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# Transcriptome and co-expression network analyses provide insights into red and blue light promote the differentiation of 'Red Globe' grape flower buds

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**Abstract**

The aim of this research is to address the issue of poor differentiation of grape flower buds at the 'Red Globe' facility. In this work, greenhouse light was employed as a control to examine the responses of flower bud differentiation in the 'Red Globe' grape to red light (R), blue light (B), and proportions of red light: blue light were 2:1 (R2B1), 4:1 (R4B1), and 6:1 (R6B1). The R4B1 treatment was observed to significantly enhance the differentiation of the flower buds, elevate the content of carbohydrates, flavonoid, auxin (IAA), abscisic acid (ABA) and giberellins (GAs), while concurrently reducing the levels of cytokinins (CKs) in flower buds in comparison to the control. A weighted gene co-expression network analysis (WGCNA) was used to establish a red and blue light regulatory network, with the MYB transcription factor *MYB93* identified as a potential co-expressed partner with genes involved in plant hormone signal transduction, flavonoid biosynthesis and starch and sucrose metabolism. The dual luciferase reporter (DLR) ssay yielded confirmation that *MYB93* activates the expression of several genes, including the plant hormone signal transduction gene *BAK1*, the flavonoid biosynthesis gene *THS1* and the starch and sucrose metabolism gene *otsB*. In conclusion, the evidence supports the effectiveness of the R4B1 treatment in accelerating flower bud differentiation in the 'Red Globe' facility, and the hub gene *MYB93* has been confirmed as a key gene in response to red and blue light.

*Keywords:* 'Red Globe'; MYB transcription factor; Red and blue light; Flower bud differentiation

## 1. Introduction

In viticulture, flower bud differentiation has a direct and substantial impact on the quality and quantity of grapes(Sawicki et al., 2015). The differentiation of flower bud in the grapevine is a multifaced process entailing a series of physiological, biochemical and morphological alterations. Ultimately, the apical meristematic tissue will differentiate into either an inflorescence or a tendril(Monteiro et al., 2021). The application of a shed film in the facility can lead to a reduction in light intensity and duration, which can lead to a reduction in the number of flower bud differentiations(Zhang et al., 2017). The cultivar 'Red Globe' is distinguished by its propensity for poor flower bud differentiation in facility production, which represents a significant challenge in this context.

As an energy source and a fundamental component of growth and development process in plants, light regulates a range of biological functions, among them photosynthesis, morphogenesis, growth and development, and metabolism(Jiao et al., 2007). Photoreceptors are capable of sensing light conditions in the surrounding environment, regulating growth and development of plant, controlling reproductive growth(Tripathi et al., 2019). The primary photoreceptors that drive morphological and developmental changes in plants are the photosensitive pigments and cryptochromes(Quail et al., 1995). The activation of different photoreceptors and gene expression is contingent upon the wavelength of light, with red and blue light exhibiting the highest relative quantum efficiency for plant photosynthesis(Wu et al., 2024).

Previous studies has shown that red light suppresses plant flowering by regulating the expression of *PHY* gene, whereas blue light regulates the expression of *CRY* gene to promote flowering(Lopez et al., 2020) . *Arabidopsis thaliana* exposure to red light inhibits and delays the expression of the flowering gene *FT*, while exposure to blue light promotes *FT* expression and thus flowering(Tsukamoto et al., 2016), with a similar effect in petunia(Fukuda et al., 2016). Strawberry plants under blue LEDs developed the most flower clusters, while those under red LEDs developed the least(Magar et al., 2018). However, some researchers have come to the opposite conclusion, suggesting that red light can promote early flowering in saffron by regulating plant hormones(Zhu et al., 2022). The research indicates that blue light suppresses apple flowering by increasing melatonin levels, whereas the impact of red light is insignificant (Zhang et al., 2019). The application red light lead to an earlier flowering phase and an increased quantity

of flower buds in Jasminum, whereas blue light has the opposite result(Chen et al., 2020).The effect of red and blue light on plant flowering therefore depends on the species involved. As understanding of the consequences of monochromatic light spectra on plant physiological processes increases, there is a growing recognition that mixed light is a vital factor in stimulating growth and development(Liu et al., 2020). The accumulation of carbohydrate in *Hippeastrum hybridum* was stimulated by a ratio of 1:9 between red and blue light, which in promotes flowering(Wang et al., 2022). Studies have found that when the ratio of red light to blue light is 2:1 promotes the formation of cucumber flower buds and accelerates flowering through hormonal regulation(Song et al., 2018). Moreover, supplementing red and blue light improved the amount of flower bud differentiation in pitay, and effect is most effective when the red and blue light ratio is 4: 1(Chen et al., 2019). Consequently, it is necessary to study the optimal proportions of red to blue light for the differentiation of plant flower buds.

Despite the widespread employment of red and blue light in the regulation in horticulture, there is a paucity of detailed studies on this process in grapes. The 10-year-old 'Red Globe' grape was employed as the research subject in this study, and different proportions of red and blue light LED lighting were applied. Flower buds were sectioned and analysed for differentiation status. Additionally, the nutrient and plant hormone content of the flower buds were measured and analyzed. Furthermore, differentially expressed genes (DEGs) in distinct treatments were identified through the utilization of RNA-seq transcriptomes and WGCNA to construct the red and blue light-mediated flower bud differentiation regulatory network. Candidate hub genes, including the MYB transcription factor (TF) *MYB93*, were identified and confirmed by a DLR assay. The purpose of this research was to explore the effect of varying proportions of red and blue light on the differentiation of grape flower buds and to determine the potential molecular regulations associated with red and blue light, in support of stable yield cultivation of facility grapes.

## 2. Materials and methods

### *2.1 Plant materials and light treatment*

The ten-year-old *Vitis vinifera* cv. 'Red Globe' grape, which was subjected to light treatment and located in the solar greenhouse of the Horticultural Industrial Park of Helan County, Yinchuan City, was selected as the test material. The test was designed with five light ratios of red and blue light, including red light (R), blue light (B), 2: 1 red and blue light (R2B1), 4: 1 red and blue light (R4B1), and 6: 1 red and blue light (R6B1), of LED plant lamps, as well as greenhouse light as a control (CK). The various treatments were separated by blackout film. These lamps emitted red and blue light at wavelength of 620 and 435 nm (Fig. S1), and were positioned 30 cm overhead. Light intensity was kept at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the level of the leaves, with the lighting treated from 8: 00 to 22: 00 each day.

### *2.2 physiological analysis*

The light treatment commenced upon the breaking of the buds on 5 April and continued until the fruit reached maturity on 15 September. The flower bud situated at the fifth node was selected, excised with a sharp scalpel, and, following the removal of the outer scales of the flower bud, placed in an FAA solution (formaldehyde-acetic acid-ethanol) for the preparation of paraffin sections. The remaining flower buds were flash-frozen in liquid nitrogen and stored in a refrigerator set to -80°C for subsequent testing.

The dehydrated flower buds were encapsulated in paraffin and sliced using a slicer (Leica RM2245). Subsequently, the sections were colored with safranin-solid green, and then sealed with neutral gum(Fan et al., 2018). The specimens were analysed using an optical microscope (Olympus BX53) and pictures were obtained via an Olympus SC180 image analysis system. The soluble sugars and starch content were quantified at 620 nm through the utilisation of the sulfuric acid-anthrone method(Yu et al., 2020). The colorimetric method was employed for the determination of flavonoid content(Islam et al., 2017).

### *2.3 Plant hormone extraction and quantitative analysis*

The flower buds were extracted with 1 $mmol \cdot L^{-1}$ butylated hydroxytoluene (BHT) and 80% methanol with a small amount of cross-linked polyvinylpyrrolidone (PVPP) added, by crushing the flower buds for four hours in a refrigerator at 4°C. The supernatant obtained by centrifugation was collected and employed for the removal of pigments via a C18 column. Following this, the solution was transferred to

centrifuge tubes and subsequently dried on nitrogen at 40-50°C and fixed. The content of IAA, GAs, CKs and ABA was determined using an enzyme-immunoassay(Qin et al., 2019).

*2.4 RNA-seq data analysis*

The OminiPlant RNA kit (ComWin, Beijing, China) was used to the extract total RNA from flower buds. Following the assessment of RNA quality, RNA-seq sequencing was conducted on the Illumina HiSeq 2500 sequencing platform. The HISAT2 software (Kim et al., 2019) was employed , and a reference genome (http://plants.ensembl.org/Vitis_vinifera/Info/Index). The DESeq2 was applied to identify differentially expressed genes under different light treatments, with the conditions being |log2 fold changes| ≥ 1 along with adjusted P-value ≤ 0.05.

*2.5 qRT-PCR verification*

The results of the transcriptome were confirmed by qRT-PCR. The ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) was selected for use in the experiments, and the primers and reference gene sequences are provided in Tabe S1. The following reaction programme was implemented: 5 min at 50℃, 30s at 95℃, 10 s at 95℃, 30 s at 60℃, 40 cycles. Data were calculated using the $2^{-\Delta\Delta Ct}$ method(Mo et al., 2022).

*2.6 WGCNA analysis*

In order to identify candidate genes associated with red and blue light-mediated differentiation of flower bud, WGCNA was utilized(Langfelder and Horvath, 2008). Calculate the topological overlap measure value of the gene network and use hierarchical clustering to divide the genes to generate a gene cluster tree. Once the minimum number of genes has been established, a one0step construction method is employed to identify closely interrelated modules, which are assigned distinct nomenclature and colors. The following parameters were used: soft power was 9, minModelSize was 30, minKMEtoStay was 0.3, and mergeCutHeight was 0.25. Modules with similar co-expression patterns were merged. The co-expression network of core genes was presented via the software Cytoscape (v3.8.0)(Han et al., 2020).

*2.7 Dual-Luciferase reporter assay*

Transformed the reporter plasmids (*BAK1: LUC*, *THS1: LUC* and *otsB: LUC*) and the effector plasmid (*MYB93*) into the *Agrobacterium strain* GV3101. A variety of bacterial combinations were injected into the leaves of 6-week-old *N. benthamiana* plants(Yang et al., 2023). Three days later, the firefly luciferase in the leaves was detected utilizing a Dual Luciferase Reporter Assay Kit (Vazyme, Nanjing, China), and the primers are shown in Table S1. The DLR assay was conducted with six biological replicates.

*2.8 Statistical analysis*

There were at least three biological replicates for each experiment, and means ± SD of the calculated data was used. The statistical analysis of the experiment data used the Student's t-test in the SPSS software (v26.0), and a $P < 0.05$ or $< 0.01$ indicated a significant difference.

**3. Results**

*3.1 Response of 'Red Globe' flower buds to different red and blue light treatments*

To investigate the role of red and blue light in the differentiation of flower buds on ‘Red Globe’, a series of red and blue light treatments were carried out. It was observed that the red-blue light treatments R4B1 and R6B1 significantly increased the flower formation rate, with R4B1 being the highest, 16% higher than the control (Fig. 1A). Furthermore, the paraffin section images revealed that the R4B1 and R6B1 treatments resulted in the formation of well-developed flower buds and a faster differentiation process compared to the control. In contrast, the R and B treatments produced a lack of differentiation in the flower buds (Fig. S2). The R4B1 and R6B1 treatments exhibited markedly elevated soluble sugar, starch, and flavonoid concentrations in the flower buds relative to the control (Fig. 1B-D). In comparison to the control, the B and R2B1 treatments did not result in a notable increase in starch and flavonoid content.

Conversely, a decline in flavonoid content was observed in the R treatment. These results suggest that by enhancing the carbohydrate and flavonoid levels of the flower buds, the R4B1 and R6B1 treatments have positively influenced flower formation.

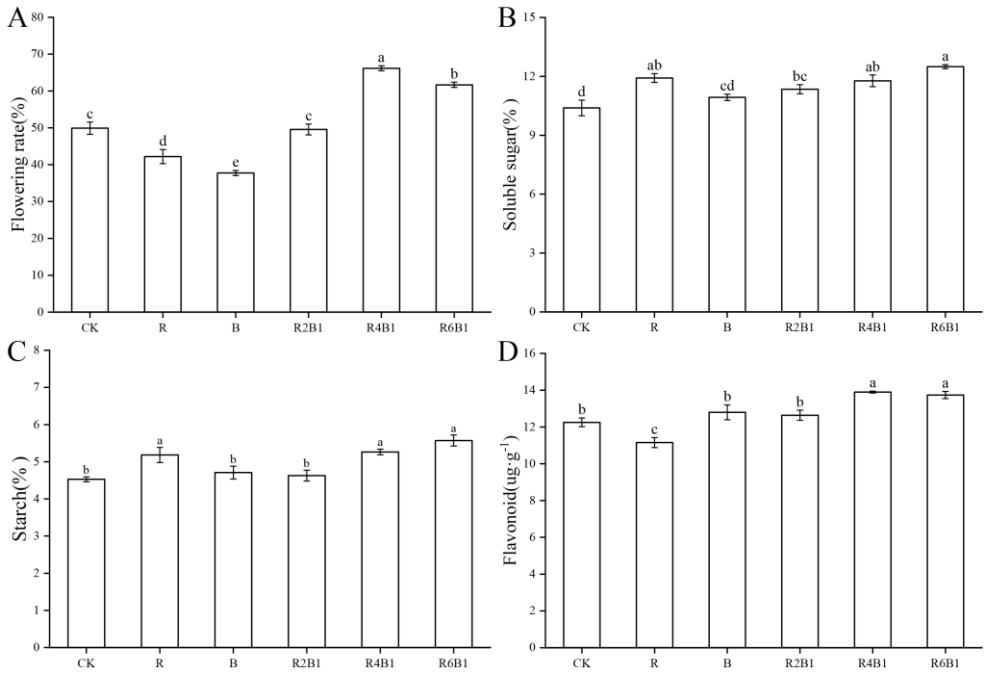



**Fig. 1.** Flower formation rate and physiological characteristics of 'Red Globe' under different treatments. (A) Flowering rate; (B) soluble sugar; (C) starch; (D) flavonoid. Vertical bars illustrate the mean ± SD (n = 3), while lowercase letters demonstrate a statistically significant at $p < 0.05$.

The differentiation of plant buds is intimately associated with the activity of plant hormones. Accordingly, the hormone values in flower buds were determined following treatments with red and blue light. The data demonstrated that the IAA concentration was markedly elevated in comparison to the control following the various light treatments, exhibiting the highest concentration following the B treatment (Fig. 2A). Figure 2B and C indicate that the ABA level was significantly increased following the red-blue light treatments in comparison to the control and other treatments, and the GAs content showed similar results. It is noteworthy that the level of CKs in the flower buds of the R4B1 was considerably lower than that observed in the control and remaining (Fig. 2D). These findings suggest distinct light treatments have influenced the hormonal composition of the flower buds, consequently impacting the rate of 'Red Globe' flower formation.

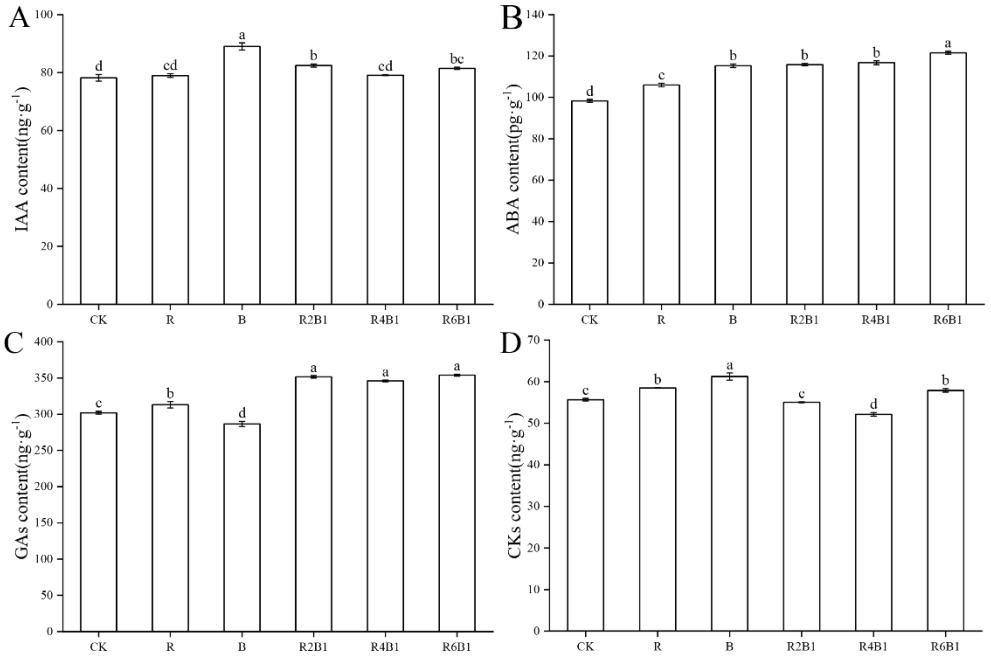



**Fig. 2.** The levels of plant hormone in flower buds with light treatments. (A) IAA; (B) ABA; (C) GAs; (D) CKs. Vertical bars illustrate the mean ± SD (n = 3), while lowercase letters demonstrate a statistically significant at $p < 0.05$.

### *3.2 RNA-seq analyses of flower buds treated with red and blue light*

To obtain insight regarding the molecular regulations of the differentiation of 'Red Globe' flower buds induced by red and blue light, RNA-seq analyses were performed, resulting in the acquisition of clean data of over 5.93 Gb for each sample (Table S2). The PCA demonstrated that the samples harvested in this project exhibited high consistency (Fig. 3A).

The statistics of DEGs in flower buds of 'Red Globe' under different treatments are shown in Fig. 3A. A total of 2498, 1578, 1612, 1702 and 1416 DEGs were found in the R, B, R2B1, R4B1 and R6B1 treatments compared to control. In comparison to the control, the expression of 1453 and 1222 genes was increased in both the R4B1 and R6B1, while the expression of 249 and 194 genes was decreased.

The Gene Ontology (GO) enrichment assay demonstrated that the terms significantly enriched for DEGs in the R4B1 relative to the control were biological regulation, catalytic activity, carbohydrate metabolic process, transcriptional regulator activity, signal transduction (Table S3). The GO terms that were overrepresented in R6B1 treatment were molecular function, catalytic activity, biological regulation, signal transduction, hormone metabolic process. As shown in Table S4, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis identified that the DEGs in both R4B1 and R6B1 treatments were overrepresented in flavonoid biosynthesis, plant hormone signal transduction, circadian rhythms-plant, starch and sucrose metabolism and MAPK signaling pathway-plant.

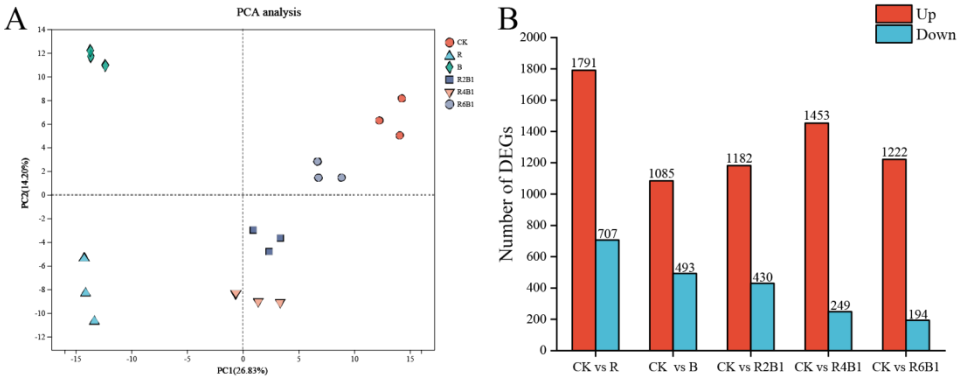



**Fig. 3.** Identification of DEGs in this study. (A) PCA analysis between samples; (B) number of DEGs in different treatments.

### *3.3 Light-induced molecular response of flower buds*

To further analyse the influence of varying red-blue light on flower bud differentiation, a Venn analysis of DEGs was conducted (Fig. 4A). The results demonstrated that 194 DEGs exhibited a conserved role across different treatments. Additionally, 499 and 435 unique DEGs were identified in R4B1 and R6B1. The expression profiles of the 194 DEGs revealed that over half of these genes were upregulated in the R and B (Fig. 4B), whereas almost all of them demonstrated low expression levels in the control. These DEGs were predominantly linked to the functional categories of germacradienol synthase activity, regulation of biological process regulation of cellular process, aldehyde catabolic process and biological regulation (Fig. 4C). The KEGG enrichment analysis revealed that the flavonoid biosynthesis, Phosphatidylinositol signaling system, circadian rhythm-plant and MAPK signaling pathway-plant pathways were significantly represented (Fig. 4D).

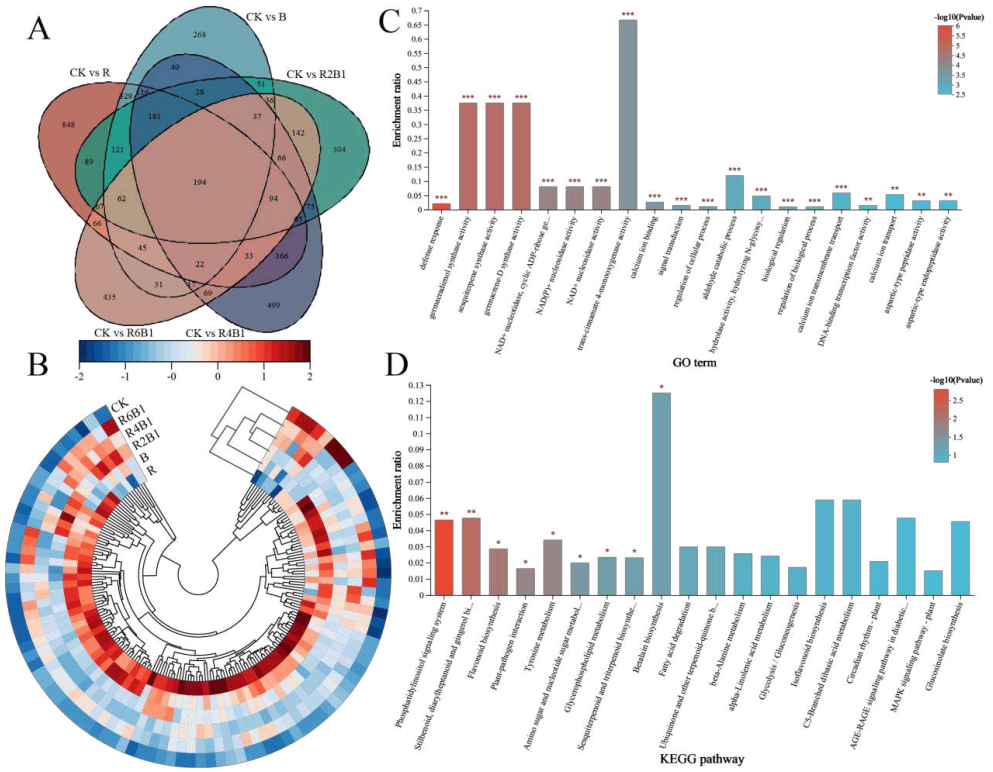



**Fig. 4.** The DEGs shared under different light treatments. (A)the Venn analysis the DEGs of different treatments; (B) clustering heat map of 194 DEGs shared in different red and blue light treatments; (C) GO enrichment for 194 DEGs; (D) KEGG enrichment for 194 DEGs.

The enrichment of the 499 specific DEGs related to the R4B1 revealed a significant representation of pathways associated with cutin, biosynthesis of various secondary metabolites, phenylpropanoid biosynthesis, DNA replication and fatty acid elongation (Fig. S3). The enrichment of 435 specific DEGs in response to R6B1 treatment revealed that they were significantly enriched in pathways including flavonoid biosynthesis, riboflavin metabolism and circadian rhythm – plant (Fig. S4). The evidence suggests that the response of red-blue light to flower bud differentiation in 'Red Globe' has a conserved role of 194 DEGs. Additionally, more specific genes promote flower bud differentiation through the plant hormone signal transduction, flavonoid biosynthesis, MAPK signaling pathway-plant and other signaling pathways.

The growth and development of plants, along with their responsiveness to light, are facilitated by plant hormones(Wit et al., 2016). A total of 48 DEGs were found as being implicated in the plant hormone signal transduction pathway in differentiation of flower buds in different light treatments. The majority of them were abundant in the auxin, ethylene, abscisic acid and brassinosteroid pathways (Fig. 5). In the auxin signaling pathway, the *IAA* genes were upregulated expression in R4B1 and R6B1, while *SAUR* genes were upregulated expression in R4B1 and R. Conversely, *SAUR* genes exhibited decreased expression in the R6B1 treatment. The expression of *ETR*, *EBF1/2*, *EIN3* and *ERF1* genes in the ethylene signaling pathway were downregulated in the control, R2B1 and R6B1, while remaining treatments upregulated. In the abscisic acid pathway, the *SNRK2* and *PP2C* genes increased expression in the control

and decreased in the R4B1 and R6B1. In the brassinosteroid pathway, the *TCH4* gene demonstrated elevated expression in the R4B1 and R6B1 treatments and reduced expression in the control, R and B treatments.

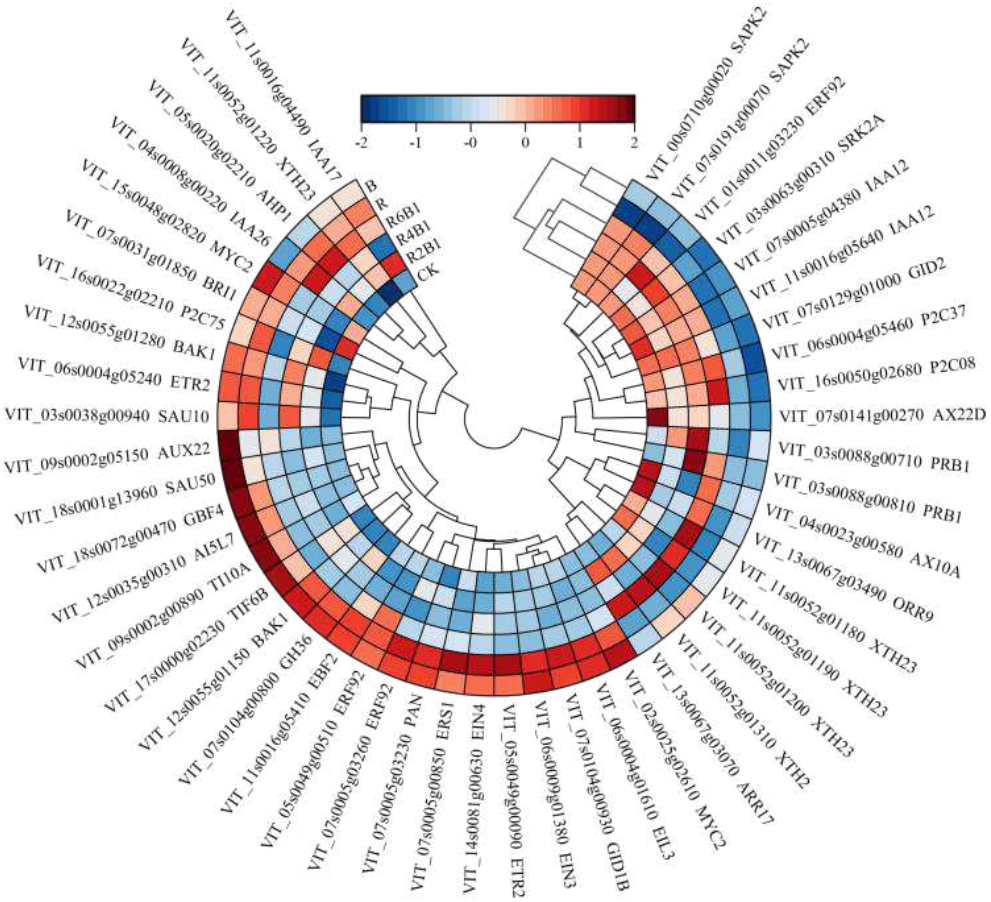



**Fig. 5.** Clustering heat map of genes correlated with plant hormone signal transduction.

The transcription of genes participating in flavonoid biosynthesis is subjected to rigorous regulatory control throughout the developmental processes associated with flowering(Yue et al., 2018). In the course of this study, 47 DEGs linked to flavonoid biosynthesis pathways were revealed (Fig. 6). These included 30 genes from the *CHS* family, four genes from the *E2* family, and two genes from the *CYP73A* family. In comparison to the control, the *CHS* family genes *THS4*, *THS5* and *THS2* were found to be upregulated and expressed in the R4B1 and R6B1 treatments. Additionally, *THS1* was observed to be upregulated in the R and B treatments. The *E2* family genes *HST* and *SHT* were identified as being downregulated and expressed in the R2B1 and R4B1 treatments. Furthermore, the *CYP73A* family gene *C7A10* was found to be upregulated and expressed in the R, B and R4B1 treatments.

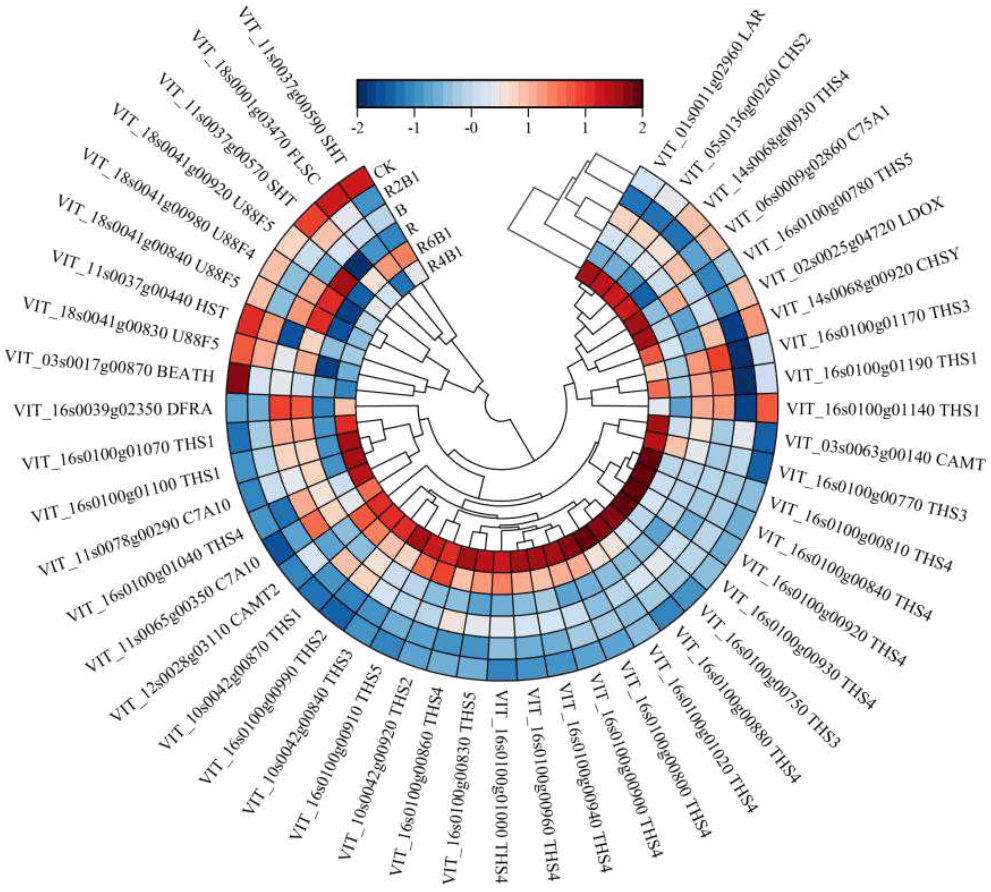



**Fig. 6.** Clustering heat map of genes correlated with flavonoid biosynthesis.

The metabolic process of carbohydrate encompasses the synthesis and decomposition of starch and sugars, which serve pivotal roles in the regulatory mechanisms governing flower formation(Lebon et al., 2005). 23 DEGs were found in the starch and sucrose metabolism pathways. Of these, the sucrose convertase gene *INV*, terpene synthase gene *TPS*, α-amylase gene *AMY*, β-glucosidase gene *BG* and ubiquitin ligase gene *E3* were found to be up-regulated in the R and B treatments. Conversely, the glycogen branching enzyme gene *GBE1* and glycogen synthase gene *GLGA* were down-regulated. Conversely, the sucrose convertase gene *INV*, terpene synthase gene *TPS*, β-glucosidase gene *BG* and ubiquitin ligase gene *E3* exhibited a reduction in expression, whereas the glycogen branching enzyme gene *GBE1* and glycogen synthase gene *GLGA* demonstrated an increase in expression in the R2B1, R4B1 and R6B1 treatments. Furthermore, the hexokinase gene *HK* exhibited a notable decline in expression levels in the control, while demonstrating an elevation in expression values in the B, R2B1, and R4B1 treatments.

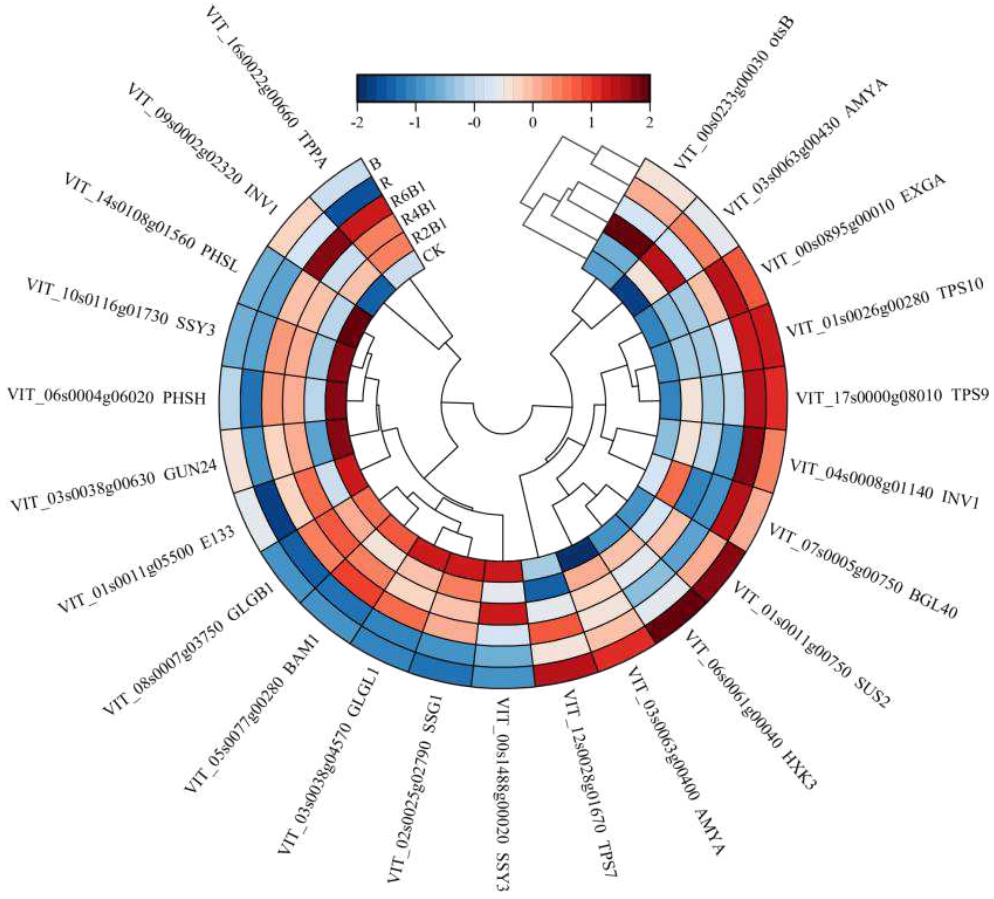



**Fig. 7.** Clustering heat map of genes correlated with starch and sucrose metabolism.

### *3.4 WGCNA to identify genes related to flower bud differentiation*

In order to determine light-regulated genes associated with the differentiation of flower buds in 'Red Globe', the WGCNA method was employed for the analysis of gene expression changes under different treatments. As illustrated in Figure 8A and B, the MEred (892 genes), MEblank (622 genes) and MEblue (1662 genes) modules exhibited a positive correlation with the flowering rate, carbohydrate and flavonoid content. These findings indicate that said genes are implicated in the process of differentiation of flower buds following red and blue light. It is typically the case that genes belonging to the MYB, bHLH and MADS TFs exert a principal regulatory influence over the process of flower development. Consequently, determine the specific gene responsible for regulating flower bud differentiation in relation to red and blue light in 'Red Globe', the gene network of these transcription factor families was subjected to analysis. As illustrated in Figure 8C, 63 MYBs, 20 bHLHs and 6 MADS genes were identified as being implicated in the flower bud differentiation. Among these MYBs, the WGCNA results also demonstrated that *MYB93* (VIT12s0055g01150) was co-expressed with 738 genes. Of these, 278 genes were successfully annotated and found to be associated with flavonoid biosynthesis, plant hormone signal transduction, MAPK signaling pathway-plant and starch and sucrose metabolism. In particular, *MYB93* was found to be co-expressed with MYB, ERF, bHLH, bZIP and WRKY transcription factors (Fig. 8D and Table S5), indicating that the MYB93-mediated gene network promotes flower bud differentiation in a transcriptionally regulated manner. Additionally, genes associated with ethylene (VIT12s0057g00690, VIT06s0004g05240, VIT07s0005g03230, VIT07s0005g03260), brassinosteroids (VIT12s0055g011), auxin (VIT14s0030g01240, VIT05s0020g01070), jasmonic acid (VIT17s0000g02230) and ethylene (VIT12s0057g00690, VIT06s0004g05240, VIT07s0005g03230, VIT07s0005g03260) related genes were expressed in correlation with MYB93, suggests that the hormone is also involved in the light response to flower bud differentiation. Furthermore, MYB93 was found to be associated with flavonoid biosynthesis (VIT11s0065g00350, VIT16s0039g02350, VIT16s0100g01070, VIT16s0100g01100, VIT16s0100g01110), MAPK signaling pathway-plant (VIT09s0002g08640, VIT10s0003g02930, VIT10s0003g04870, VIT10s0116g01240,

VIT02s0025g00510) and genes related to starch and sucrose metabolism (VIT12s0028g01670, VIT14s0006g00520, VIT17s0000g08010, VIT03s0063g01510, VIT06s0009g01650, VIT00s0233g00030) were found to be co-expressed. In conclusion, these results support that *MYB93* may act as a pivotal regulator of plant hormone transduction, TFs, starch and sucrose metabolism, flavonoid biosynthesis and MAPK signaling pathway-plant following red and blue light, thereby facilitating flower bud differentiation.

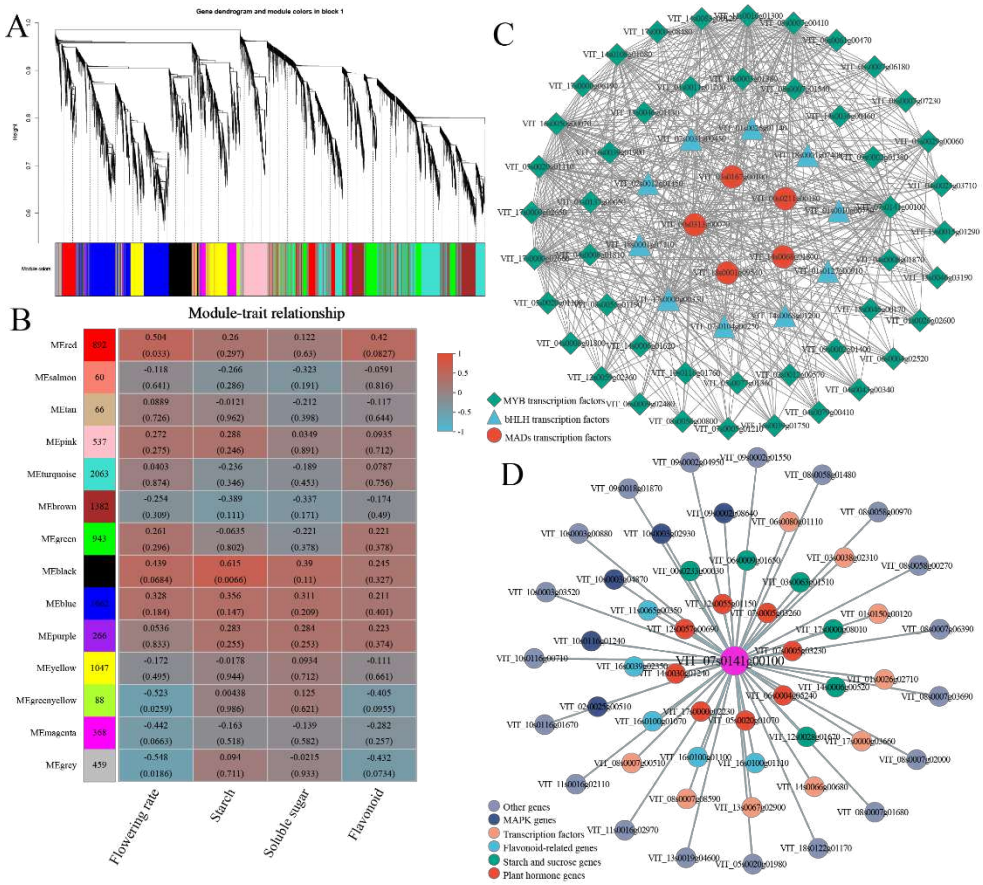



**Fig. 8.** WGCNA analysis of DEGs under different red and blue light treatments. (A) hierarchical cluster tree of co-expression modules; (B) the correlation between gene and traits, with the number of genes in each module shown by numbers on the left; (C) transcriptional network composed of MYB, bHLH and MADs transcription factor genes; (D) co-expression network of the hub gene *MYB93*.

### *3.5 MYB93 activating BAK1, otsB and THS4*

To corroborate the WGCNA findings, the expression and regulatory target genes of the MYB transcription factor *MYB93* were characterised using qRT-PCR and DLR assa. As illustrated in Figure 8A, *MYB93* exhibited increased expression in response to combined red and blue light. The DLR results demonstrated that *MYB93* markedly activated the expression of the plant hormone transduction gene *BAK1*, the flavonoid biosynthesis gene *THS4*, and the starch and sucrose genes *otsB* (Fig. 9B-D). These findings suggest that red-blue light induce the expression of *MYB93*, which in turn activates the expression of plant hormone transduction, flavonoid biosynthesis and starch and sucrose metabolism, thereby promoting flower bud differentiation.

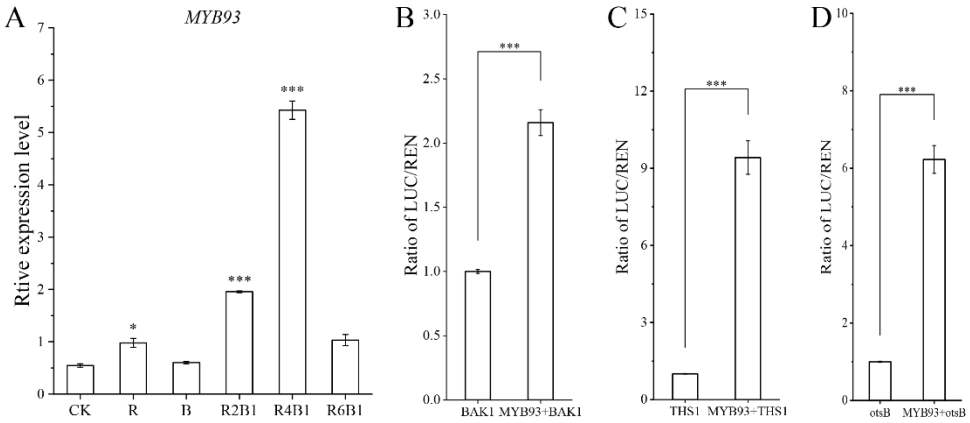



**Fig. 9.** *MYB93* transcriptionally activates the expression of *BAK1*, *THS4* and *otsB* by DLR. (A) Expression levels of *MYB93* under different treatments; (B-D) *MYB93* activates the expression of *BAK1*, *THS4* and *otsB*. Data are illustrated as mean ± SD with 6 biological replicates (* $P < 0.05$, *** $P < 0.001$).

### *3.6 qRT–PCR validates the gene expression profles*

This study randomly selected 9 genes from the pathways with higher functional enrichment for qRT-PCR validation analysis. The expression trend was consistent with the RNA-seq results, and the correlation coefficient was R = 0.79 (Fig. S5). The validation experiment showed that the RNA-seq data were credible.

## 4. Discussion

Light is a requisite condition for the formation of flower buds. The quality of light can influence the accumulation of photosynthetic substances, which regulate the nutritional and reproductive balance of the branch tips(Bennett et al., 2005). The differentiation of grape flower buds represents a pivotal turning point from vegetative growth to reproductive growth, and this process is controlled by the content of carbohydrates(Lebon et al., 2008). Sugar plays an an energetic substance and a signaling molecule that positively regulates the transition to flowering in grapevine(Rolland et al., 2006). The level of organic nutrition in the plant body is represented by starch, which is mobilised or accumulated according to the needs of the plant(Li et al., 2019). The differentiation of apple flower buds is dependent on the presence of elevated levels of both sugar and starch(Xing et al., 2015), as is the key for grape(Lebon et al., 2004) . Flavonoid are secondary metabolites by plants through the process of photosynthesis. They are present in almost all plant tissues and exert a significant influence at various stages of plant growth(Albert et al., 2023). The present work examined the effects of red-blue light on the ‘Red Globe’ facility. The results demonstrated markedly elevated rate of flower formation and augmented carbohydrate and flavonoid content in the R4B1 and R6B1 in comparison to the control. KEGG analyses revealed that the DEGs in the R4B1 and R6B1 were enriched for starch and sucrose metabolism pathways. It has been demonstrated that this pathway genes are instrumental in the differentiation of flower bud(Jing et al., 2020). Previous transcriptome sequencing studies have identified genes involved in the flavonoid biosynthesis pathway as being associated with flower bud differentiation(Shalom et al., 2012). In this work, genes in the flavonoid biosynthesis pathway were identified as being differentially expressed in response to different red and blue light treatments. These DEGs are postulated to promote nutrient accumulation and flower bud differentiation through transcriptional activation.

A number of classical plant hormones, including auxins, abscisic acid, gibberellins and cytokinins, have been evidenced to exert a role in the process of flower formation(Davis, 2009). Results showed that ABA, GAs and IAA levels increased, while CKs levels decreased in the R4B1. Transcriptome analysis of flower buds in different light treatments using RNA-seq revealed a large number of DEGs involved in plant hormonal signal transduction. During the process of bud differentiation, the presence of low levels of IAA within the bud will result in the inhibition of the formation of high-quality flower buds within the vine(Wang et al., 2014). The findings of this study indicate a considerable number of DEGs were enriched in plant hormone signal transduction, with the auxin pathway being the most prevalent. The expression of *IAA* was found to be downregulated in the R and B, whereas it was upregulated in the R4B1 and R6B1. Abscisic acid is a vital regulator of flower initiation and flower development in long-

day plants(Kazan and Lyons, 2016). The current study has revealed that numerous genes implicated in the ABA signaling pathway exhibited reduced expression levels in R, while displaying increased expression levels in R4B1 and R6B1. This finding is in accordance with the results of previous studies (Cui et al., 2013). It is widely accepted that ethylene can impede the formation of flowers and influence the progression of floral development(Iqbal et al., 2017). The negative effect of ethylene on flowering is caused by a reduction in gibberellin levels, which results in an increase in the negative regulatory factor *DELLA*(Achard et al., 2007). Gibberellins facilitate the lateral meristematic tissue differentiation and inhibit the inflorescences development. This is achieved through the signaling of GID1, DELLA protein and DELLA protein degradation regulatory factor(Conti, 2017). The study revealed that the expression of ethylene genes was upregulated in red and blue light treatments alone, while a contrasting trend was the was noted in the mixed red and blue light and control treatments, which including *EBF1/2*, *EIN3* and *ERF*. These results suggest that red and blue light regulate 'Red Globe' flower bud formation by mediating the plant hormone signal transduction pathways.

Transcription factors bind specifically to DNA sequences to activate or inhibit gene expression.(Zhao et al., 2018). It has been demonstrated that MYB transcription factors perform a crucial function responding to biotic and abiotic stresses. Additionally, research has indicated that the differential of expression of these factors is a crucial factor in the induction of flower formation(Amini et al., 2019). *MYB15* has been shown to upregulating *FT* and *SOC1* to promote flowering(Ma et al., 2015). *MYB21* has been demonstrated to negatively regulates genes involved in jasmonate biosynthesis, reducing jasmonic acid levels and controlling flower development(Reeves et al., 2012). To define the genes involved in flower bud differentiation and development by analysing the gene networks of the MYB, bHLH and MADs transcription factor families. Analysis has shown that 63, 20 and 6 genes in the MYB, bHLH and MADS families respectively, are involved in this process. This suggests that MYB transcription factors are of significant importance. The WGCNA demonstrated that red and blue light resulted in the co-expression of *MYB93* (VIT12s0055g01150) and genes related to plant hormone signal transduction, flavonoid biosynthesis, and starch and sucrose metabolism. The activation of the expression of the plant hormone signal transduction gene *BAK1*, the flavonoid biosynthesis gene *THS1* and the starch and sucrose metabolism gene *otsB* by MYB93 was subsequently confirmed by DLR. This implies that *MYB93* is a candidate master regulator of the biological process of red and blue light-regulated flower bud differentiation.

## 5. Conclusion

The objective of this research was to examine the impact of varying ratios of red-blue light on the differentiation of 'Red Globe' buds through physiological and molecular basis. The findings showed that the red red-blue light could enhance nutrient accumulation and facilitate flower bud differentiation. Transcriptome sequencing revealed that genes related to plant hormone signal transduction, starch and sucrose metabolism and flavonoid biosynthesis were involved in the red-blue light-mediated differentiation of flower buds in 'Red Globe'. Furthermore, the hub gene *MYB93* was employed to construct a red-blue light-mediated regulatory network, which was confirmed to activate the expression levels of the plant hormone signal transduction gene *BAK1*, the flavonoid biosynthesis gene *THS1*, and the starch and sucrose metabolism gene *otsB*. Inconclusion, the key gene identified in this work will prove invaluable genetic resources for enhanced flower bud differentiation, while also advancing our comprehension of the molecular mechanisms underlying red and blue light-mediated flower bud differentiation.

## Data availability

All pertinent data that substantiate the findings and conclusions of the study are incorporated into the article and supplementary files. The transcriptome data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) and can be accessed via the following accession number: The data can be accessed via the following link: PRJNA848984 (http://www.ncbi.nlm.nih.gov/sra).

## Ethics approval and consent to participate

There is no ethics approval and consent to participate in this manuscript. All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation. The collection of 'Red Globe' in this work has been granted permission by Helan County Gardening Industrial Park, Yinchuan City.

**Consent for publication**

Not applicable.

**Conflict of interest**

The authors have no competing interests to declare.

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**Author contributions**

The project was conceived and designed by Y.Z. and X.L. The research was conducted by Y.Z., X.L., M.Y., J.Z. and Q.L. Y.Z., X.L., M.Y. and Q.L. conducted an in-depth analysis and discussion of the data. The manuscript was prepared by Y.Z., Q.L. and X.L. All authors provided an interpretation of the data and contributed to the drafting of the manuscript. All authors have read and approved the final version of the manuscript for publication.

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