Cloning, Subcellular Location and Expression Analysis of Grape $MYB$ Gene

Guirong Li (liguirong10@163.com)
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology

Ran Quan
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology

Pengwei Jing
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology

Meng Wang
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology

Wenwen Xu
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology

Huiling Hu
School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology

Research Article

Keywords: Grape, MYB gene, Peel color, Resistance to stress, Hormone induction, gene expression

Posted Date: March 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-258098/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

MYB gene plays an important role in plant growth, development and response to abiotic stress. In this study, the Eurasian grape (*Vitis vinifera* L.) cultivar ‘Yatomi Rosa’ was used as the test material. Two MYB genes, VvMYBB1 gene and VvMYBA3 gene, were obtained by homologous cloning, and their subcellular location, different organs, and expression patterns under stress treatment and hormone induction were obtained. It was found that VvMYBB1 gene and VvMYBA3 gene proteins were located in the nucleus and belonged to nuclear proteins. VvMYBB1 gene and VvMYBA3 gene were highly expressed in roots and flowers. VvMYBB1 gene and VvMYBA3 gene might have a negative effect on the formation of peel color. VvMYB1 gene and VvMYBA3 gene might play an important role in drought resistance and salt stress resistance in grapes. Under the induction of exogenous hormones, the expression of VvMYBB1 gene was higher than that of VvMYBA3 gene under the treatment of IAA, ETH, SA and MeJA. Expression of VvMYBB1 gene was lower than that of VvMYBA3 gene under the treatment of 6-BA, GA3 and ABA. It showed that MYB gene played an important role in the development of different organs of grapes, fruit coloring and response to abiotic stress. This would provide reference for the utilization of grape MYB gene resources.

Introduction

MYB gene is most researched in the field of plants, and it is widely involved in various processes of plant growth and development\(^1\)\(^-\)\(^2\). Arce-Johnson & Tornielli\(^3\) found that the phenylpropanoid pathway was controlled at different branches by a set of R2R3-MYB C2 repressors in grapevine. Li et al.\(^4\) found that three R2R3 MYB gene transcription factor genes from *Capsicum annuum* showing differential expression during fruit ripening. Azuma et al.\(^5\) found the genomic and genetic analysis of MYB-related genes that regulated anthocyanin biosynthesis in grape berry skin. Studies have shown that plant MYB genes exhibited specific temporal and spatial expression characteristics in different organs and tissues\(^6\).

When plants encounter abiotic stress or hormone induction, the MYB gene will also be expressed accordingly, which will initiate a series of physiological and biochemical reactions, and ultimately reduce or eliminate the damage to plants from adversity\(^6\). Studies have shown that most MYB genes are involved in the process of response to abiotic stress and hormone signals\(^7\). The qRT-PCR results of Chai et al.\(^8\) showed that the expression level of the 17G121000 and GLYMA genes in soybeans would change under drought, dehydration, salt and abscisic acid (ABA) stress. Oh et al.\(^9\) found that AtMYB60 could adapt to arid environment by regulating the opening and closing of stomata and root growth. Seo et al.\(^10\) found that AtMYB enhanced the ability of Arabidopsis to resist drought stress by integrating auxin and ABA signals. Apple MdMYB88 and MdMYB124 regulated the expression of low-temperature response genes through CBF-dependent signaling pathways and enhanced the low-temperature resistance of apples\(^11\). 35.02% and 56.85% of Arabidopsis MYB genes were up-regulated and down-regulated under salt stress, respectively\(^12\). Liao et al.\(^13\) identified 156 GmMYB genes in soybeans, of which 43 genes changed their expression levels under ABA, salt, drought or low temperature stress. With the deepening of
research, more and more MYB genes have been proved to be involved in the regulation of plant abiotic stress and hormone induction.

Grapes are one of the most important fruit trees in the world. The widely cultivated grape cultivars are mainly Eurasian grapes (Vitis Vinifera L.). These grapes have excellent quality and processing properties, but their disease resistance and stress resistance are poor. In grapevine, 108 MYB genes have been identified from the genome of Vitis vinifera cv. Pinot Noir, which play an important role in the grape growth and development. This study is to explore the role of the new grape MYB gene in fruit color and response to abiotic stress. Two MYB genes, VvMYBB1 gene and VvMYBA3 gene, were cloned using the RT-qPCR method, and their expression patterns under different organ development and peel color, abiotic stress and different hormone induction were analyzed to understand the effect of grape MYB gene in grape fruit coloration and response to abiotic stress. The role in the process provides a reference for the utilization of grape MYB gene resources.

Results And Analysis

Gene Cloning and Sequence Analysis

Gene Cloning

Using homologous cloning, two MYB gene fragments of 1680 bp and 1104 bp size were cloned using the cDNA of the leaves of the European grape ‘Yatomi Rose’ as the template (Fig. 1). Both contained a complete open reading frame with a sequence of 100% similarity to grape VvMYBB1 gene and VvMYBA3 gene (GenBank XM_002275810 and NP_001267927, respectively). ORF total length was 1350 bp and 834 bp. VvMYBB1 gene total length was 762 bp, coding 254 amino acids, and the isoelectric point was 28.45 kD. The total length of the VvMYBA3 gene was 477 bp, coding 159 amino acids, and the isoelectric point was 18.53 kD (Fig. 2).

VvMYBB1 (1091) 7908922-7910012 bp

133, 145, 130, 184, 499

VvMYBA3 (683) 12516756-12517438

115, 87, 118, 130, 232

Neighbor-joining method in Mega 7.0 software was used to construct the phylogenetic tree between two grape MYB sequences and 16 other plant MYB proteins (Fig. 3). After cluster analysis, it was found that two large subclasses were clustered, The first category included dates (XP_015876153), oranges, woody cotton, apples (XP_008346342), lotus, Populus euphratica, castor, cocoa (XP_007010686), VvMYBB1 gene. The second category included legumes, eucalyptus grandis, hybrids, soybeans, wild soybeans, apples (AAZ20438), cocoa (XP_007051069), dates (XP_015891841), VvMYBA3 gene.
To construct two fusion expression vectors of \textit{MYB} genes, id est \text{pBI221-VyMYBB1} and \text{pBI221-VyMYBA3} and confirm \textit{VvMYB} subcellular localization of two proteins, \text{pBI221-VvMYBB1-GFP} and \text{pBI221-VvMYBA3GFP} plasmids were transformed into Arabidopsis protoplasts. The results showed that the \text{pBI221-VvMYBB1-GFP} and \text{pBI221-VvMYBA3GFP} fusion proteins were localized in the nucleus, while the control GFP proteins covered the whole cell (Fig.2) (Fig.4).

**Analysis of \textit{VvMYB} gene expression**

**Expression specificity analysis in different organs**

Fig. 5 showed that the expression of \textit{VvMYBB1} gene was the highest in root, followed by tendrils, leaves and flowers, the lowest in stem. The expression of root was 50 times of that of stem. \textit{VvMYBA3} gene expression was the highest in flowers, followed by roots, tendrils and leaves, and the lowest in stems. The expression of flower was 100 times of that of stem. These results indicated that \textit{VvMYBB1} gene and \textit{VvMYBA3} gene played an important role in the development of grape roots and flower organs of ‘Yatomi Rose’ grape.

The contents of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene were the lowest in the early stage of fruit development, and then increased gradually, and reached the highest value 8 weeks after flower development. This showed that with the prolongation of fruit development period, the two genes played an important role in fruit maturation. Compared with the relative expression of control VVUFG, the relative expression levels of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene were 5 and 14 times higher than that of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene at 8 weeks after flowering, respectively. It is speculated that \textit{VvMYBB1} gene and \textit{VvMYBA3} gene have a negative effect on the formation of grape epidermis color.

The expression of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene under stress treatment was shown in Fig. 6. Under drought treatment, \textit{VvMYBB1} gene expression increased gradually, reaching the highest value at 9 d, about 12 times of 0 h. The expression of \textit{VvMYBA3} gene increased gradually, reaching the highest value at 9 days, about 6.5 times of 0 h. Under the treatment of low temperature (4 °C), the expression of \textit{VvMYBB1} gene was higher than that of \textit{VvMYBA3} gene, and the expression of \textit{VvMYBB1} gene increased gradually, reaching the highest value in 72 h, about 12 times of 0 h. \textit{VvMYBA3} gene expression changes were not obvious. Under salt treatment, the expression of \textit{VvMYBB1} gene increased first and then decreased, reaching the highest value in 12 h, about 8 times of 0 h. The expression of \textit{VvMYBA3} gene reached the highest value at 24 h, about 2 times of 0 h. The expression of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene was not obvious in control water treatment. These results show that \textit{VvMYBB1} gene may play a role in the response of ‘Yatomi Rose’ grapes to external drought, low temperature and high salt abiotic stress, \textit{VvMYBA3} gene mainly in response to external drought and high salt abiotic stress. These results suggest that \textit{VvMYB1} gene and \textit{VvMYBA3} gene may play an important role in grape drought resistance and salt stress resistance.

The expression of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene under exogenous hormone treatment was shown in Fig. 7. \textit{VvMYBB1} gene expression was higher than \textit{VvMYBA3} gene expression under Indole-3-Acetic
Acid (IAA), ethylene (ETH), salicylic acid (SA), methyl jasmonate (MeJA) treatment, and lower than that under 6-Benzyladenine (6-BA), Gibberellin A3 (GA3), abscisic acid (ABA) treatment. Under IAA treatment, the expression of \textit{VvMYBB1} gene increased first and then decreased. At 12 h, the expression reached the highest, about 5 times of 0 h. \textit{VvMYBA3} gene expression showed a downward trend. Under GA3 treatment, the \textit{VyMYBB1} gene and \textit{VyMYBA3} gene expression increased first and then decreased, and the \textit{VyMYBB1} gene expression reached the maximum value in 12 h, about 10 times of 0 h. The expression of \textit{VyMYBA3} gene reached the maximum at 9 h, about 2.2 times of 0 h. Under the treatment of 6-BA, the expression of \textit{VyMYBB1} gene and \textit{VyMYBA3} gene increased first and then decreased, and the \textit{VyMYBB1} gene expression reached the maximum at 9 h, about 6 times of 0 h. The expression of \textit{VyMYBA3} gene reached the maximum in 3 h, about 1.2 times of 0 h. Under the treatment of ETH, the \textit{VyMYBB1} gene expression increased first and then decreased, and the \textit{VyMYBB1} gene expression reached the maximum at 48 h, about 9 times of 0 h. \textit{VyMYBA3} gene expression showed a downward trend. The \textit{VyMYBB1} gene expression of ABA increased first and then decreased, and the \textit{VyMYBB1} gene expression reached the maximum value in 12 h, about 4 times of 0 h. \textit{VyMYBA3} gene expression showed an upward trend. \textit{VyMYBA3} gene expression reached the maximum at 72 h, about 11 times of 0 h. Under the treatment of SA, \textit{VyMYBB1} gene expression increased first and then decreased reached the maximum at 9 h, about 8 times of 0 h. \textit{VyMYBA3} gene expression decreased. Under MeJA treatment, \textit{VyMYBB1} gene expression increased first and then decreased. At 12 h, the expression reached the highest, about 7.3 times of 0 h. \textit{VvMYBA3} gene expression showed a downward trend. The expression levels of \textit{VyMYBB1} gene and \textit{VyMYBA3} gene were not obvious in control water treatment at different times. These results indicate that \textit{VyMYBB1} gene and \textit{VyMYBA3} gene play a very important role in physiological activities regulated by exogenous hormones in 'Yatomi Rose' grape.

**Discussion**

Plants in response to abiotic stress such as drought, chilling injury and salt, plant defense response can itself quickly in order to survive, the induced stress response genes, antioxidants such as adversity the expression of related genes to change its metabolic processes to maintain normal stress under the condition of cells, to ensure the growth of plants\textsuperscript{14}. Plant \textit{MYB} genes are widely involved in response to abiotic stress, and they directly or indirectly regulate the expression of the corresponding stress response genes to further enhance the ability of resistance to abiotic stress\textsuperscript{6}.

Most of the \textit{MYB} genes in plants control different development processes and stress tolerance\textsuperscript{6}. The cloned \textit{VyMYBB1} gene and \textit{VvMYBA3} gene encoded proteins contained two R domains. The transient transformation of \textit{VvMYB} gene in onion epidermal cells showed that these two genes were localized in the nucleus, indicating that \textit{VvMYBB1} gene and \textit{VvMYBA3} gene proteins play a role in regulating the transcription level of other genes in the nucleus. It is consistent with the research results of Xie et al.\textsuperscript{16}, Liu et al.\textsuperscript{17} and Zhu et al.\textsuperscript{15}, gene are usually expressed in the nucleus and can specifically bind to cis-acting elements in the promoter region of eukaryotic genes to regulate gene expression.
Plant MYB gene shows specific spatiotemporal expression characteristics in different organs and tissues. In this study, the expression of VvMYBB1 gene and VvMYBA3 gene were specific in different tissues, with the highest expression of VvMYBB1 gene in roots and the highest expression of VvMYBA3 gene in flowers. Zhu et al. also found that VvMYBC2L2 gene was shown to be strongly expressed in root, flower and seed tissue, but Cavallini et al. found in grape vine 'Corvina', MYBC2L2 showed very low expression levels in almost all organs including the berry and seed. These results indicated that MYB gene expression was different in different organs of grape, suggesting that these two genes played a role in root and flower development of grape, respectively. In addition, by measuring the expression levels of VvMYBB1 gene and VvMYBA3 gene in grape peel of 'Yatomi Rose' at different stages of fruit development, compared with the relative expression levels of control VVUFG, the content of VvMYBB1 gene and VvMYBA3 gene decreased, suggesting that VvMYBB1 gene and VvMYBA3 gene had a negative effect on the formation of grape peel color of 'Yatomi Rose'. There are many MYB genes involved in the regulation of anthocyanin synthesis in grape, such as VvMYBA1 gene, VvMYBA2 gene, VvMYBA6 gene. Zhu et al. found VvMYBC2L2 gene weakly expressed during the fruit development in grapevine, it suggested that VvMYBC2L2 gene played a role as a negative function of anthocyanin biosynthesis. Ni et al. studied that Ethylene-activated PpERF105 induced the expression of PpMYB140 to inhibit anthocyanin biosynthesis, and then the PpMYB140 gene played a negative function in red pear fruit. The results of these studies are consistent with the results of this study, suggesting that VvMYBB1 gene and VvMYBA3 gene have a negative effect on the formation of grape skin color of 'Yatomi Rose' grape.

The MYB gene family plays an important role in many physiological processes in plants, such as cell cycle, environmental response, and stress response. With further research, some of the MYB genes involved have been gradually discovered. MYB gene is involved in various aspects of plant response to abiotic stress. AtMYB96 in Arabidopsis thaliana regulates drought stress responses by integrating ABA and IAA signals. The expression of SCMYBAS1 gene affects the response of sugarcane to drought and salt stress. Yang et al. showed that the expression of OsMYB2 could be induced by salt stress, cold and drought in rice. Hao et al. showed that MdMYB308L acted as a positive regulator in low temperature stress. In this study, VvMYBB1 gene and VvMYBA3 gene expressions were changed in 'Yatomi Rose' grape under three stress conditions (low temperature, high salt and drought). VvMYBB1 gene had the strongest response to different stress conditions. This suggests that VvMYBB1 gene may play an extremely important role in drought, low temperature and salt stress. Our results also indicate that MYB gene is involved in various aspects of response to abiotic stress in grape.

Previous studies also found that MYB gene was involved in the plant hormone response process. For example, in Arabidopsis, multiple MYB gene were found to be involved in the response of auxin, ethylene and cytokinin, and the AtMYB2 gene in Arabidopsis was induced by ABA, which was the first MYB gene induced by ABA in Arabidopsis. Chen et al. cloned 163 MYB genes in Arabidopsis thaliana and studied the treatment with exogenous hormones ABA, ETH, GA, IAA, JA and SA; The results showed that the expression levels of half of the MYB genes were changed when treated with SA, and 44% of them
were upregulated. The expression of \textit{TAMYB4} gene in wheat was regulated by SA, ABA and MeJA\textsuperscript{30}. \textit{PacMYBA} expression in sweet cherry (\textit{Prunus avium}) was induced by salt, MeJA and SA\textsuperscript{31}. In this study, exogenous hormones IAA, GA, 6-BA, ABA, Eth, SA and MeJA treated the material, exogenous hormones induced the expression of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene changed at different times, and \textit{VvMYBB1} gene induced the expression of different exogenous hormones was higher. These results indicate that plant \textit{MYB} gene is widely involved in the regulation of exogenous hormones in plant growth. Other expression patterns and interactions of the two genes need to be further studied and utilized.

**Conclusion**

In this study, two new \textit{MYB} genes were cloned from the Eurasian cultivar ‘Yatomi Rose’ grape. Fluorescence microscopy showed that \textit{VvMYBB1} gene and \textit{VvMYBA3} gene proteins were located in the nucleus and belonged to nuclear proteins. The expression levels of \textit{VvMYBB1} gene and \textit{VvMYBA3} gene were high in both roots and flowers, which promoted fruit ripening and had a negative effect on pericarp coloring. \textit{VvMYB1} gene and \textit{VvMYBA3} gene may play an important role in grape drought resistance and salt stress under stress conditions. Under the induction of exogenous hormones, the expression of was higher than that of \textit{VvMYBA3} gene under the treatment of IAA, ETH, SA and MeJA, while the expression of \textit{VvMYBB1} gene under the treatment of 6-BA, GA3 and ABA was lower than that of \textit{VvMYBA3} gene. These results indicate that \textit{VvMYBB1} gene and \textit{VvMYBA3} gene play an important role in the development of different organs, fruit coloration and response to abiotic stress in grape. This study provides reference for the utilization of \textit{MYB} gene resources in grape.

**Materials And Methods**

**Test materials and their treatment**

The experimental material was 4-year-old European grape (\textit{Vitis Vinifera} L.) ‘Yatomi Rose’, which was stored in the Grape Germplasm Resource Nursery of Henan Institute of Science and Technology. New roots, tendrils, shoots and stems, mature leaves and flowers with caps are collected in May. The skins came from grapes at 2, 3, 5, 7 and 8 weeks after flowering. Hormones and stress treatment materials were obtained from 1-year-old cuttings, which were stored in a greenhouse. Plastic pot size was 25 cm high and diameter was 30 cm. The cultivation substrate was garden soil: peat soil =1:1, placed in the plant incubator or incubator at 25-28 °C, light intensity 3 000 lx, light duration 14 h light /10 h darkness.

Abiotic stress treatment: The control group was sprayed with sterile water, covered with white plastic bags, and the leaves were harvested at 0, 1, 2, 3, 4, 5, 7 and 9 days after treatment; Dryness treatment started when the water content of basin soil was 70%, and leaves were harvested 0, 1, 2, 3, 4, 5, 7 and 9 days after treatment; Under low temperature treatment, potted seedlings were placed in 4 °C light incubator, and the leaves were harvested at 0, 3, 6, 9, 12, 24, 48 and 72 h after treatment; High-salt treatment, with 0.1mol /L NaCl irrigation once, until the basin bottom solution outflow, leaves were harvested at 0, 3, 6, 9, 12, 24, 48 and 72 h after treatment.
Hormone treatment: Four to six leaves of lower tip were uniformly sprayed with 7 kinds of hormones in a spray can. The control group was sprayed with sterile water and covered with a white plastic bag. The leaves were harvested at 0, 3, 6, 9, 12, 24, 48 and 72 h after treatment. The growth regulator concentration is: 500 mmol/L IAA (purity >=99%), 120 μmol/L GA (purity >=95%), 100 μmol/L 6-BA (purity >=99%), 3mmol/L Eth (purity >=85%), 100 μmol/L ABA (purity >=99%), 100 μmol/L SA (purity >=99%), 50 μmol/L MeJA (purity >=95%) solution.

All the treated materials were immediately frozen in liquid nitrogen and stored in -80 °C refrigerator. All the above experiments selected ‘Yatomi Rose’ grape plants with the same growth potential. Each treatment was repeated for 3 times, and each pot was used as a replicate.

Methods

RNA extraction and reverse transcription

RNA extraction kit (OMEGA) was used to extract total RNA from European grape leaves, with DNase I to purify DNA for 1% agarose gel electrophoresis and concentration measurement, to ensure the integrity of RNA pollution-free (OD260/280 > 1.8, OD260/280 > 2.0). The first strand of cDNA was synthesized by MMLV reverse transcriptase (PrimeScript™ 1st Strand cDNA Synthesis Kit) (TaKaRa, Dalian, China).

Cloning of full-length VvMYB gene

According to the European Grape Pinot Gris Genomic Database, Primer 5.0 was used to design the primers:

VvMYBB1-F 5’ ggcctgctgatcgggtgtaagttc 3’
VvMYBB1-R 5’ ggggtcacctcaggctggggtggacgt 3’
VvMYA3-F 5’ gggccatggaaaataaggggaatgtgctg3’
VvMYA3-R 5’ ggggtcacctcaagaagaatgaacctgcag3’

Using cDNA from the leaves of the European grape ‘Yatomi Rose’ as a template, primerstar GXL DNA Polymerase (TAKARA) was used for PCR amplification. The reaction procedure was denatured at 95 °C for 2 min; 94°C denaturation for 30s, 60°C annealing for 30s, extension for 2 min, 30 cycles; the total extension was 10 min at 72 °C. PCR products were recovered and sent to Shanghai Sangon Biotechnology Co., Ltd for sequencing.

Subcellular localization

The sequence 3 ‘and 5’ of VvMYBB1 gene and VvMYBA3 gene amplified by PCR contained XBAI and KPNI restriction sites and did not contain the open reading frame sequences of the stop codon. After
digestion, it was inserted into the expression vector pBI221-GFP to construct pBI221-\textit{VvMYB1}-GFP and pBI221-\textit{VvMYBA3}-GFP.

The inner epidermis of onion scales were exfoliated and placed on hypertonic solid MS medium for 4 h at 28 °C. Preparation of gene gun microprojectile: Weighed 0.4-0.8 mg gold powder, disinfected with 70% ethanol, and washed with sterile water; Add 50% glycerin and set aside. Add 3-5 g plasmid carrying target gene into 6 L gold powder suspension and mix; At the same time, 4 L of spermidine 0.1M and 6 L of 2.5M CaCl$_2$ were added to vortex for 2-3min. Ice bath for 15 min. Centrifuged at 12000rpm for 10s, supernatant was discarded, and 20 L anhydrous ethanol was added for resuspended precipitation; 20µL microprojectiles were taken with pipetting gun and evenly and rapidly coated on the bearing film. Ethanol was allowed to evaporate under natural conditions. Refer to the PSD-1000 manual for the transformation of gene gun. Fluorescence microscopy (LSM510; Carl Zeiss Thornwood, NY, USA) were observed and images were collected. The green fluorescence excitation wavelength was 488nm.

**Real-time quantitative PCR**

Primers designed using Primer 5.0:

\[
\begin{align*}
\textit{VvMYB1-F} & \quad \text{gagaagaagaggataccatcattg3'} \\
\textit{VvMYB1-R} & \quad \text{cttttccagggttgtgtgccagac3'} \\
\textit{VvMYA3-F} & \quad \text{cagatgtcctgtgattgcgggtag3'} \\
\textit{VvMYA3-R} & \quad \text{tggttttagaatgtgtttggggtt3'} \\
\textit{VvUFGT-R} & \quad \text{gatatggcagcagagatgggg3'} \\
\textit{VvUFGT-F} & \quad \text{tgcgtgagaagagcgagttta3'} \\
\textit{VvActin-F} & \quad \text{ctggattctggtgatggtgtgagt3'} \\
\textit{VvActin-R} & \quad \text{cagcaaggtcaggaaggatag3'}
\end{align*}
\]

Real-time fluorescent quantitative PCR adopts TaKaRa company SYBR® Premix Ex Taq™ kit, System as follows: SYBR Premix Ex Taq 10 µL, the upstream and downstream primers were 0.8 µL each, and the cDNA template was 150 ng. Finally, the cDNA template was supplemented with double steamed water to 20µL, and each treatment had three biological replicates. A two-step method was performed on a CFX96 fluorescent quantitative PCR instrument (BioRad). The procedure was denatured at 95 °C for 30 s. There were 40 cycles of denaturation at 95 °C for 10 s and annealing extension at 60°C for 30 s. The Actin gene of grape was used as internal reference. The relative expression level was calculated by 2$^{-}\Delta\Delta^{ct}$ method. The fluorescence values at each time point were repeated by three techniques, and the average value was taken to make the graph.
Declarations

Author contributions

Guirong Li, Ran Quan and Huiling Hu conceived and designed the experiments. Guirong Li, Ran Quan, Huiling Hu, Wenwen Xu, Pengwei Jing, Meng Wang conducted the experiments, participated in the data analysis, data interpretations and investigation. Guirong Li wrote the manuscript and revised the original manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Statement

The trial was conducted in full compliance with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

References

1. Schmitz, G., Tillmann, E. & Carriero, F. The tomato Blind gene encodes a MYB transcription factor that controls the formation of lateral meristems. *PNAS*. 99(2), 1064-1069 (2002).

2. Qi, Y. T. et al. Identification of the Eutrema salsugineum EsMYB90 gene important for anthocyanin biosynthesis. *BMC Plant Biol*. 20(12), 176-185 (2020).

3. Arce-Johnson, P. & Tornielli, G. B. The phenylpropanoid pathway is controlled at different branches by a set of R2R3-MYB C2 repressors in grapevine. *Plant Physiol*. 167(4), 1448–1470. (2015).

4. Li, J. G., Li, H. L. & Peng, S. Q. Three R2R3 MYB transcription factor genes from capsicum annuum showing differential expression during fruit ripening. *African J. of Biotechnol*. 10(42), 8267-8274 (2011).

5. Azuma, A. et al. Genomic and genetic analysis of Myb-related genes that regulate anthocyanin biosynthesis in grape berry skin. *Theoretical Appl. Genetics*. 117(6), 1009-1019. https://doi.org/10.1007/s00122-008-0840-1 (2008).

6. Jiang, C. K. & Rao, G. Y. (2020). Insights into the diversification and evolution of R2R3-MYB transcription factors in plants. *Plant Physiol*. 183(2), 637-655. https://doi.org/10.1104/pp.19.01082 (2020).

7. Denekamp, M. & Smeekens, S. C. Integration of wounding and osmotic stress signals determines the expression of the AtMYB102 transcription factor gene. *Plant Physiol*. 132(3), 1415-1423 (2003).

8. Chai, C. et al. Soybean transcription factor ORFeome associated with drought resistance: a valuable resource to accelerate research on abiotic stress resistance. *BMC Genomics*. 16(1), 596 (2015).

9. Oh, J. E. et al. A dual role for MYB60 in stomata regulation and root growth of Arabidopsis thaliana under drought stress. *Plant Mol. Biol*. 77(1-2), 91-103 (2011).
10. Seo, P. J. et al. The MYB96 transcription factor mediates abscisic acid signaling during drought stress response in Arabidopsis. *Plant Physiol.* **151**(1), 275-289 (2009).

11. Xie, Y. P. et al. An atypical *R2R3MYB* transcription factor increases cold hardiness by CBF-dependent and CBF-independent pathways in apple. *New phytologist.* **218**(1), 201-218 (2018).

12. Katiyar, A. et al. Genome-wide classify action and expression analysis of *MYB* transcription factor families in rice and Arabidopsis. *BMC Genomics.* **13**, 544 (2012).

13. Liao, Y. et al. Soybean *GmMYB76*, *GmMYB92*, and *GmMYB177* genes confer stress tolerance in transgenic Arabidopsis plants. *Cell. Res.* **18**(10), 1047-1060 (2008).

14. Yu, Y. H. et al. *Vitis vinifera bZIP14* functions as a transcriptional activator and enhances drought stress resistance via suppression of reactive oxygen species. *J. Berry Res.* **10**(4), 1-12. https://doi.org/10.3233/JBR-200523 (2020).

15. Zhu, Z. G. et al. A *R2R3-MYB* Transcription Factor, *VvMYBC2L2*, Functions as a transcriptional repressor of Anthocyanin biosynthesis in grapevine (*Vitis vinifera*). *Molecules.* **24**(1), 92-1-92-13. https://doi.org/10.3390/molecules24010092 (2019).

16. Xie, R. J. et al. Genome-wide analysis of citrus *R2R3MYB* genes and their spatiotemporal expression under stresses and hormone treatments. *PLoS One.* **9**(12), e113971 (2014).

17. Liu, Y. F. et al. 2017. Expression differences of pigment structural genes and transcription factors explain flesh coloration in three contrasting Kiwifruit cultivars. *Frontiers in Plant Sci.* **8**, 1507. https://doi.org/10.3389/fpls.2017.01507 (2017).

18. Cavallini, E. et al. The phenylpropanoid pathway is controlled at different branches by a set of R2R3-MYB C2 repressors in grapevine. *Plant Physiol.* **167**(4), 1448-470 (2015).

19. Cutanda-perez, M. C. et al. Ectopic expression of *VlmybA1* in grapevine activates a narrow set of genes involved in anthocyanin synthesis and transport. *Plant Mol. Biol.* **69**(6), 633-648. https://doi.org/10.1007/s11103-008-9446-x (2009).

20. Kobayashi, S., Goto-yamamotog, N. & Hirochika, H., 2004. Retrotransposon-induced mutations in grape skin color. *Sci.* **304**(5673), 982. https://doi.org/10.1126/science.1095011 (2004).

21. Sun, L. et al. Transcriptome analysis of genes involved in anthocyanins biosynthesis and transport in berries of black and white spine grapes (*Vitis davidii*). *Hereditas.* **17**(1), 153. https://doi.org/10.1186/s41065-016-0021-1 (2016).

22. Ni, J. B., et al. Ethylene-activated PpERF105 induces the expression of the repressor-type *R2R3-MYB* gene PpMYB140 to inhibit anthocyanin biosynthesis in red pear fruit. *The Plant J.* **105**(1) (2020).

23. Xu, Q., He, J., Dong, J. H., Hou, X. J. & Zhang, X. 2018. Genomic survey and expression profiling of the *MYB* gene family in watermelon. *Horticultural Plant J.* **4**(1), 1-15 (2018).

24. Seo, P. J. & Park, C. M. MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in Arabidopsis. *New Phytologist.* **186**(2), 471-483 (2010).

25. Prabu & Prasad D. T. Functional characterization of sugarcane *MYB* transcription factor gene promoter (PScMYBAS1) in response to abiotic stresses and hormones. *Plant Cell Reports.* **4**, 661-669
26. Yang, A., Dai, X. Y. & Zhang, W. H. A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. *J. of Experimental Botany*. **63**(7), 2541-2556 (2012).

27. Hao, Y. J. *et al.* An apple MYB transcription factor regulates cold tolerance and anthocyanin accumulation and undergoes MIEL1-mediated degradation. *Plant Biotechnol. J.* **18**(2), 337-353 (2020).

28. Abe, H. *et al.* Role of arabidopsis MYC and MYB ehomologs in drought and abscisic acid-regulated gene expression. *Plant Cell*. **9**, 1859-1868 (1997).

29. Chen, Y. H. *et al.* The MYB transcription factor superfamily of arabidopsis: expression analysis and phylogenetic comparison with the rice *MYB* *Plant Mol. Biol.* **60**(1), 107-24. [https://doi.org/10.1007/s11103-005-2910-y](https://doi.org/10.1007/s11103-005-2910-y) (2006).

30. Nashaat, A. M., Wang, X. J., Abou-attia, M. A., Duan, X. Y. & Kang, Z. S. A novel TaMYB4 transcription factor involved in the defense response against *Puccinia striiformis* f. sp. tritici and abiotic stresses. *Plant Mol. Biol*. **84**(5), 589-603 (2014).

31. Shen, X. J. *et al.* PacMYBA, a sweet cherry *R2R3-MYB* transcription factor, is a positive regulator of salt stress tolerance and pathogen resistance. *Plant Physiol. and Biochemistry*. **112**, 302-311 (2017).

Figures

**Figure 1**

Cloning of VvMYB two genes in grapevine.
Figure 2

Sequence analysis of two genes MYB grape.

Figure 3

Phylogenetic tree of VvMYBB1 gene and VvMYBA3 gene of the grapevine with other species MYB proteins.
Figure 4

Subcellular localization of VvMYB6 protein. Bright field (a) DAPI (b) Dil (c) GFP (d) GFP + DAPI merge (e) GFP + Dil merge (f). Bars correspond to 10 μm.

Figure 5

Expression analysis of VvMYB two genes.
Figure 6

The relative expression of VvMYBB1 gene and VvMYBA3 gene treated with abiotic stress.
Figure 7

The relative expression of VvMYBB1 gene and VvMYBA3 gene treated with hormones. *: Significant differences of genes compared with 0 h (P<0.05).