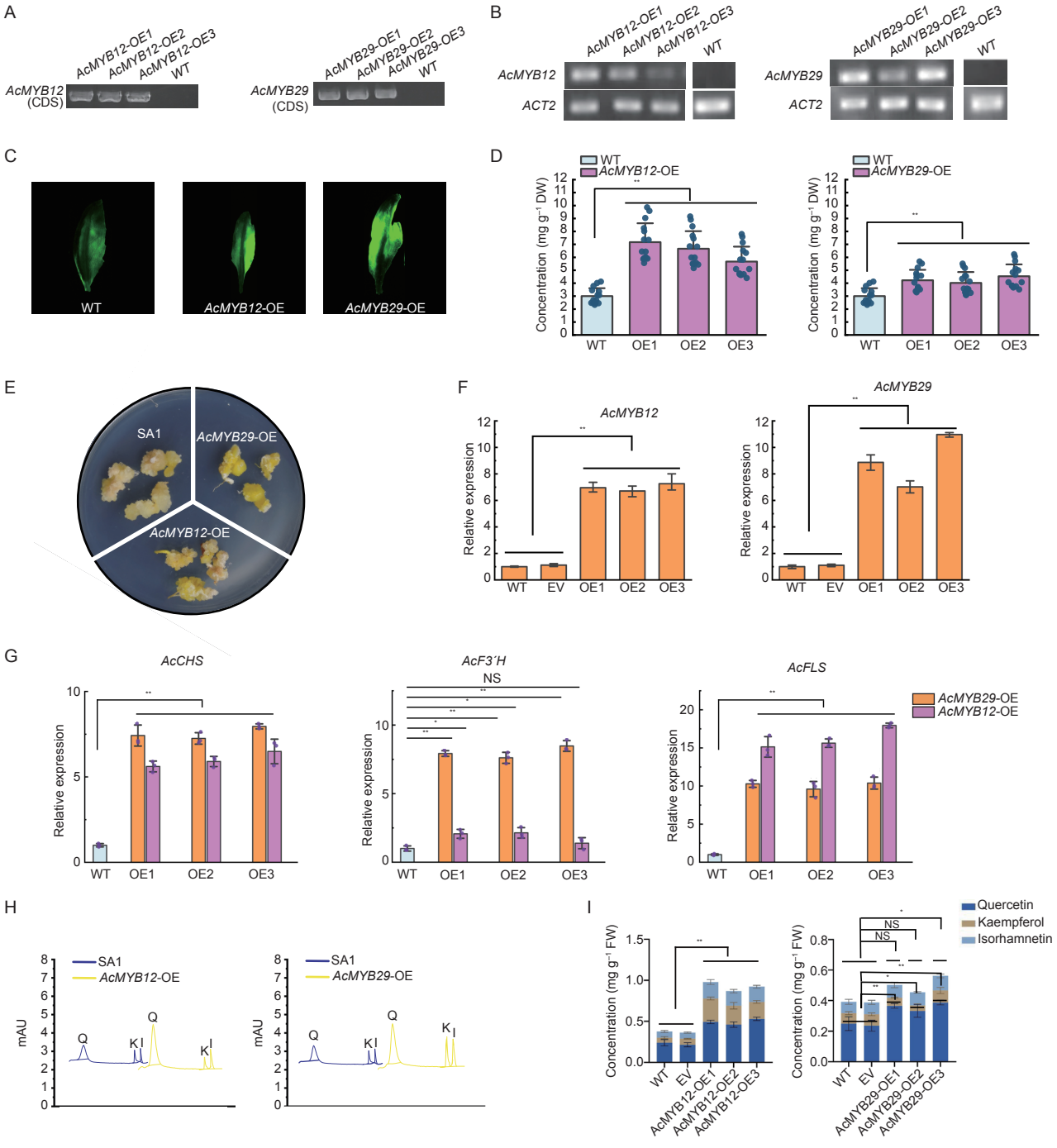


Fig. 7 Overexpression of *AcMYB12* and *AcMYB29* significantly enhanced flavonol content in *Arabidopsis thaliana* and onion callus tissue. A, identification of heterologously expressing *MYB29, MYB12*. DNA from candidate *Arabidopsis* plants (T_3) was extracted and utilized as templates for PCR reactions using full-length primers of *AcMYB29, AcMYB12*. B, semiquantitative PCR. The cDNA from WT and overexpression plants served as template with quantitative primers for *AcMYB29* and *AcMYB12*, *β-actin* as reference gene, primers for internal reference quantification were designed for PCR. C, *in situ* DPBA staining quantified flavonols in *Arabidopsis* leaves. D, flavonol content in leaves of overexpressed and WT was determined by HPLC. E, wild-type and overexpressed onion callus. SA1 (Wild-type): callus with no gene imported and grown under normal conditions, served as a control group. F, RT-qPCR of *AcMYB29* and *AcMYB12* was conducted in WT and overexpressed onion callus. Data are means±SD (*n*=3). EV, the empty vector is transferred to callus. G, relative expression of flavonol pathway genes in WT and overexpression callus. Data are means±SD (*n*=5). H and I, determination of flavonol content in WT and overexpressed callus. H, peak area map of the substance, and substance concentration and peak area are positively correlated. Among them, 'Q' represents quercetin, 'K' represents kaempferol, and 'I' represents isorhamnetin. For panels D and F–I statistically significant differences were determined by the two-tailed Student's *t*-test (*, $P<0.05$; **, $P<0.01$).

proportion of quercetin in total flavonols was notably higher in *AcMYB29*-OE compared to *AcMYB12*-OE. These results demonstrate that both AcMYB12 and AcMYB29 positively regulate flavonol biosynthesis in onion, while

AcMYB29 specifically activates transcription of *AcF3´H*, leading to variations in quercetin accumulation.

3.11. AcMYB12, AcMYB29 can promote the transcription of *AcCHS*, *AcF3´H*, and *AcFLS*

Transcription activation assays were conducted to validate the molecular mechanism through which AcMYB12 and AcMYB29 regulate flavonol biosynthesis. Both transcription factors significantly activated the expression of *AcCHS* and *AcFLS*. However, only AcMYB29 demonstrated significant activation of *AcF3´H* transcription, while AcMYB12 showed no effect (Fig. 8-B). LUC fluorescence imaging tests in tobacco leaves corroborated these findings (Fig. 8-D). This observation potentially explains AcMYB29's preference for regulating quercetin synthesis. In conclusion, AcMYB12 and AcMYB29 promote the transcriptional activation of *AcCHS*, *AcFLS* to regulate flavonol biosynthesis. AcMYB29's specific regulation of quercetin accumulation may result from its distinct transcriptional activation of *AcF3´H*. This was verified in experiments where *AcMYB29* and *AcF3´H* were silenced simultaneously (unpublished).

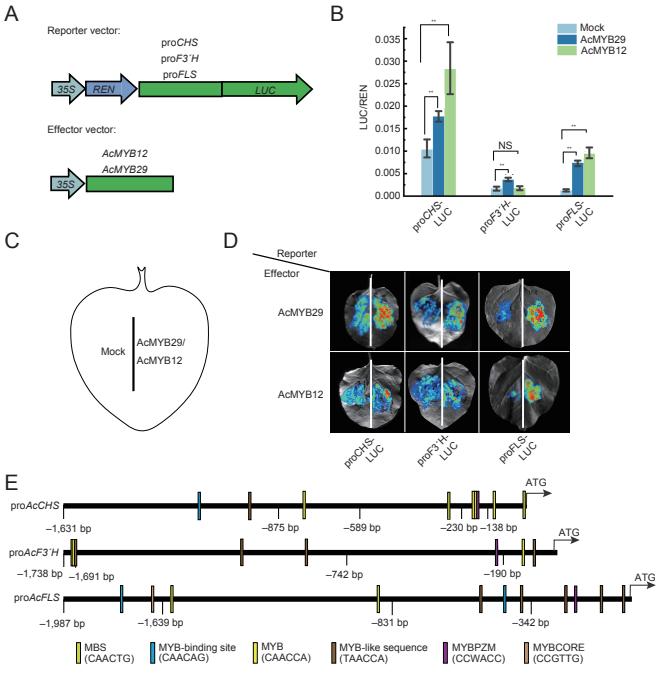


Fig. 8 Transcriptional activation analysis of AcMYB12 and AcMYB29, and category analysis of MYB binding elements in the promoters pro*AcCHS*, pro*AcF3´H* and pro*AcFLS*. A, a schematic representation of the structure of the vector used in the transcriptional activation assay. B, detection of the transcriptional activation capacity of AcMYB29 and AcMYB12. Mock: empty reporter vector+effector vector-MYB29/12. AcMYB12/29: reporter vector-pro*CHS*/ *F3´H*/*FLS*+effector vector-AcMYB29/12. REN, Renilla luciferase; LUC, luciferase. Data are means±SD (n=3). Statistically significant differences were determined by the two-tailed Student's t-test (*, P<0.05; **, P<0.01). C and D, the fluorescence intensity was used to visualize the transcriptional activation ability of the (B) results. E, visualization of the *cis*-elements of the promoters. Statistical analysis of the number and distribution of *cis*-elements was conducted using PlantCARE for the initiation of flavonol pathway genes.

3.12. AcMYB12 and AcMYB29 directly bound to the promoter of *AcCHS, AcF3´H*, and *AcFLS*

To elucidate the mechanism underlying differences in transcriptional regulatory capacity, this study examined the interactions between specific transcription factors and gene promoters. Studies have demonstrated that SG7-MYB transcription factors directly bind to flavonol pathway gene promoters to enhance transcription (Shan *et al.* 2020). The present investigation selected specific upstream regions of *AcCHS*, *AcF3´H* and *AcFLS* to analyze their interaction with AcMYB12 and AcMYB29. A yeast one-hybrid assay confirmed the interaction between these transcription factors and the selected regions (Fig. 9-B). The observed variations in yeast growth suggested differences in binding strength, potentially explaining the distinct transcription levels of the two genes involved in flavonol regulation.

The binding of transcription factors to promoters is primarily mediated by *cis*-elements. The distinct regulatory effects of AcMYB12 and AcMYB29 may be attributed to differences in their binding to *cis*-elements. Analysis of the promoters revealed numerous MYB *cis*-elements across all three promoters, with notable variations in both type and frequency (Fig. 8-E). To investigate this phenomenon, promoter truncation experiments were performed. For the pro*CHS* promoter, AcMYB29 exhibited significant differences in transcriptional activation between fragments 1 and 2 and between fragments 3 and 4, while AcMYB12 showed differences only between fragments 3 and 4 (Fig. 9-A). In the pro*FLS* promoter, both transcription factors demonstrated variations between fragments 3 and 4, with AcMYB12 additionally showing differences between fragments 1 and 2 (Fig. 9-A). These findings suggest that the differential transcriptional activity of AcMYB12 and AcMYB29 may result from their distinct binding affinities to *cis*-elements.

Additional analysis demonstrated that both transcription factors interact with MBS, MYBPZM, and MYBCORE *cis*-elements. However, AcMYB29 specifically interacted with a MYB-like sequence, while AcMYB12 did not (Fig. 9-C). These observations indicate that variations in their binding to *cis*-elements result in different transcriptional regulatory capacities and ultimately lead to distinct patterns in quercetin accumulation.

4. Discussion

4.1. Flavonols have potential functions in onion response to abiotic stress

Flavonoids are ubiquitous plant compounds that serve essential roles in growth and development (Petrussa *et al.* 2013; Ramaroson *et al.* 2022). Anthocyanins provide flowers with vibrant colors that attract pollinators, facilitating pollination (Tanaka *et al.* 2008). These compounds also enhance plant resistance to both biotic and abiotic stress. For instance, *Arabidopsis* enhances its freezing tolerance through anthocyanin synthesis during cold acclimation (Li *et al.* 2021). Flavonols, another class of flavonoids, play vital roles in plant development. They contribute to yellow flower coloration (Czemmel *et al.* 2009; Takahashi *et al.* 2007; Kim *et al.* 2017) and function as copigments for anthocyanins, generating

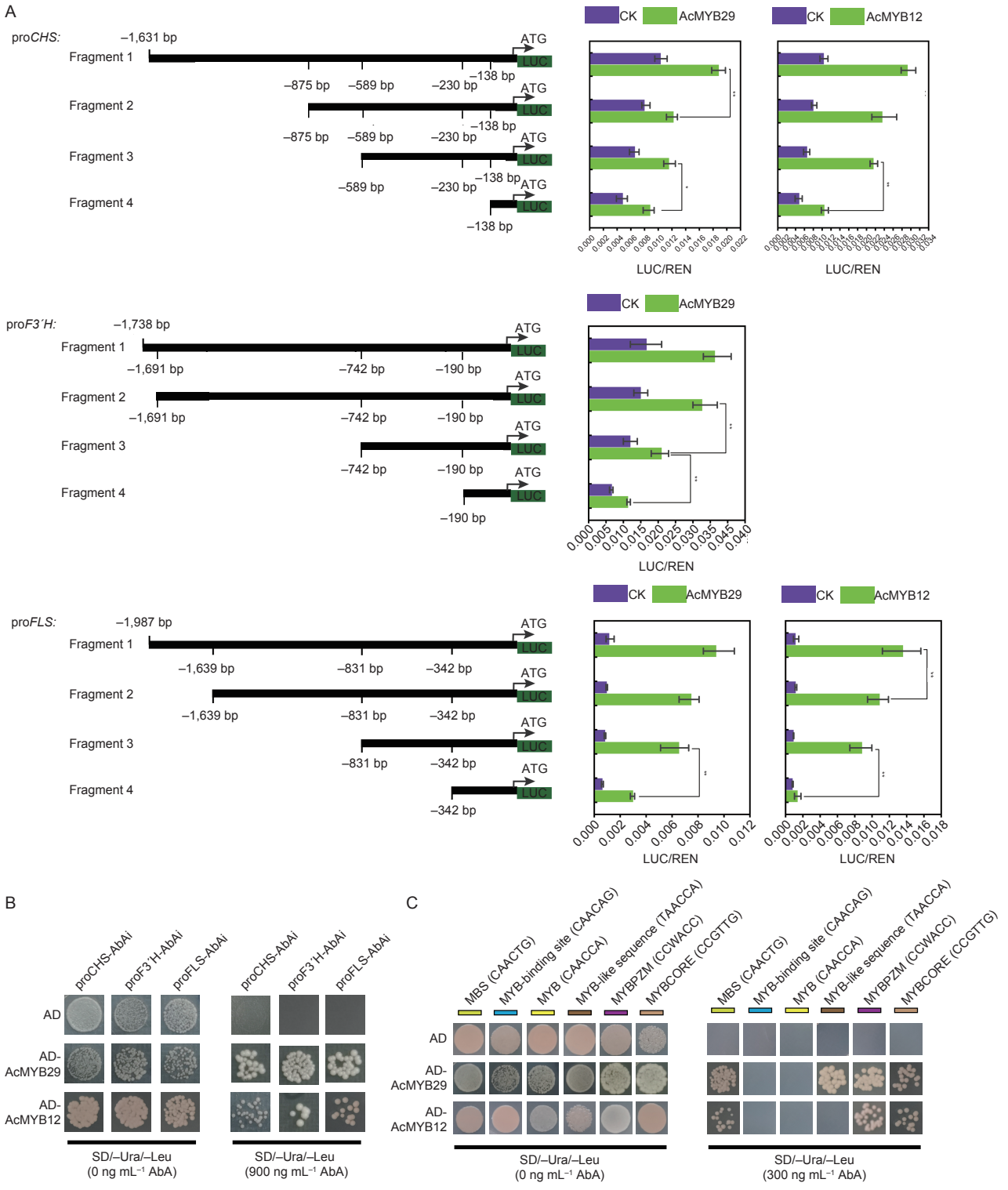


Fig. 9 Analysis of the transcriptional activation differences of AcMYB12 and AcMYB29 on the promoters pro*AcCHS*, pro*AcF3′H* and pro*AcFLS*, and the binding activity of MYB binding elements. A, detection of the transcriptional activation capacity of MYB29 and MYB12. Data are means±SD (n=3). Statistically significant differences were determined by the two-tailed Student's t-test (*, P<0.05; **, P<0.01). B and C, yeast one-hybrid assay. At least three biological replicates were performed for each result. Among them, B examines the binding of the full-length promoter to *AcMYB29* and *AcMYB12* and C demonstrates the binding test of the *cis*-elements in the promoter to *AcMYB29* and *AcMYB12*.

diverse flower colors. Additionally, they provide protection against various environmental stresses. For example, plants accumulate flavonols in response to UV-B exposure (Zhao *et al.* 2021; Yoo *et al.* 2013). Flavonol production increases under various stress conditions, including physical injury, cold stress, and drought (Todesco *et al.* 2022; Bhatia *et al.* 2018). In transient silencing experiments, control seedlings exhibited increased flavonol synthesis, presumably

in response to mechanical damage during root cutting. However, seedlings with silenced *AcMYB12* and *AcMYB29* demonstrated impaired growth compared to controls, likely due to reduced flavonol levels (Appendix J). The precise physiological mechanism requires further investigation.

4.2. Cluster-29851.120213, Cluster-29851.111946, Cluster-29851.119571 are key enzymes in flavonol biosynthesis of onion

While enzymes involved in onion flavonol biosynthesis, including AcCHS, AcCHI, AcF3H, AcF3´H, and AcFLS, have been identified, their functions require additional validation in onions. This investigation employed transient silencing techniques to verify their functions. CHS serves as a crucial enzyme for flavonol production (Li *et al.* 2015). In onions, two CHS forms, AcCHS-A and AcCHS-B, regulate skin color (Kim *et al.* 2005). Transcriptome analysis revealed that Cluster-29851.120211 and Cluster-29851.120213 share 89.31% amino acid similarity and both serve essential roles in flavonol biosynthesis. However, Cluster-29851.120213 demonstrates stronger association with flavonol accumulation patterns (Fig. 3-B). AcF3´H is essential for synthesizing quercetin and its precursor, dihydroquercetin (Masuzaki *et al.* 2006). In asODN-*AcF3´*H, quercetin levels decreased significantly, confirming AcF3´H's role in quercetin synthesis. FLS represents another critical enzyme, with AcFLS-H6 and AcFLS-HRB identified in onions (Park *et al.* 2020). In 'SA1', amino acid variations were observed between Cluster-29851.119571 and AcFLS-H6, AcFLS-HRB. Although Cluster-29851.119571 exhibited more variation compared to AcFLS-HRB, the two site mutations did not occur in the critical functional domain of AcFLS-HRB. This mutation aligns with previous studies, suggesting it does not affect substrate affinity. Therefore, Cluster-29851.119571 exhibits close relation to AcFLS-HRB. *In vitro* studies demonstrate that AcFLS-HRB shows greater efficiency in producing quercetin than AcFLS-H6 (Park *et al.* 2017). Although the roles of flavonol pathway genes have been investigated, the precise molecular mechanisms underlying quercetin accumulation in onions remain unclear. This research provides novel insights into the mechanism at the transcriptional level.

4.3. *AcMYB12* and *AcMYB29* positively regulate flavonol biosynthesis in onion and are functionally conserved in *Arabidopsis*

Although flavonol biosynthesis has been elucidated, the regulatory mechanisms remain incompletely understood. Transient silencing and overexpression experiments demonstrated that *AcMYB12* and *AcMYB29* positively regulated flavonol biosynthesis. In overexpressed *Arabidopsis*, despite the different distribution of *cis*-elements in the flavonol pathway gene promoter compared to onion, they contain identical binding active *cis*-elements as in onion, enabling their regulation of flavonol in *Arabidopsis* (Appendix L).

4.4. Light is a potential regulator of flavonol biosynthesis, and *AcMYB12* and *AcMYB29* may be mediators

In this investigation, flavonol content and types vary among onion varieties at different developmental stages and in different tissues (Appendix N) (Yoo *et al.* 2013; Wang *et al.* 2018a). The findings indicate that transcriptional regulation plays a predominant role in this process. The expression of *AcMYB12* and *AcMYB29* demonstrates strong correlation with flavonol biosynthesis in various varieties and tissues (Appendix N). Previous studies revealed that outer bulb layers contain higher concentrations of secondary metabolites, including anthocyanins, than inner layers (Li *et al.* 2022). The observed difference in flavonol content may be attributed to light exposure, particularly UV-B. Mature bulbs possess 10–12 layers, and the outer layers, exposed to UV-B, provide protection for the inner layers (Yoo *et al.* 2013). Light-responsive *cis*-elements were identified in the promoters of flavonol-related genes (Fig. 3-E), indicating that light influences their transcription, resulting in elevated flavonol levels in outer layers. This phenomenon warrants further investigation.

4.5. Differences in the binding of AcMYB12 and AcMYB29 to the *cis*-element of the flavonol pathway gene promoter led to differences in their regulatory capacity

Although SG7-MYB family exhibits limited membership, distinct variations in number and function across species. In *Arabidopsis*, the promoter of *AtF3´H* remains unregulated by AtMYB11, AtMYB12, and AtMYB111. In freesias, among four SG7-MYB family members, only FhMYBF3 demonstrated specific activation of *FhFLS1*. The underlying molecular mechanisms of these variations remain incompletely understood. This investigation identified two transcription factors, AcMYB12 and AcMYB29, that regulate flavonol biosynthesis. These factors exhibit notable differences in specific regulatory functions. While both AcMYB12 and AcMYB29 regulate *AcCHS* and *AcFLS*, AcMYB29 predominantly influences quercetin synthesis, whereas AcMYB12 regulates total flavonol synthesis. The variation in *cis*-elements among flavonol synthesis gene promoters contributes to differences in flavonol accumulation. These findings advance our understanding of SG7-MYB family members' roles in flavonol biosynthesis (Fig. 10).

5. Conclusion

This study identified key flavonol biosynthesis genes in onions, including *AcCHS, AcF3´H*, and *AcFLS*. More significantly, the transcription factors AcMYB12 and AcMYB29 were characterized. Although these factors demonstrate functional redundancy in regulating *CHS* and *FLS*, they exhibit substantial differences in regulating *F3´H*, which correlates with the diversity of *cis*-elements in the promoters and variations in the binding of AcMYB12 and AcMYB29 to the MYB-like sequence. These discoveries enhance our understanding of the SG7-MYB family's role in regulating flavonol biosynthesis.

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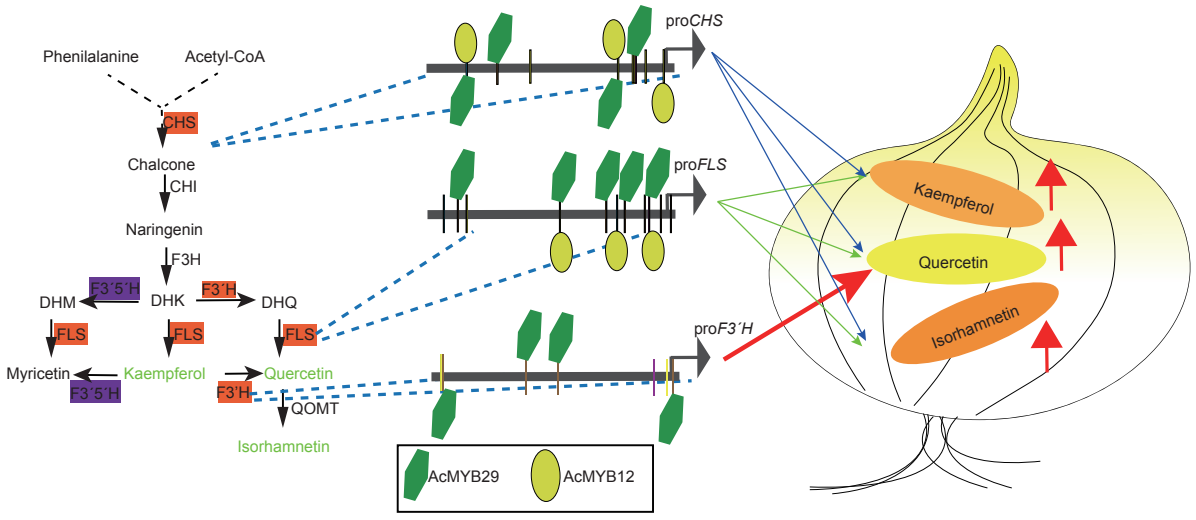


Fig. 10 A proposed mechanistic model illustrating how AcMYB29 and AcMYB12 regulate flavonol biosynthesis in onion. DHM, dihydromyricetin; DHK, dihydrokaempferol; DHQ, dihydroquercetin; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3´H, flavonoid 3´-hydroxylase; F3´5´H, flavonoid-3´,5´-hydroxylase; FLS, flavonol synthase; QOMT, quercetin *O*-methyltransferase.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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References

Bhatia C, Pandey A, Gaddam S R, Hoecker U, Trivedi P K. 2018. Low temperature-enhanced flavonol synthesis requires light-associated regulatory components in *Arabidopsis thaliana*. *Plant & Cell Physiology*, **59**, 2099–2112.

Czemmel S, Stracke R, Weisshaar B, Cordon N, Harris N N, Walker A R, Robinson P, Bogs J. 2009. The grapevine R2R3-MYB transcription factor VvMYBF1 regulates flavonol synthesis in developing grape berries. *Plant Physiology*, **151**, 1513–1530.

Jia Q W, Yin Y Q, Gai S T, Tian L, Zhu Z H, Qin L, Wang Y. 2024. Onion cryptochrome 1 (AcCRY1) regulates photomorphogenesis and photoperiod flowering in *Arabidopsis* and exploration of its functional mechanisms under blue light. *Plant Physiology and Biochemistry*, **206**, 108300.

Kim B, Cho Y, Kim S. 2017. Identification of a novel DFR-A mutant allele determining the bulb color difference between red and yellow onions (*Allium cepa* L.). *Plant Breeding and Biotechnology*, **5**, 45–53.

Kim S, Yoo K S, Pike L M. 2005. The basic color factor, the c locus, encodes a regulatory gene controlling transcription of chalcone synthase genes in onions (*Allium cepa* L.). *Euphytica*, **142**, 273–282.

Ko E Y, Nile S H, Sharma K, Li G H, Park S W. 2015. Effect of different exposed lights on quercetin and quercetin glucoside content in onion (*Allium cepa* L.). *Saudi Journal of Biological Sciences*, **22**, 398–403.

Lee R, Baldwin S, Kenel F, McCallum J, Macknight R. 2013. *FLOWERING LOCUS T* genes control onion bulb formation and flowering. *Nature Communications*, **4**, 2884.

Li X J, Cao L J, Jiao B B, Yang H F, Ma C S, Liang Y. 2022. The bHLH transcription factor AcB2 regulates anthocyanin biosynthesis in onion (*Allium cepa* L.). *Horticulture Research*, **9**, uhac128.

Li Y P, Shi Y T, Li M Z, Fu D Y, Wu S F, Li J G, Gong Z Z, Liu H T, Yang S H. 2021. The CRY2-COP1-HY5-BBX7/8 module regulates blue light-dependent cold acclimation in Arabidopsis. *The Plant Cell*, **33**, 3555–3573.

Li Y, Kim J I, Pysh L, Chapple C. 2015. Four isoforms of Arabidopsis 4-Coumarate:CoA ligase have overlapping yet distinct roles in phenylpropanoid metabolism. *Plant Physiology*, **169**, 2409–2421.

Liang T, Shi C, Peng Y, Tan H J, Xin P Y, Yang Y, Wang F, Li X, Chu J F, Huang J R, Yin Y H, Liu H T. 2020. Brassinosteroid-activated BRI1-EMS-SUPPRESSOR 1 inhibits flavonoid biosynthesis and coordinates growth and UV-B stress responses in plants. *The Plant Cell*, **32**, 3224–3239.

Liu H N, Su J, Zhu Y F, Yao G F, Allan A C, Ampomah-Dwamena C, Shu Q, Wang K L, Zhang S L, Wu J. 2019. The involvement of *PybZIPa* in light-induced anthocyanin accumulation via the activation of *PyUFGT* through binding to tandem G-boxes in its promoter. *Horticulture Research*, **6**, 134.

Ma D W, Constabel C P. 2019. MYB repressors as regulators of phenylpropanoid metabolism in plants. *Trends in Plant Science*, **24**, 275–289.

Masuzaki S, Shigyo M, Yamauchi N. 2006. Direct comparison between genomic constitution and flavonoid contents in allium multiple alien addition lines reveals chromosomal locations of genes related to biosynthesis from dihydrokaempferol to quercetin glucosides in scaly leaf of shallot (*Allium cepa* L.). *Theoretical & Applied Genetics*, **112**, 607–617.

Meng X Y, Li Y Q, Zhou T T, Sun W, Shan X T, Gao X, Wang L. 2019. Functional differentiation of duplicated flavonoid 3-*O*-glycosyltransferases in the flavonol and anthocyanin biosynthesis of *Freesia hybrida*. *Frontiers in Plant Science*, **10**, 1330.

Park S, Kim D H, Lee J Y, Ha S H, Lim S H. 2017. Comparative analysis of two flavonol synthases from different-colored onions provides insight into flavonoid biosynthesis. *Journal of Agricultural and Food Chemistry*, **65**, 5287–5298.

Park S, Kim D H, Yang J H, Lee J Y, Lim S H. 2020. Increased flavonol levels in tobacco expressing *AcFLS* affect flower color and root growth. *International Journal of Molecular Sciences*, **21**, 1011.

Perez-Gregorio M R, Regueiro J, Simal-Gándara J, Rodrigues A S, Almeida D P F. 2014. Increasing the added-value of onions as a source of antioxidant flavonoids: A critical review. *Critical Reviews in Food Science & Nutrition,* **54**, 1050–1062.

Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S, Vianello A. 2013. Plant flavonoids — biosynthesis, transport and involvement in stress responses. *International Journal of Molecular Sciences*, **14**, 14950–14973.

Qin L, Ma H L, Zhang X, Zhang Z H, Zhang X, Wang Y. 2023.

Metabolomics and transcriptomics analyses provides insights into S-alk(en)yl cysteine sulfoxides (CSOs) accumulation in onion (*Allium cepa*). *Scientia Horticulturae*, **310**, 111727.

Ramaroson M L, Koutouan C, Helesbeux J J, Le Clerc V, Hamama L, Geoffriau E, Briard M. 2022. Role of phenylpropanoids and flavonoids in plant resistance to pests and diseases. *Molecules* (Basel, Switzerland), **27**, 8371.

Rodrigues A S, Pérez-Gregorio M R, García-Falcón M S, Simal-Gándara J, Almeida D P F. 2011. Effect of meteorological conditions on antioxidant flavonoids in portuguese cultivars of white and red onions. *Food Chemistry*, **124**, 303–308.

Schwinn K E, Ngo H, Kenel F, Brummell D A, Albert N W, McCallum J A, Pither-Joyce M, Crowhurst R N, Eady C, Davies K M. 2016. The onion (*Allium cepa* L.) *R2R3-MYB* gene *MYB1* regulates anthocyanin biosynthesis. *Frontiers in Plant Science*, **7**, 1865.

Shan X T, Li Y Q, Yang S, Yang Z Z, Qiu M, Gao R F, Han T T, Meng X Y, Xu Z Y, Wang L, Gao X. 2020. The spatio-temporal biosynthesis of floral flavonols is controlled by differential phylogenetic MYB regulators in *Freesia hybrida*. *The New Phytologist*, **228**, 1864–1879.

Shen, N, Wang T F, Gan Q, Liu S, Wang L, Jin B. 2022. Plant flavonoids: classification, distribution, biosynthesis, and antioxidant activity. *Food Chemistry*, **383**, 132531.

Slimestad R, Fossen T, Vagen I M. 2007. Onions: a source of unique dietary flavonoids. *Journal of Agricultural and Food Chemistry*, **55**, 10067–10080.

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.

Sun W, Cao Z Y, Li Y, Zhao Y X, Zhang H. 2007. A simple and effective method for protein subcellular localization using agrobacterium-mediated transformation of onion epidermal cells. *Biologia*, **62**, 529–532.

Takahashi R, Githiri S M, Hatayama K, Dubouzet E G, Shimada N, Aoki T, Ayabe S, Iwashina T, Toda K, Matsumura H. 2007. A single-base deletion in soybean flavonol synthase gene is associated with magenta flower color. *Plant Molecular Biology*, **63**,125–135.

Tanaka Y, Sasaki N, Ohmiya A. 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, **54**, 733–749.

Todesco M, Bercovich N, Kim A, Imerovski I, Owens G L, Dorado Ruiz Ó, Holalu S V, Madilao L L, Jahani M, Légaré J S, Blackman B K, Rieseberg L H. 2022. Genetic basis and dual adaptive role of floral pigmentation in sunflowers. *eLife*, **11**, e72072.

Wang S L, Chu Z H, Jia R, Dan F, Shen X L, Li Y, Ding X H. 2018a. SlMYB12 regulates flavonol synthesis in three different cherry tomato varieties. *Scientific Reports*, **8**, 1582.

Wang Z L, Wang S, Kuang Y, Hu Z M, Qiao X, Ye M. 2018b. A comprehensive review on phytochemistry, pharmacology, and flavonoid biosynthesis of *Scutellaria baicalensis*. *Pharmaceutical Biology*, **56**, 465–484.

Williams J S, Thomas M, Clarke D J. 2005. The gene *stlA* encodes a phenylalanine ammonia-lyase that is involved in the production of a stilbene antibiotic in photorhabdus luminescens *tt01*. *Microbiology*, **151**, 2543–2550.

Xie Z P, Sundström J F, Jin Y K, Liu C L, Jansson C, Sun C X. 2014. A selection strategy in plant transformation based on antisense oligodeoxynucleotide inhibition. *The Plant Journal*, **77**, 954–961.

Yao P F, Huang Y J, Dong Q X, Wan M, Wang A H, Chen Y W, Li C L, Wu Q, Chen H, Zhao H X. 2020. FtMYB6, a light-induced SG7 R2R3-MYB transcription factor, promotes flavonol biosynthesis in tartary buckwheat (*Fagopyrum tataricum*). *Journal of Agricultural and Food Chemistry*, **68**, 13685–13696.

Yoo K S, Lee E J, Patil B S. 2013. Changes in quercetin glucoside concentrations of onion bulbs by scales, during storage, and in sprouting leaves exposed to UV. *Postharvest Biology & Technology*, **83** , 65–71.

Zhai R, Zhao Y X, Wu M, Yang J, Li X Y, Liu H T, Wu T, Liang F F, Yang C Q, Wang Z G, Ma F W, Xu L F. 2019. The MYB transcription factor PbMYB12b positively regulates flavonol biosynthesis in pear fruit. *BMC Plant Biology*, **19**, 85.

Zhang X B, Abrahan C, Colquhoun T A, Liu C J. 2017. Proteolytic regulator controlling chalcone synthase stability and flavonoid biosynthesis in Arabidopsis. *The Plant Cell*, **29**, 1157–1174.

Zhang Y, He Y, Zhao H Y, Wang Y, Wu C L, Zhao Y Z, Xue H N, Zhu Q D, Zhang J L, Ou X Q. 2024. The 14-3-3 protein BdGF14a increases the transcriptional regulation activity of *BdbZIP62* to confer drought and salt resistance in tobacco. *Plants* (Basel, Switzerland), **13**, 245.

Zhao X C, Zeng X S, Lin N, Yu S W, Fernie A R, Zhao J. 2021. CsbZIP1-CsMYB12 mediates the production of bitter-tasting flavonols in tea plants (*Camellia sinensis*) through a coordinated activator-repressor network. *Horticulture Research*, **8**, 110.

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