

A novel R2R3-MYB transcription factor PbMYB1L of *Pyrus bretschneideri* regulates cold tolerance and anthocyanin accumulation

Xuan Zhou (✉ 2819315975@qq.com)

Sichuan Agricultural University - Chengdu Campus <https://orcid.org/0009-0003-4559-6549>

Diya Lei

Sichuan Agricultural University - Chengdu Campus

Wantian Yao

Sichuan Agricultural University - Chengdu Campus

shangyun Li

Sichuan Agricultural University - Chengdu Campus

Haiyan Wang

Sichuan Agricultural University - Chengdu Campus

Jiayu Lu

Sichuan Agricultural University - Chengdu Campus

Yunting Zhang

Sichuan Agricultural University - Chengdu Campus

Yuanxiu Lin

Sichuan Agricultural University - Chengdu Campus

Yan Wang

Sichuan Agricultural University - Chengdu Campus

Wen He

Sichuan Agricultural University - Chengdu Campus

Mengyao Li

Sichuan Agricultural University - Chengdu Campus

Qing Chen

Sichuan Agricultural University - Chengdu Campus

Ya Luo

Sichuan Agricultural University - Chengdu Campus

Xiaorong Wang

Sichuan Agricultural University - Chengdu Campus

Haoru Tang

Sichuan Agricultural University - Chengdu Campus

Yong Zhang

Research Article

Keywords: Pear, PbMYB1L, Cold tolerance, Anthocyanin accumulation

Posted Date: October 5th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3383542/v1>

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

[Read Full License](#)

Version of Record: A version of this preprint was published at Plant Cell Reports on January 11th, 2024.
See the published version at <https://doi.org/10.1007/s00299-023-03117-3>.

A novel R2R3-MYB transcription factor PbMYB1L of *Pyrus bretschneideri* regulates cold tolerance and anthocyanin accumulation

Xuan Zhou[†], Diya Lei[†], Wantian Yao, Shangyun Li, Haiyan Wang, Jiayu Lu, Yunting Zhang, Yuanxiu Lin, Yan Wang, Wen He, Mengyao Li, Qing Chen, Ya Luo, Xiaorong Wang, Haoru Tang and Yong Zhang*

College of Horticulture, Sichuan Agricultural University, Chengdu, 611130, China

*Correspondence: zhyong@sicau.edu.cn

[†] These authors contributed equally to this work

Abstract

Key Message *PbMYB1L* enhances the cold tolerance and anthocyanin accumulation of transgenic Arabidopsis by regulating the expression of genes related to the cold-responsive genes pathway and anthocyanin synthesis pathway.

Abstract MYB transcription factors (TFs) have been demonstrated to play diverse roles in plant growth and development. In the present study, we identified a novel R2R3-MYB transcription factor, *PbMYB1L*, from the peel of 'Red Zaosu' pear (*Pyrus bretschneideri*), which was induced by cold stress and acted as a positive regulator in anthocyanin biosynthesis. Notably, the transgenic Arabidopsis lines exhibited enhanced tolerance to cold stress. Compared to the Arabidopsis wild-type plants, the transgenic lines displayed increased chlorophyll levels, longer primary roots, and reduced reactive oxygen species (ROS) levels including O²⁻, hydrogen peroxide (H₂O₂), and malondialdehyde (MDA). Furthermore, significant upregulation of key cold-responsive genes *AtCBF1*, *AtCBF2*, *AtCBF3*, *AtCBF4*, and *AtKIN1* was observed in the transgenic plants under cold stress conditions compared to wild type. Arabidopsis plants overexpressing *PbMYB1L* had significant anthocyanin accumulation in leaves after cold treatment with quantitative results indicating higher expression of anthocyanin structural genes compared to wild type. These finding suggests that *PbMYB1L* not only plays a vital role in conferring cold tolerance but also acts as a crucial regulator of anthocyanin biosynthesis.

Keywords: Pear; PbMYB1L; Cold tolerance; Anthocyanin accumulation

Introduction

As the most extensive family of transcription factors (TFs), MYB TFs play a crucial role in regulating plant growth and development processes as well as mediating plant responses to biotic and abiotic stresses (Zhang et al. 2014). MYB TFs are composed of 1-4 MYB repeats, which play crucial roles in transcriptional regulation and interacting with other proteins. Based on the number of conserved MYB domains present in the N-terminus, the MYB TFs can be divided into 1R-MYB, R2R3-MYB, R1R2R3-MYB, and 4R-MYB (Dubos et al. 2010). The R2R3-MYB TFs is the most predominant among them, and certain members participate in regulating secondary metabolism, plant development, and abiotic stress (Stracke et al. 2001). Numerous studies have shown that R2R3-MYB is involved in environmental stress response of higher plants. MYB transcription factors are important regulators of plant tolerance to cold stress in many species. *MYB15* in *Arabidopsis* can reduce its plant freezing tolerance (Agarwal et al. 2006). In Apple, *MdMYB23*, *MdMYB73*, *MdMYB88* and *MdMYB124* improves calli resistance to cold (An et al. 2018; Zhang et al. 2016; Xie et al. 2018). Moreover, recent studies have revealed that R2R3-MYB TFs can regulate the expression of downstream COR (cold-responsive) genes by regulating the expression of CBFs (C-repeat binding factors), a key transcription factor in cold reactions, forming a complex regulatory network to further enhance its regulatory capabilities (Wang et al. 2021). Together, these findings highlight the critical importance of R2R3-MYB TFs in regulating stress tolerance in plants and underscore their potential for use in biotechnological applications aimed at improving crop yield and quality.

Cold stress, a devastating abiotic factor, can induce the generation of reactive oxygen species (ROS), resulting in oxidative damage (Suzuki and Mittler, 2006; Gill and Tuteja, 2010) and inhibiting plant growth and crop production (Shi et al. 2018). To cope with cold stress, plants have developed efficient mechanisms to sense and respond to low temperatures. In response to cold stress, over 1000 genes are induced, among which at least 170 encode TFs, including MYB, bZIP, and AP2/ERF families (Chinnusamy et al. 2007; Jakoby et al. 2002; Jaglo-Ottosen et al. 1998). The CBF TFs belonging to the AP2/ERF family have been reported to play important roles in response to cold stress (Stockinger et al. 1997). The modulation of CBF genes in response to cold stress has been extensively investigated, and MYB TFs have been demonstrated to promote the transcription of CBF genes and enhance cold tolerance. In *Arabidopsis*, *AtMYB15* suppresses the expression of CBF genes and thus reduces

freezing tolerance (Agarwal et al. 2006). In Apple, *MdMYB23* improves calli resistance to cold by directly binding to the promoters of *MdCBF1* and *MdCBF2*, activating their expression. In apple calli, overexpressed by *MdMYB73*, the expression of *MdCBF3*, *MdCBF4* and *MdCBF5* increased significantly, indicating that *MdMYB73* improved cold tolerance through the CBF cold reaction pathway, while *MdMYB88* and *MdMYB124* targeted *MdCSP3* and *MdCCA1* genes, respectively, positively regulating the expression of cold tolerance and cold response genes of apples (An et al. 2018; Zhang et al. 2016; Xie et al. 2018).

Anthocyanins, in their glycosylated form, are abundant natural water-soluble plant pigments that are synthesized as part of the phenylpropanoid pathway in plants. The content and composition of anthocyanins vary among different plant organs, resulting in a diverse array of colors exhibited by flowers, leaves, and fruits. Anthocyanins are synthesized through the phenylpropanoid metabolic pathways and primarily regulated by structural and regulatory genes. The anthocyanin biosynthesis structural genes, encoding enzymes involved in the anthocyanin biosynthesis pathway, include chalcone synthase (CHS), chalcone isomerase (CHI), flavonone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), anthocyanidin synthase (ANS), and UDP-glucose: flavonoid 3-O-glycosyltransferase (UGT). The regulatory genes can control the expression of structural genes (Xu et al. 2014). The regulatory network of anthocyanin biosynthesis is predominantly governed by R2R3-MYB transcription factors, whose conserved binding motif can interact with other transcription factors to modulate the accumulation and deposition of anthocyanins. MYB TFs have been shown to play important roles in the anthocyanin synthesis pathway. Several studies have successfully identified and characterized numerous MYB TFs in fruit plants. In grapes, the first MYB transcription factor, *VvMYB1*, promotes anthocyanin synthesis in various grape tissues (Jeong et al. 2006). Similarly, *MdMYB1* in apples, *MrMYB1* in bayberry and *VaMYB1* in blueberries have been found to positively regulate anthocyanin synthesis (Takos et al. 2006; Niu et al. 2010; Die et al. 2020). In a recent study, it was discovered that the *MdMYB1* transcription factor, a key regulator of anthocyanin biosynthesis in apples, directly activates the expression of the *MdDHAR* gene. This activation, in turn, promotes the activity of the DHAR enzyme and leads to increased accumulation of Ascorbic acid (AsA) (An et al. 2021). Similarly, the *PpMYB1* transcription factor isolated from peaches has been shown to regulate fruit resistance against *Rhizopus stolonifer* infection (Li et al. 2023b). In conclusion, MYB transcription factors play pivotal roles not only in the

anthocyanin synthesis pathway but also in the synthesis of various other substances and in enhancing stress resistance.

Most MYB TFs play important roles in anthocyanin biosynthetic pathways, but it is unclear whether or how they are involved in plant resistance to abiotic stress. In apple, overexpression of *MdMYB308L* and *MdMYB2* led to improved cold tolerance and increased anthocyanin accumulation (An et al. 2020; Jiang et al. 2022). In this study, a low temperature-responsive R2R3-MYB TF *PbMYB1L* was characterized in pear. Further studies showed that *PbMYB1L* positively regulates the cold tolerance and cold response gene expression of Arabidopsis. The enhanced cold tolerance of *PbMYB1L* may be attributed to its positive regulation on the expression of the CBF gene family. Overexpression of *PbMYB1L* led to increased anthocyanin accumulation in transgenic Arabidopsis after low temperature treatment, indicating that *PbMYB1L* also plays an important role in anthocyanin accumulation. Taken together, we have identified and characterized a novel MYB TF that regulates the cold tolerance and anthocyanin accumulation.

Materials and methods

Plant materials and treatments

The fruits of ‘Red Zaosu’ pear (*Pyrus bretschneideri* Rehd.) were selected as materials from Ya’an, Sichuan, China, in 2022. Arabidopsis seeds were germinated on 1/2MS medium at a temperature of 23°C with a photoperiod of 16 hours of light and 8 hours of darkness. For the cold treatment, seedlings were placed in a growth incubator set at 4°C for 0, 1, 3, 6, 12, 24 h. Each treatment involved a minimum of 30 seedlings, and leaves were sampled from five randomly selected seedlings at specific time points and pooled as a material sample. Three technical replicates were used for each sample, and the data are shown as means $\pm$ standard errors (SE; $n = 3$). Three biological replicates were used for each of the wild type (WT) and over expressed lines (OE). Tissues from all of the treatments were harvested and immediately frozen in liquid nitrogen, and stored at -80°C until further use.

Bioinformatics analysis

The amino acid sequences of R2R3-MYB transcription factors were obtained from the NCBI database (<https://www.ncbi.nlm.nih.gov>). A phylogenetic tree was

generated with the maximum likelihood method (1000 bootstrap replicates) of the MEGA 6.0 program. Alignment of *PbMYB1L* and other R2R3-MYB transcription factor sequences was performed using DNAMAN software. Promoter cis-acting elements are analyzed using an online website (PlantCARE, a database of plant promoters and their cis-acting regulatory elements).

Subcellular localization

A vector containing pCAMBIA1300-*PbMYB1L* plasmid, along with an empty vector as control, was constructed and then transformed into *Agrobacterium tumefaciens* strain GV3101. The *A. tumefaciens* cells carrying the pCAMBIA1300-*PbMYB1L* plasmid and empty vector were separately injected into *N. benthamiana* leaves. Fluorescence signals were detected by an A1 confocal laser scanning microscope (Nikon, Tokyo, Japan) at 36-72 h after the injection. The primers used are listed in Supplementary Table S1.

GUS staining assays

The promoter of *PbMYB1L* (-2000 bp) was cloned from genomic DNA of 'Red Zaosu' pear into the pCAMBIA1301 vector upstream of the GUS gene, creating the reporter vector *Agrobacterium* (*A. tumefaciens* GV3101) were transiently expressed in *N. benthamiana* using 4-week-old tobacco plants as described previously (Alabd et al. 2022). The primers used are listed in Supplementary Table S1. After injection, tobacco is left under dark conditions for 12 h, then treated with low temperature, after which samples are taken for GUS staining and determination of GUS activity. The GUS staining and GUS activity using staining and assay kits (Beijing Coolaber Biotechnology).

RNA extraction and quantitative real-time PCR

Total RNA was extracted according to a previously described cetyltri methylammonium bromide method (Zhang et al. 2011). The quantitative real-time (qRT)-PCR assays were completed with the CFX Connect™ Real-time PCR system (Bio-Rad, Hercules, CA, USA) as previously described (Ni et al. 2019). Details

regarding the qRT-PCR primers are listed in Supplementary Table S2.

Transformation of Arabidopsis

The coding sequence (CDS) of *PbMYBIL* was inserted into *A. tumefaciens* GV1301 cells, under the control of the cauliflower mosaic virus 35S promoter (*PbMYBIL*-OE). The primers used are listed in Supplementary Table S1. The transformation of Arabidopsis plants according to the floral dip method (Clough and Bent, 1998). The transformation process was repeated after one week. The transgenic plant seeds were collected and screened on 1/2 MS medium supplemented with 50 mg/L hygromycin. The *PbMYBIL*-overexpressing lines were selected based on PCR. The next experiments were conducted using the third generation of transgenic Arabidopsis lines. Additionally, 7-day-old screened seedlings were transferred to the growth medium and incubated under controlled environmental conditions (23°C and 16 h light: 8 h dark).

Measurement of MDA content, O²⁻ and H₂O₂

The MDA content in Arabidopsis leaves was assayed following the method of Zhang et al (Zhang et al. 2009). To preliminarily detect O²⁻ and H₂O₂ accumulation, histochemical staining assays were performed using nitro blue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB). Place the three-week-old plants in a 4°C incubator for 1 h after processing for staining. The process and operating steps were described in a previous publication (Wang et al. 2017; Shi et al. 2010). The content of O²⁻ and H₂O₂ content were measured using analytical kits (JiangSu Grace Biotechnology).

Anthocyanin measurement

Total anthocyanin content was determined using a previously reported method (Shin et al. 2007; Lim et al. 2017). Briefly, 0.1 g of liquid nitrogen-ground samples was accurately weighed and added to 600 µL of buffer containing 1% methanol hydrochloride. The samples were then extracted overnight in a gentle, dark, and cold (4°C) shaker. After extraction, 200 µL double-distilled water and 200 µL chloroform was added to each sample, and the mixture centrifuged at 12,000 g for 10 min, and

200 μ L supernatant was aspirated to detect absorbance values at 530 nm and 657 nm
on a multifunctional full-wavelength microplate reader (Varioskan, LUX, Singapore).

Results

Identification and characterization of PbMYB1L

MYB transcription factors has important function in the modulation of plant growth and development processes, including participation stress response and anthocyanin biosynthesis. We identified an R2R3-MYB transcription factor from the peel of the ‘Red Zaosu’ pear, which encoded a 361-amino acids protein. Phylogenetic analysis revealed its close relationship to PbMYB1, and upon aligning its amino acid sequence with PbMYB1, a similarity of 70.84% was observed, leading to its designation as PbMYB1L (Figure 1a, b). The subcellular localization of PbMYB1L was determined by fusing it with the green fluorescent protein (GFP) reporter gene, and introducing 35S::PbMYB1L-GFP into tobacco leaves. The results revealed that PbMYB1L exhibited nuclear localization (Figure 1c).

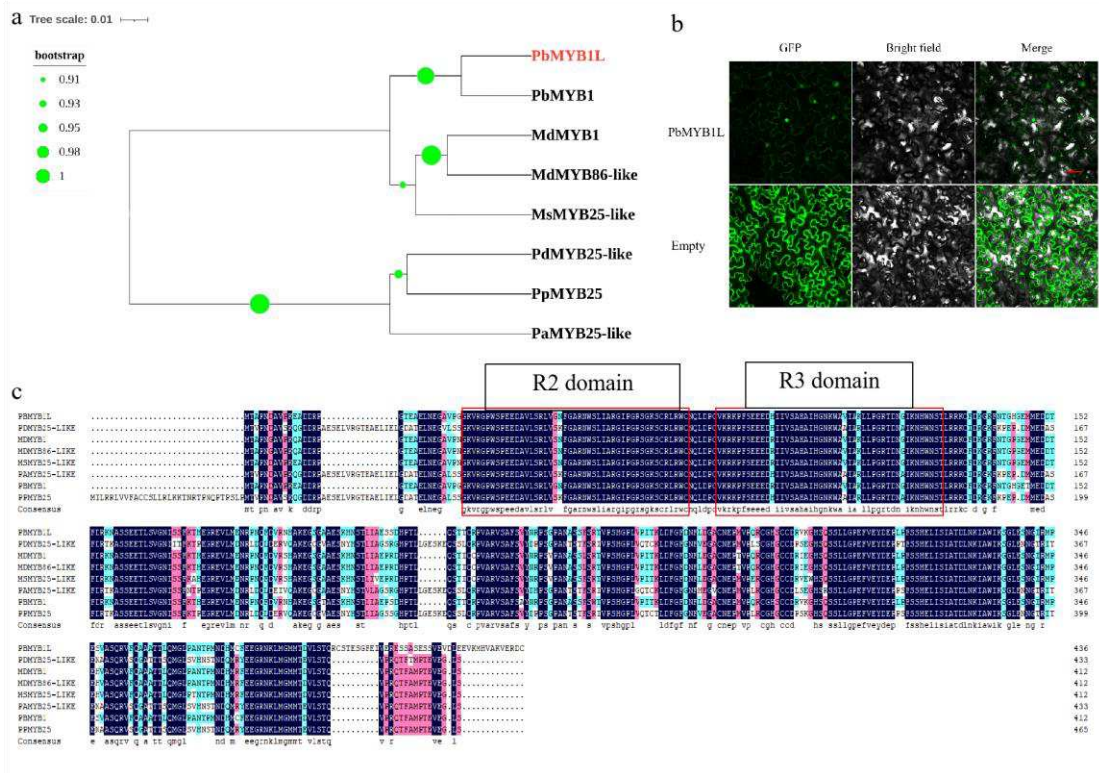


Fig. 1 Bioinformatics analysis and identification analysis of PbMYB1L.

(a) Phylogenetic analysis of the R2R3 domain proteins from pear and other plant species. Pear (*Pyrus bretschneideri*): PbMYB1L(>XP_009344337.2); Apple (*Malus sieversii*): MsMYB25-like (>XP_050157282.1); Apple (*Malus domestica*): MdMYB1 (>ABK20308.1);

MdMYB86-like (>NP_001288045.1); Cherry (*Prunus avium*): PaMYB25-like (>XP_021821784.1), Peach (*Prunus persica*): PpMYB25 (>XP_007218805.2); Almond (*Prunus dulcis*): PdMYB25-like (>XP_034204384.1). **(b)** Subcellular localization of *PbMYB1L* expressed in tobacco leaf cells. **(c)** Sequence alignment of *PbMYB1L*, PbMYB1, MsMYB25-like, MdMYB1, MdMYB86-like, PaMYB25-like, PpMYB25 and PdMYB25-like proteins. The R2 and R3 domains are indicated by the red lines around the sequences.

***PbMYB1L* is responsive to low-temperature**

To further elucidate the functionality of *PbMYB1L*, we successfully cloned and meticulously examined its promoters. Our comprehensive promoter analysis of *PbMYB1L* unveiled the presence of three low-temperature response elements (LTR) within its promoter region (Figure 2a), which potentially play a pivotal role in mediating the cold response of *PbMYB1L*. We conducted reverse transcription quantitative PCR (RT-qPCR) analysis to investigate the expression pattern of *PbMYB1L* in ‘Red Zaosu’ calli subjected to low-temperature stress treatment (4°C). The result revealed a rapid and transient upregulation of *PbMYB1L* expression, with peak levels observed at 3 h following exposure to low temperature stress (Figure 2b). These findings provided preliminary evidence suggesting that low temperature can induce the expression of *PbMYB1L*. To further validate the prediction, we generated a fusion construct by integrating the *PbMYB1L* promoter (2000 bp) with a β -glucuronidase (GUS) reporter gene. This construct was then introduced into tobacco leaves and subjected to low temperature treatment. Subsequent GUS staining analysis demonstrated that Pro*PbMYB1L*:GUS injected tobacco leaves exhibited significantly higher GUS activity following cold treatment (Figure 2c, d), providing evidence for the activation of *PbMYB1L* transcription upon exposure to cold stress.

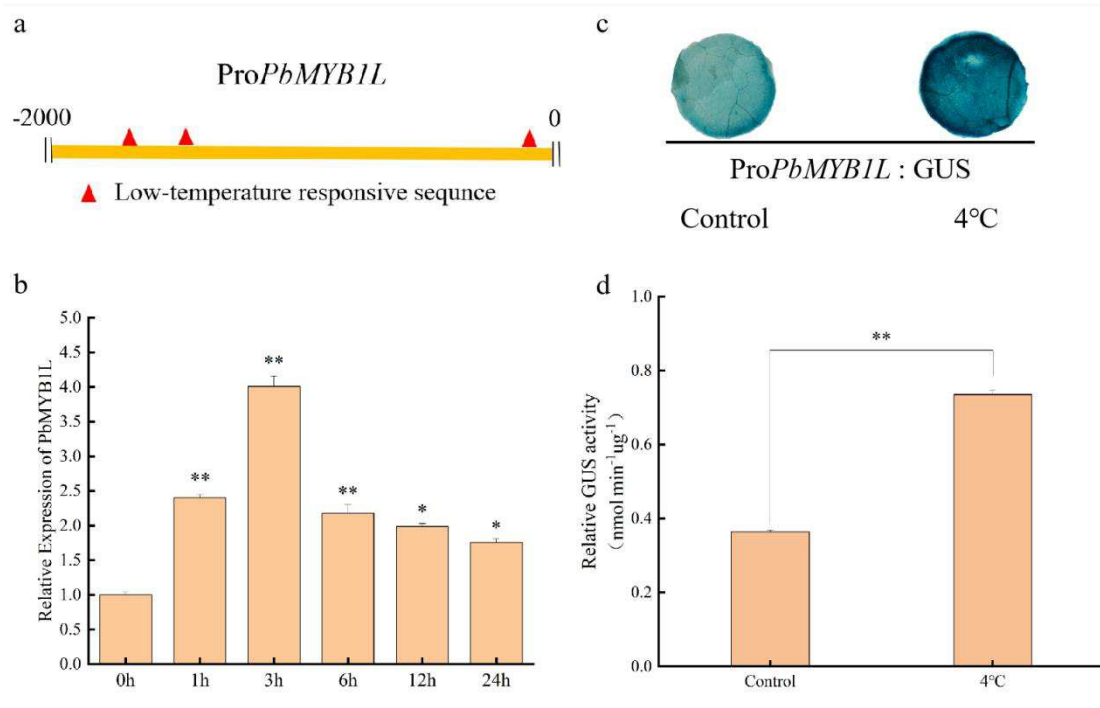


Fig. 2 *PbMYB1L* responds to low temperatures.

(a) The position of LTR in the *PbMYB1L* promoter. (b) RT-qPCR analysis of *PbMYB1L* exposed to 4°C treatment. (c) GUS staining analysis of the *PbMYB1L* promoter using Pro*PbMYB1L*:GUS injectable tobacco leaves exposed to 4°C. Tobacco leaves are left dark for 24 h after injection and then processed. Control: GUS staining for 3 h at 24°C; 4°C: cold treatment for 3 h at 4°C. (d) GUS activities of the injectable tobacco leaves are shown. Asterisks indicate the meaning determined by the t-test: **P* < 0.05 and ***P* < 0.01. There is no special explanation later, and the meaning of the asterisk is the same as above.

***PbMYB1L* improves the root growth phenotype of the plant at low temperatures**

To investigate the role of *PbMYB1L* in cold tolerance, we generated a vector harboring the full coding sequence (CDS) of *PbMYB1L* driven by the cauliflower mosaic virus 35S promoter (*PbMYB1L*-OE), which was subsequently introduced into wild-type *Arabidopsis* (WT). Six transgenic *Arabidopsis* lines were individually obtained (Fig. 3a). Following a 7-day growth on 1/2 MS medium, *Arabidopsis* seedlings (OE and WT) were transferred to vertical positions and subjected to incubation at both 4°C and 23°C. After a duration of 14 days, root lengths were measured. The primary root length of OE seedlings was significantly longer than that of WT at 4°C, whereas no significant difference was observed in the control group (Fig. 3b, c). Furthermore, we also assessed the chlorophyll content in plant leaves and found a moderate increase in transgenic plants following low temperature treatment

(Fig. 3d). Therefore, we propose that over expression of *PbMYBIL* enhances the cold tolerance of transgenic Arabidopsis, leading to a modest increase in primary root length under low temperature conditions.

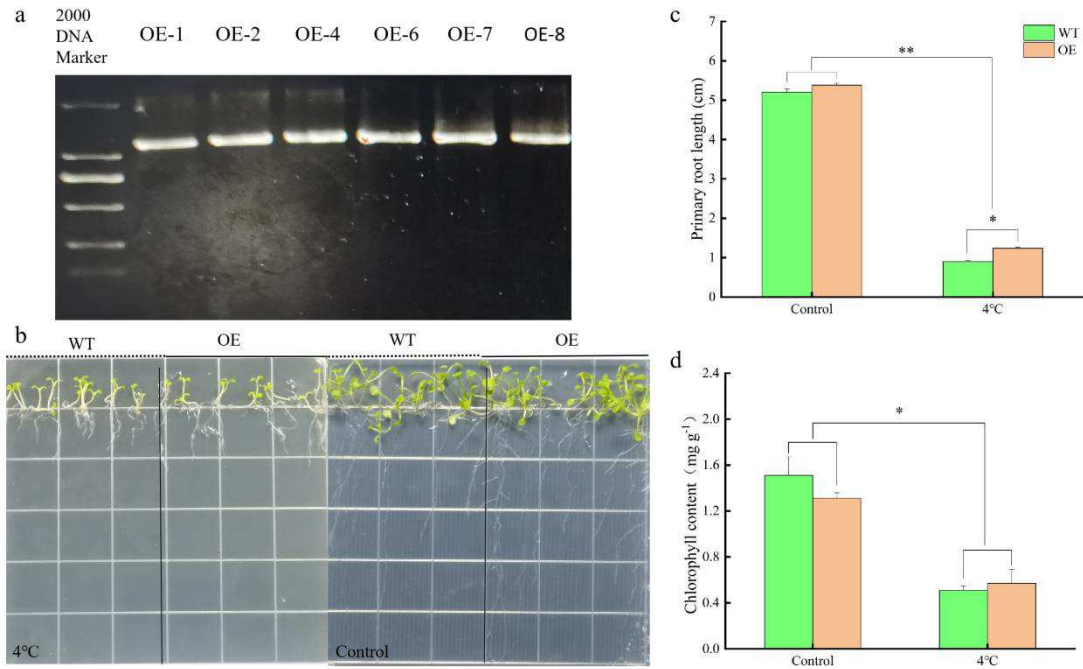


Fig. 3 Primary root growth phenotype of Arabidopsis under cold stress.

(a) PCR identification of transgenic Arabidopsis. **(b)** Root growth patterns of WT and OE plants. **(c)** Primary root length analysis. **(d)** Chlorophyll content analysis. Primary root length and Chlorophyll content was measured after 14 days of growth.

Involvement of *PbMYBIL* in the regulation of the cold tolerance of plants

To further investigate the cold stress resistance of transgenic Arabidopsis, we subjected 14-day-old *PbMYBIL* transgenic and control plants (OE and WT) to a 4°C incubator for 1 h. Subsequently, we performed DAB and NBT on Arabidopsis after low temperature treatment, followed by quantification of O²⁻, H₂O₂ and MDA levels in leaves. The histochemical staining results revealed a significant increase in reactive oxygen species (ROS) levels under cold stress, both in the transgenic and control plants. Importantly, the wild-type (WT) plants exhibited significantly higher levels accumulation of H₂O₂ and O²⁻ compared to the transgenic lines. After exposure to cold stress, the transgenic plants exhibited lower accumulation of blue sediment (NBT staining) compared to the WT plants, while there was no significant difference in the brown sediment (DAB staining) (Figure 4a). To further validate these findings, we treated 14-day-old seedlings overexpressing *PbMYBIL* and control plants at 4°C. We measured the content of H₂O₂ and O²⁻ in the leaves at different time points within a

24-hour period. The results demonstrated that after 24 hours of treatment, the accumulation of H_2O_2 and O_2^- in the leaves of WT plants was significantly higher than that in the transgenic plants (Figure 4b, c). Furthermore, the MDA content of the plants was also quantified. As the duration of treatment increased, a gradual rise in MDA content was observed, with WT plants exhibiting significantly higher levels compared to transgenic plants after three hours of treatment (Figure 4d).

These results demonstrate that the over expression of *PbMYB1L* in Arabidopsis leads to a reduction in ROS accumulation under cold stress conditions, as evidenced by decreased levels of H_2O_2 , O_2^- , and blue sediment in the transgenic plants compared to the WT plants. Moreover, analysis of MDA content indicated that the membrane damage caused by cold stress was more pronounced in the WT plants than in the transgenic lines. *PbMYB1L* overexpression enhanced root growth and antioxidant capacity in response to cold stress. Collectively, these findings underscore the pivotal role of *PbMYB1L* in conferring tolerance to cold stress.

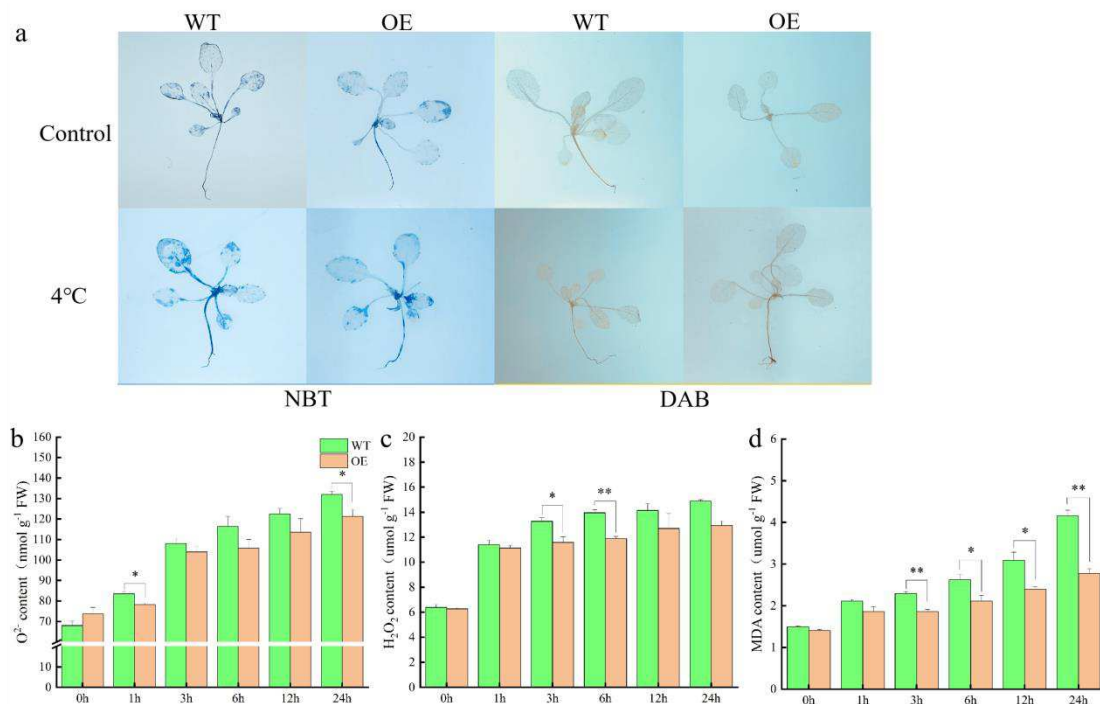


Fig. 4 Phenotype and related physiological indexes of Arabidopsis under cold stress.

(a) Histochemical staining with DAB and NBT of WT and OE plants. Two-week old plants from WT and OE were treated with cold for 1 h and then subjected to histochemical DAB and NBT solution for assessing the accumulation of H_2O_2 and O_2^- in leaves, respectively. **(b)** Quantitative measurement of O_2^- activity in WT and OE plants. **(c)** Quantitative measurement of H_2O_2 in WT and OE plants. **(d).** Quantitative measurement of MDA in WT and OE plants.

***PbMYB1L* promotes Arabidopsis cold tolerance by regulating cold responsive gene expression**

To elucidate the molecular mechanisms underlying *PbMYB1L*-mediated cold tolerance, we assessed the expression of cold-responsive genes in *PbMYB1L*-overexpressing (OE) and wild-type (WT) Arabidopsis plants subjected to cold stress. Furthermore, we investigated the potential involvement of the CBF pathway in *PbMYB1L*-mediated cold tolerance by examining the expression of CBF genes in WT and *PbMYB1L*-OE Arabidopsis. Our results revealed that, at 1 hour after cold stress, the expression levels of *AtCBF1*, *AtCBF2*, *AtCBF3*, *AtCBF4*, *AtICE1*, *AtKIN1*, *AtKIN2* and *AtRD29A* were significantly higher in *PbMYB1L*-OE Arabidopsis compared to WT plants. Furthermore, in the transgenic lines, the expression of these cold-responsive genes peaked after 6 hours of cold treatment and remained significantly elevated compared to WT plants. Importantly, both WT and *PbMYB1L*-OE Arabidopsis exhibited an upward trend in the expression of these genes indicating that *PbMYB1L* positively regulates their expression thereby influencing Arabidopsis's cold tolerance. These findings are consistent with the observed enhancement in freezing tolerance of *PbMYB1L*-OE plants suggesting that *PbMYB1L* promotes the expression of these cold-responsive genes under conditions of cold stress in Arabidopsis.

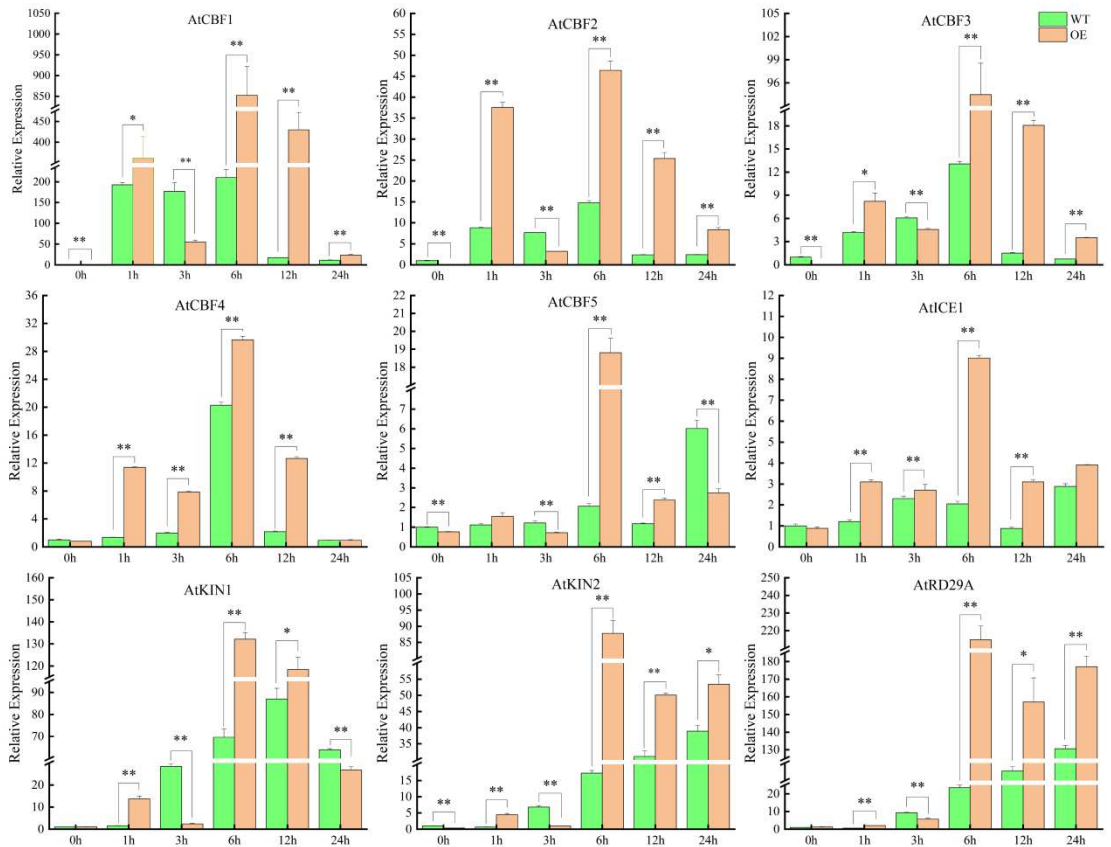


Fig. 5 Transcript levels of cold-responsive genes in WT and OE Arabidopsis plants under cold stress. Transcript levels of endogenous cold-responsive genes of C-REPEAT BINDING FACTOR 1 (*AtCBF1*), C-REPEAT BINDING FACTOR 2 (*AtCBF2*), C-REPEAT BINDING FACTOR 3 (*AtCBF3*), C-REPEAT BINDING FACTOR 4 (*AtCBF4*), C-REPEAT BINDING FACTOR 5 (*AtCBF5*), INDUCER OF CBF EXPRESSION 1 (*ICE1*), COLD INDUCED1(*KIN1*), COLD INDUCED2(*KIN2*), *RD29A* in transgenic plants under control or cold conditions.

Cold stress induce an increase in the expression of anthocyanin biosynthesis in transgenic Arabidopsis

After subjecting Arabidopsis to low-temperature treatment, a distinct purplish-red coloration was observed on the leaves of *PbMYB1L*-overexpressing plants, which starkly contrasted with the wild-type Arabidopsis leaves. Consequently, we postulate that cold treatment potentially triggers anthocyanin accumulation in transgenic plants. Following a 14-days growth period, Arabidopsis plants were subjected to an additional 14 days of cold stress at 4°C. As illustrated in Figure 6a, a significant increase in anthocyanin content was evident on the leaves of *PbMYB1L*-OE Arabidopsis plants, while no substantial accumulation was detected in the WT counterparts. To further verify validate the promotion of anthocyanin accumulation in OE Arabidopsis under cold stress, we quantified expression levels of six structural genes (*AtCHS*, *AtCHI*, *AtDFR*, *AtF3H*, *AtANS* and *AtUFGT*) involved in anthocyanin biosynthesis as well as three transcription factors (*AtMYB10*, *AtbHLH3*, *AtWD40*). Our results demonstrated a significant upregulation of these genes in OE Arabidopsis compared to WT at six time points within 24 hours. These findings provide evidence that cold stress induces the expression of key genes associated with transgenic Arabidopsis anthocyanins biosynthesis and thereby facilitates anthocyanin production.

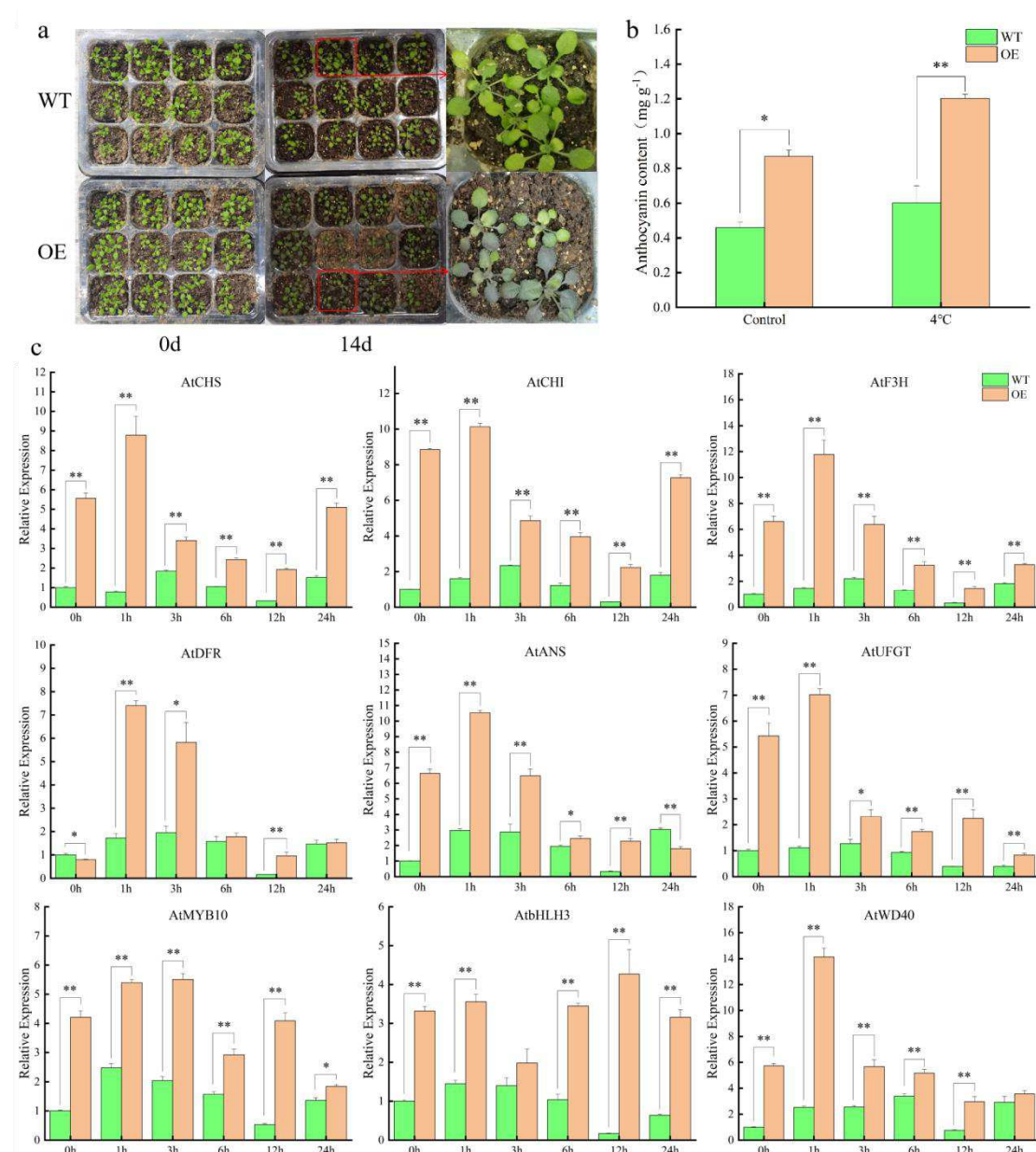


Fig. 6 Quantitative analysis of phenotype, anthocyanin content and genes related to anthocyanin synthesis in Arabidopsis at low temperatures. (a) WT and OE of plant phenotypes at cold conditions. (b) Anthocyanin content of Arabidopsis leaves after cold treatment. (c) Expression patterns of anthocyanin biosynthetic genes and transcription factors in Arabidopsis at different time points under 4°C treatment. Two-week old plants from WT and OE were growth in 4°C for 14 days and then observed the plant phenotype and determined leaf anthocyanin content.

Discussion

Numerous studies have demonstrated that MYB proteins play pivotal roles in regulatory networks governing diverse biological processes, encompassing secondary

metabolite biosynthesis and stress response. For instance, the overexpression of *AcMYB10* in kiwifruit leads to an augmentation in anthocyanin synthesis (Peng et al. 2019). Conversely, *CmMYB012* is induced under prolonged exposure to high temperatures and acts as a suppressor of flavonoid and anthocyanin biosynthesis (Zhou et al. 2021). Similarly, within the Rosacea family, the overexpression of *FvMYB24* in *Arabidopsis* upregulates the expression of stress-related genes, thereby enhancing salt tolerance in transgenic *Arabidopsis* (Wang et al. 2021). Recent investigations have revealed that *McMYB4* participates in apple response to temperature fluctuations by modulating phenylpropan metabolism and hormone signaling (Hao et al. 2021). *MdMYB23* and *MdMYB73* improve cold tolerance of transgenic calli through the CBF pathway in apples, while *VvMYBA6* enhances ROS scavenging system capability and improves cold tolerance of transgenic *Arabidopsis* by promoting anthocyanin synthesis (An et al. 2018; Zhang et al. 2016; Sun et al. 2017). Moreover, the upregulation of *MdMYB308L* and *MdMYB2* has been demonstrated to enhance both cold tolerance and anthocyanin accumulation (An et al. 2020; Jiang et al. 2022). These findings imply that MYB transcription factors exhibit distinct regulatory roles in secondary metabolite synthesis and abiotic stresses responses, indicating their diverse functions in governing various biological processes. Therefore, it is crucial to identify MYB genes capable of regulating both cold stress responses and anthocyanins synthesis for a comprehensive understanding of the cold signaling pathways associated with cold tolerance and anthocyanin production, as well as for providing potential candidate genes for genetic manipulation.

In this study, we identified a MYB gene, *PbMYB1L*, in *Pyrus bretschneideri*. Despite the extensive characterization of MYBs in field and cash crops, their functions in pears remain largely unexplored. To gain insights into the evolutionary relationship of *PbMYB1L*, we constructed a phylogenetic tree using sequences from pear, apple, peach, cherry, and almond. The analysis revealed that *PbMYB1L* is closely related to *PbMYB1*. Furthermore, motif scanning analysis indicated a high degree of homology in the R2R3 domain of *PbMYB1L*. Cold treatment induced the expression of *PbMYB1L*. Interestingly, the expression patterns of *PbMYB1L* resemble those observed for *MdMYB23* and *MdMYB73* which are known to positively regulate cold-tolerance and cold-response genes through CBF-dependent pathways (An et al. 2018; Zhang et al. 2016). These findings underscore the potential role of *PbMYB1L* in cold stress responses and provide valuable insights into the regulatory mechanisms associated with cold tolerance in pears.

We conducted an analysis of the *PbMYB1L* promoter and identified cis-acting elements associated with the response to cold stress. In order to investigate the role of *PbMYB1L* in enhancing cold hardiness, transgenic Arabidopsis strains were subjected to cold stress conditions. The stress tolerance assay demonstrated that the selected transgenic lines exhibited improved tolerance towards cold stress, as evidenced by measurements of MDA levels, chlorophyll content, primary root length, and phenotypic observations. These findings provide evidence that overexpression of *PbMYB1L* confers enhanced tolerance to cold stress. Our results are consistent with previous studies in apples which have shown that *MYB88* and *MYB124* positively regulate the expression of genes related to both cold resistance and response in apples and Arabidopsis (Xie et al. 2018). Overexpression of MYB genes has been demonstrated to enhance abiotic stress tolerance in transgenic plants, highlighting their potential for genetic engineering approaches aimed at improving stress tolerance (Peng et al. 2019).

To further investigate the cold tolerance of Arabidopsis transgenics, we conducted a comprehensive set of physiological, molecular, and biochemical analyses to elucidate the underlying mechanisms. One aspect that received particular attention was the examination of physiological differences between overexpressing (OE) and wild type (WT) plants under cold stress conditions. In plants, metabolic imbalances can trigger intracellular oxidative stress by promoting the production and accumulation of reactive oxygen species (ROS). The level of ROS reflect the extent of damage to cellular components (Suzuki et al. 2012). Membrane lipid peroxidation, as indicated by malondialdehyde (MDA) levels, is associated with membrane systems' integrity (Christine et al. 1994). Our results demonstrated lower MDA levels in WT plants compared to transgenic strains, suggesting that they may experience more severe oxidative stress. To further explore this phenomenon, histochemical staining was performed to determine O_2^{2-} and H_2O_2 accumulation in plants after exposure to cold stress. The staining intensity observed through DAB and NBT staining was significantly weaker in transgenic lines than in WT plants (Fig 4b). These findings indicate that the transgenic strains possess an enhanced ROS clearance system which plays a crucial role in protecting them from various forms of damage. This improved ROS metabolism may be a key component contributing to the conferred cold tolerance resulting from *PbMYB1L* overexpression in transgenic plants.

Furthermore, we observed that cold stress induced a significant accumulation of anthocyanins in transgenic Arabidopsis plants. Through quantitative analysis of key

structural genes and transcription factors involved in anthocyanin synthesis pathway, we found a significantly higher expression level of these genes in the transgenic strains compared to WT plants following cold treatment. This suggests that the upregulation of these genes in the transgenic plants contributes to an enhanced accumulation of anthocyanins in response to cold stress. These results provide valuable insights into the mechanisms underlying the improved cold tolerance observed in *PbMYB1L*-overexpressing transgenic Arabidopsis plants, highlighting the significance of an enhanced ROS clearance system and an upregulated anthocyanin synthesis pathway for conferring cold tolerance.

Previous study have demonstrated the crucial role of CBFs in regulating cold stress responses (Liu, et al. 2018). In apple, *MdMYB23*, *MdMYB108L* have been reported to enhance cold tolerance by positively modulating the transcription of CBF genes (An, et al. 2018; Wang, et al. 2019). In this study, we investigated the impact of overexpressing *PbMYB1L* on the response to cold stress in Arabidopsis. We observed that the expression levels of *AtCBF1*, *AtCBF2*, *AtCBF3*, *AtCBF4*, *AtICE1* *AtKIN1*, *AtKIN2* and *AtRD29A* were significantly higher in *PbMYB1L* transgenic Arabidopsis compared to wild type plants at 1 h after exposure to cold stress. Furthermore, all these aforementioned cold-responsive genes reached their peak expression levels in transgenic strains after six hours of cold treatment and remained significantly higher than those in wild type plants. It is worth noting that MYB transcription factors are predominantly associated with anthocyanin synthesis in many fruit trees. In grapes, the first MYB transcription factor, *VvMYBA1*, promotes anthocyanin synthesis in various grape tissues (Jeong et al. 2006). Similarly, *MdMYB1* in apples and *MrMYB1* in bayberry have been found to positively regulate anthocyanin synthesis (Tako et al. 2006; Niu et al. 2010). In blueberries, the *VaMYB1* transcription factor plays a pivotal role in enhancing anthocyanin synthesis within the fruit (Die et al. 2020). Additionally, *LcMYB1* in litchi contributes to anthocyanin accumulation in hairy roots (Li et al. 2023a). To further validate the ability of *PbMYB1L* to promote anthocyanin accumulation, we quantified both structural genes and transcription factors involved in anthocyanin biosynthesis in OE and WT Arabidopsis after cold treatment. The results demonstrated significantly higher expression levels of these structural genes and transcription factors at six time points within a 24-hour period compared to those observed in WT plants. These findings indicate that cold stress induces upregulation of transgenic Arabidopsis genes associated with anthocyanin biosynthesis, thereby facilitating enhanced production of anthocyanins. Overall, our results suggest that

PbMYBIL may function as a transcriptional regulator by promoting the upregulation of endogenous regulatory genes such as CBF genes which subsequently activate downstream target genes involved not only in cold stress response but also in anthocyanin biosynthesis. Further investigations are warranted to identify interacting downstream genes regulated by *PbMYBIL* and elucidate their roles underlying *PbMYBIL*-mediated cold tolerance mechanisms. Moreover, future studies should explore the precise involvement of *PbMYBIL* within the pathway regulating anthocyanin synthesis. This study provides valuable insights into the potential contribution of *PbMYBIL* towards improving plant tolerance against cold stress while emphasizing on comprehending molecular interactions crucial for this process.

In conclusion, *PbMYBIL* emerges as a pivotal regulator governing anthocyanin biosynthesis and cold stress response in pear cultivars, thereby offering promising prospects for the advancement of biotechnological strategies aimed at enhancing cold stress tolerance.

Author contribution statement Conceptualization: XZ, DYL, and YZ. XZ, HYW, JYL conducted the experiment. Data analysis: XZ, WTY. Formal analysis: XRW. Methodology: SYL, YTZ and YXL. Software: YW, WH and QC. Visualizations: XZ, DYL, and MYL. Writing - first draft, XZ and DYL. Writing - Reviews and Editing, YZ, and YL. Project administration: HRT. Funding acquisition, YZ. All authors have read and agreed to the published version of the manuscript.

Acknowledgments This work was supported by grants from the Science and Technology Plan Project of Sichuan Province (Grant No. 2021YFYZ0023-03) and the Double Support Project of Discipline Construction of Sichuan Agricultural University (03573134).

Data availability statement The data that support the findings of this study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Reference

- Agarwal M, Hao YJ, Kapoor A, Dong CH, Fujii H, Zheng XW, Zhu JK (2006) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J Biol Chem*, 281(49): 37636-37645. <https://doi.org/10.1074/jbc.M605895200>
- Alabd A, Ahmad M, Zhang X, Gao HY, Peng L, Zhang L, Ni JB, Bai SL, Teng YW (2022) Light-responsive transcription factor PpWRKY44 induces anthocyanin accumulation by regulating PpMYB10 expression in pear. *Hort Res*, 9: 199. <https://doi.org/10.1093/HR/UHAC199>
- An JP, Li R, Qu FJ, You CX, Wang XF, Hao YJ (2018) R2R3-MYB transcription factor MdMYB23 is involved in the cold tolerance and proanthocyanidin accumulation in apple. *Plant J*, 96(3): 562-577. <https://doi.org/10.1111/tpj.14050>
- An JP, Wang XF, You CX, Hao YJ (2021) The anthocyanin biosynthetic regulator MDMYB1 positively regulates ascorbic acid biosynthesis in apples. *Front Agric Sci Eng*, 8(2): 231-235. <https://doi.org/10.15302/J-FASE-2020367>
- An JP, Wang XF, Zhang XW, Xu HF, Bi SQ, You CX, Hao YJ (2020) An apple MYB transcription factor regulates cold tolerance and anthocyanin accumulation and undergoes MIEL1-mediated degradation. *Plant Biotechnol. J*, 18(2): 337-353. <https://doi.org/10.1111/pbi.13201>
- Chinnusamy V, Zhu JH, and Zhu JK (2007) Cold stress regulation of gene expression in plants. *Trends Plant Sci*, 12, 444–451. <https://doi.org/10.1016/j.tplants.2007.07.002>
- Christine HF, Maud L, Karl JK (1994) Photooxidative stress in plants. *Physiol Plant*, 92(4): 696-717. <https://doi.org/10.1111/j.1399-3054.1994.tb03042.x>
- Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis*. *Plant J*, 16(6): 735-743. <https://doi.org/10.1046/j.1365-313x.1998.00343.x>
- Die JV, Jones RW, Ogden EL, Ehlenfeldt MK, Rowland LJ (2020) Characterization and Analysis of Anthocyanin-Related Genes in Wild-Type Blueberry and the Pink-Fruited Mutant Cultivar ‘Pink Lemonade’: New Insights into Anthocyanin Biosynthesis. *Agron*,

10(9): 1296-1313. <https://doi.org/10.3390/agronomy10091296>

Dubos C, Stracke R, Grotewold E, Weisshaar B, Martin C, Lepiniec L (2010) MYB transcription factors in Arabidopsis. Trends in Plant Sci, 15(10): 573-581. <https://doi.org/10.1016/j.tplants.2010.06.005>

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem, 48(12): 909-930. <https://doi.org/10.1016/j.plaphy.2010.08.016>

Hao SX, Lu YF, Peng Z, Wang EY, Chao LK, Zhong SL, Yao YC (2021) McMYB4 improves temperature adaptation by regulating phenylpropanoid metabolism and hormone signaling in apple. Hort Res, 8(1): 182. <https://doi.org/10.1038/S41438-021-00620-0>

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998) Arabidopsis CBF1 Overexpression Induces COR Genes and Enhances Freezing Tolerance. Science, 280(5360): 104-106. <https://doi.org/10.1126/science.280.5360.104>

Jakoby M, Weisshaar B, Dr oge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in Arabidopsis. Trends Plant Sci. 7, 106–111. [https://doi.org/10.1016/S1360-1385\(01\)02223-3](https://doi.org/10.1016/S1360-1385(01)02223-3)

Jeong ST, Goto YN, Hashizume K, Kobayashi S, Esaka M (2006) Expression of VvmybA1 gene and anthocyanin accumulation in various grape organs. Am J Enol Vitic, 57(4): 507-510. <https://doi.org/10.5344/ajev.2006.57.4.507>

Jiang H, Zhou LJ, Gao HN, Wang XF, Li ZW, Li YY (2022) The Transcription Factor MdMYB2 Influences Cold Tolerance and Anthocyanin Accumulation by Activating SUMO E3 Ligase MdSIZ1 in Apple. Plant Physiol, 189: 2044-2060. <https://doi.org/10.1093/PLPHYS/KIAC211>

Li S, Qin YQ, Jing SQ, Wang D, Zhang ZK, Qin YH, Hu GB, Zhao JT (2023a) Metabolome and transcriptome analyses reveal the molecular mechanisms of LcMYB1 regulating anthocyanin accumulation in litchi hairy roots. Plant Physiol Biochem, 200: 107749-107757. <https://doi.org/10.1016/J.PLAPHY.2023.107749>

Li YF, Zuo XX, Ji NN, Zhang JL, Wang KT, Jin P, Zheng YH (2023b) PpMYB1 and PpNPR1 interact to enhance the resistance of peach fruit to *Rhizopus stolonifer* infection. Plant Physiol Biochem, 198: 107682-107692. <https://doi.org/10.1016/J.PLAPHY.2023.107682>

- Lim SH, Kim DH, Kim JK, Lee JY, Ha SH (2017) A Radish Basic Helix-Loop-Helix Transcription Factor, RsTT8 Acts a Positive Regulator for Anthocyanin Biosynthesis. *Front Plant Sci*, 8: 1917. <https://doi.org/10.3389/fpls.2017.01917>
- Liu JY, Shi YT, Yang SH (2018) Insights into the regulation of C-repeat binding factors in plant cold signaling. *J Integr Plant Biol*, 60(09): 780-795. <https://doi.org/10.1111/jipb.12657>
- Ni JB, Bai SL, Zhao Y, Qian MJ, Tao RY, Yin L, Gao L, Teng YW (2019) Ethylene response factors Pp4ERF24 and Pp12ERF96 regulate blue light-induced anthocyanin biosynthesis in 'Red Zaosu' pear fruits by interacting with MYB114. *Plant Mol Biol*, 99(1-2): 67-78. <https://doi.org/10.1007/s11103-018-0802-1>
- Niu SS, Xu CJ, Zhang WS, Zhang B, Li X, Kui LW, Ferguson IB, Allan AC, Chen KS (2010) Coordinated regulation of anthocyanin biosynthesis in Chinese bayberry (*Myrica rubra*) fruit by a R2R3 MYB transcription factor. *Planta*, 231(4): 887-899. <https://doi.org/10.1007/S00425-009-1095-Z>
- Peng YY, Kui LW, Cooney JM, Wang TC, Espley RV, Allan AC (2019) Differential regulation of the anthocyanin profile in purple kiwifruit (*Actinidia species*). *Hort Res*, 6(1): 3. <https://doi.org/10.1038/s41438-018-0076-4>
- Shi J, Fu XZ, Peng T, Huang XS, Fan QJ, Liu JH (2010) Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidative capacity and stomatal response. *Tree physiol*, 30(7): 914-922. <https://doi.org/10.1093/treephys/tpq030>
- Shi YT, Ding YL, Yang SH (2018) Molecular Regulation of CBF Signaling in Cold Acclimation. *Trends Plant Sci*, 23(7): 623-637. <https://doi.org/10.1016/j.tplants.2018.04.002>
- Shin J, Park E, Choi G (2007) PIF3 regulates anthocyanin biosynthesis in an HY5-dependent manner with both factors directly binding anthocyanin biosynthetic gene promoters in Arabidopsis: Functional relationship between PIF3 and HY5. *Plant J*, 49(6): 981-994. <https://doi.org/10.1111/j.1365-313X.2006.03021.x>
- Stockinger EJ, Gilmour SJ, Thomashow MF (1997) Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a

cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. PNAS, 94(3): 1035-1040. <https://doi.org/10.1073/pnas.94.3.1035>

Stracke R, Werber M, Weisshaar B (2001) The R2R3-MYB gene family in Arabidopsis. Curr Opin Plant Biol, 4(5): 447-456. [https://doi.org/10.1016/S1369-5266\(00\)00199-0](https://doi.org/10.1016/S1369-5266(00)00199-0)

Sun M, Feng XX, Gao JJ, Peng RH, Yao QH, Wang LJ (2017) VvMYBA6 in the promotion of anthocyanin biosynthesis and salt tolerance in transgenic Arabidopsis. Plant Biotechnol Rep, 11(5): 299-314. <https://doi.org/10.1007/s11816-017-0452-9>

Suzuki N, Mittler R (2006) Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiol Plant. 126, 45–51. <https://doi.org/10.1111/j.0031-9317.2005.00582.x>

Suzuki N, Koussevitzky S, Mittler R, Miller G (2012) ROS and redox signalling in the response of plants to abiotic stress. Plant Cell Environ, 35(2): 259-270. <https://doi.org/10.1111/j.1365-3040.2011.02336.x>

Takos AM, Jaffé FW, Jacob SR, Bogs J, Robinson SP, Walker AR (2006) Light-induced expression of a MYB gene regulates anthocyanin biosynthesis in red apples. Plant Physiol, 142(3): 1216-1232. <https://doi.org/10.1104/PP.106.088104>

Wang K, Zhong M, Wu YH, Bai ZY, Liang QY, Liu QL, Pan YZ, Zhang L, Jiang BB, Jia Y, Liu GL (2017) Overexpression of a chrysanthemum transcription factor gene DgNAC1 improves the salinity tolerance in chrysanthemum. Plant Cell Rep, 36(4): 571-581. <https://doi.org/10.1007/s00299-017-2103-6>

Wang SH, Shi MY, Zhang Y, Xie XB, Sun PP, Fang CB, Zhao J (2021) FvMYB24, a strawberry R2R3-MYB transcription factor, improved salt stress tolerance in transgenic Arabidopsis. Biochem Biophys Res Commun, 569: 93-99. <https://doi.org/10.1016/J.BBRC.2021.06.085>

Wang XP, Niu Y, Zheng Y (2021) Multiple Functions of MYB Transcription Factors in Abiotic Stress Responses. Int J Mol Sci, 22(11): 6125. <https://doi.org/10.3390/IJMS22116125>

Xie YP, Chen PX, Yan Y, Bao CN, Li XW, Wang LP, Shen XX, Li HY, Liu XF, Niu CD, Zhu C, Fang N, Shao Y, Zhao T, Yu JT, Zhu JH, Xu LF, Steven VN, Ma FW, Guan QM (2018)

An atypical R2R3 MYB transcription factor increases cold hardiness by CBF-dependent and CBF-independent pathways in apple. *New Phytol*, 218(1): 201-218. <https://doi.org/10.1111/nph.14952>

Xu WJ, Damaris G, Sophie B, Gourrierc JL, Johanne T, Zsolt K, Lepiniec L, Dubos C (2014) Complexity and robustness of the flavonoid transcriptional regulatory network revealed by comprehensive analyses of MYB-bHLH-WDR complexes and their targets in *Arabidopsis* seed. *New Phytol*, 202(1): 132-144. <https://doi.org/10.1111/nph.12620>

Zhang D, Yu B, Bai JH, Qian MJ, Shu Q, Su J, Teng YW (2011) Effects of high temperatures on UV-B/visible irradiation induced postharvest anthocyanin accumulation in ‘Yunhongli No. 1’ (*Pyrus pyrifolia* Nakai) pears. *Sci Hortic*, 134: 53-59. <https://doi.org/10.1016/j.scienta.2011.10.025>

Zhang L, Tian LH, Zhao JF, Song Y, Zhang CJ, Guo Y (2009) Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis. *Plant physiol*, 149(2): 916-928. <https://doi.org/10.1104/pp.108.131144>

Zhang QY, Yu JQ, Wang JH, Hu DG, Hao YJ (2016) Functional characterization of MdMYB73 reveals its involvement in cold stress response in apple calli and *Arabidopsis*. *J Integr Agric*, 16(10): 2215-2221. [https://doi.org/10.1016/S2095-3119\(17\)61723-4](https://doi.org/10.1016/S2095-3119(17)61723-4)

Zhang Y, Butelli E, Martin C (2014) Engineering anthocyanin biosynthesis in plants. *Curr. Opin. Plant Biol*, 19: 81-90. <https://doi.org/10.1016/j.pbi.2014.05.011>

Zhou LJ, Geng ZQ, Wang YX, Wang YG, Liu SH, Chen CW, Song AP, Jiang JF, Chen SM, Chen FD (2021) A novel transcription factor CmMYB012 inhibits flavone and anthocyanin biosynthesis in response to high temperatures in chrysanthemum. *Hort Res*, 8(1): 248. <https://doi.org/10.1038/s41438-021-00675-z>

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.docx](#)