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SmMYB113 integrates ABA signaling with ICE-CBF transcriptional synergy to enhance low-temperature adaptation in eggplant

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Abstract

The MYB transcription factor family represents the largest class of transcription factors in plants, playing crucial roles in regulating plant growth, development, and stress defense responses. This study demonstrates that overexpression of *SmMYB113* in eggplant cultivar '108' significantly enhances cold tolerance. The results showed that *SmMYB113* enhances cold tolerance through a dual regulatory mode: it binds to the promoter of the ABA biosynthetic gene *SmAAO4-1*, upregulating its expression and significantly increasing ABA content; meanwhile, it physically interacts with SmICE2, enhancing SmICE2's transcriptional activation capability on *SmCBF2*, thereby elevating the expression of downstream cold-responsive genes to combat low-temperature stress. These results indicate that *SmMYB113* regulates eggplant cold tolerance through a multi-layered cold resistance mechanism integrating hormone signaling and transcriptional cascades.

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Introduction

Low-temperature stress is a key environmental factor limiting the yield of solanaceous crops. Plants respond to low temperatures via intricate regulatory networks[1], in which hormone signaling pathways, including ABA[2], and transcription factor pathways, such as CBF and MYB[3–5], play pivotal roles. ABA, as a key stress-response hormone, not only regulates stomatal closure and osmotic balance but also enhances cold resistance by activating cold-responsive genes[6]. Concurrently, the ICE-CBF pathway serves as a conserved cold adaptation route, driving the expression of downstream cold-responsive proteins (e.g., Cold-Regulated genes, COR)[7]. However, the cross-talk between these two major systems, particularly how transcription factors integrate hormone signaling with transcriptional cascades, remains relatively understudied. Elucidating this regulatory network holds significant theoretical value for designing cold-tolerant crops with synergistic multi-pathway regulation.

MYB transcription factors exhibit remarkable functional polymorphism in diverse plant processes, including secondary metabolism, cell differentiation, and abiotic stress responses[8–10]. Studies indicate that Subgroup 2 R2R3-MYB proteins participate in low-temperature responses through various modes, enhancing plant cold tolerance by mediating the ABA signaling pathway. For instance, oil palm *EgMYB111* enhances cold tolerance by controlling the transcription of key ABA regulatory genes *EgSnRK2.1*, *EgSnRK2.3*, and *EgSnRK2.5*[11]. Tomato SlMYB15 binds to upstream regulatory elements of ABA biosynthesis and signaling transduction genes *SlNCED1* and *SlABF4*, leading to their upregulation and increased tomato cold tolerance[12]. Additionally, MYB transcription factors form heterodimers with bHLH transcription factors to activate the anthocyanin biosynthesis pathway, alleviating photo-inhibition damage. For example, the citrus R2R3 MYB transcription factor *CsRuby1* is activated by two ethylene response factors, subsequently increasing citrus cold tolerance by upregulating anthocyanin synthesis[13]. Apple MdMYB308L interacts with MdbHLH33, acting as a positive regulator in apple cold tolerance and anthocyanin accumulation[14].

SmMYB113 is a key transcription factor responsible for compositional variation of anthocyanin and color diversity among eggplant peels[15]. Current evidence establishes that *SmMYB113* participates in the anthocyanin biosynthetic pathway in eggplant[15]. Under low-temperature conditions, SmCBFs proteins interact with SmMYB113, thereby indirectly activating key anthocyanin biosynthesis genes including chalcone synthase (*CHS*), and dihydroflavonol 4-reductase (*DFR*), which consequently enhances anthocyanin production[16]. Based on our previous findings, *SmMYB113*-OE lines exhibited enhanced cold tolerance under low-temperature conditions, coupled with a marked upregulation of *SmMYB113* expression and a significant increase in anthocyanin accumulation. These collective observations led to the selection of *SmMYB113* as the primary focus of this study.

As a key signaling molecule in plant stress responses, ABA dynamically regulates stomatal movement, osmolyte synthesis, and stress memory formation by activating the SnRK2 (SNF1-related protein kinase 2) kinase cascade[17–20]. Low-temperature stress significantly induces endogenous ABA accumulation[21], while exogenous ABA application enhances cold tolerance[22], suggesting a profound interaction between ABA signaling and cold adaptation mechanisms. Studies show that ABI1 enhances ICE1 protein stability by regulating OST1 (Open Stomata 1) kinase-mediated phosphorylation, promoting CBF expression[23]. On the other hand, the ICE-CBF pathway, as a conserved axis for cold response, has been extensively validated in model plants such as *Arabidopsis* and tomato[24,25]. Upon cold exposure, ICE acts upstream in the cold

response cascade as one of the earliest signals[26], driving the expression of CBF transcription factors by binding to the canonical MYC cis-element (CANNTG) in the CBF3/DREB1A promoter, thereby activating downstream cold-responsive genes like COR and RD (responsive to dehydration)[27,28].

Eggplant is an important protected vegetable crop. Its year-round production mode ensures a stable food supply but poses the challenge of maintaining normal growth under low-temperature conditions. In this study, it was found that *SmMYB113* likely enhances eggplant cold resistance through a dual regulatory mode: directly activating ABA biosynthesis and interacting with core components of the ICE-CBF pathway, forming a synergistic hormone-transcription network. Therefore, *SmMYB113* plays a crucial role during cold stress in eggplant, providing novel insights for improving low-temperature-tolerant eggplant varieties through molecular breeding.

Materials and methods

Plant materials and growth conditions

The eggplant (*Solanum melongena* L.) materials used were the inbred line '108' (wild-type, WT) and T2 generation seeds of *SmMYB113* overexpression lines, maintained by self-pollination in our laboratory. Eggplant seeds were surface-sterilized, soaked in warm water for 6–8 h at room temperature, and germinated in darkness at 28 °C until radicle emergence. Germinated seeds were sown in plug trays filled with a peat moss : vermiculite (3:1, v/v) substrate mix and cultivated in a glass greenhouse at Shandong Agricultural University (Shandong, China) under natural light. Seedlings at the two-true-leaf stage were transplanted into pots containing the same substrate mix. Plants were irrigated with Yamazaki eggplant nutrient solution for water and nutrient management; other cultivation practices followed standard protocols.

When seedlings reached the 5–6 true-leaf stage, uniform and robust plants were selected and subjected to low-temperature treatment in a growth chamber. Low-temperature conditions were set at a constant 5 °C with a 16 h light/8 h dark photoperiod. Samples were collected at 0, 1, and 9 d after the initiation of low-temperature treatment. For exogenous ABA treatment, eggplant seedlings were sprayed with 200 μM ABA solution, one day before low-temperature exposure and then transferred to the growth chamber for cold treatment. Samples were collected at 0, 1, and 9 d after cold treatment initiation. All samples were flash-frozen in liquid nitrogen and stored at −80 °C until use.

Determination of physiological and biochemical indices

The physiological and biochemical indices were determined as follows. Electrolyte leakage (EL) was measured by sampling leaves from the same position. Uniform leaf discs were punched, weighed to 0.2 g, and completely immersed in 50 mL centrifuge tubes containing 20 mL of deionized water. The samples were shaken at 200 rpm and 28 °C for 1–2 h. The initial electrical conductivity (EC0) of the deionized water and the conductivity after shaking (EC1) were recorded using a digital conductivity meter. Subsequently, the samples were boiled at 95 °C for 15 –20 min, cooled to room temperature, and the final conductivity (EC2) was measured. Relative electrolyte leakage (REL, %) was calculated as [(EC1 − EC0) / (EC2 − EC0)] × 100%, with three biological replicates performed and the mean value reported. Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) method. Hydrogen peroxide (H_2O_2) content was quantified with a Hydrogen Peroxide Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Superoxide anion ($O_2^{\cdot-}$) content was assessed using the hydroxylamine oxidation method. Histochemical staining for H_2O_2 was carried out using 3,3′-diaminobenzidine (DAB), and for $O_2^{\cdot-}$ using nitro blue tetrazolium (NBT); representative images of DAB and NBT staining in wild-type leaves under low-temperature conditions are provided for reference in Supplementary Fig. S1. ABA content was measured with an enzyme-linked immunosorbent assay (ELISA) kit. Antioxidant enzyme activities were evaluated as follows: superoxide dismutase (SOD) activity was determined by the NBT reduction method, peroxidase (POD) activity by the guaiacol method, catalase (CAT) activity by the ammonium molybdate colorimetric assay[29], and ascorbate peroxidase (APX) activity according to the method described by Nakano & Asada[30].

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using TaKaRa MiniBEST Universal RNA Extraction Kit (Takara, Tokyo, Japan). cDNA synthesis was performed using the TransScript® One-Step gDNA Removal and cDNA Synthesis Super Mix kit (TRANSGEN, Beijing, China) according to the manufacturer's instructions. qRT-PCR was performed using Vazyme® AceQ qPCR SYBR Green Master Mix (Without ROX) on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, USA). The *SmACT7* (*Actin 7*) gene was used as the internal reference gene for normalization. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_T}$ method[31].

Transcriptome sequencing and analysis

Total RNA was extracted using an RNA extraction kit (Takara, Tokyo, Japan). RNA quality was assessed using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), 1.2% agarose gel electrophoresis, and an Agilent 2100 Bioanalyzer. mRNA library construction, sequencing, and bioinformatic analysis were performed following the methods described by Li et al.[32].

Yeast one-hybrid (Y1H) and yeast two-hybrid (Y2H) assays

Y1H

The *SmMYB113* coding sequence was fused into the pGADT7 vector. The *SmAAO4-1* promoter sequence was fused into the pABAi vector. Both constructs were sequentially co-transformed into the yeast strain Y1H Gold. Similarly, the *SmICE2* coding sequence was fused into pGADT7, and the *SmCBF1* and *SmCBF2* promoter was fused into pABAi, then co-transformed into Y1H Gold. Positive clones were selected on SD/-Leu medium with or without Aureobasidin A (AbA, 100 ng·mL^{-1}).

Y2H

SmMYB113 and *SmICE2* coding sequences were cloned into pGADT7 and pGBKT7 vectors, respectively. Empty vectors served as controls. Recombinant plasmids were co-transformed into the yeast strain Y2H Gold. Transformants were plated on SD/-Trp/-Leu (-T/-L) medium and incubated at 28 °C for 2 d. Single colonies were then streaked onto SD/-Ade/-His/-Leu/-Trp (-T/-L/-H/-A) medium (Clontech, Palo Alto, USA) and incubated at 28 °C for 2 d to identify positive interacting clones.

Bimolecular fluorescence complementation (BiFC) assay

The BiFC assay was performed in *Nicotiana benthamiana* leaves. *Agrobacterium tumefaciens* strains harboring the relevant constructs were grown to an optical density of 600 (OD 600) ≈1.5, centrifuged at 5,000 rpm for 5 min, and the pellets were resuspended in infiltration buffer (MS salts + 1.5% sucrose + 10 mM MES pH 5.6 + 10 mM $MgCl_2$ + 150 μM acetosyringone). Suspensions were infiltrated into the abaxial side of tobacco leaves using a 1.5 mL syringe. Infiltrated plants were grown under normal conditions for 2 d. Lower epidermal peels were then examined for fluorescence signals using confocal microscopy.

Firefly luciferase complementation imaging (LCI) assay

Healthy *N. benthamiana* plants were prepared. *Agrobacterium* cultures were grown, pelleted, and resuspended in infiltration buffer as described for BiFC. Suspensions were infiltrated into the abaxial side of tobacco leaves using a 1.5 mL syringe. Plants were grown under normal light conditions for 2 d. Luminescence signals at the infiltration sites were detected and imaged using a low-light cooled CCD imaging apparatus (NightOWL II LB983, Berthold).

Results

Overexpression of *SmMYB113* enhances cold tolerance

To investigate the relationship between *SmMYB113* and eggplant cold tolerance, *SmMYB113* overexpression (OE) lines were generated via *Agrobacterium*-mediated leaf disc transformation. Positive transgenic seedlings (Supplementary Fig. S2) were identified, and T2 seeds were harvested. Two OE lines (OE1, OE4) and WT seedlings were subjected to low-temperature (5 °C) treatment. Results (Fig. 1a) showed that after 1 d of cold treatment, no significant differences were observed between lines. However, after 9 d, WT seedling growth points died, while OE line seedlings maintained intact upper leaves and growth points without wilting. Metabolic characteristics are key indicators of stress resistance. Measurements of electrolyte leakage (REL) (Fig. 1d), and malondialdehyde (MDA) content (Fig. 1e) revealed that both were significantly lower in OE lines compared to WT after cold treatment. Furthermore, hydrogen peroxide (H_2O_2) (Fig. 1f), and superoxide anion ($O_2^{\cdot-}$) (Fig. 1g) contents were significantly lower in OE lines than in WT after cold treatment. Consistent results were obtained from NBT and DAB staining (Fig. 1b, c). Additionally, antioxidant enzyme activities (SOD, POD, CAT, APX) were significantly higher in OE lines than in WT after cold treatment (Fig. 1h–k). These findings indicate that *SmMYB113* overexpression confers stronger cold tolerance compared to WT.

Transcriptomics reveals a synergistic pathway network

Through PCA and correlation analysis, it was detected that the three biological replicates within the same group exhibited tight clustering, confirming the reliability of the data for downstream analysis (Supplementary Fig. S3). Differentially expressed genes (DEGs) were identified in leaves of OE1, OE4, and WT plants at 0 h and 12 h after cold treatment initiation, using thresholds of FDR ≤ 0.001 and $|\log_2 FC| \geq 2$. The selection of 0 h and 12 h was based on the observation that the expression of *SmMYB113* in WT plants peaked at 12 h under low-temperature treatment (Supplementary

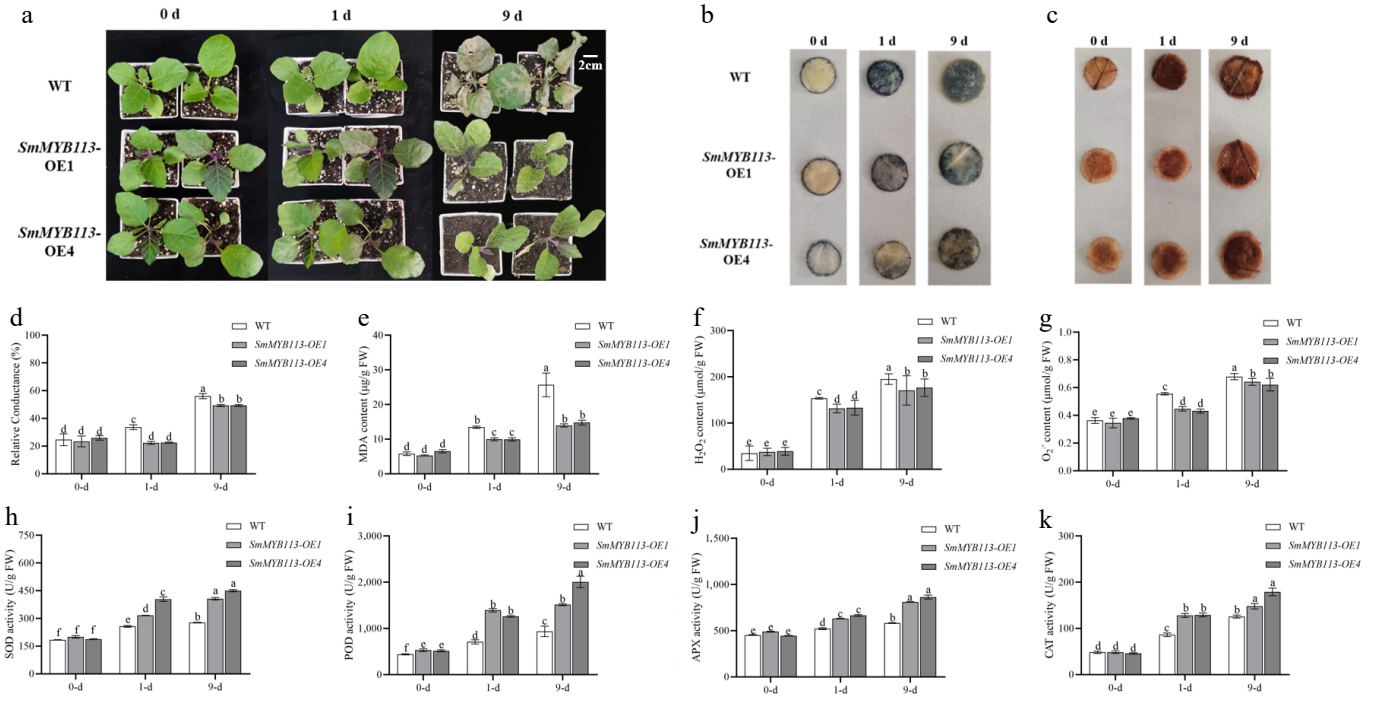

Fig. 1 Phenotypic and physiological differences between WT and SmMYB113-OE plants under low-temperature stress. (a) Phenotypic differences under 5 °C (16 h light/8 h dark) at 0, 1, and 9 d. (b) DAB staining for H_2O_2 localization. (c) NBT staining for $O_2^{\cdot-}$ localization. (d) Relative electrolyte leakage (REL). (e) MDA content. (f) Colorimetric quantification of H_2O_2 content. (g) Hydroxylamine oxidation assay for $O_2^{\cdot-}$ content. (h)–(k) Activities of antioxidant enzymes SOD, POD, APX, and CAT. Values represent means ± SD of three independent biological replicates (n = 3). Significant differences were determined by one-way ANOVA followed by Tukey's test ($p < 0.05$). Different lowercase letters indicate significant differences ($p < 0.05$).

Fig. S4). As shown in Fig. 2b, 7,670, 9,424, and 7,368 DEGs were identified in the WT, OE1, and OE4 groups, respectively. A total of 4,133 DEGs were common to all three groups. For focused analysis, DEGs present in both OE1 and OE4 groups but absent in the WT group were selected, yielding 1,113 DEGs potentially involved in eggplant low-temperature stress response.

GO enrichment analysis of the 1,113 DEGs (Fig. 2a) revealed significant enrichment in processes including 'lipid metabolic process', 'organic acid metabolic process', 'carboxylic acid metabolic process', 'oxoacid metabolic process', and 'lipid biosynthetic process'. KEGG pathway analysis (Fig. 2c) showed enrichment in 20 pathways, primarily 'glycerophospholipid metabolism', 'glycerolipid metabolism', 'terpenoid backbone biosynthesis', 'fructose and mannose metabolism', 'sesquiterpenoid and triterpenoid biosynthesis', 'photosynthesis-antenna proteins', 'glycine, serine, and threonine metabolism', 'isoquinoline alkaloid biosynthesis', 'alanine, aspartate, and glutamate metabolism', and 'carotenoid biosynthesis'.

Key DEGs of interest (Supplementary Table S1) included genes involved in ABA synthesis (*SmAAO4-1*, SMEL4.1_12g014560.1), cold response factors (*SmICE2*, SMEL4.1_03g029410.1), and CBF transcription factors (*SmCBF1*, SMEL4.1_12g001770.1; *SmCBF2*, SMEL4.1_03g007630.1; *SmCBF3*, SMEL4.1_10g023430.1). qRT-PCR confirmed that SmAAO4-1 and *SmCBFs* were upregulated in OE lines, consistent with transcriptomic trends (Fig. 2d–f). Further analyses investigated the interaction between *SmMYB113* and these genes.

SmMYB113 enhances cold adaptation via ABA biosynthesis

To study the regulatory relationship between *SmMYB113* and ABA, ABA content was measured in OE1, OE4, and WT leaves at 1 and 9 d post-cold treatment. As shown in Fig. 3d, ABA content was significantly higher in OE1 and OE4 than in WT at 0 d. ABA levels increased in all lines at 1 and 9 d, but the increase was greater in OE lines. Compared to WT, ABA content in OE1 increased by 22.59%, 11.51%, and 21.26% at 0, 1, and 9 d, respectively. In OE4, it increased by 14.0%, 22.36%, and 21.56%. This indicates that *SmMYB113* overexpression enhances ABA synthesis in transgenic lines.

To confirm ABA's role in enhancing cold tolerance, WT plants were sprayed with water or exogenous ABA (200 μM). Results (Fig. 3a) showed that after 9 d of cold treatment, untreated (CK) and water-sprayed (A1) plants exhibited yellowing of lower leaves and wilting of growth points. In contrast, ABA-sprayed (A2) plants maintained intact leaves and healthy growth points. Measurements of REL and MDA content (Fig. 3e, f) showed no significant differences among treatments before cold stress. At 1 d post-stress, MDA

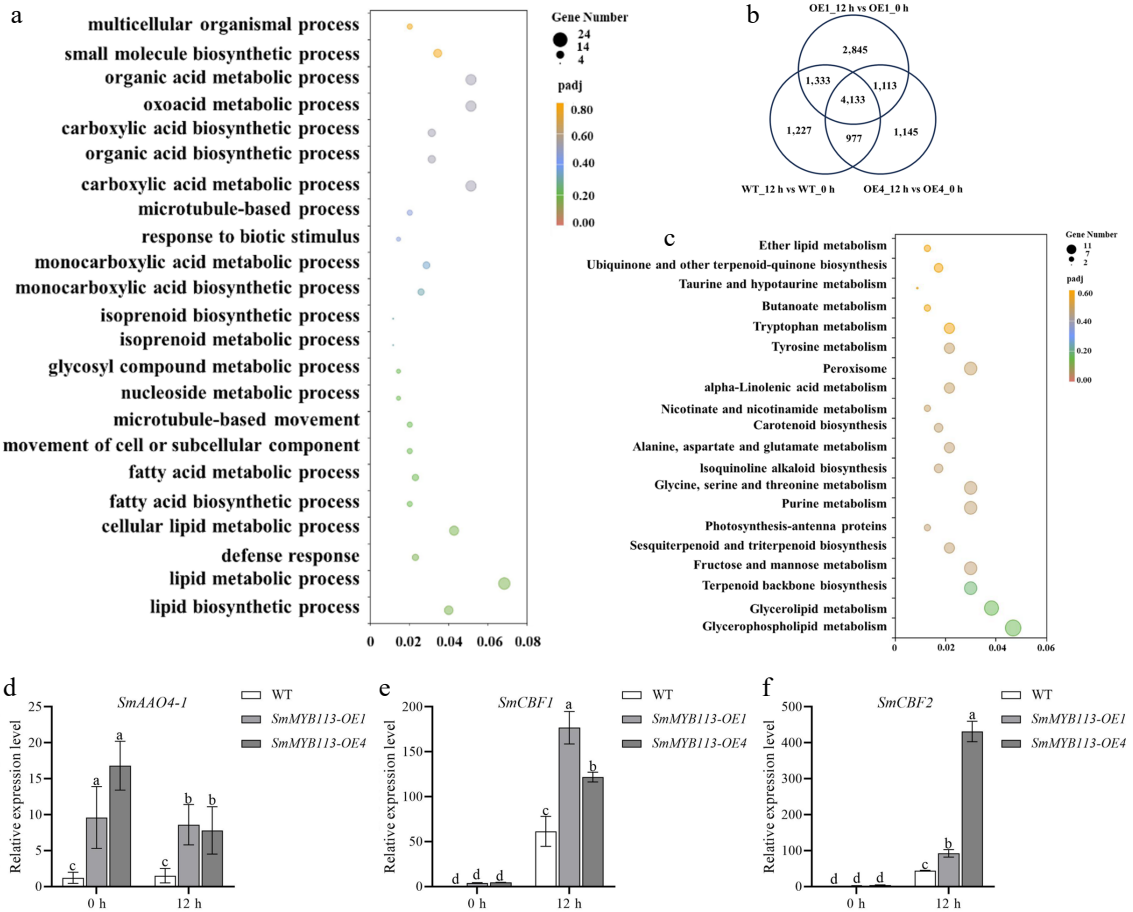

Fig. 2 Transcriptomic analysis reveals synergistic cold response pathways. (a) Gene Ontology (GO) enrichment analysis of the 1,113 DEGs common to OE lines but absent in WT. (b) Venn diagram of DEGs identified in WT, OE1, and OE4 plants under cold stress. (c) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the 1,113 DEGs. (d)–(f) Expression levels of key genes in OE and WT plants under 5 °C (16 h light/8 h dark) at 0 and 12 h: (d) ABA biosynthetic gene *SmAAO4-1*, and cold response factors (e) *SmCBF1*, and (f) *SmCBF2*. Values represent means ± SD of three independent biological replicates ($n = 3$). Significant differences were determined by one-way ANOVA followed by Tukey's test ($p < 0.05$). Different lowercase letters indicate significant differences ($p < 0.05$).

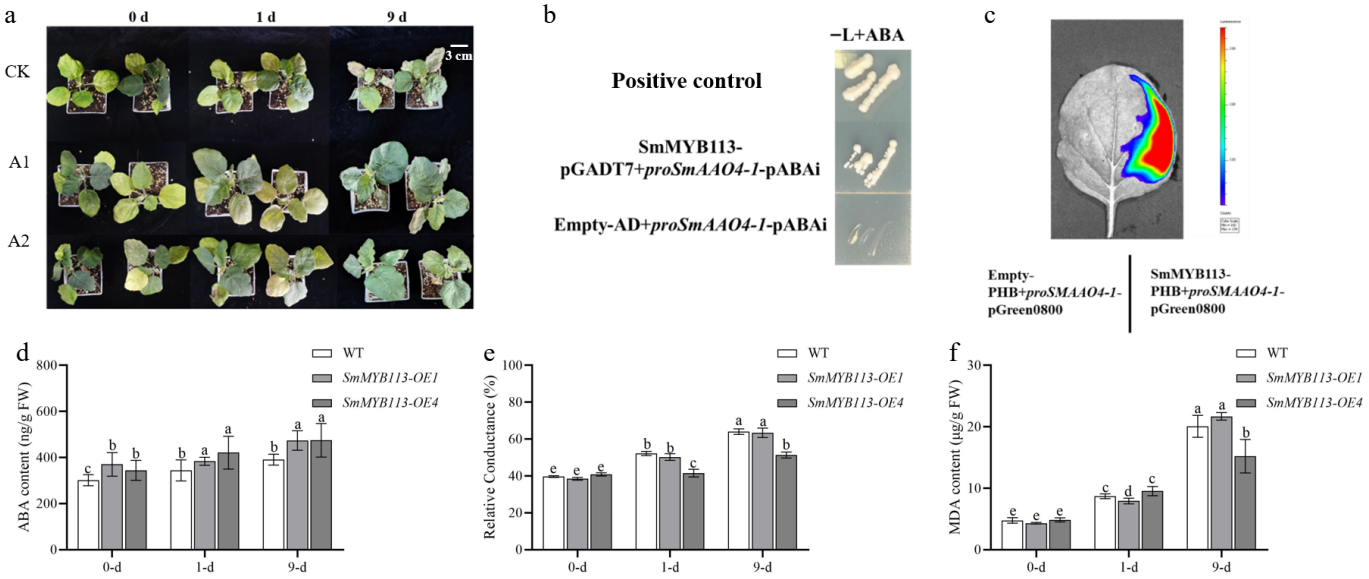

Fig. 3 Role of ABA in cold tolerance and regulation of *SmAAO4-1* by *SmMYB113*. (a) Phenotype of WT plants under 5 °C (16 h light/8 h dark) at 0, 1, and 9 d after pretreatment: CK (no pretreatment), A1 (water spray), A2 (ABA spray). (b) Y1H assay showing interaction of SmMYB113 with the *SmAAO4-1* promoter. Yeast growth on SD/-Leu medium with AbA (100 ng·mL^{-1}). (c) LCI assay in tobacco leaves confirming SmMYB113 binding to the *SmAAO4-1* promoter and activating luciferase expression. Physiological indices in WT, OE1, and OE4 leaves under 5 °C (16 h light/8 h dark) at 0, 1, and 9 d: (d) ABA content, (e) Relative electrolyte leakage (REL), (f) MDA content. Values represent means ± SD of three independent biological replicates (n = 3). Significant differences were determined by one-way ANOVA followed by Tukey's test (p < 0.05). Different lowercase letters indicate significant differences (p < 0.05).

content differences were non-significant, but REL was significantly lower in ABA-treated plants. By 9 d, both REL and MDA were significantly lower in ABA-treated plants compared to controls. This demonstrates that exogenous ABA application alleviates cold damage and enhances cold tolerance in eggplant. Furthermore, the phenotype of ABA-treated WT plants closely resembled that of *SmMYB113*-OE plants, indirectly supporting that *SmMYB113* enhances cold adaptation by upregulating ABA synthesis.

SmAAO4 is a key gene for ABA biosynthesis in eggplant. To verify if *SmMYB113* enhances ABA biosynthesis, Y1H assay showed that yeast co-transformed with SmMYB113-pGADT7 and proSmAAO4-1-pABAi grew on SD/-Leu medium containing AbA (100 ng·mL^{-1}) (Fig. 3b). Furthermore, LCI assay confirmed that SmMYB113 binds to the *SmAAO4-1* promoter and activates luciferase expression in tobacco leaves (Fig. 3c). Meanwhile, evidence was obtained that SmMYB113 forms homomultimers (Supplementary Fig. S5). These results demonstrate that SmMYB113 binds to the *SmAAO4-1* promoter and activates its expression. In summary, SmMYB113 increases ABA content and enhances cold tolerance by activating SmAAO4-1 expression.

Physical interaction between SmMYB113 and SmICE2

The interaction between SmMYB113 and SmICE proteins was investigated. BiFC assay, with SmMYB113 fused to YFPC and SmICE2 fused to YFPN, showed fluorescence signal in the nucleus of tobacco leaf cells (Fig. 4a), indicating interaction. Y2H assay (Fig. 4b) demonstrated that only yeast co-transformed with SmMYB113 and SmICE2 grew on stringent SD/-Trp/-Leu/-His/-Ade (-T/-L/-H/-A) medium, while controls did not, confirming interaction in yeast. These results collectively demonstrate that SmMYB113 directly interacts with SmICE2.

To identify the key interaction domain in SmMYB113, online prediction indicated that residues 49-348 are crucial. Deletion of this 300 bp region (SmMYB113Del, Fig. 4c) abolished the interaction in Y2H assay (Fig. 4d). Consistently, LCI assay showed no fluorescence signal when SmMYB113Del and SmICE2 were co-expressed in tobacco leaves (Fig. 4f), unlike the strong signal observed with full-length SmMYB113 and SmICE2 (Fig. 4e). This confirms that the 49–348 bp region is essential for SmMYB113-SmICE2 interaction.

SmMYB113-SmICE2 interaction enhances SmICE2-mediated transcriptional activation of *SmCBF2*

To validate SmICE2's ability to activate *SmCBF* transcription, SmICE2 was fused to the effector vector (pGreenII 62-SK), and the *SmCBF2* promoter was fused to the reporter vector (pGreenII 0800-LUC). LCI assay (Fig. 5a) showed that SmICE2 binds the *SmCBF2* promoter and activates luciferase expression. Y1H assay (Fig. 5b), with SmICE2-pGADT7 and pro*SmCBF2*-pABAi, confirmed that SmICE2 activates *SmCBF2* expression.

Crucially, co-expression of SmMYB113 and SmICE2 significantly enhanced the luciferase activity driven by the *SmCBF2* promoter compared to expression of SmICE2 alone (Fig. 5c, d). Quantitative analysis of luminescence intensity confirmed this synergistic enhancement (Fig. 5d). These results indicate that the SmMYB113-SmICE2 interaction enhances SmICE2's binding capability and transcriptional activation potential on the *SmCBF2* promoter.

Discussion

This study demonstrates that overexpression of *SmMYB113* in the eggplant cultivar '108' significantly enhances cold tolerance. Y1H and LCI assays revealed that *SmMYB113* transcriptionally activates SmAAO4-1 expression, promoting ABA biosynthesis and thereby enhancing cold tolerance. Furthermore, Y2H and BiFC assays showed that *SmMYB113* physically interacts with SmICE2, enhancing SmICE2's transcriptional activation of *SmCBF2*, thus

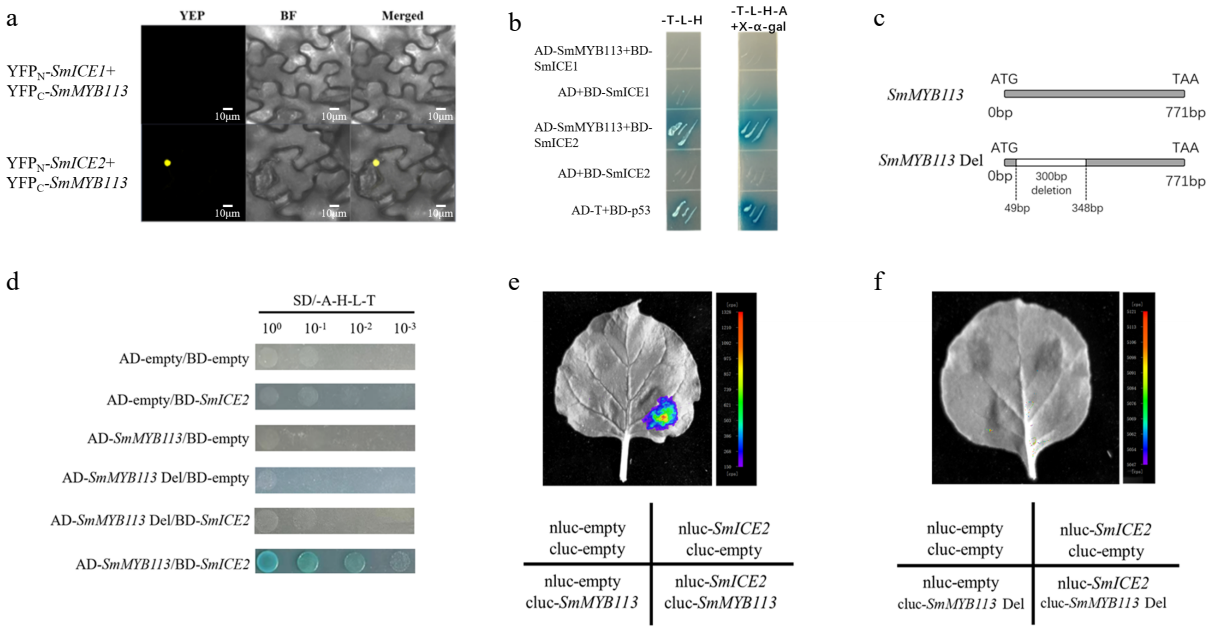

Fig. 4 Validation of SmMYB113-SmICE2 interaction and identification of the interaction domain. (a) BiFC assay showing SmICE2-SmMYB113 interaction in tobacco leaf nuclei. SmICE1/YFPN and SmICE2/YFPN were paired with SmMYB113/YFPC. (b) Y2H assay confirming SmICE2-SmMYB113 interaction on SD/-T/-L/-H/-A medium. (c) Schematic diagram of SmMYB113 deletion mutant (SmMYB113Del, lacking residues 49–348). (d) Y2H assay showing loss of interaction between SmMYB113Del and SmICE2. (e) LCI assay showing strong fluorescence signal upon co-expression of full-length SmMYB113 and SmICE2. (f) LCI assay showing loss of fluorescence signal upon co-expression of SmMYB113Del and SmICE2.

participating in the ICE-CBF pathway to boost cold tolerance. This synergistic regulatory mode ensures enhanced cold tolerance under low-temperature stress while amplifying cold stress signaling efficiency through the integration of hormone and transcriptional regulatory mechanisms.

Anthocyanins, as important secondary metabolites, function as effective antioxidants that mitigate oxidative damage by utilizing their hydroxyl groups to neutralize free radicals and scavenge reactive oxygen species (ROS), thereby maintaining osmotic balance and cellular redox homeostasis. Consequently, plants often synthesize anthocyanins to reduce oxidative injury induced by low-temperature stress[33]. Overexpression of the MYB transcription factor *Rosea1* in tobacco has been demonstrated to enhance anthocyanin accumulation and significantly improve cold stress tolerance[34]. Interestingly, *SmMYB113* has been previously reported to regulate anthocyanin accumulation[15]. Following low-temperature treatment, it was observed that the ROS content in eggplant leaves of the *SmMYB113*-OE lines was significantly lower than in WT lines. This indicates that anthocyanins accumulated in *SmMYB113*-OE eggplant leaves effectively scavenge cold-induced ROS, thereby conferring enhanced tolerance to low-temperature stress.

Plant hormones play vital roles in abiotic stress responses. Transcriptomic analysis of *SmMYB113*-OE and WT plants after cold stress revealed that DEGs were primarily enriched in secondary metabolite pathways and hormone synthesis/signaling pathways, with significant changes in ABA synthesis and signaling genes. ABA is one of the most important stress hormones, crucial in various physiological processes including seed dormancy, germination, stomatal movement, fruit development, and responses to biotic/abiotic stresses. Cellular ABA levels fluctuate dynamically in response to physiological and environmental cues, and these concentration changes determine ABA's role in plant physiology and development[35–37]. As a primary hormone in stress response, ABA is induced by multiple abiotic stresses like drought, salinity, and cold. Cold treatment induces ABA accumulation in plants like *Arabidopsis* and tomato[38]. The present measurements showed significantly higher ABA levels in OE lines compared to WT at the same time points under cold stress. Y1H and LCI assays proved that SmMYB113 binds to the SmAAO4-1 promoter, activating its expression. To cope with stress, plants accumulate more ABA in leaves, promoting stomatal closure, enhancing water balance, and inducing antioxidant defense systems to mitigate oxidative damage. Moreover, ABA can activate numerous cellular responses through signaling pathways and induction of HSPs and CBFs, promoting stress tolerance[39,40]. The present results collectively indicate that *SmMYB113* overexpression increases ABA biosynthesis and accumulation in eggplant, positively regulating cold tolerance.

Exogenous ABA application enhances cold adaptation in various plants[41]. To further verify ABA's positive role in eggplant cold tolerance, exogenous ABA was applied. Cold stress duration increased membrane damage, reflected in rising REL and MDA. However, exogenous ABA application slowed this increase in REL compared to controls. ABA-treated plants also exhibited significantly lower $O_2^{\cdot-}$ and H_2O_2 levels than WT, indicating ABA reduces membrane damage and improves low-temperature stress tolerance.

Numerous studies have identified MYB transcription factors regulating plant cold tolerance via interaction with ICE to modulate downstream CBF expression. For example, *AtMYB15* in *Arabidopsis* negatively regulates cold tolerance by repressing *CBF* expression; *AtMYB15* mutants show elevated *CBF* expression and enhanced cold tolerance. *AtMYB15* also interacts with ICE1 to regulate *CBF* expression[42]. The ICE-CBF-COR transcriptional cascade is the primary pathway for cold response in plants. Upon cold induction, upstream ICE transcription factors activate CBF expression, which in turn regulates downstream COR genes, enabling the plant to respond to cold stress. In *Arabidopsis*, *AtICE2* overexpression induces

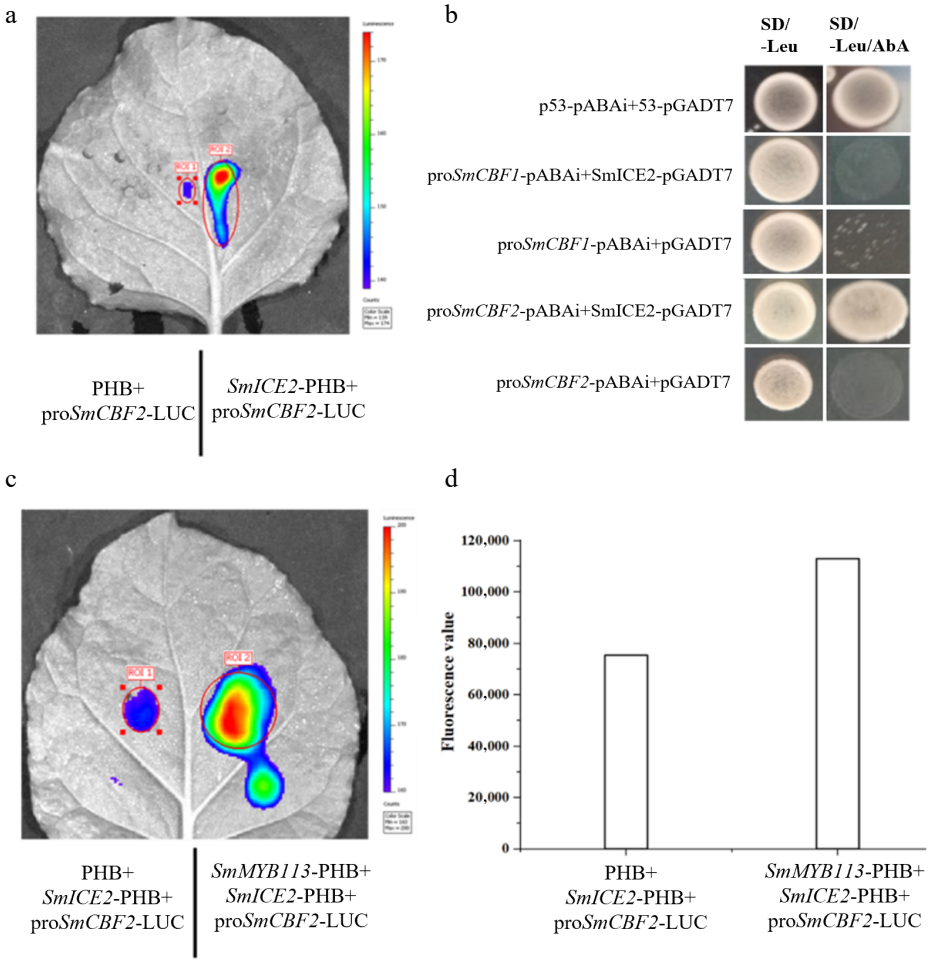

Fig. 5 SmMYB113-SmICE2 interaction enhances SmICE2-mediated transcriptional activation of *SmCBF2*. (a) LCI assay showing SmICE2 binding to and activating the *SmCBF2* promoter. (b) Y1H assay confirming SmICE2 activation of *SmCBF2* expression. (c) LCI assay demonstrating that co-expression of SmMYB113 and SmICE2 synergistically enhances luciferase activity driven by the *SmCBF2* promoter compared to SmICE2 alone. (d) Quantification of luminescence intensity from (c).

downstream cold-responsive genes AtCBF1/2, enhancing cold tolerance by increasing proline content, SOD, and CAT activity[43]. The present results are consistent: transcriptomic analysis showed significant changes in *ICE* and *CBF* expression in OE lines. Investigation of how *SmMYB113* participates in the ICE-CBF cascade revealed its interaction with SmICE2 and confirmed that SmICE2 activates *SmCBF2* expression. Further study demonstrated that the SmMYB113-SmICE2 interaction enhances SmICE2's transcriptional activation of *SmCBF2*.

In summary, this study reveals a novel mechanism by which *SmMYB113* enhances low-temperature adaptation in eggplant through the synergistic regulation of ABA biosynthesis and the ICE-CBF transcriptional cascade, forming a multi-layered network (Fig. 6). *SmMYB113* enhances cold tolerance by upregulating endogenous ABA, an effect effectively mimicked by exogenous ABA application. This provides a theoretical basis and application prospects for developing low-temperature tolerance stimulants based on ABA analogs or ABA signaling activators (e.g., for protected seedling production or early spring transplanting). For instance, Cao et al.[44] reported an ABA analog named AMF that significantly improved plant drought resistance. Future research could focus on screening highly efficient, low-cost active substances, and their optimal application protocols. Furthermore, plant stress responses often involve multiple signaling pathways. Future studies could explore whether the *SmMYB113*-mediated cold tolerance pathway interacts with other hormone signals (e.g., jasmonic acid, ethylene) or second messengers (e.g., Ca^{2+}, ROS), which would contribute to a more comprehensive understanding of the regulatory network underlying eggplant adaptation to low-temperature stress.

Conclusions

This study demonstrates that *SmMYB113* significantly enhances low-temperature tolerance in eggplant through a dual regulatory mechanism. First, SmMYB113 transcriptionally activates the ABA biosynthetic gene *SmAAO4-1*, increasing endogenous ABA accumulation. Second, SmMYB113 physically interacts with the transcription factor SmICE2, enhancing its ability to bind and activate the promoter of *SmCBF2*. Our findings reveal a multilayered regulatory role for SmMYB113 in coordinating phytohormone signaling and transcriptional reprogramming to enhance cold adaptation. This mechanism provides valuable targets for molecular breeding of cold-tolerant eggplant varieties.

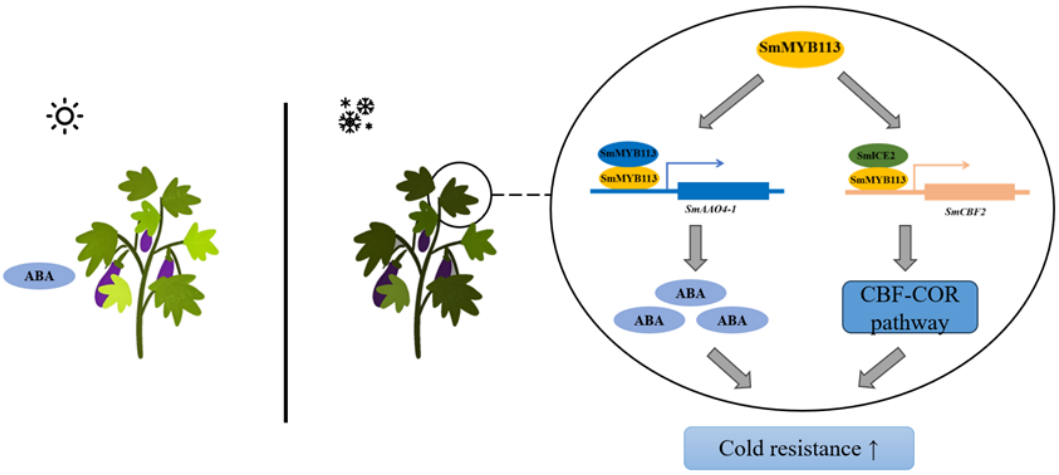

Fig. 6 Proposed model of *SmMYB113*-mediated cold tolerance in eggplant. Upon exposure to low temperature, *SmMYB113* enhances cold tolerance through two mechanisms: (1) Transcriptional activation of *SmAAO4-1*, promoting ABA biosynthesis; (2) Physical interaction with SmICE2, enhancing SmICE2's transcriptional activation of *SmCBF2*, thereby participating in the ICE-CBF pathway.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Yang F, Ji T, Li J; data collection: Yang G, Hu W, Li L, Liu W, Gao Y; analysis and interpretation of results: Yang G, Hu W, Ji T; draft manuscript preparation: Hu W, Yang G; draft review and editing: Yang F, Ji T. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw data of the sequence during the current study are available from the corresponding author upon reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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