Journal of Integrative Agriculture 2026, 25(8): 3228–3239

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

EjRabF2b enhances cold resistance in loquat (*Eriobotrya japonica*) through antioxidant regulation

Hao Fu[1, 2, 3], Qian Chen[1, 2], Shunyuan Yong[1, 2], Toru Fujiwara[3], Jiangbo Dang[1, 2], Danlong Jing[1, 2], Di Wu[1, 2], Guolu Liang[1, 2#], Qigao Guo[1, 2#]

[1] *Key Laboratory of Agricultural Biosafety and Green Production of Upper Yangtze River (Ministry of Education)/College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400715, China*

[2] *State Cultivation Base of Crop Stress Biology for Southern Mountainous Land/Academy of Agricultural Sciences, Southwest University, Chongqing 400715, China*

[3] *Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 113-8657, Japan*

Highlights

- *EjRabF2b* is significantly upregulated in loquat under cold stress, indicating its strong responsiveness to low-temperature signals.
- *EjRabF2b* influences cold tolerance by modulating antioxidant enzyme activity and stabilizing cell membrane structure.
- *EjZAT10* directly binds to the promoter of *EjRabF2b* and activates its transcription, revealing a key regulatory module in the cold stress response.

Abstract

Loquat (*Eriobotrya japonica*) is an evergreen fruit tree native to China, with a flowering period that typically occurs in winter (October to January), making it vulnerable to low-temperature stress during critical reproductive stages. However, the molecular mechanisms underlying cold tolerance in loquat remain largely unclear. In this study, transcriptome data from multiple loquat cultivars were analyzed using Weighted Gene Co-expression Network Analysis (WGCNA), identifying two gene modules (brown and turquoise modules) highly associated with cold treatment. Among the cold-responsive candidates, the Rab5 family GTPase *EjRabF2b* was consistently upregulated under low-temperature conditions. Functional validation revealed that overexpression of *EjRabF2b* in *Arabidopsis thaliana* and tomato significantly enhanced cold tolerance, while its silencing in loquat compromised stress resistance. Mechanistically, *EjRabF2b* contributed to maintaining cell membrane integrity and enhancing antioxidant enzyme activity. Promoter analysis and interaction assays further confirmed that the C2H2-type transcription factor EjZAT10 directly binds to the promoter of *EjRabF2b* and activates its transcription under cold stress. Collectively, this study uncovers a regulatory module composed of EjZAT10 and *EjRabF2b* that participates in loquat cold adaptation through vesicle-mediated antioxidant defense and membrane protection, offering a theoretical foundation and potential targets for the molecular breeding of cold-tolerant cultivars.

Keywords: loquat, cold tolerance, *EjRabF2b*, transcription factors, *EjZAT10*

1. Introduction

Loquat (*Eriobotrya japonica*) is a perennial woody fruit tree belonging to the Rosaceae family and is predominantly cultivated in subtropical regions. Originating in China, loquat has been cultivated for over 2,000 years and is now grown commercially in more than 30 countries worldwide, including Japan, the United States, France, Italy, Egypt, and Spain (Lin *et al.* 2010). In China, central and southern regions serve as the major production areas (Ali *et al.* 2021). However, in recent years, these areas have increasingly experienced extreme cold events (Qian and Zhang 2012; Cao *et al.* 2022; Wang *et al.* 2017). Loquat is inherently sensitive to low temperatures (Cai *et al.* 2006), and its flowering period, which typically spans from October and January, coincides with

Received 7 April 2025; Received in revised form 1 August 2025; Accepted 15 September 2025; Available online 31 October 2025
Hao Fu, E-mail: fh1994@126.com; #Correspondence Guolu Liang, E-mail: lianggl@swu.edu.cn; Qigao Guo, E-mail: qgguo@126.com

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2025.10.018

the coldest months (Jiang *et al.* 2019). As a result, flower and young fruit development are particularly vulnerable to frost, often leading to substantial yield losses (Guillamón *et al.* 2020; Qin *et al.* 2023). Therefore, enhancing the cold tolerance of loquat has become a pressing priority in breeding and genetic research (Zhang *et al.* 2022).

Low-temperature stress primarily affects the stability of plant cell membranes, which are considered the main targets of cold-induced injury (Hoffman *et al.* 2010; Wang *et al.* 2025). Low temperatures reduce membrane fluidity and impair membrane protein function, thereby disrupting material transport and metabolic processes (Palta *et al.* 1993; Hou *et al.* 2016). Rab GTPases are molecular switches that alternate between GTP-bound active and GDP-bound inactive states, and they play crucial roles in vesicle trafficking processes, including docking, fusion, and fission. These functions are critical for plant growth and responses to environmental stresses (Liao *et al.* 2014; Buono *et al.* 2017). In *Arabidopsis thaliana*, 57 Rab GTPases are classified into eight subfamilies: RabA-H (Rutherford and Moore 2002). Increasing evidence indicates that Rab GTPases are key regulators of abiotic stress tolerance across various species (Fu *et al.* 2024). For instance, Rab7 and Rab5 are upregulated in *Pennisetum glaucum* and mango under cold stress. Transgenic tobacco plants expressing *PgRab7* exhibit enhanced tolerance to salt and drought, likely through improved vesicle trafficking and protection of the endomembrane system (Nahm *et al.* 2003; Agarwal *et al.* 2008; Liu *et al.* 2014). Conversely, *OsRab11C1* negatively regulates cold tolerance in rice, as its knockout lines show improved resistance compared to overexpressing lines (Wang *et al.* 2021). These findings suggests functional divergence among Rab subfamilies. However, the involvement of Rab GTPases in loquat under cold stress remains uncharacterized.

The transcription factor ZINC FINGER OF *ARABIDOPSIS* THALIANA 10 (ZAT10), a member of the C2H2-type zinc finger protein family, was originally identified in *A. thaliana* (Lippuner *et al.* 1996; Catalá *et al.* 2011; Park *et al.* 2015). ZAT10 expression is rapidly induced by various abiotic stressors, including drought, cold, salt, UV-B radiation, oxidative stress, and high light intensity (He *et al.* 2019; Min *et al.* 2020; Wang *et al.* 2022). Overexpression of ZAT10 in transgenic *A. thaliana*, rice, and poplar significantly enhances plant tolerance to multiple abiotic stresses (Rossel *et al.* 2007; Xiao *et al.* 2009; Park *et al.* 2015; He *et al.* 2019). Notably, ZAT10 functions as a cold-inducible regulator that activates COR (cold-responsive) genes during low-temperature conditions (Liu *et al.* 2022). In apple, overexpression of the *MhZAT10* also improves cold tolerance (Li *et al.* 2023). Moreover, ZAT10 is involved in a CBF-independent signaling pathway acting downstream of MAPKs and upstream of reactive oxygen species (ROS)-scavenging enzymes such as ascorbate peroxidases (APX) and catalases (CAT) (Shi *et al.* 2018; Gong *et al.* 2020), highlighting its central role in abiotic stress responses. Despite these findings, the regulatory role of ZAT10 in loquat and its downstream targets under cold stress remain unexplored. Although Rab small GTPases like *EjRabF2b* are known to be involved in stress adaptation (Fu *et al.* 2024), their transcriptional regulation by ZAT10 has not yet been established.

In this study, we performed a comparative transcriptomic analysis of the loquat before and after low-temperature treatment and identified a cold-responsive Rab small GTPase gene, *EjRabF2b*. We validated its critical role in cold stress response in *A. thaliana* and loquat, and further confirmed its function in Micro-Tom tomato and yeast systems. In addition, we found that the transcription factor EjZAT10 directly binds to the promoter of *EjRabF2b* and activates its expression in response to cold stress. These findings suggest that the EjZAT10–*EjRabF2b* module participates in redox regulation and membrane stabilization, contributing to cold tolerance in loquat.

2. Materials and methods

2.1. Plant materials, growth condition and cold treatments

Arabidopsis thaliana lines used in this study included the wild-type Col-0, the *rabf2b* mutant (SALK_090266C), and *EjRabF2b*-overexpressing (OE) transgenic lines in both Col-0 and *rabf2b* backgrounds. For Micro-Tom tomato, both wild-type and *EjRabF2b*-OE lines were included. In loquat, wild-type and virus-induced gene silencing (VIGS) lines targeting *EjRabF2b* were used. All plants were cultivated under controlled conditions at 23°C–25°C with a 16 h light/8 h dark photoperiod. Cold treatment protocols were designed according to the species-specific cold tolerance and experimental objectives. Specifically, 4-week-old *A. thaliana* seedlings were treated at 1°C for 7 d; Micro-Tom plants were exposed to 4°C for 72 h; and loquat samples were subjected to –3°C for 48 h. Phenotypic changes were recorded after each cold treatment.

2.2. Construction of co-expression network and identification of key genes

Transcriptome data used in this study were obtained from Liu (2021). A total 18 samples for WGCNA analysis were collected from hybrid seedlings of three cultivars, GZ23×B431 (triploid), GZ23 (diploid), and B431 (tetraploid), following cold treatment at –3°C for 72 h; untreated plants maintained at 25°C served as controls. Each group included three biological replicates.

Gene co-expression networks were constructed using the WGCNA package (v1.63) in R (Langfelder and Horvath 2008). Based on expression quartiles and filtering criteria, 19,877 genes with first quartile (Q1) expression and FPKM values greater than three were selected. The blockwiseModules function was used to construct the co-expression network with the following parameters: minModuleSize=200, power=10 (correlation coefficient threshold=0.8), TOMType="unsigned", mergeCutHeight=0.25 (i.e., modules within eigengenes correlation>0.75 were merged), verbose=3, and maxBlockSize=20,000. As a result, 19,877 genes were clustered into nine co-expression modules, with 103 outliers genes assigned to the grey module. Module Eigengenes were calculated and correlated with cold-responsive phenotypic traits. Hub genes were defined as those with gene significance (GS)>0.96, high module membership (MM),

and a weighted correlation index >0.93, indicating strong association with cold treatment. Functional annotation of key genes was conducted using Gene Ontology (GO) (http://www.geneontology.org), and pathway enrichment analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa and Goto 2000).

2.3. *EjRabF2b* isolation and sequence analysis

The coding sequence (CDS) of *Eja14G002720* was retrieved from the Loquat Coding Sequence Database (https://ngdc.cncb.ac.cn/gwh/). Gene-specific primers was designed based on this sequences were identified using the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/; accessed on 10 February 2022). Sequence alignments between *EjRabF2b* and its homologs in *Pyrus×communis* (*RabF2b*, KAB2637060.1) and *A. thaliana* (*AtRabF2b*, NP_193699.1) were performed using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). Phylogenetic analysis was conducted using MEGA11 (Kumar *et al.* 2016). Total RNA was extracted from loquat and *A. thaliana* tissues using the Plant Total RNA Purification Kit (Tiangen, Beijing, China). First-strand cDNA was synthesized with a reverse transcription kit that includes genomic DNA removal (TaKaRa, Japan). Quantitative real-time PCR (qRT-PCR) was conducted using Novostar SYBR Supermix (Novoprotein, Suzhou, China). *EjACTIN*, *AtACTIN* and *SlACTIN* served as internal reference genes. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), with all reactions performed in triplicate. The primer sequences are listed in the Appendix A.

2.4. Subcellular localization analysis

The full-length *EjRabF2b* coding sequence was fused in-frame with enhanced green fluorescent protein (eGFP) and inserted into the pCAMBIA2300 vector under the control of the CaMV35S RNA promoter. The recombinant plasmid was introduced into *A. tumefaciens* GV3101 *via* transformation. The bacterial cultures were centrifuged and resuspended in infiltration buffer, then infiltrated into *Nicotiana benthamiana* leaves using a needleless syringe (Sparkes *et al.* 2006). After infiltration, the plants were incubated in the dark at 22–24°C for 48 h in a greenhouse. eGFP fluorescence signals were observed using a confocal laser scanning microscope (LSM780, Zeiss, Germany).

2.5. *Arabidopsis thaliana* and Micro-Tom transformation

Transgenic *A. thaliana* plants were generated using the floral dip method (Clough and Bent 1998) with wild-type Col-0 and *rabf2b* mutant lines serving as recipient plants to generate the T_0 transgenic plants. The recombinant plasmid pCAMBIA2300 containing *the EjRabF2b* gene was introduced into Micro-Tom tomato plants *via A. tumefaciens* strain GV3101, according to the protocol described by Sun *et al.* (2006). Transgenic lines were generated by qPCR to confirm gene expression levels, and details of line identification are provided in Appendix B. Homozygous T_3 generation seeds were used for all subsequent experiments.

2.6. Fluorescence staining of transgenic *A. thaliana* roots

Plasma membrane (PM) integrity was assessed as described by (Schapire *et al.* 2008). Five-day-old wild-type Col-0 and transgenic *A. thaliana* seedlings grown under standard conditions were subjected to cold at 4°C for 12 h. Immediately after treatment, the seedlings were incubated with 5 μmol L^{-1} FM4-64 (Life Technologies, Carlsbad, USA), a lipophilic dye that labels the outer leaflet of the PM, for 5 min. Root samples were imaged using a confocal laser scanning microscope (LSM780, Zeiss, Germany) and compared to control seedlings.

2.7. Transient expression system in loquat

Virus-induced gene silencing (VIGS) in loquat was performed *via* vacuum infiltration, following the method described by (Xu *et al.* 2023). After infiltration, the treated loquat seedlings were transferred to sterilized soil and grown under controlled conditions for subsequent phenotypic and molecular analyses. Gene silencing efficiency was assessed by qRT-PCR, and transgenic identification details are provided in Appendix B.

2.8. *EjRabF2b* expression in yeast

The *EjRabF2b* gene was cloned into the pYES2 empty vector, which contains a URA3 selection marker under the control of the GAL1 promoter. The resulting recombinant plasmids, pYES2-NBT-*EjRabF2b* and the empty vector pYES2-NBT, were introduced into *Saccharomyces cerevisiae* strain BY4741 using the lithium acetate transformation method. Transformed cells were plated on SD/–Ura dropout medium and incubated at 30°C for 2–4 d to select for positive colonies. Confirmed transformants were resuspended in 2 mL of sterile water and subjected to cold stress treatments at 4°C, 0°C, –20°C, and –40°C for 12, 24, 36, and 48 h, respectively. Following treatment, the yeast suspensions were adjusted to an OD_{600} of 0.2, serially diluted from 10^0 to 10^{-4}, and spotted onto SG-Ura plates. Plates were incubated at 30°C for 3–4 d, and colony growth was recorded and photographed for comparative analysis.

2.9. Antioxidant enzymatic assay

Leaf tissue was ground into a fine powder under liquid nitrogen, and 200 mg of the powdered material was homogenized in 1.6 mL of ice-cold phosphate-buffered saline (PBS, 50 mmol L^{-1}, pH 7.8). The homogenate was centrifuged at 10,000×g for 15 min at 4°C, and the resulting supernatant was collected for subsequent antioxidant enzyme activity assays. Superoxide dismutase (SOD) activity was determined by measuring its inhibition of nitroblue tetrazolium (NBT) photoreduction, with absorbance recorded at 550 nm (Beauchamp and Fridovich 1971). Ascorbate peroxidase (APX) activity was measured following the method of Nakano and Asada (1981), with minor modifications. Peroxidase (POD) activity was assayed according to the guaiacol method as described by Muñoz-Muñoz *et al.* (2009).

2.10. Dual-luciferase assay

In the Dual-Luciferase assay, the full-length promoter of

EjRabF2b (2,252 bp; Appendix C) was cloned into the pGreen0800-Luc vector. The effector construct 35S:*EjZAT10* was introduced into *A. tumefaciens* strain GV3101. Equal volumes (1:1, v/v) of *A. tumefaciens* cultures harboring the reporter and effector plasmids were co-infiltrated into leaves of 4-week-old *N. benthamiana* plants. After incubation at 23°C for 2–3 d, luciferase activities were measured using a Dual-Luciferase Assay Kit (TransGen, Beijing, China). The ratio of firefly luciferase (LUC) to Renilla luciferase (REN) was quantified with a chemiluminescence imaging system (Clinx, Shanghai, China) to evaluate promoter activity.

2.11. Yeast-one-hybrid (Y1H) assays

The Y1H assay was performed using the Matchmaker Gold Y1H System (Clontech) according to the manufacturer's protocol. Potential *ZAT10* binding sites in the *EjRabF2b* promoter were predicted using the JASPAR database (http://www.jaspar.genereg.net), with a specific focus on the sequence CATTCCTACTC. An 86-bp fragment containing this motif was amplified and designated as Pro*EjRabF2b*. This fragment was digested with BstBI and cloned into the pAbAi bait vector, which was then transformed into the Y1HGold yeast strain and selected on SD/–Ura medium. Prey vectors were constructed by cloning *EjZAT10* fused with GAL4 activation domains into the pGADT7 vector. The resulting prey plasmid was co-transformed with the bait vector into the Y1HGold strain. Serial dilutions of transformants (OD_{600}=0.2, 0.02, 0.002, 0.0002) were spotted on SD/–Leu and SD/–Leu/AbA (200 ng mL^{-1} AbA) plates and incubated at 30°C for 3–4 d to assess protein–DNA interactions.

2.12. Electrophoretic mobility shift assay

The coding sequence of *EjZAT10* was cloned into the pGEX-4T-3 vector and transformed into *E. coli* strain BL21 (DE3). Recombinant protein expression was induced with 1 mmol L^{-1} IPTG at 16°C for 20 h, and the GST-tagged protein was subsequently purified using a GST Protein Purification Kit (Promega, Shanghai, China). Primer sequences used for cloning are listed in Appendix A. For the EMSA, biotin-labeled oligonucleotide probes were synthesized by Tsingke (Tsingke, Beijing, China) annealed to form double-stranded DNA (dsDNA) probes. The electrophoretic mobility shift assay was conducted using the LightShift Chemiluminescent EMSA Kit (Thermo, Waltham, USA). To verify the specificity of protein–DNA binding, unlabeled competitor probes (50× and 100× excess) and mutant probes were included in the binding reactions.

2.13. Determination of electrolyte leakage and petiole angle

To assess membrane integrity under low-temperature stress, 0.2 g of leaf tissue was immersed in 20 mL of distilled water and incubated overnight at room temperature. The initial conductivity (E_1) was measured using a DDS-309 conductivity meter (Chengdu Century Ark Technology Co. Ltd., Chengdu, China). Subsequently, the samples were boiled for 30 min, cooled to room temperature, and the final conductivity (E_2) was measured. Relative electrolyte leakage was calculated as (E_1/E_2)×100%. For petiole angle measurements, the angle between the main stem and the petiole was determined using a standard protractor.

2.14. Statistical analysis

A Shapiro-Wilk test was used to assess data normality. For non-normally distributed data, a $\log_{10}$ transformation was applied to analysis. One-way analysis of variance (ANOVA), followed by Tukey's HSD post hoc test (P<0.05) where appropriate, was used to assess treatment effects on *EjRabF2b* expression and antioxidant enzyme activities. Independent sample *t*-tests and ANOVA (P<0.05) were employed to compare LUC/REN activity. All statistical analyses and data visualization were performed using GraphPad Prism v10.0.

3. Results

3.1. Construction of weighted gene co-expression network and identification of key genes from brown and turquoise modules

In recent years, our team has made substantial progress in elucidating cold tolerance mechanisms in loquat and has acquired valuable transcriptomic datasets (Liu 2021). Based on this resource, we conducted weighted gene co-expression network analysis (WGCNA). Hierarchical clustering of 18 samples (Appendix D) revealed two distinct groups, indicating that cold treatment markedly altered global gene expression patterns. A soft-thresholding power of 10 was determined based on a correlation coefficient threshold of 0.78 (Appendix D), and was subsequently applied to construct the co-expression network.

Dynamic tree cutting and module merging identified nine distinct gene modules. Among them, the brown (r^2=0.95, P=1e–9) and turquoise (r^2=–0.97, P=7e–11) modules showed the strongest correlations with cold treatment (Fig. 1-A), suggesting that genes within these modules are highly responsive to cold stress. These modules comprised 1,286 and 2,866 genes, respectively. The expression heatmaps (Fig. 1-B) and scatterplots of module membership (MM) versus gene significance (GS) (Fig. 1-C) further support their association with cold stress response.

To identify hub genes, we selected 386 genes from the brown and turquoise modules, each with MM>0.93 and GS>0.96. GO enrichment analysis (Fig. 1-D) and KEGG pathway enrichment (Appendix E) indicated significant involvement of these genes in vesicle-mediated transport and endocytosis. Among these, *EjRabF2b* was prioritized due to its high MM and GS values and its annotation as a Rab small GTPase involved in intracellular vesicle trafficking.

Previous studies have shown that *RabF2b* plays essential roles in endomembrane transport and plant responses to abiotic stresses (Fu *et al.* 2024). However, its function under cold stress remains unexplored, especially in loquat. Based on this hypothesis, *EjRabF2b* was considered a central regulator in cold-responsive signaling pathways and was consequently selected as the primary candidate gene for further functional validation.

3.2. Phylogenetic analyses and expression characteristics of the *EjRabF2b* gene

We conducted an in-depth investigation of the gene EVM0027287, which was identified as a putative homolog of *AtRabF2b* based on a SwissProt database search. Specifically, it matched entry sp|Q9SN68|RAF2B_ARATH, corresponding to the Ras-associated protein in *A. thaliana*. Notably, phylogenetic analysis using the NCBI database revealed that the closest homologs of *EjRabF2b* were found in *Pyrus ussuriensis*×*Pyrus communis* (KAB2637060.1), *Malus domestica* (XP 008371839.1) and *Pyrus*×*bretschneideri* (XP 009341201.2) (Fig. 2-A). Multiple sequence alignment showed high conservation of amino acid residues among *EjRabF2b* and its homologs, especially within the RAB GTPase domain (Fig. 2-B). Expression analysis revealed that *EjRabF2b* was upregulated under cold treatment in all three genetic backgrounds: the hybrid line GZ23×B431 (triploid), and its parental lines GZ23 (diploid) and B431 (tetraploid) (Fig. 2-C). Subcellular localization analysis further demonstrated that the *EjRabF2b*-eGFP fusion protein colocalized with nuclear and endoplasmic reticulum markers in *Nicotiana benthamiana* leaves, indicating its localization to both compartments (Fig. 2-D).

3.3. *EjRabF2b* regulation of antioxidant enzyme activity affects cold tolerance in *A. thaliana* and loquat

To elucidate the function of *EjRabF2b* under cold stress, we cloned the *EjRabF2b* cDNA and transformed it into both wild-type *A. thaliana* Col-0 and *rabf2b* mutant backgrounds. Three independent overexpression lines (OE-2, OE-10, and OE-12) in the Col-0 background and three transgenic lines (RT-2, RT-5, and RT-8) in the *rabf2b* mutant background were selected for further analysis. Compared with Col-0 plants, the OE lines exhibited significantly enhanced cold tolerance (Fig. 3-A), showing less wilting and dehydration symptoms and maintaining healthy leaves under stress conditions. Enzymatic assays indicated significantly increased activities of APX, SOD, and POD in OE lines relative to Col-0 (Fig. 3-B).

In contrast, *rabf2b* mutants exhibited pronounced cold sensitivity, including severe leaf curling, wilting, and necrosis after 7 d at 1°C. Partial recovery of cold tolerance was observed in the RT lines, as indicated by milder symptoms and improved antioxidant enzyme activity compared to the *rabf2b* mutants, although still less than in OE lines. These results suggest that *EjRabF2b* enhances cold tolerance in *A. thaliana*, likely through upregulation of ROS-scavenging enzyme activities.

The integrity of the PM was evaluated using FM4-64, a lipophilic fluorescent dye that labels intracellular membranes upon PM disruption (Dhonukshe *et al.* 2006; Schapire *et al.* 2008). At 23°C, root cells of both transgenic and wild-type seedlings showed intact membrane outlines, with FM4-64 localized to the cell surface (Fig. 3-C, 23°C). After cold stress at 4°C, strong FM4-64 staining was observed in the root elongation zones and tips of *rabf2b* mutants and RT lines, indicating PM damage. However, RT lines showed milder staining, with cells in RT-5 partially excluding the dye, suggesting partial restoration of *EjRabF2b* function. In Col-0, about half of the root cells were damaged, while in *EjRabF2b*-OE lines, significantly fewer cells were stained, indicating enhanced PM integrity under cold stress.

To further verify the function of *EjRabF2b* in cold tolerance, we employed VIGS to downregulate its expression in loquat. As shown in Fig. 3-D, *EjRabF2b* expression was significantly reduced in silenced lines, which displayed more severe cold-induced damage compared to WT and TRV::00 plants. Symptoms included pronounced leaf dehydration and curling, accompanied by a 20–30% increase in electrolyte leakage.

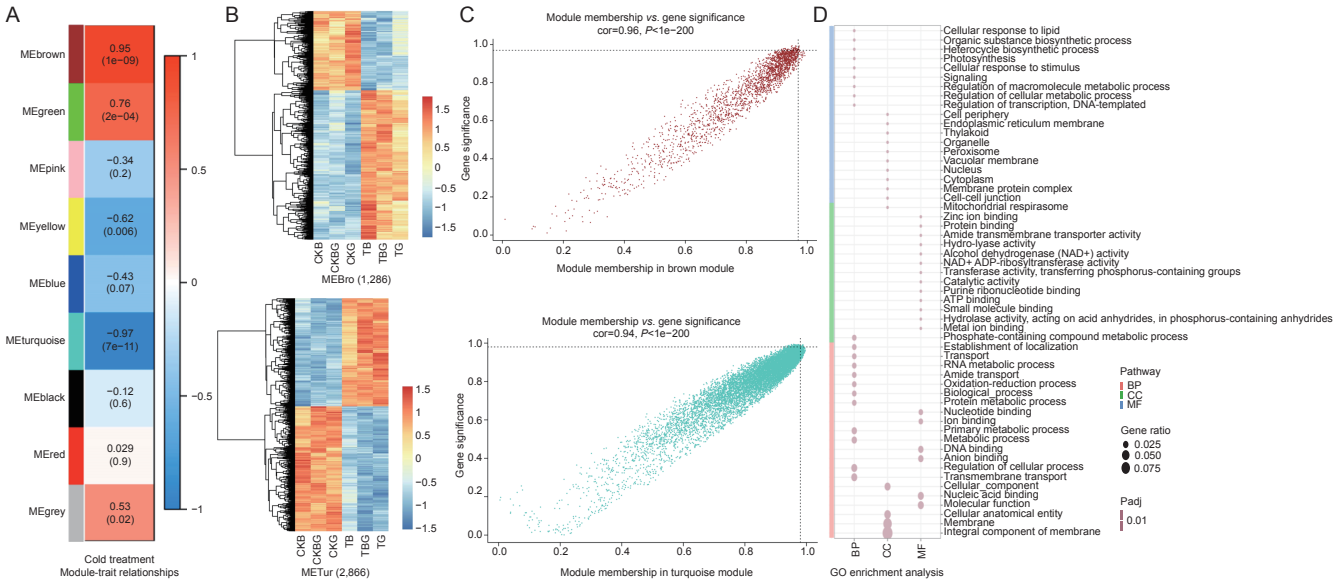


Fig. 1 WGCNA analysis of loquat low-temperature transcriptome. A, correlation analysis between module and cold treatment. Red represents positive correlation, while blue represents a negative correlation, the intensity of the color reflects the strength of the correlation. B, heatmap of gene expression for highly correlated modules along with the number of genes in each module. C, scatterplot showing GS for cold treatment versus MM in the brown and turquoise modules, respectively (each dot represents a gene). Black dotted lines are drawn at GS=0.96 and MM=0.93. D, GO enrichment analysis of genes highly correlated with the brown and turquoise modules.

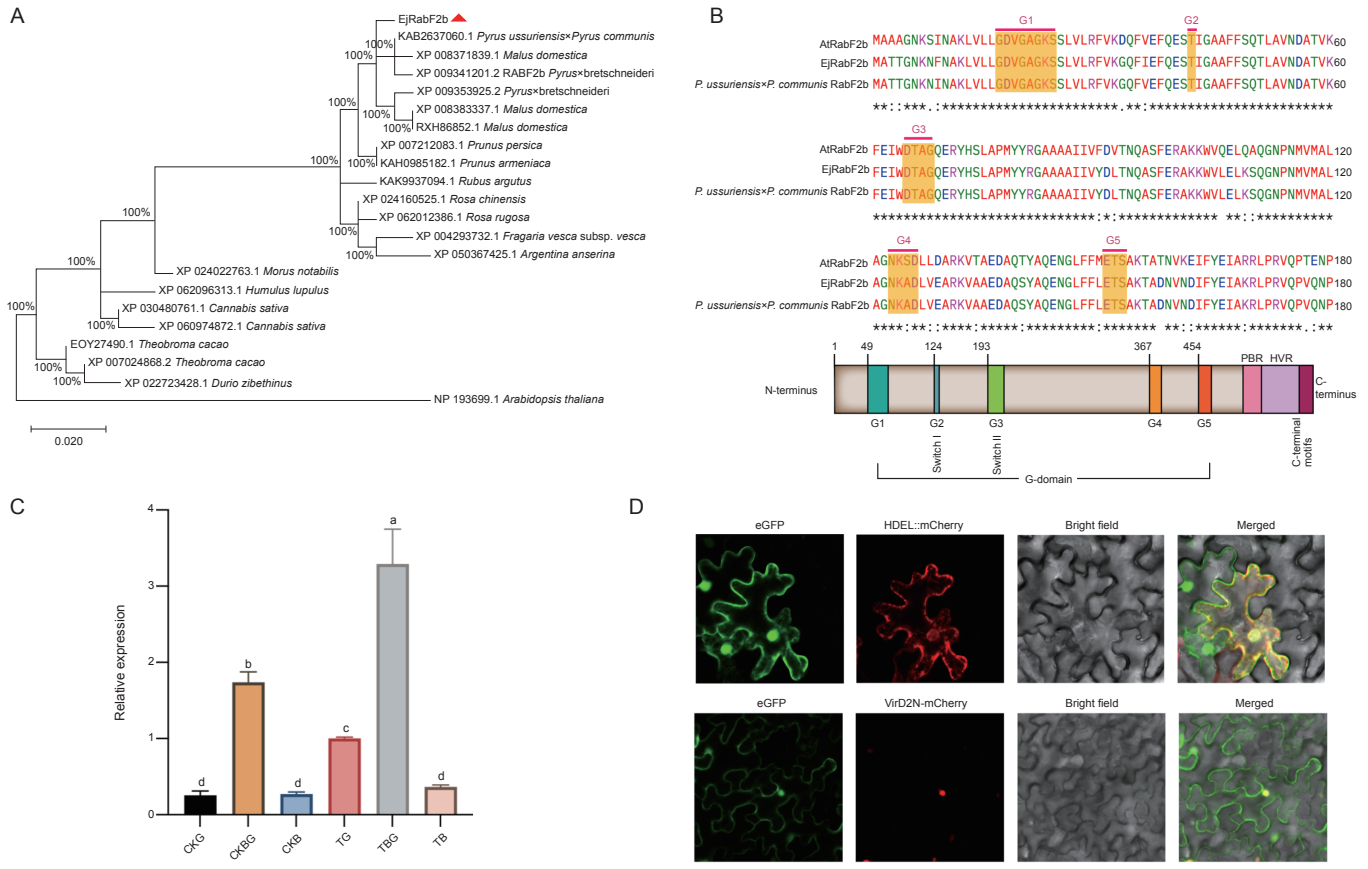


Fig. 2 Phylogenetic analyses and expression characteristics of *EjRabF2b*. A, schematic representation of the domain structure of *EjRabF2b*. B, sequence comparison of *EjRabF2b* with *AtRabF2b* (NP_193699.1) and RabF2b *of Pyrus ussuriensis×Pyrus communis, Pyrus ussuriensis×Pyrus communis* (KAB2637060.1). C, expression profiles of *EjRabF2b* in GZ23×B431 (triploid), GZ23 (diploid), and B431 (tetraploid). Error bars represent the standard deviation of three biological replicates (*n*=3). Different lowercase letters above the bars indicate significant differences among planting patterns with $P<0.05$. D, subcellular localization of *EjRabF2b* in *Nicotiana benthamiana* epidermal cells. eGFP, enhanced green fluorescent protein; HDEL::mCherry serves as an endoplasmic reticulum marker, while the NLS domain of VirD2 fused with mCherry functions as a nuclear marker.

Quantitative measurements revealed a 50–60% greater leaf angle deflection in silenced lines, indicative of turgor loss and structural collapse. Antioxidant enzyme assays showed significant reductions in APX and POD activities compared with controls, suggesting impaired ROS scavenging capacity. These results indicate that silencing *EjRabF2b* disrupts redox homeostasis under cold stress, making plants more vulnerable to oxidative damage and cryoinjury (Fig. 3-E). Collectively, these findings demonstrate that *EjRabF2b* enhances cold tolerance by promoting antioxidant enzyme activity and maintaining PM integrity, thereby mitigating cellular damage during chilling stress.

3.4. *EjRabF2b* enhances cold stress tolerance in Micro-Tom tomato and yeast

To further evaluate the role of *EjRabF2b* in cold stress response, we introduced the gene into Micro-Tom tomato plants and *Saccharomyces cerevisiae* strain BY4741 for functional validation. Under optimal conditions (23°C), both WT and OE plants exhibited normal growth with no phenotypic differences. However, after 72 h of 4°C treatment, all lines exhibited varying degrees of cold-induced injury. WT plants showed severe symptoms, including pronounced leaf dehydration, curling, and desiccation. OE-10 plants showed moderate damage, while OE-8 plants displayed minimal symptoms, with only slight leaf edge dehydration (Fig. 4-A).

Electrolyte leakage analysis revealed significantly higher ion leakage in WT plants, indicating substantial membrane damage. In contrast, OE-8 and OE-10 lines showed markedly lower leakage, suggesting improved membrane stability under chilling stress. Consistently, antioxidant enzyme assays demonstrated significantly elevated POD and APX activities in the OE lines compared to WT (Fig. 4-B), indicating enhanced ROS-scavenging capacity conferred by *EjRabF2b*.

Additionally, yeast spot assays demonstrated that yeast cells expressing *EjRabF2b* exhibited enhanced survival across all tested cold stress condition compared to the control group, as evidenced by more robust colony growth. Notably, the growth advantage was particularly evident under extreme cold stress conditions (–40 and –20°C) (Fig. 4-C), with more robust colony formation compared to vector controls.

Collectively, these results demonstrate that *EjRabF2b* overexpression enhances cold tolerance in both Micro-Tom tomato and yeast, likely through improved antioxidant

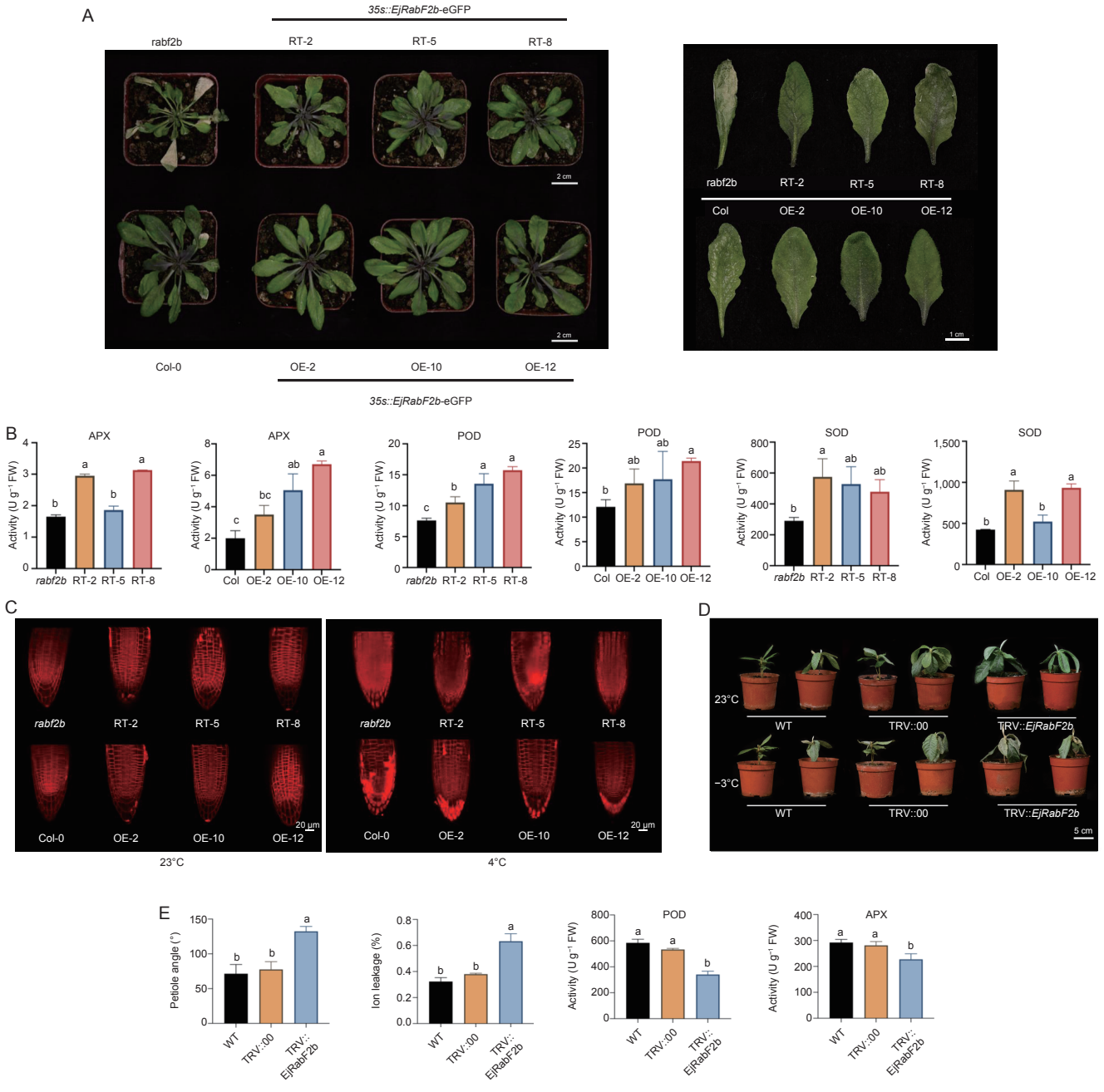


Fig. 3 Identification and phenotypes of *EjRabF2b* transgenic in *Arabidopsis thaliana* and loquat. A, the growth situation of *Arabidopsis thaliana* plants at 1°C for 7 d. Scale bars=2 or 1 cm. B, the activities of ascorbate peroxidase (APX), guaiacol peroxidase (POD) and superoxide dismutase (SOD) of *A. thaliana*. Error bars represent the standard deviation of three biological replicates (*n*=3). Different lowercase letters above the bars indicate significant differences among planting patterns with $P<0.05$. C, plasma membrane integrity in transgenic and wild-type seedlings under cold stress. Representative root images are shown. Scale bar=20 μm. D, the performance of the silenced loquat strain after 48 h at –3°C. Scale bars=5 cm. E, petiole angle, ion leakage, and the activities of POD and APX in loquat seedling leaves after 48 h of stress treatment. Error bars represent the standard deviation of three biological replicates (*n*=3). Different lowercase letters above the bars indicate significant differences among treatments with $P<0.05$.

defense and membrane stability. There finding are consistent with observations in loquat, further supporting a conserved role of *EjRabF2b* in cold stress adaptation across species.

3.5. EjZAT10 directly binds to the promoter of *EjRabF2b* and activates its transcription

To identify transcription factors regulating *EjRabF2b*, we cloned its promoter and analyzed it using JASPAR (https://jaspar.elixir.no). A putative binding site for EjZAT10 was predicted within the promoter region, located between –1,597 and –1,586 bp upstream of the transcription start site (Fig. 5-A and B). This 86-bp region was designated as Pro*EjRabF2b*. Transcriptome and qPCR analysis (Appendix F) revealed that *EjZAT10* expression significant upregulated under cold stress in loquat.

To validate the interaction between EjZAT10 and the promoter of *EjRabF2b*, a yeast one-hybrid (Y1H) assay

was conducted. All yeast strains grew well on SD/–Leu medium, and growth on selective SD/–Leu medium containing aureobasidin A (AbA, 200 ng mL^{-1}) confirmed specific protein–DNA binding. As expected, the positive control showed robust colony formation, validating system functionality. Yeast co-transformed with Pro*EjRabF2b*-AbAi+pGADT7-*EjZAT10* also grew well on AbA-containing medium, confirming that *EjZAT10* directly binds to the *EjRabF2b* promoter. No growth was observed in the negative control, supporting the specificity of this interaction (Fig. 5-C).

Electrophoretic mobility shift assays (EMSA) were further performed using purified GST-tagged *EjZAT10* protein and both wild-type and mutant DNA probes. The absence of the *EjZAT10*-GST protein, no band shift occurred. A clear shift was observed upon addition of *EjZAT10*-GST protein to the wild-type probe, confirming binding. This shift was reduced in a dose-dependent manner upon competition with 50- and 100-fold excess unlabeled wild-type probe. In contrast, the mutant probe failed to compete for binding, indicating that *EjZAT10* specifically recognizes the wild-type sequence (Fig. 5-D).

Meanwhile, a dual-luciferase reporter assay was performed to assess the transcriptional activation potential

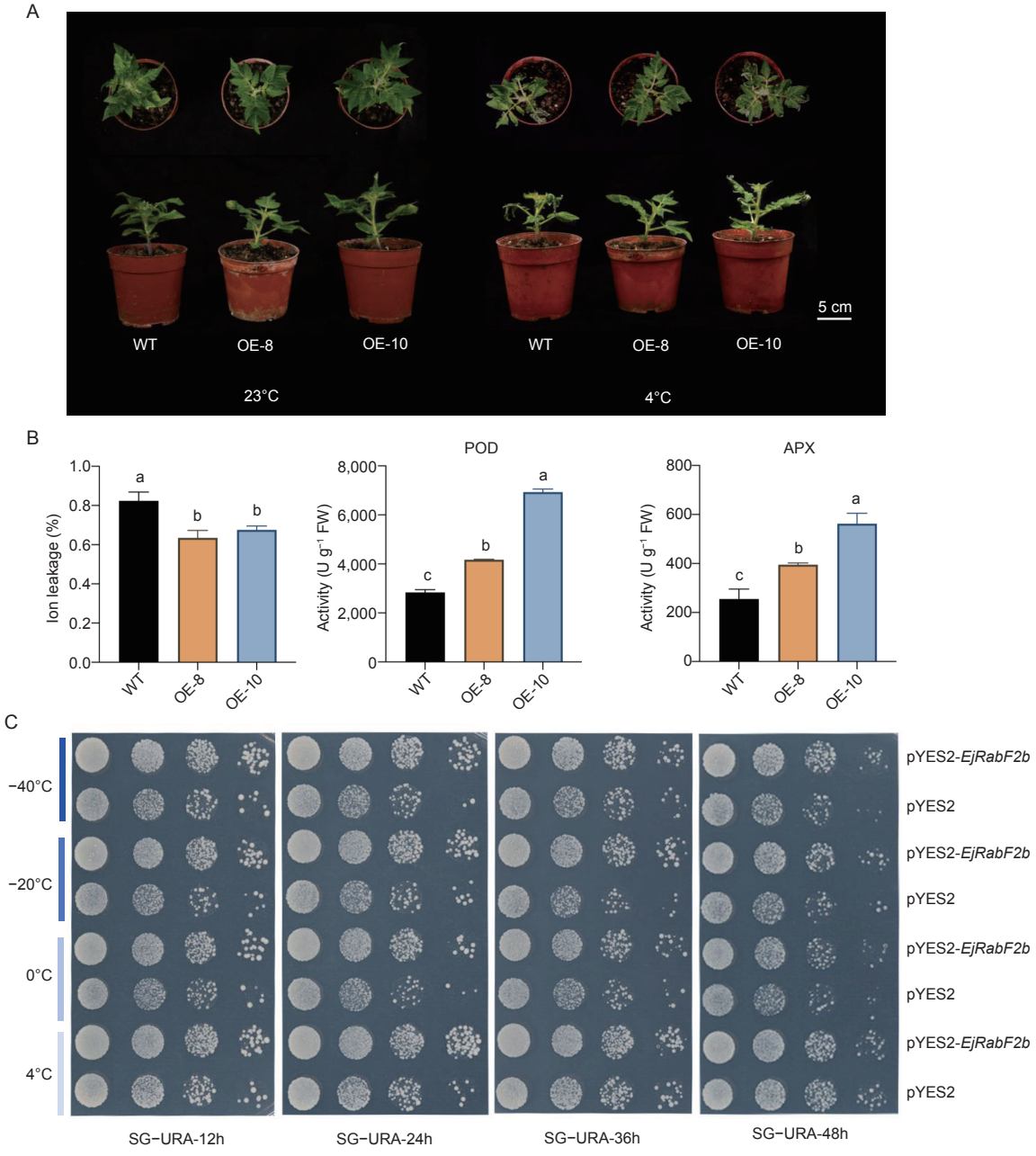


Fig. 4 Growth performance of transformed Micro-Tom and yeast under cold stress. A, phenotypic comparison of WT, OE-8, and OE-10 Micro-Tom plants under normal (23°C) and cold stress (4°C for 72 h) conditions. Scale bar=5 cm. B, quantification of ion leakage, peroxidase (POD), and ascorbate peroxidase (APX) enzyme activities in WT, OE-8, and OE-10 plants. Error bars represent the standard deviation of three biological replicates (n=3). Different lowercase letters above the bars indicate significant differences among treatments with P<0.05. C, yeast grown with serial dilutions on SD–URA solid medium under –40°C, –20°C, 0°C, and 4°C stress for 12, 24, 36, 48 h.

of *EjZAT10*. Co-infiltration of *EjZAT10* with the *EjRabF2b* promoter–LUC construct (2,253 bp) into tobacco leaves resulted in a significant increase (~3-fold) in LUC/REN activity compared to the control (Fig. 5-E), indicating that EjZAT10 activates *EjRabF2b* transcription in plants.

4. Discussion

4.1. Conserved cold-responsive role of *EjRabF2b* and its potential involvement in vesicle-mediated stress adaptation

Through a systematic evaluation of cold tolerance across loquat germplasm with different ploidy levels and transcriptomic analysis using WGCNA. We identified two gene modules strongly correlated with cold treatment. Among the 386 candidate genes derived from these modules, *EjRabF2b* was prioritized due to its marked upregulation in response to cold stress, suggesting its potential role in cold tolerance in loquat. As a member of the Rab5 family, *EjRabF2b* is involved in vesicle trafficking and endocytosis, processes that are crucial for maintaining membrane integrity and mediating stress-responsive signal transduction.

Although functional studies of Rab5-related genes in

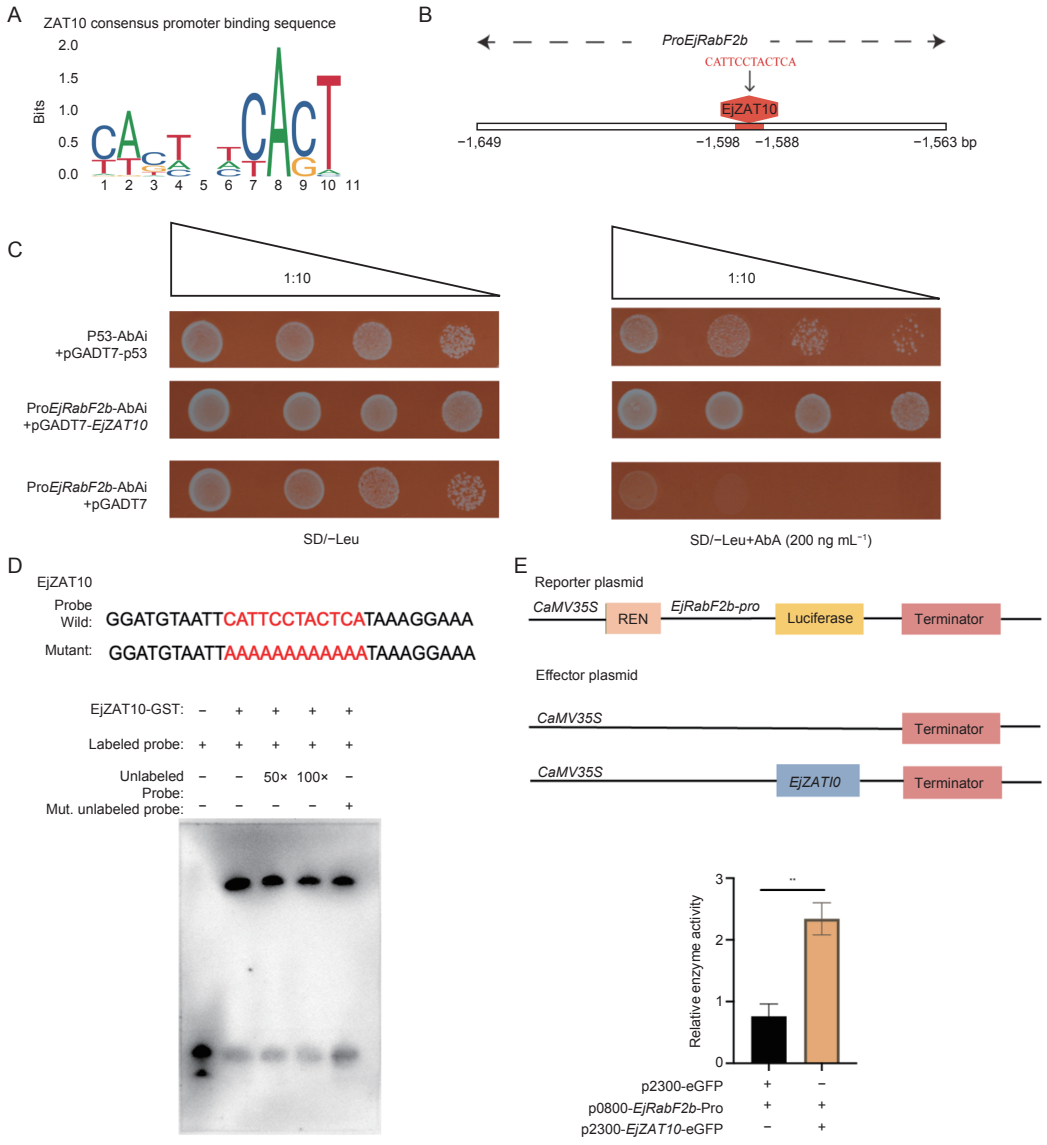


Fig. 5 *EjZAT10* directly binds to the promoter region of *EjRabF2b* and activates the transcriptional activity of the *EjRabF2b* promoter. A, consensus DNA-binding sequence for *EjZAT10* identified from the JASPAR database. B, *EjZAT10* binding sites on the Pro*EjRabF2b* promoter (–1,586 to –1,597 bp). C, yeast one-hybrid assay showing *EjZAT10* binding to the *EjRabF2b* promoter. Aureobasidin A (AbA, 200 ng mL^{-1}) was used as a screening marker. P53-AbAi+pGADT7-p53 served as a positive control, while Pro*EjRabF2b*-AbAi+pGADT7 served as a negative control. D, electrophoretic mobility shift assay (EMSA) showing the specific binding of *EjZAT10* to the *EjRabF2b* promoter probe. Purified *EjZAT10*-GST protein was incubated with biotin-labeled, unlabeled (cold), or mutant probes as indicated (+/−). The biotin-labeled probe carried a 3′ biotin tag, whereas the unlabeled and mutant competitors did not. Binding specificity was confirmed by the reduced signal intensity upon addition of excess unlabeled competitor (50× and 100×). E, dual-luciferase assay showing *EjZAT10* activation of the *EjRabF2b* promoter. Conducted in *Nicotiana benthamiana* leaves, LUC/REN activity measurements indicate promoter activation (2,252 bp). Error bars represent the standard deviation of three biological replicates (n=3). **, P<0.01.

Rosaceae species remain limited, emerging evidence suggests that vesicle trafficking-associated Rab GTPases play important roles in cold stress responses within this family. For example, in pear (*Pyrus bretschneideri*), *PbrRabGAP10* regulates pollen tube growth through vesicle-mediated cellulose deposition, indicating its potential involvement in stress adaptation (Kou *et al.* 2024). In apple (*Malus domestica*), Rab family members such as Rab11A and Rab18 are upregulated during cold storage, suggesting their participation in maintaining membrane stability under low-temperature conditions (Karagiannis *et al.* 2018). These findings underscore the conserved role of Rab GTPase-mediated vesicle dynamics in the cold tolerance mechanisms of Rosaceae fruit trees.

Beyond Rosaceae species, Rab5 proteins have also been implicated in cold and salt stress responses in other plants. For example, in *Arabidopsis*, the *Arabidopsis*-specific Rab5 protein ARA6 regulates endosomal membrane transport and supports stress adaptation by modulating vesicle trafficking (Yin *et al.* 2017). Similarly, Rab5 expression is upregulated under salinity and low-temperature stress in *Mesembryanthemum crystallinum* and *Mangifera indica* (Bolte *et al.* 2000; Liu *et al.* 2014). Consistently, in this study, *EjRabF2b* showed a conserved cold-responsive pattern across loquat cultivars with different ploidy levels, supporting the conserved role of Rab GTPases in abiotic stress tolerance (O'Mahony and Oliver 1999; Ebine *et al.* 2011; Yin *et al.* 2017; Zhang *et al.* 2023).

4.2. *EjRabF2b* regulates vesicle trafficking to enhance antioxidant defense and membrane integrity

Recent studies suggest a link between Rab proteins and regulation of antioxidant enzymes in plant responses to abiotic stresses. Rab-mediated vesicle transport supports membrane stability, which helps regulate ROS levels and preserve cellular function (Bolte *et al.* 2000; Liu *et al.* 2014). In particular, Rab5 and Rab7 have been shown to be upregulated under cold or salt stress, which correlates with enhanced activities of antioxidant enzymes such as SOD, APX, and POD, thereby mitigating oxidative damage (Yin *et al.* 2017; Zhang *et al.* 2022).

Our results suggests that *EjRabF2b* enhances cold tolerance in loquat by significantly promoting the activities of APX, SOD, and POD under low-temperature conditions (Fig. 3). These enzymes play crucial roles in scavenging ROS that accumulate during chilling stress and would otherwise compromise membrane integrity and disrupt cellular functions (Mu *et al.* 2021). Members of the Rab5 family have been shown to regulate membrane dynamics and vesicle recycling. Therefore, *EjRabF2b* is likely involved in modulating vesicle trafficking dynamics and endocytosis, thereby maintaining redox homeostasis under stress conditions (Bolte *et al.* 2000; Liu *et al.* 2014; Yin *et al.* 2017).

Maintenance of PM integrity is essential during cold stress, as it prevents excessive ion leakage and preserves signaling capacity (Zhou *et al.* 2021). In our study, overexpression of *EjRabF2b* in *A. thaliana* significantly reduced FM4-64 uptake in root tip cells under chilling conditions, indicating enhanced PM integrity and supporting a protective role of *EjRabF2b*. This suggests *EjRabF2b* may facilitate the vesicle-mediated delivery, recycling, or anchoring of antioxidant enzymes to specific subcellular sites, such as the PM or peroxisomes, enabling localized ROS detoxification. Alternatively, *EjRabF2b* may modulate antioxidant gene expression *via* retrograde signaling pathways triggered by vesicle or membrane stress (Kamal *et al.* 2020). Together, these findings support a model in which *EjRabF2b* integrates vesicle trafficking with redox regulation to enhance plant cold tolerance.

4.3. Cold-induced transcriptional regulation of *EjRabF2b* by EjZAT10 in loquat

C2H2 zinc finger proteins represent an important class of transcription factors that have been extensively characterized in *A. thaliana*. Numerous members of the C2H2 family, including *ZAT7*, *ZAT10*, and *ZAT12*, are known to play pivotal roles in plant responses to abiotic stresses (Kielbowicz-Matuk 2012; Liu *et al.* 2017; Li *et al.* 2023). Notably, *ZAT10* functions independently of the *ICE-CBF-COR* pathway and can induce the expression of *COR* genes, such as *COR15a*, *COR47*, and *KIN1* in response to cold, underscoring its crucial role in cold stress tolerance (Park *et al.* 2015; Nguyen *et al.* 2016; Liu *et al.* 2022).

In this study, we demonstrated that the transcription factor EjZAT10 not only responds to cold stress but also directly binds to the promoter of *EjRabF2b*, thereby enhancing its expression (Fig. 5-E). These findings reveal a previously uncharacterized regulatory mechanism contributing to cold tolerance in loquat.

Nevertheless, the broader physiological functions of EjZAT10 under cold stress remain to be fully elucidated. Furthermore, whether the EjZAT10–*EjRabF2b* regulatory module is evolutionarily conserved among other Rosaceae species remains an open question. Addressing this may provide new insights into the evolution of cold-responsive transcriptional networks in perennial fruit crops.

5. Conclusion

In this study, *EjRabF2b*, a key gene related to cold tolerance, was identified in loquat (Fig. 6). Its expression significantly enhances the activity of antioxidant enzymes, thereby maintaining cellular integrity and improving cold tolerance. Additionally, the transcription factor EjZAT10 was shown to directly bind to the promoter of *EjRabF2b* and activates its transcription under cold stress conditions. Together, these findings deepen our understanding of the molecular mechanisms governing cold stress responses in loquat and offer valuable insights for future research and potential breeding strategies targeting abiotic stress resilience.

Acknowledgements

Dr. Hao Fu acknowledges the support of the China Scholarship Council Program (202406990071). This work was supported by the National Key R&D Program of China (2023YFD1600800), the National Natural Science Foundation of China (32470386), the Chongqing Science and Technology Commission, China (cstc2021jscx-gksbX0010

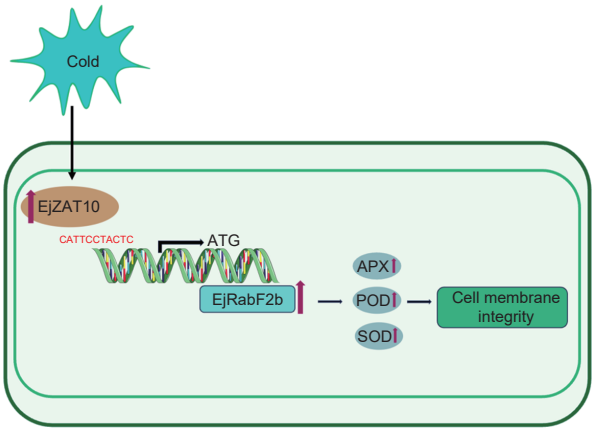


Fig. 6 A graphical summary of the role of *EjZAT10* and *EjRabF2b* in cold tolerance regulation in loquat.

and cstc2024ycjh-bgzxm0202), Characteristic Fruit Industry and Technology System Innovation Team of Chongqing Agriculture and Rural Affairs Commission, China (2020[3]01), and the Chongqing Postgraduate Research and Innovation Program, China (CYB22133).

Declaration of competing interest

The authors declare that they have no conflict of interest.

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

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

Appendices associated with this paper are available at https://doi.org/10.1016/j.jia.2025.10.018

References

Agarwal P K, Agarwal P, Jain P, Jha B, Reddy M K, Sopory S K. 2008. Constitutive overexpression of a stress-inducible small GTP-binding protein PgRab7 from *Pennisetum glaucum* enhances abiotic stress tolerance in transgenic tobacco. *Plant Cell Reports*, **27**, 105–115.

Ali M M, Rizwani H M, Yousef A F, Zhi C, Chen F X. 2021. Analysis of toxic elements in leaves and fruits of loquat by inductively coupled plasma-mass spectrometry (ICP-MS). *Acta Scientiarum Polonorum-Hortorum Cultus*, **20**, 33–42.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase — improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*, **44**, 276–287.

Bolte S, Schiene K, Dietz K J. 2000. Characterization of a small GTP-binding protein of the rab 5 family in *Mesembryanthemum crystallinum* with increased level of expression during early salt stress. *Plant Molecular Biology*, **42**, 923–936.

Buono R A, Leier A, Paez-Valencia J, Pennington J, Goodman K, Miller N, Ahlquist P, Marquez-Lago T T, Otegui M S. 2017. Escrt-mediated vesicle concatenation in plant endosomes. *Journal of Cell Biology*, **216**, 2167–2177.

Cai C, Xu C J, Shan L L, Li X, Zhou C H, Zhang W S, Ferguson I, Chen, K S. 2006. Low temperature conditioning reduces postharvest chilling injury in loquat fruit. *Postharvest Biology and Technology*, **41**, 252–259.

Cao Y Y, Zhang Q H, Sun J Z, Li R M, Huang Y H, Zhuang J, Xu J, Chen Y. 2022. Effects of weather conditions on the public demand for weather information via smartphone in multiple regions of China. *Weather Climate and Society*, **14**, 813–822.

Catalá R, Medina J, Salinas J. 2011. Integration of low temperature and light signaling during cold acclimation response in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 16475–16480.

Clough S J, Bent A F. 1998. Floral dip: A simplified method for agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant Journal*, **16**, 735–743.

Dhonukshe P, Baluska F, Schlicht M, Hlavacka A, Samaj J, Friml J, Gadella T W J. 2006. Endocytosis of cell surface material mediates cell plate formation during plant cytokinesis. *Developmental Cell*, **10**, 137–150.

Ebine K, Fujimoto M, Okatani Y, Nishiyama T, Goh T, Ito E, Dainobu T, Nishitani A, Uemura T, Sato M H, Thordal-Christensen H, Tsutsumi N, Nakano A, Ueda T. 2011. A membrane trafficking pathway regulated by the plant-specific RAB GTPase ARA6. *Nature Cell Biology*, **13**, 853–859.

Fu H, Chen Q, Yong S, Dang J, He Q, Jing D, Wu D, Liang G, Guo Q. 2024. The potential role of vesicle transport-related small GTPases rabs in abiotic stress responses. *Plant Physiology and Biochemistry*, **219**, 109411.

Gong Z Z, Xiong L M, Shi H Z, Yang S H, Herrera-Estrella L R, Xu G H, Chao D Y, Li J R, Wang P Y, Qin F, Li J J, Ding Y L, Shi Y T, Wang Y, Yang Y Q, Guo Y, Zhu J K. 2020. Plant abiotic stress response and nutrient use efficiency. *Science China-Life Sciences*, **63**, 635–674.

Guillamón J G, Prudencio A S, Yuste J E, Dicenta F, Sánchez-Pérez R. 2020. Ascorbic acid and prunasin, two candidate biomarkers for endodormancy release in almond flower buds identified by a nontargeted metabolomic study. *Horticulture Research*, **7**, 203.

He F, Li H G, Wang J J, Su Y Y, Wang H L, Feng C H, Yang Y L, Niu M X, Liu C, Yin W L, Xia X L. 2019. PeSTZ1, a C2H2-type zinc finger transcription factor from *Populus euphratica*, enhances freezing tolerance through modulation of ROS scavenging by directly regulating *PeAPX2*. *Plant Biotechnology Journal*, **17**, 2169–2183.

Hoffman L, DaCosta M, Ebdon J S, Watkins E. 2010. Physiological changes during cold acclimation of perennial ryegrass accessions differing in freeze tolerance. *Crop Science*, **50**, 1037–1047.

Hou Q C, Ufer G D, Bartels D. 2016. Lipid signalling in plant responses to abiotic stress. *Plant Cell and Environment*, **39**, 1029–1048.

Jiang Y Y, Peng J R, Zhu Y M, Su W B, Zhang L, Jing Y, Lin S Q, Gao Y S. 2019. The role of *EjSOC1s* in flower initiation in *Eriobotrya japonica*. *Frontiers in Plant Science*, **10**, 253.

Kamal M M, Ishikawa S, Takahashi F, Suzuki K, Kamo M, Umezawa T, Shinozaki K, Kawamura Y, Uemura M. 2020. Large-scale phosphoproteomic study of membrane proteins reveals early signaling events in response to cold. *International Journal of Molecular Sciences*, **21**, 8631.

Kanehisa M, Goto S. 2000. Kegg: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, **28**, 27–30.

Karagiannis E, Michailidis M, Tanou G, Samiotaki M, Karamanoli K, Avramidou E, Ganopoulos I, Madesis P, Molassiotis A. 2018. Ethylene-dependent and -independent superficial scald resistance mechanisms in 'Granny Smith' apple fruit. *Scientific Reports*, **8**, 11436.

Kielbowicz-Matuk A. 2012. Involvement of plant C_2H_2-type zinc finger transcription factors in stress responses. *Plant Science*, **185**, 78–85.

Kou X B, Cao P, Zhao Z, Zhang X, Dai Y, Wang K, Wu J Y, Zhang S L. 2024. Comparative genomic analysis of the *RabGAP* gene family in seven Rosaceae species, and functional identification of *PbrRabGAP10* in controlling pollen tube growth by mediating cellulose deposition in pear. *International Journal of Biological Macromolecules*, **256**, 128498.

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870–1874.

Langfelder P, Horvath S. 2008. WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*, **9**, 559.

Li X L, Meng D, Li M J, Zhou J, Yang Y Z, Zhou B B, Wei Q P, Zhang J K. 2023. Transcription factors MhDREB2A/MhZAT10 play a role in drought and cold stress response crosstalk in apple. *Plant Physiology*, **192**, 2203–2220.

Liao J L, Wu C X, Burlak C, Zhang S, Sahm H, Wang M, Zhang Z Y, Vogel K W, Federici M, Riddle S M, Nichols R J, Liu D L, Cookson M R, Stone T A, Hoang Q Q. 2014. Parkinson disease-associated mutation R1441H in LRRK2 prolongs the "active state" of its GTPase

domain. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, 4055–4060.
Lin S, Sharpe R H, Janick J. 1998. loquat: botany and horticulture. In: Jules J, ed., *Horticultural Reviews.* John Wiley & Sons, New Jersey, USA. pp. 233–276.
Lippuner V, Cyert M S, Gasser C S. 1996. Two classes of plant cDNA clones differentially complement yeast calcineurin mutants and increase salt tolerance of wild-type yeast. *Journal of Biological Chemistry*, **271**, 12859–12866.
Liu M X. 2021. Screening strong cold-hardiness and high-light-efficiency triploid loquat (*Eriobotrya japonica*) and study on the underlying mechanisms. Ph D thesis, Southwest University, China. (in Chinese)
Liu Y H, Khan A R, Gan Y B. 2022. C2H2 zinc finger proteins response to abiotic stress in plants. *International Journal of Molecular Sciences*, **23**, 2370.
Liu Y H, Liu D D, Hu R, Hua C M, Ali I, Zhang A D, Liu B H, Wu M J, Huang L L, Gan Y B. 2017. AtGIS, a C2H2 zinc-finger transcription factor from *Arabidopsis* regulates glandular trichome development through GA signaling in tobacco. *Biochemical and Biophysical Research Communications*, **483**, 209–215.
Liu Z L, Luo C, Dong L, Toan C V, Wei P X, He X H. 2014. Molecular characterization and expression analysis of a GTP-binding protein (MiRab5) in *Mangifera indica*. *Gene*, **540**, 86–91.
Livak K J, Schmittgen T D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, **25**, 402–408.
Min X Y, Liu Z P, Wang Y R, Liu W X. 2020. Comparative transcriptomic analysis provides insights into the coordinated mechanisms of leaves and roots response to cold stress in common vetch. *International Journal of Molecular Sciences*, **23**, 8477.
Mu J Q, Fu Y J, Liu B C, Zhang Y, Wang A Y, Li Y X, Zhu J B. 2021. SiFBA5, a cold-responsive factor from *Saussurea involucrata* promotes cold resilience and biomass increase in transgenic tomato plants under cold stress. *BMC Plant Biology*, **21**, 75.
Muñoz-Muñoz J L, García-Molina F, García-Ruiz P A, Arribas E, Tudela J, García-Cánovas F, Rodríguez-López J N. 2009. Enzymatic and chemical oxidation of trihydroxylated phenols. *Food Chemistry*, **113**, 435–444.
Nahm M Y, Kim S W, Yun D J, Lee S Y, Cho M J, Bahk J D. 2003. Molecular and biochemical analyses of *OsRab7*, a rice *Rab7* homolog. *Plant and Cell Physiology*, **44**, 1341–1349.
Nakano Y, Asada K. 1981. Hydrogen-peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. *Plant and Cell Physiology*, **22**, 867–880.
Nguyen X C, Kim S H, Hussain S, An J, Yoo Y, Han H J, Yoo J S, Lim C O, Yun D J, Chung W S. 2016. A positive transcription factor in osmotic stress tolerance, ZAT10, is regulated by MAP kinases in *Arabidopsis*. *Journal of Plant Biology*, **59**, 55–61.
O'Mahony P J, Oliver M J. 1999. Characterization of a desiccation-responsive small GTP-binding protein (Rab2) from the desiccation-tolerant grass *Sporobolus stapfianus*. *Plant Molecular Biology*, **39**, 809–821.
Palta J P, Whitaker B D, Weiss L S. 1993. Plasma-membrane lipids associated with genetic-variability in freezing tolerance and cold-acclimation of *Solanum* species. *Plant Physiology*, **103**, 793–803.
Park S, Lee C M, Doherty C J, Gilmour S J, Kim Y, Thomashow M F. 2015. Regulation of the *Arabidopsis* CBF regulon by a complex low-temperature regulatory network. *Plant Journal*, **82**, 193–207.
Qian W H, Zhang Z J. 2012. Precursors to predict low-temperature freezing-rain events in southern China. *Chinese Journal of Geophysics-Chinese Edition*, **55**, 1501–1512. (in Chinese)
Qin M F, Li H D, Guo Z T, Zhu Y L, Wang R R, Zhang M, Zhang Q, Xu Y, Song J, Huang Z, Xu A X. 2023. Phenotypic damage and transcriptomic responses of flower buds in rapeseed (*Brassica napus* l.) under low-temperature stress. *Industrial Crops and Products*, **198**, 116669.
Rossel J B, Wilson P B, Hussain D, Woo N S, Gordon M J, Mewett O P, Howell K A, Whelan J, Kazan K, Pogson B J. 2007. Systemic and intracellular responses to photooxidative stress in *Arabidopsis*. *Plant Cell*, **19**, 4091–4110.
Rutherford S, Moore I. 2002. The *Arabidopsis* Rab GTPase family: Another enigma variation. *Current Opinion in Plant Biology*, **5**, 518–528.
Schapire A L, Voigt B, Jasik J, Rosado A, Lopez-Cobollo R, Menzel D, Salinas J, Mancuso S, Valpuesta V, Baluska F, Botella M A. 2008. *Arabidopsis* synaptotagmin 1 is required for the maintenance of plasma membrane integrity and cell viability. *Plant Cell*, **20**, 3374–3388.
Shi Y, Ding Y, Yang S. 2018. Molecular regulation of CBF signaling in cold acclimation. *Trends in Plant Science*, **23**, 623–637.
Sparkes I A, Runions J, Kearns A, Hawes C. 2006. Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nature Protocols*, **1**, 2019–2025.
Sun H J, Uchii S, Watanabe S, Ezura H. 2006. A highly efficient transformation protocol for Micro-Tom, a model cultivar for tomato functional genomics. *Plant and Cell Physiology*, **47**, 426–431.
Wang X, Liu F, Jiang D. 2017. Priming: A promising strategy for crop production in response to future climate. *Journal of Integrative Agriculture,* **16**, 2709–2716.
Wang J, Wei M, Wang H Y, Mo C J, Zhu Y C, Kong Q S. 2025. A time-course transcriptome reveals the response of watermelon to low-temperature stress. *Journal of Integrative Agriculture*, **24,** 1786–1799.
Wang D, Liu Z, Xiao Y H, Liu X L, Chen Y, Zhang Z, Kang H X, Wang X L, Jiang S, Peng S S, Tan X Q, Zhang D Y, Liu Y, Wang G L, Li C G. 2021. Association mapping and functional analysis of rice cold tolerance QTLs at the bud burst stage, *Rice*, **14**, 98.
Wang X Y, Wu Y, Sun M D, Wei X, Huo H Y, Yu L L, Zhang J X. 2022. Dynamic transcriptome profiling revealed key genes and pathways associated with cold stress in castor (*Ricinus communis* l.). *Industrial Crops and Products*, **178**, 114610.
Xiao B Z, Chen X, Xiang C B, Tang N, Zhang Q F, Xiong L Z. 2009. Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. *Molecular Plant*, **2**, 73–83.
Xu H X, Meng D, Yang Q, Chen T, Qi M, Li X Y, Ge H, Chen J W. 2023. Sorbitol induces flower bud formation via the MADS-box transcription factor EjCAL in loquat. *Journal of Integrative Plant Biology*, **65**, 1241–1261.
Yin C F, Karim S, Zhang H S, Aronsson H. 2017. *Arabidopsis* RabF1 (ARA6) is involved in salt stress and dark-induced senescence (DIS). *International Journal of Molecular Sciences*, **18**, 309.
Zhang C B, Wu F, Yan Q, Duan Z, Wang S S, Ao B, Han Y Y, Zhang J Y. 2023. Genome-wide analysis of the *Rab* gene family in melilotus albus reveals their role in salt tolerance. *International Journal of Molecular Sciences*, **24**, 126.
Zhang J Y, An H S, Zhang X Y, Xu F J, Zhou B Q. 2022. Transcriptomic analysis reveals potential gene regulatory networks under cold stress of loquat (lindl.). *Frontiers in Plant Science*, **13**, 944269.
Zhou S Q, Chen L, Chen G, Li Y X, Yang H Q. 2021. Molecular mechanisms through which short-term cold storage improves the nutritional quality and sensory characteristics of postharvest sweet potato tuberous roots: A transcriptomic study. *Foods*, **10**, 2079.

Executive Editor-in-Chief Sanwen Huang
Managing Editor Lingyun Weng