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Research Article

The Effects of Chilling on Antioxidant Enzyme System and Related Gene Expression Levels in Sweet Corn Seeds with Different Germination Characteristics

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Abstract

Sweet corn is a vegetable and grain dual-use crop with high economic value and industrial advantages. Low temperature stress significantly reduces the germination rate of sweet corn seeds, which has a negative impact on both quality and yield. This study used the chilling sensitive sweet corn inbred line 20hi111 and the chilling tolerant sweet corn inbred line T135 as experimental materials to measure the MDA (malondialdehyde) and H_2O_2 content, CAT, POD, and SOD enzyme activities, and enzyme gene expression patterns during seed germination under low temperature (10 ℃) and normal temperature (25 ℃) treatments. The research results indicated that during low-temperature germination, the H_2O_2 content and CAT activity of 20hi111 were generally higher than those of T135, while the POD and SOD activities and MDA content were generally lower than those of T135. There was no strict consistency between gene expression and enzyme activity. At low temperature, the expression of *ZmCAT1* and *ZmCAT3* in 20hi111 was significantly higher than T135, while there was no significant difference in *ZmPOD1*. In 20hi111, *ZmPOD3* was first lower and then higher than T135, and *ZmSOD3* and *ZmSOD9* were lower than T135 under low temperature treatment. In this study, the activity of antioxidant enzymes and the expression of antioxidant enzyme-related genes in sweet corn inbred lines with different germination characteristics under low temperature were analyzed, which provided some theoretical basis for cultivating sweet corn varieties with low temperature tolerance.

Keywords

Sweet Corn, Germination, Low Temperature, Antioxidant Enzyme Activity

1. Introduction

Sweet corn (*Zea mays* convar. *saccharata*) is widely planted worldwide and is a new crop that integrates vegetables, fruits, grains, and feed [1]. As sweet corn is a warm loving crop, its growth and development process are sensitive to low temperature [2]. Due to the short growth cycle and high multiple cropping index of sweet corn, people usually choose staggered planting in order to increase economic value. Therefore, early spring, late autumn, and winter planting will be affected by low temperature, resulting in seedling shortages, dead seedlings, weak growth, and other phenomena, affecting yield and quality [3, 4]. It is of great significance in production to study the low-temperature germination of sweet corn.

Low temperature stress can disrupt the balance of reactive

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oxygen species and normal metabolic system in cells during the early stages of crop germination, affecting the germination process of seeds [5]. The antioxidant pathway, as one of the important regulatory mechanisms for improving crop tolerance to low temperature stress, reduces the damage of cell membranes to reactive oxygen species (ROS) by regulating the production and activity of antioxidant enzymes [6]. The enzymatic defense system in plants includes antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which can directly or indirectly regulate the production and elimination of ROS [7]. The decrease in temperature causes changes in the activity and quantity of antioxidant enzymes in corn, leading to an imbalance in ROS content and affecting plant growth and development. Studies have shown that low temperatures can increase the content of antioxidant enzymes in maize, but decrease enzyme activity, and there are significant differences among different varieties [8]. The effect of low temperature on sweet corn is much greater than that on glutinous corn, and the activities of SOD and POD are significantly reduced [9]. The transcription levels of antioxidant enzymes in two different genotypes of maize under low temperature stress were measured. The results showed that the expression of key antioxidant enzymes in maize varieties tolerant to low temperature stress was significantly enhanced under low temperature conditions to cope with environmental discomfort [10].

In our preliminary research, we conducted low-temperature germination experiments on 100 sweet corn inbred lines and ultimately selected a chilling sensitive inbred line 20hi111 and a chilling tolerant inbred line T135. The germination rate of 20hi111 is as high as 92.2% under room temperature treatment (RT), but it does not germinate under low temperature treatment (LT). T135 has a germination rate of 100% under RT and the highest germination rate among 100 inbred lines under LT, reaching up to 94.4%. Meanwhile, through whole genome association analysis, we have identified several genes related to antioxidant activity that may play a role in the temperature tolerant germination of sweet corn [11]. On this basis, we conducted physiological indicators related to the antioxidant enzyme system on two sweet corn inbred lines with different germination characteristics in this study, and measured the expression pattern of antioxidant enzyme genes, in order to explore the physiological and molecular mechanisms of low-temperature germination of sweet corn and provide technical reference for the development and production of chilling tolerant sweet corn varieties.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Sweet corn inbred line T135 and 20hi111 were used as the experimental materials. Seeds of consistent size and plump grain shape are selected. After soaking and disinfecting in 0.2% NaCl solution for 20 minutes, the seeds were rinsed clean with distilled water. Thirty seeds from each inbred line were placed in sterile culture dishes with three layers of filter paper, and then place them in artificial climate chambers at 10 ℃ (low temperature) and 25 ℃ (room temperature) under dark conditions. An appropriate amount of distilled water was regularly added. Each process was repeated three times. At room temperature, samples were taken on days 0, 1, 2, 3, and 4 after germination began. And at low temperature, samples were taken on days 0, 1, 3, 5, 7, 10, and 14 after germination began. Approximately 3g of seeds were collected each time, which were repeated three times and were stored at -80 ℃ for subsequent experiments.

2.2. Extraction of Total RNA and Quantitative Real-time PCR Analysis (qRT-PCR)

ZmSOD3, *ZmSOD9*, *ZmPOD1*, *ZmPOD3*, *ZmCAT1*, and *ZmCAT3* are six antioxidant enzyme genes screened by transcriptome sequencing [12]. Samples were taken at 0, 1, 3, and 5 days after germination at 10 ℃ (low temperature) and 25 ℃ (room temperature) for qRT-PCR detection. Quick RNA Isolation kit (Huayueyang Biotech, Beijing, China) and *TransScript*® Uni All-in-One First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China) were used for total RNA extraction and reverse transcription synthesis of cDNA. Primer 5.0 was used for primer design (Table 1). qRT-PCR was carried out with Taq Pro Universal SYBR qPCR Master Mix (Vazyme Bio, Nanjing, China) and the QuantStudio 5 Real-Time PCR Instrument (Thermo Fisher Scientific, Waltham, MA, USA). The amplification programs were 95 ℃ for 30 s, followed by 40 cycles at 95 ℃ for 10 s, 60 ℃ for 10 sec, and 72 ℃ for 15 s. *Zmubiquitin2* was used as internal reference gene. The $2^{-\Delta\Delta CT}$ method was used as data analysis.

Table 1. *The primer list.*

Primer name	Primer sequence (5'-3')
qubi2-F	TGGTTGTGGCTTCGTTGGTT
q*ubi2*-R	GCTGCAGAAGAGTTTTGGGTACA
q*ZmCAT1*-F	GAGATCCAAATGGTACGGTATT
q*ZmCAT1*-R	CAGGCTGTCGTGAGAAGTGC
q*ZmCAT3*-F	GGGAGAAGGCAACCATAC
q*ZmCAT3*-R	TGGGTGTCCGAGCGAGT
q*ZmPOD1*-F	GCCTCCACTTCCACGACT
q*ZmPOD1*-R	CTTCTTCTCCGACAACCAGC
q*ZmPOD3*-F	CATCATCGGTGGCACTAAC
q*ZmPOD3*-R	ACTTTCAGGACCAAGTTTGC

Primer name	Primer sequence (5'-3')
q*ZmSOD3*-F	TTTGGTTCATTTGAGGCACT
q*ZmSOD3*-R	CTCCCAGATCAATCCC
q*ZmSOD9*-F	CGGAGACATCGTGAAATAAA
qZmSOD9-R	CCACAAAGCACATCGAAAC

2.3. Determination of SOD, CAT, POD Activity, and MDA, H_2O_2 Content

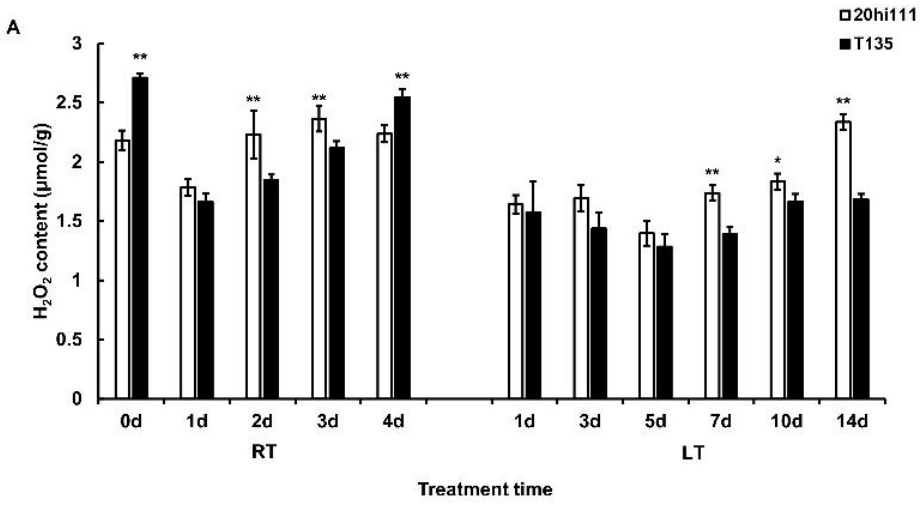
The determination of enzyme activities and substance contents related to the antioxidant system were carried out using reagent kits (Soleibao Biotechnology Co., Ltd., Beijing, China). The determination of SOD was carried out using a superoxide dismutase (SOD) activity detection kit (Item number: BC0175). The determination of CAT was carried out using a Catalase (CAT) detection kit (Item number: BC0205). The determination of POD was carried out using a Peroxidase (POD) detection kit (Item number: BC0095). The determination of MDA was carried out using a Malondialdehyde (MDA) content detection kit (Item number: BC0025). The determination of H_2O_2 was carried out using a Hydrogen peroxide (H_2O_2) content detection kit (Item number: BC3595). According to the manufacturer's instructions, the sample was weighed 1g, added with 10mL of extraction solution, homogenized in an ice bath, centrifuged at 8000g for 1 minute at 4 ℃, and the supernatant was taken. Reagents were added to a 96 well plate according to the steps in the kit, using a microplate reader to read the absorbance values at the wavelengths corresponding to each indicator, and calculate the CAT, POD, SOD enzyme activities and MDA, H_2O_2 content based on the absorbance values.

3. Results

3.1. The Effect of Chilling on H_2O_2 Content and CAT Activity During the Germination of Sweet Corn Seeds

The 20hi111 is a chilling-sensitive sweet corn inbred line, while T135 is a chilling-tolerant sweet corn inbred line. Under RT (room temperature treatment), the H_2O_2 content of 20hi111 and T135 first decreased and then increased, both of which began to rise on the second day. The H_2O_2 content of T135 was higher than 20hi111 on days 2 and 3, lower than 20hi111 on days 0 and 4, and showed no difference on day 1. Under LT (low temperature treatment), the H_2O_2 content of 20hi111 and T135 also decreased first and then increased, both starting to rise on the 7th day. The H_2O_2 content of T135 showed no difference compared to 20hi111 before the 5th day, but significantly increased from the 7th day onwards (Figure 1A).

Under RT, the CAT activity of 20hi111 and T135 first increased and then decreased. Overall, the CAT activity of 20hi111 was higher than that of T135, only lower on the second day. Under LT, the CAT activity of 20hi111 and T135 gradually increased, with 20hi111 being significantly higher than T135. It can be summarized that the H_2O_2 content and CAT activity increased with prolonged germination time, with significantly higher levels observed in 20hi111 compared to T135 (Figure 1B). Interestingly, we found that the H_2O_2 content and CAT activity in 20hi111 were both higher than those in T135, which may be related to the promotion of CAT activity by H_2O_2.



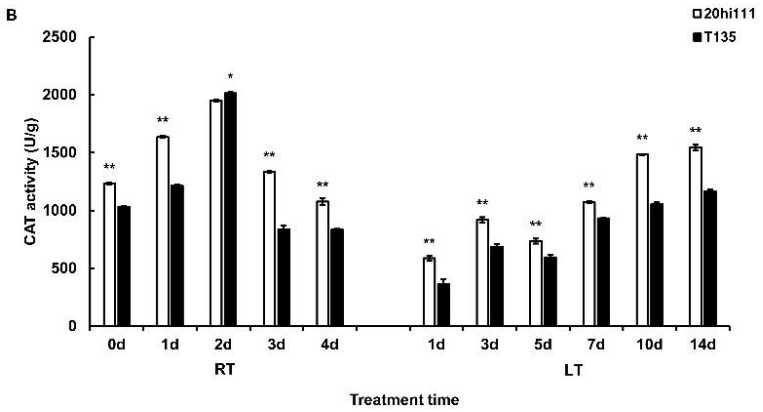
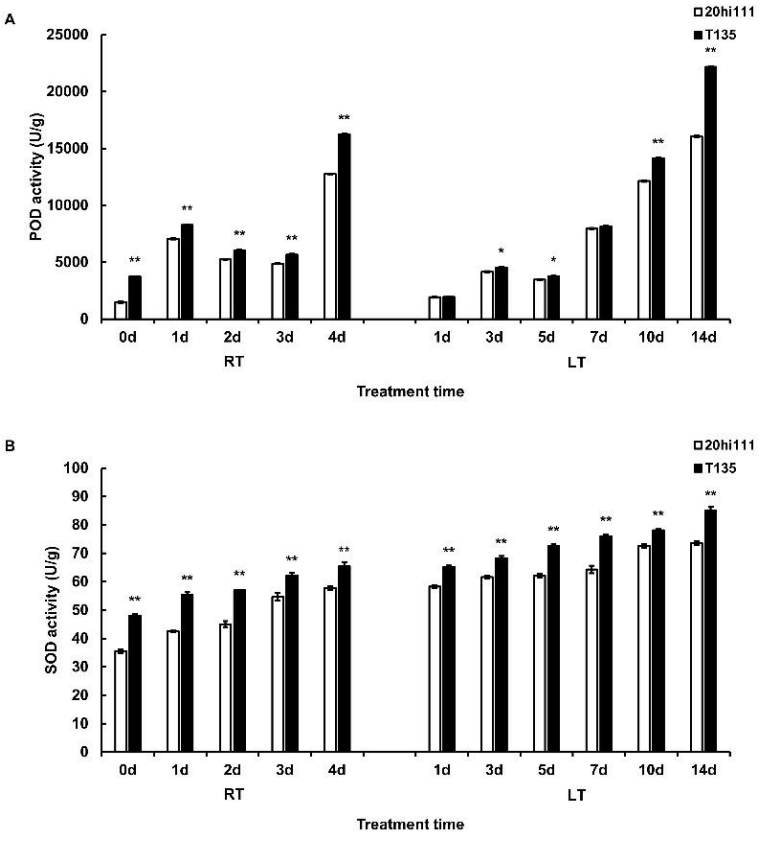
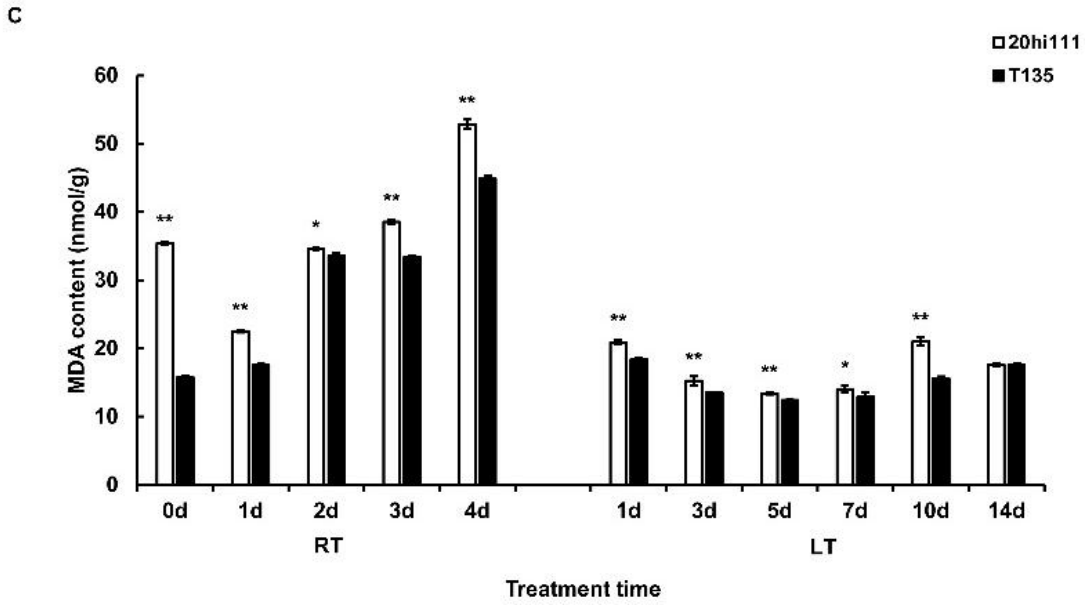


Figure 1. *H_2O_2 content and CAT activity during seed germination of T135 and 20hi111 sweet corn inbred lines under RT and LT. RT: room temperature treatment (25 ℃). LT: Low temperature treatment (10 ℃). (A) Determination of H_2O_2 content. (B) Determination of CAT activity. Standard error is indicated. Asterisks represent Student's t test significance compared with the control ($^{*}P$ <0.05, $^{**}P$ <0.01).*

3.2. The Effect of Chilling on POD, SOD Activity and MDA Content During the Germination of Sweet Corn Seeds

Whether under RT or LT, POD activity first increased, then decreased, and then increased in both 20hi111 and T135, with T135 being significantly higher than 20hi111. Under RT, POD activity increased on the first day, decreased slightly on the second and third days compared to the first day, and strongly increased on the fourth day. Under LT, POD activity increased on the first day, slightly decreased on the second day, and began to rise sharply on the seventh day (Figure 2A). For SOD activity, whether under RT or LT, it slowly increased with the extension of germination time, and the activity of T135 was significantly higher than that of 20hi111 (Figure 2B). Under RT, the MAD content first decreased and then increased in 20hi111, gradually increased in T135, and was significantly higher in 20hi111 than in T135. Under LT, the MDA content first decreased and then increased in 20hi111 and T135, with a significant increase in 20hi111 compared to T135 (Figure 2C).



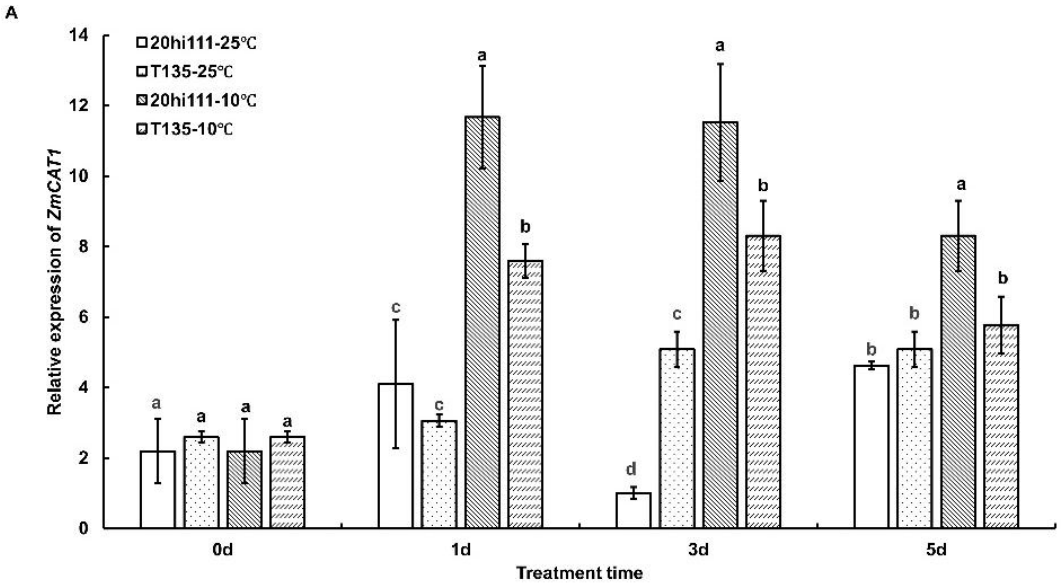


Standard error is indicated. Asterisks represent Student's t test significance compared with the control (*p<0.05,**P<0.01).

Figure 2. *POD, SOD activity and MDA content during seed germination of T135 and 20hi111 sweet corn inbred lines under RT and LT. (A) Determination of POD activity. (B) Determination of SOD activity. (C) Determination of MDA content.*

3.3. The Effect of Chilling on CAT Gene Expression During the Germination of Sweet Corn Seeds

The expression of *ZmCAT1* and *ZmCAT3* were basically the same, and the overall expression level increased after germination. On the 0th day of treatment, there was no significant difference among the four groups, which named 20hi111-25 ℃, T135-25 ℃, 20hi111-10 ℃, and T135-10 ℃. After one day of treatment, significant differences were observed among the four groups. *ZmCAT1* and *ZmCAT3* had the highest expression levels in 20hi111-10 ℃, followed by T135-10 ℃, and the lowest expression levels in T135-25 ℃ and 20hi111-25 ℃ (Figure 3A, B). In summary, the expression of *CAT* genes was higher under LT than under RT, and was also higher in 20hi111 than in T135.



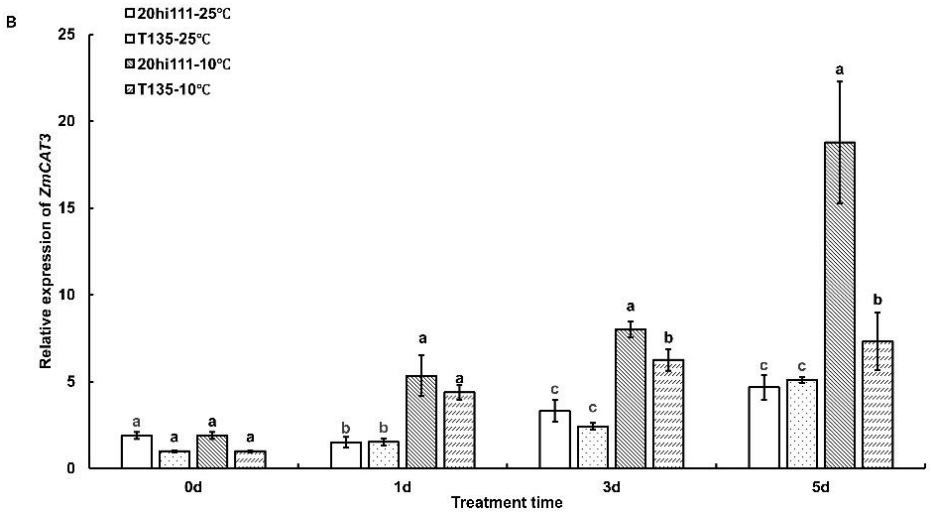
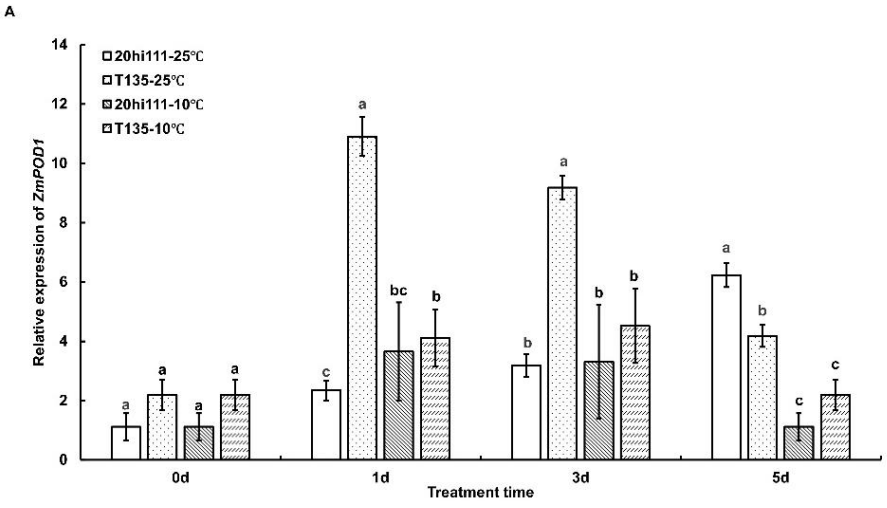


Figure 3. *The expression patterns of CAT genes during seed germination of T135 and 20hi111 sweet corn inbred lines under RT (25 ℃) and LT (10 ℃). (A) The expression pattern of ZmCAT1. (B) The expression pattern of ZmCAT3. Different letters represent significant differences as determined using one-way ANOVA followed by Duncan's test (P <0.05).*

3.4. The Effect of Chilling on POD Gene Expression Patterns During the Germination of Sweet Corn Seeds

During the treatment, the expression level of *ZmPOD1* showed a gradual increase in 20hi111-25 ℃, and showed an initial increase followed by a decrease in T135-25 ℃, 20hi111-10 ℃, and T135-10 ℃. On the 0th day of treatment, there was no significant difference among the four groups. After one day treatment, the expression level was highest at T135-25 ℃, followed by 20hi111-10 ℃ and T135-10 ℃, and was lowest at 20hi111-25 ℃. On the 3th day, the expression level was still highest at T135-25 ℃, and there was no significant difference observed at 20hi111-10 ℃, T135-10 ℃, and 20hi111-25 ℃. On the 5th day, the expression level was highest at 20hi111-25 ℃, followed by T135-25 ℃, and 20hi111-10 ℃ and T135-10 ℃ showed no significant difference (Figure 4A).

During the treatment, the expression level of *ZmPOD3* showed a gradual increase in 20hi111-25 ℃, T135-25 ℃, and 20hi111-10 ℃, and showed an initial increase followed by a decrease in T135-10 ℃. On the 0th day, there was no significant difference among the four group. On the 1th day, the expression levels of T135-10 ℃, 20hi111-10 ℃, and T135-25 ℃ increased, with the highest expression level observed at T135-10 ℃. And there was no difference in expression between 20hi111-10 ℃ and T135-25 ℃. On the 3th day, the expression level was highest at T135-10 ℃ and 20hi111-10 ℃, and was still lowest at 20hi111-25 ℃. On the 5th day, the expression level of 20hi111-10 ℃ suddenly increased, and it was the highest among the four groups. On the contrary, the expression level of T135-10 ℃ sharply decreased to the lowest among the four groups (Figure 4B). The expression patterns of *ZmPOD1* and *ZmPOD3* were not exactly the same. Overall, the expression of *POD* gene in T135 was slightly higher than that in 20hi111.



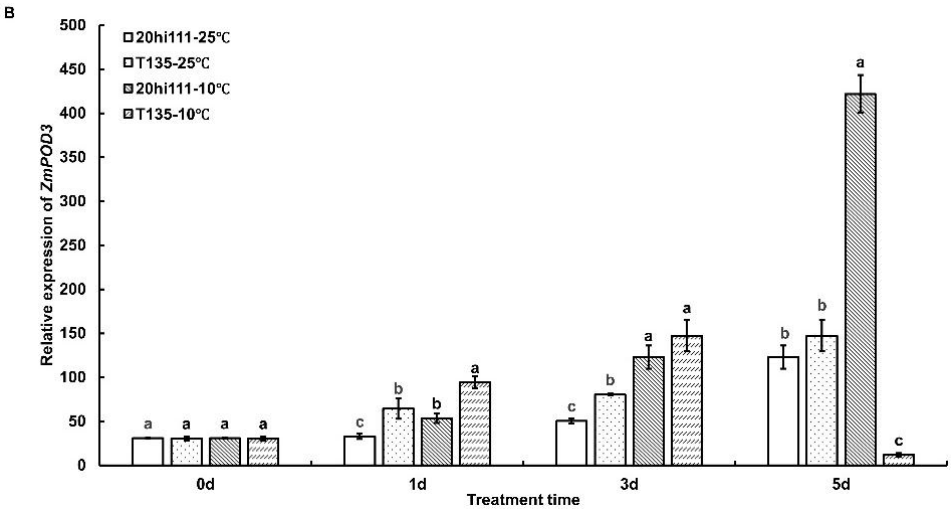
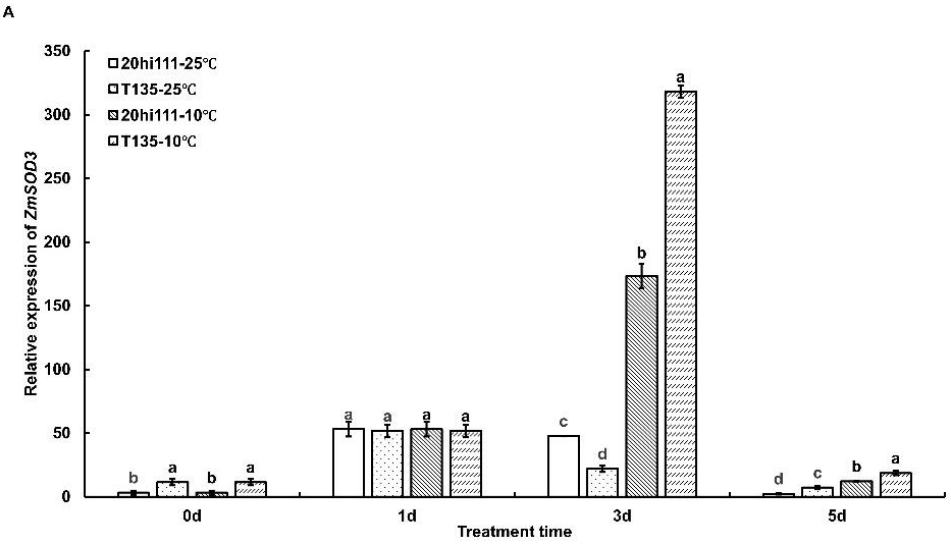


Figure 4. *The expression patterns of POD genes during seed germination of T135 and 20hi111 sweet corn inbred lines under RT (25 ℃) and LT (10 ℃). (A) The expression pattern of ZmPOD1. (B) The expression pattern of ZmPOD3. Different letters represent significant differences as determined using one-way ANOVA followed by Duncan's test (P <0.05).*

3.5. The Effect of Chilling on SOD Gene Expression Patterns During the Germination of Sweet Corn Seeds

The *ZmSOD3* expression showed a trend of first increasing and then decreasing in all four groups. On the 0th day, the expression level of *ZmSOD3* was significantly higher in T135 than in 20hi111. On the 1th day, the expression levels of all four groups increased, but there was no significant difference between the four groups. On the 3th day, the expression levels of T135-10 ℃ and 20hi111-10 ℃ increased significantly, showing the highest expression level at T135-10 ℃, followed by 20hi111-10 ℃. And the expression level at 20hi111-25 ℃ was significantly higher than that at T135-25 ℃. On the 5th day, the expression level sharply decreased. The order of expression level was as follows: T135-10 ℃, 20hi111-10 ℃, T135-10 ℃, and 20hi111-10 ℃ (Figure 5A).

The expression level of *ZmSOD9* showed a gradual increase in 20hi111-25 ℃, T135-25 ℃, and 20hi111-10 ℃, and showed an initial increase followed by a decrease in T135-10 ℃. There was no significant difference between the four groups on the 0th and 5th day. On the 1th and 3th day, the order of expression level was as follows: T135-10 ℃, T135-25 ℃, T135-25 ℃, and 20hi111-10 ℃ (Figure 5B). Overall, the expression of *SOD* gene under LT was significantly higher than that under RT, and the expression level in T135 was significantly higher than that in 20hi111.



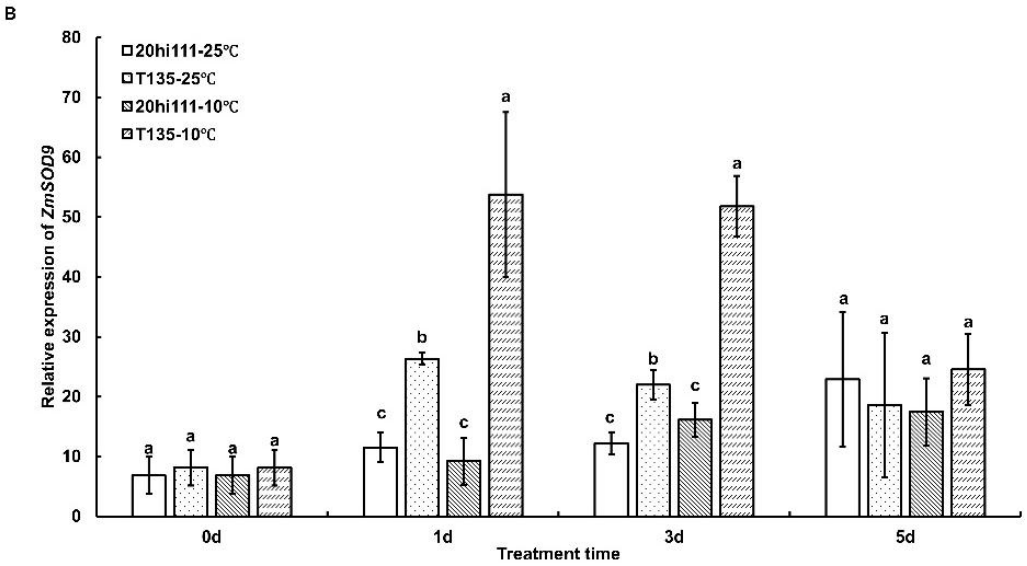


Figure 5. *The expression patterns of SOD genes during seed germination of T135 and 20hi111 sweet corn inbred lines under RT (25 ℃) and LT (10 ℃). (A) The expression pattern of ZmSOD3. (B) The expression pattern of ZmSOD9. Different letters represent significant differences as determined using one-way ANOVA followed by Duncan's test (P <0.05).*

4. Discussion

Sweet corn has thin skin, high sugar content, and good palatability, which are attributed to the absence of genes that convert sugar into starch in the endosperm [13]. Although these characteristics are beneficial for commercialization for human consumption, they hinder the production of high-quality seeds due to the lack of starch, reduced skin thickness, and high sugar content, making seeds less prone to germination, and more susceptible to mechanical damage, biological invasion, and spoilage [14, 15]. Under this premise, if sweet corn is sown at low temperatures, it will further exacerbate the poor germination rate [16]. Low temperature stress is one of the main abiotic stresses that limit plant productivity growth and geographical distribution [17]. Plants can increase antioxidant enzyme activity to eliminate excessive reactive oxygen species in the body when subjected to low temperature stress [18, 19]. MDA is a membrane permeable substance that is a product of membrane lipid oxidation. Its content can indicate the strength of plant cold resistance [20]. This study first detected the content of H_2O_2 and MDA, and found that the content in the low-temperature sensitive sweet corn inbred line 20hi111 was significantly higher than that in the low-temperature tolerant T35 sweet corn inbred line (Figure 1A, Figure 2C). The content of H_2O_2 and MDA showed a trend of first decreasing and then increasing during germination, accompanied by an increase and then decrease in enzyme activities such as CAT and POD (Figures 1, 2). This is consistent with previous studies, where an increase in MDA content is usually accompanied by an enhancement in enzyme activity. In the early stage, antioxidant enzyme activity is strong and has a certain clearing effect on oxidative products. As the low temperature time prolongs, oxidative products accumulate, and high MDA content will in turn inhibit the activity of these oxidases [21]. H_2O_2 and MDA showed the same trend. Research showed that at low concentrations, reactive ROS act as second messengers corresponding to extracellular signal transduction from membrane receptors and intracellular regulatory systems. However, when the level of reactive oxygen species increases above a certain threshold, oxidative stress response can accompany cell survival processes, which is harmful to cells [22, 23]. Therefore, it can be seen from the MDA and H_2O_2 content that T135 has stronger cold resistance than 20hi111.

SOD is the first line of defense for plants involved in low temperature stress, converting superoxide anions into H_2O_2, which is further converted into water and oxygen using CAT and POD [24]. There are 11 different mRNA transcripts of 4 types of SODs in maize, with SOD3 encoding mitochondrial Mn-SOD and SOD9 encoding leaf green Fe-SOD [25]. Overexpression of *SOD* gene can significantly improve plant cold tolerance [26, 27]. Whether at room temperature or low temperature, the SOD content in T135 is always higher than that in 20hi111 (Figure 2B), and the overall expression levels of *ZmSOD3* and *ZmSOD9* are always higher than that in 20hi111 in T135 (Figure 5). The high activity and gene expression level of *ZmSOD* may be one of the reasons for T135's low-temperature germination tolerance.

For POD, the POD activity in T135 was always higher than that in 20hi111. During germination, POD activity showed a trend of first increasing, then decreasing, and then rapidly increasing. Overall, after germination, POD activity was enhanced (Figure 2A). The expression level of *ZmPOD1* was also higher than that of 20hi111 in T135, but the expression level of *ZmPOD3* was higher than that of 20hi111 in T135 during the

early stage of germination and lower than that of 20hi111 in the later stage, showing no significant correlation with the maize variety (Figure 4). Our results are consistent with previous studies, showing that POD enzyme activity is closely related to low-temperature germination, but not highly correlated with gene expression [12]. It is also possible that the *ZmPOD3* gene is not a key low-temperature tolerant germination gene, as similar findings have been made in other studies [28].

CAT is located in the mitochondria [29], and during the cold acclimation period of maize, its increased activity is mainly concentrated in this organelle. Under low temperature conditions, the reactive oxygen species in mitochondria increase, and CAT can clear the reactive oxygen species in mitochondria, preventing the respiratory function of maize from being blocked [30, 31]. CAT enzyme has been reported to play an important role in cold acclimation and cold resistance of rice [32]. Low temperature stress increases the activities of SOD and CAT in cold resistant tobacco and increases their gene expression levels [33]. The isoenzymes of CAT mainly include CAT1, CAT2, CAT3, etc. Among them, maize CAT3 is a key enzyme that improves the cold resistance of maize and sequence analysis shows that CAT1 has a target sequence for peroxides [34]. The expression of *CAT3* is enhanced during plant cold treatment, maintaining low levels of H_2O_2 in the body [35]. Our research shows that CAT activity first increases and then decreases during the germination process of sweet corn at room temperature, while CAT activity gradually increases at low temperatures (Figure 1B). Surprisingly, in 20hi111 at low temperatures, both CAT activity and *CAT* gene expression levels were higher than T135 (Figures 1, 3), showing results opposite to previous studies [9]. We speculate that this may be related to the stress response of plants to H_2O_2, inducing the expression of protective enzyme genes to clear reactive oxygen species induced by cold stress [36]. There are research reports that in plants, SOD enzyme is mainly used to clear peroxide anions, thereby producing oxygen and H_2O_2. H_2O_2 is further decomposed by POD and CAT [37]. At different stages of germination, different types of oxidases may contribute relatively differently to the clearance of reactive oxygen species [38]. Therefore, it is possible that CAT enzyme activity and gene expression may be lower than 20hi111 during the pre-germination or even germination period.

5. Conclusions

This study measured the MDA and H_2O_2 content, CAT, POD, and SOD enzyme activities, and the gene expression patterns of *ZmSOD3*, *ZmSOD9*, *ZmPOD1*, *ZmPOD3*, *ZmCAT1*, and *ZmCAT3* during seed germination of chilling sensitive sweet corn inbred line 20hi111 and chilling tolerant sweet corn inbred line T135 under low temperature (10 ℃) and room temperature (25 ℃) treatments. The results showed that low-temperature treatment significantly increased the content of H_2O_2 and MDA in 20hi111 compared to T135. In 20hi111, the activities of POD and SOD were also significantly higher than T135, but the activity of CAT was significantly lower than T135. In terms of gene expression, at low temperatures, the expression levels of *ZmSOD1* and *ZmSOD3* were significantly lower than T135 in 20hi111, while there was no difference in *ZmPOD1*. In 20hi111, *ZmPOD3* was first higher and then lower than T135, while *ZmCAT1* and *ZmCAT3* were significantly higher than T135. In this experiment, the content of H_2O_2 and MDA, as well as the activities of SOD and POD, can be used as identification indicators to determine the low-temperature germination tolerance of sweet corn seeds. Low levels of oxidation and high levels of SOD enzyme activity and gene expression may be the main reasons for T135's strong cold tolerance. Our research is beneficial for screening and creating seed resources that are tolerant to low temperature germination, which will contribute to the improvement of sweet corn breeding. In addition, our research provides a crucial theoretical basis for understanding the physiological and molecular biology mechanisms of low-temperature germination of sweet corn.

Abbreviations

MDA	Malondialdehyde
CAT	Catalase
POD	Peroxidase
SOD	Superoxide Dismutase
ROS	Reactive Oxygen Species
RT	Room Temperature Treatment
LT	Low Temperature Treatment
qRT-PCR	Quantitative Real-time PCR Analysis

Author Contributions

Tingzhen Wang: Writing – original draft, Writing – review & editing
Jianjian Chen: Software, Validation
Zhenxing Wu: Investigation, Resources
Fangjian Li: Resources, Writing – review & editing
Guihua Lv: Conceptualization, Supervision, Validation

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Data Availability Statement

The data are contained within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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