

Article

The Transcription Factor *AcMYC2* Alleviates Chilling Injury by Improving Cold Resistance of Kiwifruit ‘Taishan 1’

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Abstract

Kiwifruit, classified as a respiratory climacteric fruit, faces challenges due to its limited resistance to storage and transportation. Although low-temperature storage is a cost-effective and widely used method, the cold injury it induces poses significant hurdles to industrial development. In this study, we selected ‘Taishan 1’, the dominant kiwifruit cultivar in Shandong Province, as the experimental material. Through transcriptome sequencing, we identified the key gene *AcMYC2*, which plays a crucial role in the kiwifruit’s response to low-temperature stress. Subsequently, virus-induced gene silencing (VIGS) was performed on ‘Taishan 1’ kiwifruit, and gene overexpression was validated in tomatoes. The results demonstrated that *AcMYC2* enhances cold tolerance in kiwifruit accompanied by multiple physiological processes, including antioxidant activity, lipid metabolism, and cell wall degradation. These findings offer significant insights into mitigating cold injury during low-temperature storage of kiwifruit and provide a theoretical foundation for advancing postharvest preservation techniques.

Keywords: kiwifruit (*Actinidia chinensis*); *AcMYC2*; low temperature storage; chilling injury

1. Introduction

Kiwifruit, a perennial climbing plant within the genus *Actinidia* Lindl. of the Actinidiaceae family, is renowned for its rich nutritional profile, including dietary fiber, vitamins, trace elements, alkaloids, and flavonoids, earning it the title of the ‘king of fruits’. China is home to a diverse array of kiwifruit resources. Globally, there are 54 species and 21 varieties of kiwifruit, with all but the Nepal and white-backed kiwifruits being cultivated in China [1]. Currently, the predominant method for post-harvest preservation of kiwifruit is low-temperature storage, which effectively delays fruit softening and prolongs shelf life. However, extended periods of low-temperature storage can lead to physiological and biochemical disorders, resulting in cold injury and a decline in fruit quality [2].

Low-temperature storage can decrease the respiration rate and slow down metabolism. Meanwhile, it inhibits ethylene synthesis by suppressing the activities of the ethylene precursor (ACC, 1-Aminocyclopropane-1-Carboxylate), ACC synthase, and ACC oxidase, thereby extending the shelf life of fruits [3]. Although existing techniques, such as 1-MCP (1-Methylcyclopropene) treatment, can inhibit ethylene synthesis [4], they are unable to specifically target and regulate the antioxidant system and lipid metabolism. Kiwifruit, as



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a climacteric fruit, experiences delayed ripening under low-temperature storage; however, chilling injury caused by its cold sensitivity, such as softening, browning, and off-odors, remains a significant bottleneck hindering industrial development [5]. Membrane lipid peroxidation [6], reactive oxygen species (ROS) accumulation [7], and cell wall degradation [8] triggered by chilling injury during low-temperature storage remain central research topics. Kim et al. discovered that the metabolism of primary phenolic compounds in kiwifruit are strongly influenced by temperature during postharvest storage, decreasing with rising storage temperature and duration [9]. Jeong et al. found that throughout the storage period, low-temperature treatment of kiwifruit effectively preserves the contents of total phenols and flavonoids, as well as its antioxidant capacity [10]. The *AaCBF4* gene in kiwifruit promotes soluble sugar accumulation and participates in cold injury response by regulating the expression of *AaBAM3.1* (β -amylase gene) [11]. Therefore, investigating the molecular mechanisms underlying cold tolerance in postharvest kiwifruit stored at low temperatures is crucial for reducing storage losses and promoting the healthy development of the kiwifruit industry.

Jasmonic acid (JA), a plant hormone derived from fatty acids as a hydroxylipin, plays a crucial role in plant growth, development, and stress responses [12]. In plants, MYC2 serves as a pivotal transcription factor within the JA signaling pathway, exerting regulatory effects by forming the COI/JAZs/MYC2 complex and participating in various hormone signal transduction processes [13]. MYC2 positively regulates *AchnFAR* and facilitates the ABA (Absciscic Acid)-mediated formation of primary alcohols during the wound-freezing process in kiwifruit [14]. *MdMYC2* interacts with the G-box in the *MdCBF1* promoter to modulate the freezing tolerance of apples [15]. Under MeJA (Methyl Jasmonate) mediation, the interaction between *MaMYC2s* and *MaICE1* enhances the cold tolerance of banana fruit and reduces the incidence of chilling injury [16]. The upregulation of MYC2 gene expression leads to the increased expression of genes encoding polyphenol oxidase, phenylalanine ammonia-lyase, and other resistance-related genes, thereby aiding the fruit in coping with external biological stress and effectively delaying fruit softening and decay [17].

Kiwifruit, as a climacteric fruit, presents additional challenges for storage and transportation due to its thin peel. Currently, low-temperature storage is the primary method for preserving kiwifruit. However, despite its benefits, this approach poses risks because kiwifruit is sensitive to cold and prone to chilling injury. Therefore, balancing the need to delay ripening while avoiding chilling damage is crucial for maintaining the fruit's quality during storage and transportation. Although the transcription factor MYC2 has been confirmed to play a crucial role in plant stress resistance, hormone signal transduction, and growth and development, the molecular mechanisms underlying kiwifruit's response to low-temperature storage have not been reported. Compared with 'Xuxiang' and 'Hongyang', the predominant kiwifruit cultivar 'Taishan 1' in Shandong Province, exhibits relatively poor storage tolerance and cold sensitivity, frequently suffering from chilling injury during low-temperature storage. Consequently, this study has chosen this cultivar for research purposes. Through combined transcriptomic and metabolomic analyses, the key gene *AcMYC2* responding to low-temperature stress was identified. Subsequently, its function and molecular mechanisms were investigated in both 'Taishan 1' kiwifruit and tomato fruits.

2. Results

2.1. Transcriptome Data Analysis

To identify genes associated with low-temperature stress in kiwifruit, transcriptome sequencing was conducted on 'Taishan 1' kiwifruit from three different treatment groups using the Illumina HiSeq system (Illumina Company, San Diego, CA, USA). A total of

52.0 G clean reads were obtained, with the effective data volume for each sample ranging from 5.96 G to 6.79 G. The Q20 base distribution ranged from 97.30% to 97.52%, the Q30 base distribution ranged from 92.63% to 93.02%, and the average GC content was 46.64% (Table S1). Differentially expressed genes in ‘Taishan 1’ kiwifruit fruits after low-temperature stress were identified through gene expression analysis using the DESeq2 (version 1.20.0) software. The screening criteria were $|\log_2(\text{Fold Change})| > 1$ and a $\text{padj} < 0.05$. The treatment conditions included: fresh fruit left untreated, fruit treated at 2 °C for 7 days, and fruit treated at 25 °C for 7 days (CK vs. CT vs. NT). The results revealed that a total of 22,480 genes were expressed in the kiwifruit across the three treatment groups. In the CT vs. NT comparison group, there were 10,202 differentially expressed genes, comprising 5011 up-regulated and 5191 down-regulated genes. In the CT vs. CK comparison group, 9186 differentially expressed genes were identified, including 4163 up-regulated and 5023 down-regulated genes. Meanwhile, in the NT vs. CK comparison group, 13,242 differentially expressed genes were found, with 5959 up-regulated and 7283 down-regulated genes (Figure 1A,B). Figure 1C showed the genes expression in partial transcriptome sequencing.

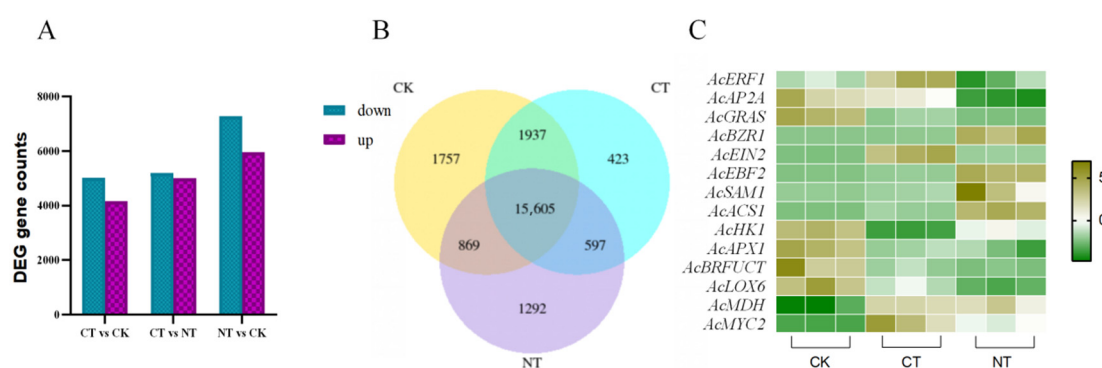


Figure 1. Analysis of differentially expressed genes. (A) Volcanic map of differentially expressed genes. (B) Venn diagram of differentially expressed genes. (C) Heatmap of differentially expressed gene clustering.

2.2. Analysis of DEGs Among Three Comparison Groups

In the CK versus CT comparison group, KEGG enrichment analysis revealed that the differentially expressed genes were primarily enriched in pathways related to plant hormone signal transduction, MAPK (Mitogen-Activated Protein Kinase) plant signaling pathways, zeatin biosynthesis, and phosphonate and hypophosphite metabolism (Figure 2A). GO functional classification indicated that, within cellular components (CCs), the enriched differentially expressed genes were involved in membrane-enclosed lumen and organelle lumen, while in molecular functions (MFs), they participated in DNA-binding transcription factor activity and transcription regulator activity (Figure 2B).

In the CT versus NT comparison group, KEGG enrichment analysis showed that the differentially expressed genes were mainly enriched in carbon metabolism, plant hormone signal transduction, amino acid biosynthesis, ribosome, and starch and sucrose metabolism pathways (Figure 2C). GO functional classification revealed that, in terms of molecular functions (MFs), differentially expressed genes were primarily enriched in transcriptional regulator activity, glycosyltransferase activity, transmembrane transporter activity, and so on. Regarding biological processes (BPs), these genes were mainly concentrated in cellular responses to stimuli, lipid metabolism processes, ion transport, and other processes. Within the cellular components (CCs) category, differentially expressed genes were predominantly enriched in organelle parts, intracellular organelle parts, non-membrane-bound organelles, and other components (Figure 2D).

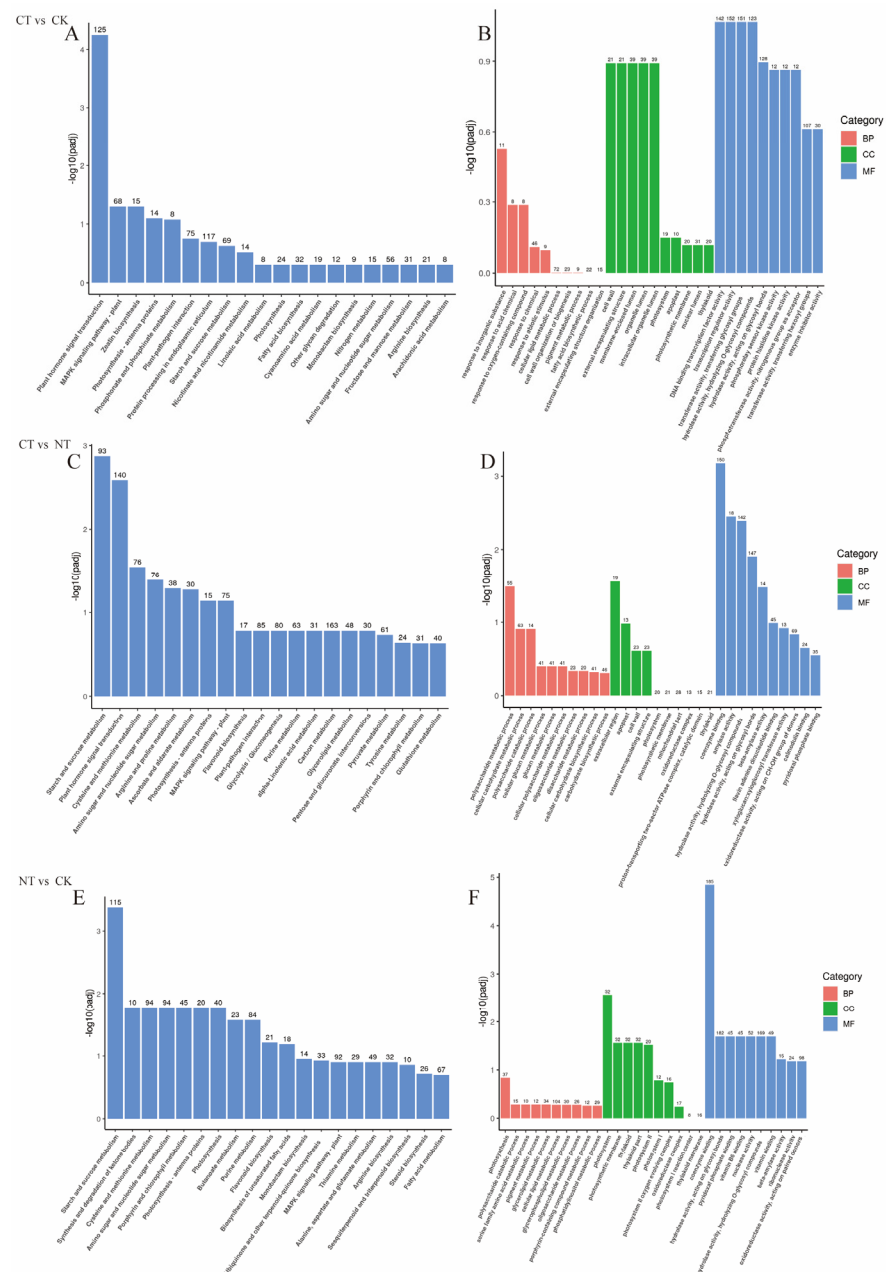


Figure 2. Histogram of the enrichment of the three differentially expressed genes KEGG and GO. The Y-axis represents the significance level of term enrichment, denoted as $-\log_{10}(\text{padj})$. Different colors indicate distinct functional classifications, and the numerical values atop the bars in the histogram represent the gene counts. **(A)** Histogram of KEGG enrichment analysis of differentially expressed genes in CT vs. CK. **(B)** Histogram of GO enrichment analysis of differentially expressed genes in CT vs. CK. **(C)** Histogram of KEGG enrichment analysis of differentially expressed genes in CT vs. NT. **(D)** Histogram of GO enrichment analysis of differentially expressed genes in CT vs. NT. **(E)** Histogram of KEGG enrichment analysis of differentially expressed genes in NT vs. CK. **(F)** Histogram of GO enrichment analysis of differentially expressed genes in NT vs. CK.

In the comparison group of NT and CK, KEGG enrichment analysis indicated that the differentially expressed genes were mainly enriched in pathways such as starch and sucrose metabolism, ketone body synthesis and degradation, and cysteine and methionine metabolism (Figure 2E). GO functional analysis further revealed that, in biological processes (BPs), differentially expressed genes were primarily enriched in cellular lipid metabolism processes, while in molecular functions (MFs), they were mainly enriched in coenzyme binding. Additionally, we identified several differentially expressed genes

encoding transcription factors (TFs), among which the *MYC2* transcription factor exhibited significant upregulation after low-temperature storage and slight upregulation after room temperature storage (Figure 2F).

2.3. Transcriptome Data Validation

To verify the accuracy of differential gene expression levels in the transcriptome data, we selected the differentially expressed genes *AcMYC2*, *AcEIN2*, *AcERF1*, *AcJAR1*, *AcJAZ1*, *AcEBF1*, along with the internal reference gene *Actin*, for qRT-PCR relative expression analysis (Figure 3). The expression of all six genes was influenced by storage conditions. Specifically, the expression levels of *AcEIN2*, *AcEBF1*, and *AcERF1* were significantly up-regulated compared to CK following low-temperature treatment and exhibited an even greater up-regulation after storage at 25 °C. *AcJAR1* and *AcMYC2* also showed significant upregulation compared to CK after low-temperature treatment, but *AcMYC2* also demonstrated a certain degree of upregulation after treatment at 25 °C. Additionally, the expression level of *AcJAZ1* was significantly down-regulated compared to CK after low-temperature treatment. These findings were consistent with the expression trends observed in the transcriptome data.

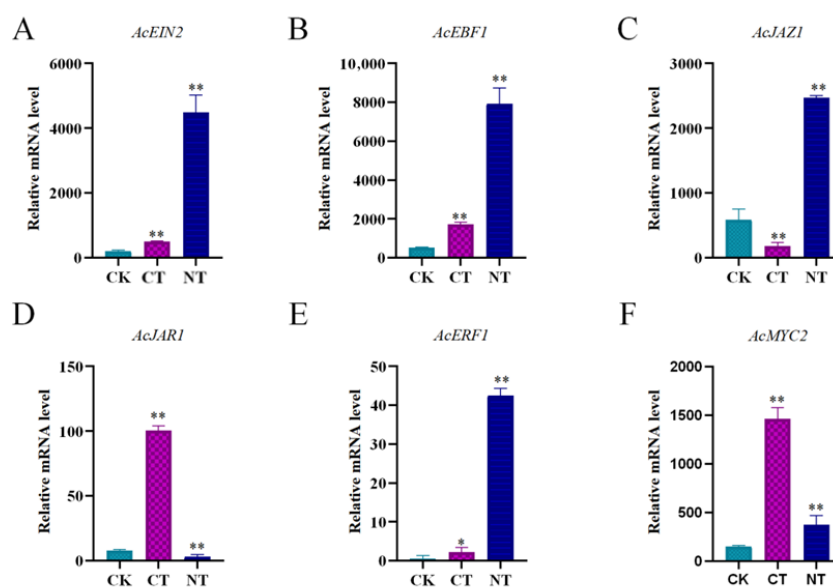


Figure 3. Differential expressed genes qRT-PCR validation (* $p < 0.05$; ** $p < 0.01$, t -test).

2.4. Silencing *AcMYC2* Accelerates the Softening Process of Kiwifruit Under Low-Temperature Storage Conditions

Using TRV-mediated virus-induced gene silencing technology, kiwifruit fruits were transiently silenced. With wild-type (WT) as the control group, the relative expression level of the *AcMYC2* gene in the silencing group decreased by 61.75% (Figure S1). The changes in fruit storability are closely related to the metabolism of cell wall substances. In this study, the soluble protein content, soluble pectin mass fraction, protopectin mass fraction, and polygalacturonase (PG) activity were measured in fruits from the control group (WT), the empty vector group (TRV2), and the silencing group (*AcMYC2*-silenced) after being treated at 25 °C and 2 °C for 7 days, respectively (Figure 4). At 2 °C, compared with the empty vector group, the soluble protein content in the *AcMYC2*-silenced kiwifruit increased by 10.1%, PG activity increased by 22.6%, and the soluble pectin mass fraction increased by 35.2%. These data indicate that reduced *AcMYC2* expression can accelerate kiwifruit softening.

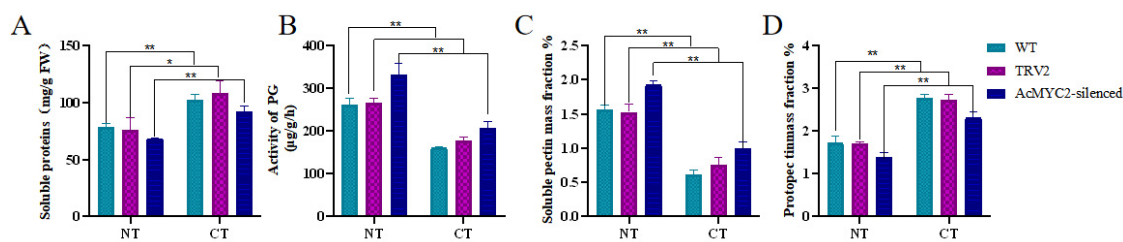


Figure 4. Determination of physiological markers of fruit softening. (A) Soluble protein content, (B) PG activity, (C) Soluble pectin fraction, (D) Mass fraction of protosoluble pectin. CT represents 7 days of storage at 2 °C, and NT represents 7 days of storage at 25 °C (* $p < 0.05$; ** $p < 0.01$, t -test).

2.5. Silencing *AcMYC2* Reduces the Antioxidant Enzyme Activity in Kiwifruit Under Low-Temperature Storage Conditions

SOD (Superoxide Dismutase), POD (Peroxidase), and APX (Ascorbate Peroxidase) are antioxidant enzymes present in fruits, playing a crucial role in scavenging reactive oxygen species within the fruit and maintaining the normal physiological metabolism of kiwifruit. To explore whether *AcMYC2* enhances antioxidant enzyme activity to eliminate the accumulation of reactive oxygen species under low-temperature storage conditions, we measured the antioxidant enzyme activities of wild-type (WT), empty-vector control (no-load group), and *AcMYC2*-silenced (silence group) kiwifruit before and after treatment at 2 °C for 7 days (Figure 5). Compared with the fruits in the no-load group, the POD, SOD, and APX activities in the *AcMYC2*-silenced kiwifruit decreased by 12.0%, 14.5%, and 11.2%, respectively, while the hydrogen peroxide content significantly increased by 28.6%. These findings indicate that silencing *AcMYC2* significantly inhibits antioxidant enzyme activity and substantially elevates hydrogen peroxide levels in ‘Taishan 1’ kiwifruit under low-temperature conditions.

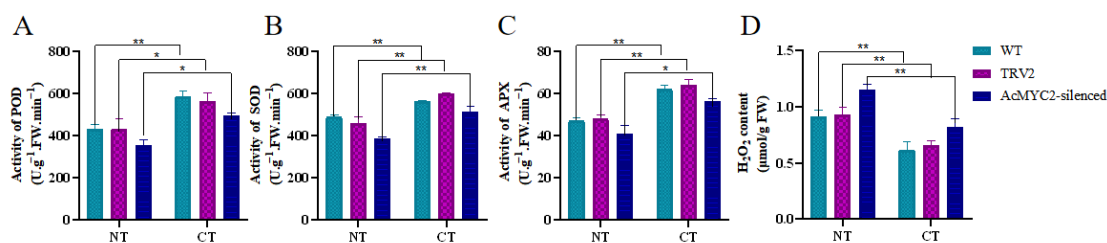


Figure 5. Antioxidant enzyme activity and H₂O₂ accumulation in the control and silent groups under two storage conditions. (A) POD activity, (B) SOD activity, (C) APX activity, (D) H₂O₂ content. CT represents 7 days of storage at 2 °C, and NT represents 7 days of storage at 25 °C (* $p < 0.05$; ** $p < 0.01$, t -test).

2.6. Generation of *AcMYC2*-Overexpressing Tomato Lines

To investigate the specific function of the *AcMYC2* gene, the constructed *AcMYC2*-GFP vector was transformed into *Agrobacterium tumefaciens* LBA4404 and subsequently used for tomato genetic transformation (Figure 6A). Genomic DNA was extracted from both wild-type and transgenic tomato plants, amplified by PCR, and identified through agarose gel electrophoresis, resulting in the identification of seven overexpression lines. To screen for the desired experimental materials, RNA was extracted from WT and the identified positive tomato seedlings, and their transcriptional expression levels were detected by qRT-PCR. It was observed that the expression levels of *OE1*, *OE2*, and *OE3* were higher compared to those of the other lines (Figure 6B,C).

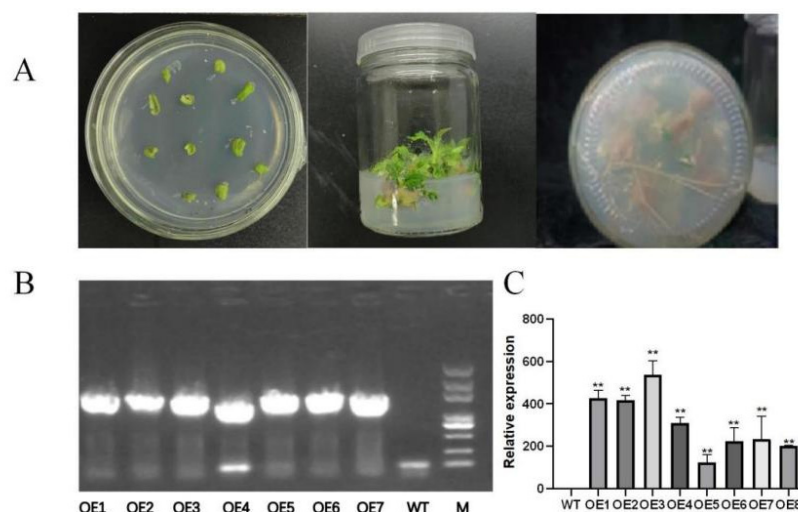


Figure 6. Acquisition and screening of *AcMYC2* overexpressing lines. (A) Genetic transformation of *AcMYC2* tomato, (B) PCR analysis of *AcMYC2* overexpressing lines, (C) Detection of transcription level in overexpressed lines (** $p < 0.01$, t -test).

2.7. Effects of Overexpressing *AcMYC2* on the Physiological Indices of Tomato Fruits During Low-Temperature Storage

After 10 days of the color-breaking period, wild-type (WT), GFP-empty, and OE-overexpressing tomato fruits were stored at 25 °C and 2 °C for 7 days, respectively. A CTX texture analyzer was employed to measure the hardness of tomato fruits subjected to both low-temperature and normal-temperature treatments. The results indicated that the hardness of tomato fruits under low-temperature treatment was significantly higher than that under normal-temperature storage (Figure 7A). The contents of soluble pectin, protosoluble pectin, and the activity of polygalacturonase (PG) were measured in WT, GFP-empty, and OE-overexpressing *AcMYC2* groups before and after low-temperature storage. At 2 °C, compared with the GFP group, the PG activity in the OE group was significantly reduced by over 21.5% (Figure 7B), the soluble pectin content decreased by 28.0% (Figure 7C), and the protosoluble pectin content increased by 17.3% (Figure 7D). These findings suggest that overexpression of *AcMYC2* enhances the firmness of tomato fruits, inhibits the degradation of protopectin, and delays fruit softening under low-temperature conditions.

Compared to normal temperature treatment, low temperature treatment significantly elevated the activities of SOD, POD, and CAT (Catalase) in tomato fruits (Figure 8A–C) and markedly reduced the H₂O₂ content in them (Figure 8D). Under low-temperature conditions, the activities of SOD, POD, and CAT in overexpressed tomato fruits were 22.9%, 15.8%, and 26.1% higher, respectively, than those in GFP control fruits. These findings indicate that *AcMYC2* can prolong the storage duration of tomato fruits by enhancing the activity of antioxidant enzymes and decreasing H₂O₂ content.

The lycopene content in WT, empty-vector, and *AcMYC2*-overexpressing tomato fruits was measured before and after low-temperature treatment (Figure 9). The results indicated that, across all groups, the lycopene content in tomato fruits subjected to low-temperature treatment was higher than that in fruits stored at room temperature. Low-temperature storage effectively slowed down the decline in lycopene content. Moreover, the lycopene content in the overexpressed transgenic tomato fruits was 22.7%, 26.2%, and 24.5% higher than that in the GFP group, respectively.

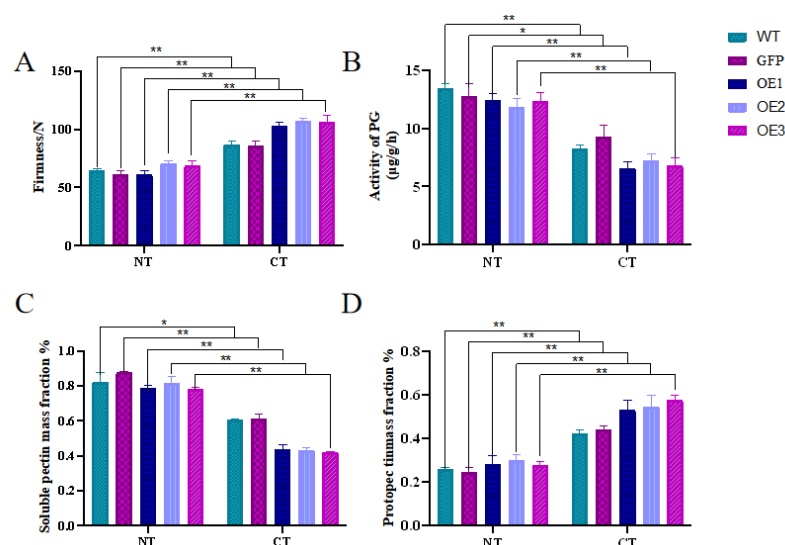


Figure 7. Determination of physiological markers of fruit softening. (A) Fruit hardness, (B) PG activity, (C) Soluble pectin mass fraction, (D) Mass fraction of protosoluble pectin. CT represents 7 days of storage at 2 °C, and NT represents 7 days of storage at 25 °C (* $p < 0.05$; ** $p < 0.01$, t -test).

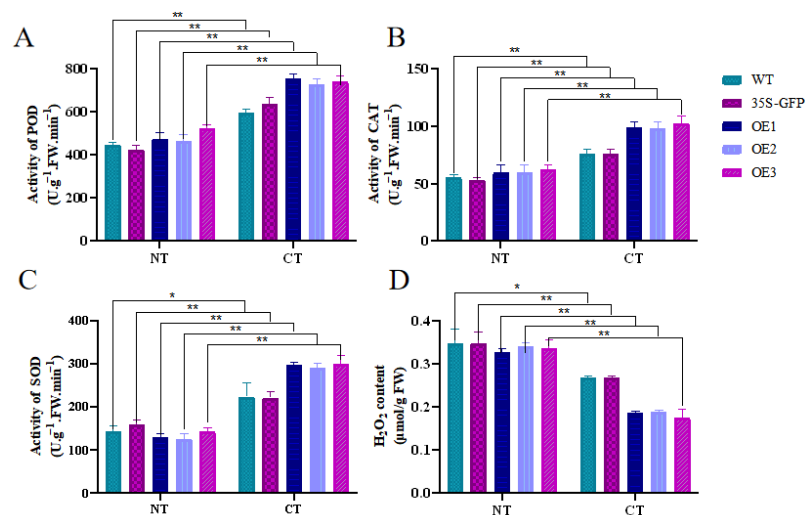


Figure 8. Antioxidant enzyme activity and H_2O_2 accumulation in tomato fruit under two storage conditions. (A) POD activity, (B) CAT activity, (C) SOD activity, (D) H_2O_2 content. CT represents 7 days of storage at 2 °C, and NT represents 7 days of storage at 25 °C (* $p < 0.05$; ** $p < 0.01$, t -test).

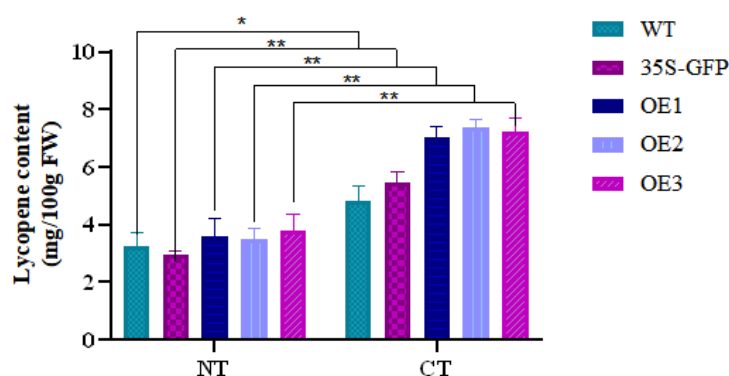


Figure 9. Changes in lycopene content in tomato fruits under two storage conditions. CT represents 7 days of storage at 2 °C, and NT represents 7 days of storage at 25 °C (* $p < 0.05$; ** $p < 0.01$, t -test).

3. Discussion

Kiwifruit is classified as a respiratory climacteric berry, making it challenging to store and transport. Low-temperature storage can effectively delay the onset of the respiratory peak. Consequently, this method is frequently employed to prolong the storage period and minimize postharvest losses, although kiwifruit is prone to chilling injury [18]. A total of 22,480 differentially expressed genes were identified in the transcriptome sequencing results of kiwifruit subjected to untreated, 2 °C, and 25 °C conditions (CK vs. CT vs. NT). KEGG and GO analyses revealed that these differential genes were predominantly enriched in pathways related to plant hormone signal transduction, carbon metabolism, and starch and sucrose metabolism, all of which are closely associated with the fruit's adaptive response to low temperatures. Sequencing indicated a significant upregulation of *AcMYC2* in kiwifruit following low-temperature treatment, a finding corroborated by qRT-PCR. It is hypothesized that the upregulation of *AcMYC2* may enhance the fruit's cold tolerance by promoting the antioxidant system and regulating lipid metabolism.

MYC2 serves as a switch within the JA signaling pathway and plays a pivotal role in JA-regulated secondary metabolism, defense responses, and the growth and development of fruits [19]. POD, SOD, CAT, and APX are crucial antioxidant enzymes in kiwifruit, which help maintain the balance of reactive oxygen species (ROS) within plants, extend the storage duration of kiwifruit, and enhance their resistance to chilling injury [20]. In this study, kiwifruit with silenced *AcMYC2* and tomato fruit with overexpressed genes were subjected to low-temperature storage experiments. It was observed that the kiwifruit with silenced *AcMYC2* exhibited lower antioxidant enzyme activity and higher ROS content, whereas the overexpressed tomato fruit demonstrated enhanced activities of POD, CAT, and SOD following low-temperature treatment. Measurement of H₂O₂ content revealed a reduced accumulation in the overexpressed tomato fruit, indicating that the significant increase in enzyme activity inhibited ROS accumulation in transgenic tomato fruit during low-temperature storage. Similarly, elevated *SIMYC2* expression augmented the activity of antioxidant enzymes in tomato fruit, bolstered the cold resistance of transgenic tomato fruit, and decreased both the incidence and severity of chilling injury, aligning with the findings of this study [21].

Research has revealed that under low-temperature storage conditions, *PpCBF2* inhibits the activity of polygalacturonase (PG) in peaches by interacting with *PpCBF3*, thereby delaying fruit softening [22]. Low temperature inhibits fruit softening in pears by suppressing pectinase activity [23]. In this study, low-temperature treatment significantly reduced both polygalacturonase activity and soluble pectin content in the fruits. After low-temperature treatment, the PG activity and soluble pectin content in kiwifruit from the *AcMYC2*-silenced group were significantly higher than those in the empty vector control group, whereas in tomatoes overexpressing *AcMYC2*, the PG activity and soluble pectin content were significantly lower than those in the control group.

Lycopene exhibits antioxidant properties and can influence cell growth, differentiation, and stress responses by modulating signal pathways and gene expression within plants. The external application of lycopene can enhance the cold tolerance of grapefruit fruits [24]. Treatment with MeJA and overexpression of *SIMYC2* inhibited the growth and photosynthesis of tomato seedlings, yet increased the contents of lycopene, carotenoids, soluble sugars, total phenols, and flavonoids, suggesting that JA signal transduction can suppress the growth of tomato seedlings and alter the fruit quality of tomatoes [25]. Our findings indicate that, under low-temperature conditions, the lycopene content in tomato fruits overexpressing *AcMYC2* was significantly higher than that in the control group.

4. Materials and Methods

4.1. Plant Materials and Growth Conditions

The ‘Taishan 1’ kiwifruit was used as the experimental material and harvested when its soluble solids content reached 8%. The sampling site was the Taidong Experimental Base of the Shandong Institute of Pomology (36°12′ 34.15″ N, 117°09′ 35.25″ E), which has an average annual rainfall of 697 mm, an annual sunshine duration of 2226 h, and a frost-free period of 205 days. Each sample group consisted of three biological replicates, with five fruits per replicate. The fresh fruits were left untreated (CK), treated at 25 °C for 7 days (NT), and treated at 2 °C for 7 days (CT). Subsequently, the pulp was separated using a blade, rapidly frozen in liquid nitrogen, and stored in a −80 °C freezer. The tomatoes (*Solanum lycopersicum*, Micro Tom) [26] utilized in this study were cultivated in a 25 °C greenhouse under a 16 h light/8 h dark cycle and a relative humidity of 60–75%.

4.2. Bacterial Strains and Vectors

The *Escherichia coli* strain used in the experiment was Trans T1; the *Agrobacterium strains* used were GV3101 [27] and LBA4404 [28]. The plant expression vectors employed included pTRV1, pTRV2, pZP211-GFP, etc.; the cloning vector used was pEASY-Blunt (simple).

4.3. Biochemical Reagents and Enzymes

The RNAprep Pure Polysaccharide Polyphenol Plant Total RNA Extraction Kit (DP441), Polysaccharide Polyphenol Plant Genomic DNA Extraction Kit (DP360), Reverse Transcription and Quantification Kit, Agarose Gel DNA Recovery Kit, and Rapid Plasmid Mini-Prep Kit were purchased from Tiangen Biotech (Beijing, China); the DNA Marker was purchased from Tiangen Biotech (Beijing, China); the high-fidelity DNA polymerase was sourced from Vazyme Biotech (Nanjing, China); and T4 DNA ligase and various restriction endonucleases were obtained from Thermo Fisher Scientific, Wilmington, DE, USA. The kits for detecting the activities of peroxidase, catalase, and superoxide dismutase were purchased from Gris Biotechnology, Suzhou, China.

4.4. Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

Based on the differences in gene expression levels among samples, representative differentially expressed genes were selected for qRT-PCR validation. Using sequences provided by the kiwifruit genome database, primers were designed via the online website (<https://www.primer3plus.com/>). With *AcActin* serving as the internal reference gene, cDNA was synthesized from the samples using the Vazyme reverse transcription kit. The diluted cDNA (threefold dilution) was then used as the template for qRT-PCR with Vazyme’s ChamQ Universal SYBR qPCR Master Mix kit (Nanjing, China). The results were calculated using the $2^{-\Delta\Delta C_t}$ method. All primers used in this experiment were synthesized by Sangon Biotech (Shanghai, China), with specific primer sequences detailed in Table S2.

4.5. Transcriptome Sequencing and Analysis

Transcriptome sequencing was performed using the Illumina HiSeq system (Illumina Company, San Diego, CA, USA), with fastp (version 0.19.7) employed for quality control. HISAT2 (version 2.2.1) software was utilized to rapidly and accurately align the clean reads against the ‘*Actinidia chinensis* (Hong Yang) v3’ [29] reference genome (<http://kiwifruitgenome.org/>), thereby obtaining the positional information of reads on the reference genome. The filtering criteria included the removal of reads with adapters, reads containing N bases, and low-quality reads. Additionally, DESeq2 (version 1.34.0) software was applied to analyze differential gene expression, with the screening criteria set as $|\log_2(\text{Fold Change})| > 1$ and $\text{padj} < 0.05$.

4.6. Virus Silencing Kiwifruit

The TRV-mediated virus silencing technique was employed [30]. Sixty ‘Taishan 1’ kiwifruit fruits, each with a soluble solids content of 8%, were selected and evenly divided into three groups to serve as infection materials. A disposable sterile syringe (1 mL) was utilized to inject the aforementioned bacterial suspension, with 100 µL evenly administered from two horizontal directions perpendicular to the fruit’s central axis, at an injection depth of approximately 2 cm. Fruits injected with sterile water and those with an empty vector served as negative controls. Repeated injections were performed at the same pinhole location on the fruit every other day, for a total of three injections. All kiwifruit were placed in a 25 °C constant-temperature incubator in the dark, with the relative humidity maintained at 70%. After seven days, intact fruits were selected, and samples of kiwifruit pulp surrounding the injection holes were collected for qRT-PCR analysis and the measurement of other physiological indicators.

4.7. Data Statistics and Analysis

All data are presented as the mean $\pm$ standard deviation derived from three biological replicates. The graphs were generated using Excel and GraphPad Prism 8.0.2 software, and significant differences in the data were analyzed using a *t*-test, with * indicating $p < 0.05$ and ** indicating $p < 0.01$. Additionally, the raw sequence data generated for this research have been submitted to the Genome Sequence Archive in the National Genomics Data Center, China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA of transcriptome sequencing data: CRA038828).

5. Conclusions

Illumina HiSeq high-throughput sequencing technology was applied to analyze low-temperature-stored kiwifruit, revealing that carbon metabolism, plant hormone signal transduction, and other pathways are crucial in regulating the fruit’s response to low-temperature storage. Through gene silencing and overexpression of *AcMYC2*, it was discovered that *AcMYC2* could enhance antioxidant enzyme activity, delay fruit softening, and thereby reduce chilling injury in kiwifruit under low-temperature storage conditions. The function of *AcMYC2* in low-temperature storage offers new insights into the mechanism of kiwifruit’s response to chilling injury and provides a theoretical foundation for improving fruit cold tolerance through genetic regulation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants15050759/s1>, Table S1: Sequencing quality control results. Table S2: PCR primers. Figure S1: *AcMYC2*-silenced fruit transcription level validation (* $p < 0.05$; ** $p < 0.01$, *t*-test). Note: WT served as the control group, TRV2 as the empty vector group, and *AcMYC2*-silenced as the TRV-mediated virus silencing group.

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Abbreviations

The following abbreviations are used in this manuscript:

VIGS	Virus-induced gene silencing
ACC	1-Aminocyclopropane-1-Carboxylate
JA	Jasmonic Acid
MeJA	Methyl Jasmonate
ABA	Absciscic Acid
MAPK	Mitogen-Activated Protein Kinase
qRT-PCR	Quantitative Reverse Transcription PCR
PG	Polygalacturonase
SOD	Superoxide Dismutase
POD	Peroxidase
APX	Ascorbate Peroxidase
CAT	Catalase
1-MCP	1-Methylcyclopropene

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