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Abscisic Acid Improves Chilling-Induced Oxidative Stress in *Trichosanthes kirilowii* Maxim Seedlings

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ABSTRACT

***Trichosanthes kirilowii*, an important economic plant in China, is sensitive to chilling. To investigate the effect of abscisic acid (ABA) treatment on the chilling tolerance of *Trichosanthes kirilowii* Maxim, different concentrations of ABA were sprayed on *Trichosanthes kirilowii* leaves and the resulting catalase (CAT) and superoxide dismutase (SOD) activities and the malondialdehyde (MDA) content in the leaves were determined. The results indicated that at 4 ºC, the CAT and SOD activities increased at first and then decreased with increasing ABA concentration. By contrast, the MDA content decreased at first and then increased. They both had an extreme value when sprayed with 5.0 or 7.5 mg L^{-1} ABA. A real-time polymerase chain reaction was performed to investigate the influence of exogenous ABA on the *CAT* gene expression of *Trichosanthes kirilowii* leaves. The results indicated that at 4 ºC, the CAT relative gene expression showed a high degree of positive correlation with the enzyme activities of CAT and SOD, and a negative correlation with MDA content. These results led to three conclusions. First, exogenous ABA exhibits significant effect on the chilling tolerance of *Trichosanthes kirilowii.* Second, exogenous ABA significantly increases the enzyme activities of CAT and SOD under cold environments. Finally, under cold environments and the effect of ABA, the relative gene expression of catalase 2 (CAT2) gene was found to play an important role in the enhancement of the chilling tolerance of *Trichosanthes kirilowii* leaves.**

Keywords: Catalase, Cold, Malondialdehyde, Real time PCR, Superoxide dismutase.

INTRODUCTION

Various tolerance mechanisms have been suggested on the basis of the biochemical and physiological changes related to chilling injury. As temperature is reduced, a specific temperature determined by the ratio of saturated to unsaturated fatty acids accelerates the conversion of lipids of a liquid-crystalline condition into that of a solid condition in plant cell membranes (Campos *et al*., 2003; Partelli *et al*., 2011). The conversion of fatty acid may give rise to chilling resistance at lower temperatures in the plant cells (Luciana *et al*., 2010).

ABA-related experiments generally take MDA as an indicator of cell damage (Hung and Kao, 2003; Bueno *et al*., 1998; Lu *et al*., 2009). In the current study, MDA was used as an indicator of chilling tolerance, in which lower MDA content indicated better chilling tolerance. ABA induces stomata cells in plant leaves to shut to reduce transpiration, which plays an important role in alleviating drought-stress (Irving *et al*., 1992; Lee *et al*., 1999; Pei *et al*., 2000; Murata *et al*., 2001). Sripinyowanich *et al*. (2010) stated that ABA can increase the salt-stress tolerance of indica rice (*Oryza sativa* L.), while Wan and Li (2006) stated that ABA can increase the water-stress tolerance of Arabidopsis. ABA also plays an important role in enhancing the chilling tolerance and the cold-stress tolerance of

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fruits and seeds (Rikin *et al*., 1979; Wang and Buta, 1994). Moreover, increased endogenous ABA content can also reduce cold stress-related damage (Wang, 1991). However, the effects of exogenous ABA on the chilling tolerance of *Trichosanthes kirilowii* in cold-stress environments have not been reported.

Large amounts of reactive oxygen species generated in plants in cold-stress environments destroy the plants' chloroplast, cell membrane, protein, and nucleic acid, among others (Hu *et al.*, 2005; Zhang *et al.*, 2009; Heidari *et al.*, 2012). Catalase (CAT) and superoxide dismutase (SOD) play important roles in prevention of the poisoning of reactive oxygen species (Lee and Lee, 2000; Mizuno *et al*., 1998). In plants, SOD helps clear up O_2^- to generate O_2 and H_2O_2, which can then be decomposed into H_2O and O_2. This process is catalyzed by ascorbate peroxidase (APX) in the chloroplast (Miyake and Asada, 1994). The effects of exogenous ABA on CAT and SOD in *Trichosanthes kirilowii* leaves have not been reported.

CAT is an iron porphyrin enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. CAT is involved in the CAT and SOD enzyme systems that clear up reactive oxygen species (Purev *et al*., 2010). Most of the higher plant species have three kinds of *CAT* genes (Iwamoto *et al*., 2000). For example, Arabidopsis has catalase1 (CAT1), catalase2 (CAT2), and catalase3 (CAT3) (Frugoli *et al*., 1996). *CAT* genes play important roles in resisting stress (Guan and Scandalios, 1993). Whether or not the three kinds of *CAT* genes found in *Trichosanthes kirilowii* play an important role in the chilling tolerance of *Trichosanthes kirilowii*, and, if so, which one is most important has not been reported.

Trichosanthes kirilowii is a type of liana that belongs to the *Cucurbitaceae* family whose root tuber is used to treat diabetes (Zhou *et al*., 2002). Moreover, *Trichosanthes kirilowii* can also effectively curb the AIDS virus and possesses some anti-cancer properties (Xie, 2000), giving it high medicinal value. However, *Trichosanthes kirilowii* has a low chilling tolerance that makes it susceptible to frost damage, which has a negative impact on the following year's yield. Enhancing the cold hardiness of *Trichosanthes kirilowii* is one of the priorities in its cultivation.

Therefore, the current study aims to reveal the following three points: (1) whether or not exogenous ABA affects the chilling tolerance of *Trichosanthes kirilowii*, (2) whether or not exogenous ABA affects CAT and SOD, and (3) whether or not the three kinds of *CAT* genes present in *Trichosanthes kirilowii* play important roles in enhancing chilling tolerance, and if so, which one is the most important.

MATERIALS AND METHODS

Biological Materials

The seeds were purchased from Sanmenxia City, China. When a *Trichosanthes kirilowii* grew its third true leaf, it was moved into a complete solution culture. After two weeks cultivation, the *Trichosanthes kirilowii* plants of approximately the same growth were selected for the subsequent experiments. The *Trichosanthes kirilowii* plants were divided into seven groups numbered 1 to 7. Group numbers 3 to 7 were sprayed thrice daily with 2.5, 5.0, 7.5, 10.0 and 5.0 mg L^{-1} of ABA, respectively. After 2 days, group numbers 2 to 6 were placed in a climate chamber (Radford Technology Company, Ningbo, China) under an environment of 4±1ºC for 3 days. Subsequently, group numbers 1 and 7 were placed in a similar climate chamber in an environment of 25±1ºC for 3 days, too. In the climate chambers, all plants were given illumination for 16 h. After 3 days in the climate chamber, the leaves were harvested for further experimentation. Treatments were divided into seven groups including CK1:

Sprayed no ABA at 25ºC; T1: Sprayed no ABA at 4ºC; T2: Sprayed 2.5 mg L^{-1} ABA at 4ºC; T3: Sprayed 5.0 mg L^{-1} ABA at 4ºC; T4: Sprayed 7.5 mg L^{-1} ABA at 4ºC; T5: Sprayed 10.0 mg L^{-1} ABA at 4ºC, CK2: Sprayed 5 mg L^{-1} ABA at 25ºC.

Enzyme Assay

MDA was measured by the barbituric acid chromogenic method (Zhao and Li, 1999). Enzyme assay of CAT and SOD was carried out according to Lee and Lee (2000).

Primer Design

Based on the sequences of homologous species, the degenerate primers were designed by the National Center for Biotechnology Information (NCBI). The length of *CAT2* gene fragment was 330 bp. F: *5'-TAATGCTCCWAAGTGTSCTCA-3'*, R: *5'-GCCTGAGWCCA-GTAYGWGATCCA-3'*. Actin2 (DQ115882) fragment, with a gene length of 117 bp, was chosen as the reference gene. The primers of Actin2 were F: *5'-GAAGGAATAACCACGCT-CAG-3'*, R: *5'-ACACAGTTCCCATCTACGAG-3'*. The primers were designed by TAKARA (Dalian, China).

Total RNA Extraction

Trichosanthes kirilowii leaves were ground in liquid nitrogen, and then lysed in the extraction buffer. Chloroform: isoamyl alcohol (24:1) was added, and the mixture centrifuged to obtain the upper phase. Absolute ethanol (2× volume) and 0.1× volume of NaCl (5M) were added. After centrifugation, the pellet was re-suspended in DEPC-treated water. An equal volume of TE-saturated phenol (pH 8.0): chloroform: isoamyl alcohol (25:24:1) was added, and the phases separated by centrifugation at room temperature. To the upper phase, 2× volume of absolute ethanol and 0.1× volume NaCl (5M) were added and the solution was centrifuged. The RNA pellet was re-suspended in DEPC-treated water.

Reverse-transcription and PCR Amplification

First-strand cDNA synthesis was performed, following the manufacturer's (Invitrogen Life Technologies, Carlsbad, CA) instructions, using 2 µg of the total RNA treated with DNaseI (TAKARA, Dalian, China), 200 units of Avian Myelobastosis Virus (AMV) reverse transcriptase (Invitrogen Life Technologies), 50 units of RNase inhibitor (RNase out, Invitrogen Life Technologies), 1 mM dNTPs, and 2.5 µM random primers (Sigma-Aldrich, St. Louis, MO). The mix for reverse transcriptase (20 µl) was incubated for 50 minutes at 37ºC. The PCR mix (25 µl) contained 2 µl of cDNA (10% of the first-strand reaction, corresponding to approximately 100 ng of the total RNA), 4 µl PCR buffer (5×, Invitrogen Life Technologies), 0.2 mM dNTPs, 0.5 µM of each primer, 1.5 mM $MgCl_2$, and 0.5 units of Taq polymerase (PlatinumTaq polymerase, Invitrogen Life Technologies). Amplifications of the target gene were conducted in a Genetic Thermal Cycler with 40 cycles of 94ºC for 30 seconds, 40ºC for 2 minutes, and 72ºC for 30 seconds, with a 5 minutes extension at 72ºC. Amplifications of the reference gene were conducted in a Genetic Thermal Cycler with 36 cycles of 95ºC for 30 seconds, 57ºC for 45 seconds, and 72ºC for 1 minute, with a 6 minute extension at 72ºC.

Real-time PCR Amplification

Degenerate primers were used to clone a *CAT2* gene fragment with length of approximately 330 bp. The sequencing results were entered into the NCBI for the homology comparison. The results were found to have 87% similarity with *Cucurbita pepo* mRNA and CAT2, ensuring that the

cloned fragment belonged to the *Trichosanthes kirilowii CAT2* gene. A semi-quantitative PCR was then performed based on the sequencing results. The 330 bp long fragment was cloned to reduce the interference of non-specific clone fragments. The specific primers of the *CAT2* gene were designed as follows: F: *5'-TAATGCTCCTAAGTGTCCTCA-3'*, R: *5'-GCCTGAGTCCAGTACGAGATCCA-3'*.

The PCR mixture contained 1 µl of diluted cDNA, 5 µl of 2× SYBR Green PCR Master Mix, and 200 nM of each gene-specific primer in a final volume of 20 µl. Real-time PCRs were performed using an ABI Prism 7,000 Sequence Detection System and the appropriate software (PE Applied Biosystems, USA). All PCRs were performed under the following conditions: 2 minutes at 95ºC, 40 cycles of 10 seconds at 94ºC, 10 seconds at 57ºC, and elongated at 72ºC for 40 seconds in 96-well optical reaction plates (Applied Biosystems, USA). The specificity of the PCR products were verified using a melting curve analysis (60 to 95ºC) after 40 cycles and agarose gel electrophoresis. The levels of CAT2 transcripts were normalized by endogenous Actin transcripts. Each set of experiments was repeated three times.

RESULTS

Determination of MDA

The concentration of MDA in leaves was very low when plants were grown under 25ºC. By contrast, this value turned to be higher when plants were moved to 4ºC for 3 days. The accumulation of MDA in the recovered plants suggests that the plasma membrane in leaves might be damaged during 4ºC condition (Figure 1).

The induction of MDA in plant leaves under 4ºC condition was repressed by 2.5 mg L^{-1} ABA treatment, and this repression was more significant in the presence of 5.0 mg L^{-1} ABA. However, further increase in the concentration of ABA did not play more effective role in reducing the MDA content as shown by the abundant value of MDA in 7.5 mg L^{-1} ABA treated plants when compared with that in 5.0 mg L^{-1} ABA treated plants, and this value was even higher in 10.0 mg L^{-1} ABA treated groups. These results suggested that the optimal

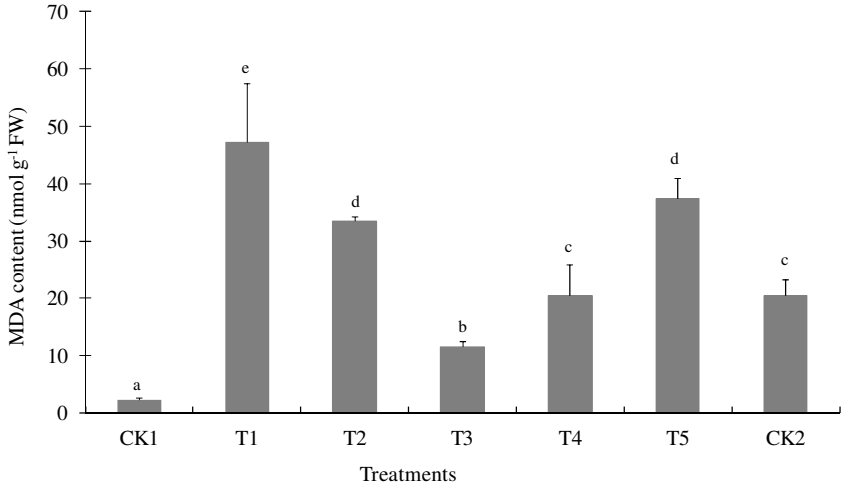

Figure 1. Measurement of MDA in extracts of leaves sprayed with different concentrations of ABA. We took MDA as indicator of chilling tolerance. The content was lower, the chilling tolerance was better. Value with the same letter are not significantly different at P 0.05 level, according to Duncan's multiple range test. Data are Means ±SD (n= 3). CK1: Sprayed no ABA at 25ºC; T1: Sprayed no ABA at 4ºC; T2: Sprayed 2.5 mg L^{-1} ABA at 4ºC; T3: Sprayed 5.0 mg L^{-1} ABA at 4ºC; T4: Sprayed 7.5 mg L^{-1} ABA at 4ºC; T5: Sprayed 10.0 mg L^{-1} ABA at 4ºC, CK2: Sprayed 5 mg L^{-1} ABA at 25ºC.

concentration of ABA in blocking the induction of MDA at 4ºC might be 5.0 mg L^{-1}.

Subsequently, we attempted to determine whether the 5.0 mg L^{-1} ABA was functioning in reducing MDA content under normal condition. The results showed that externally applied ABA increased the MDA content when grown under 25ºC condition, rather than decreasing this value (Figure 1). This suggested that the extra ABA was not effective in preventing plant from producing MDA; on the contrary, breaking the balance of ABA was even harmful for plant growth.

Catalase Activity Assay

One unit of CAT is defined as the amount of enzyme which liberates the peroxide oxygen from 1μmol H_2O_2 solution in 1 minute at 25ºC (Xing and Ding, 1981).

The CAT activity in leaves was high when plants were grown under 25 ºC, regardless of spraying 5.0 mg L^{-1} ABA or not. But, the CAT activity turned to be lower when plants were moved to 4ºC for 3 days. The CAT activity did not increase significantly when sprayed with 2.5 mg L^{-1} ABA under 4ºC condition. When the ABA concentration was increased to 5.0 mg L^{-1}, we could see a significant mounting of CAT activity (Figure 2). But the concentration increased to 7.5 mg L^{-1} could not provide a higher mounting of CAT activity than 5.0 mg L^{-1}. The treatment sprayed with 10.0 mg L^{-1} ABA appeared worse, it only had a similar mounting of CAT activity compared with the plants sprayed with 2.5 mg L^{-1} ABA. Therefore, we thought that a comfortable concentration of ABA in increasing the CAT activity at 4ºC might be 5.0 mg L^{-1}.

Total SOD Activity Assay

One unit of SOD is defined as the amount of enzyme which causes a 50% decrease of the SOD-inhibitable nitrotetrazolium blue chloride (NBT) reduction (Beyer and Fridovich, 1987).

In the plants that had not been sprayed with ABA, chilling stress did not induce a significant increase in total SOD activity; whereas in plants sprayed with ABA at 4ºC, the SOD activity gradually increased. The value reached its extreme in the concentration of 5.0 mg L^{-1}. After that, the activity was gradually decreased. Under the condition of 4ºC, the pattern of changes in

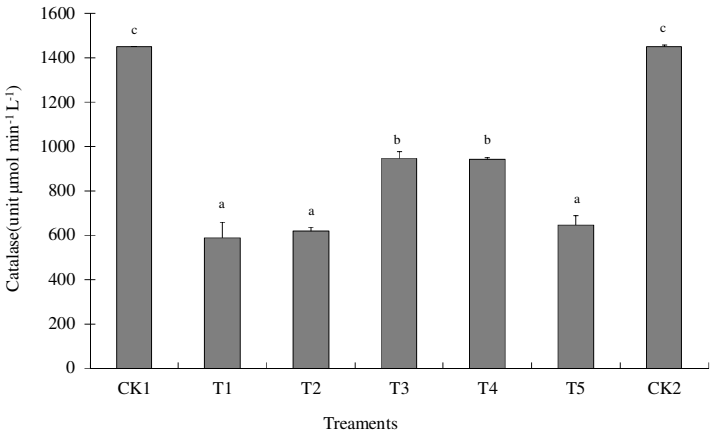

Figure 2. Activity of CAT in leaves sprayed with different concentrations of ABA. One unit of catalase is defined as the amount of enzyme which liberates the peroxide oxygen from 1μmol H_2O_2 solution in 1 minute at 25ºC. Value with the same letter are not significantly different at P 0.05 level, according to Duncan's multiple range test. Data are Means±SD (n= 3). CK1: Sprayed no ABA at 25ºC; T1: Sprayed no ABA at 4ºC; T2: Sprayed 2.5 mg L^{-1} ABA at 4ºC; T3: Sprayed 5.0 mg L^{-1} ABA at 4ºC; T4: Sprayed 7.5 mg L^{-1} ABA at 4ºC; T5: Sprayed 10.0 mg L^{-1} ABA at 4ºC, CK2: Sprayed 5 mg L^{-1} ABA at 25ºC.

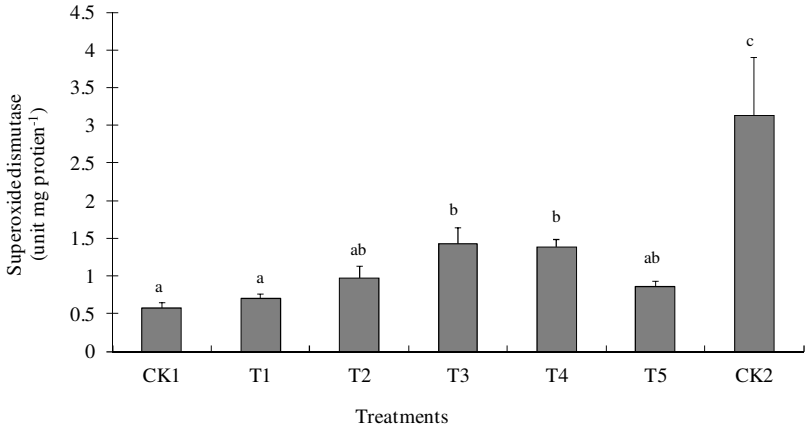

Figure 3. Activity of SOD in leaves sprayed with different concentrations of ABA. One unit of SOD is defined as the amount of enzyme which causes a 50% decrease of the SOD-inhibitable NBT reduction. Value with the same letter are not significantly different at P 0.05 level, according to Duncan's multiple range test. Data are Means ±SD (n= 3). CK1: Sprayed no ABA at 25ºC; T1: Sprayed no ABA at 4ºC; T2: Sprayed 2.5 mg L^{-1} ABA at 4ºC; T3: Sprayed 5.0 mg L^{-1} ABA at 4ºC; T4: Sprayed 7.5 mg L^{-1} ABA at 4ºC; T5: Sprayed 10.0 mg L^{-1} ABA at 4ºC, CK2: Sprayed 5 mg L^{-1} ABA at 25ºC.

the SOD activity was very similar to that of changes in the CAT activity (Figure 3). To increase the SOD activity at 4ºC, spraying 5.0 mg L^{-1} ABA would be most acceptable. However, plants sprayed with 5.0 mg L^{-1} ABA had a significantly higher SOD activity at 25ºC compared to those at 4ºC. We could conclude that chilling may be unfriendly to ABA in increasing SOD activity of *Trichosanthes kirilowii* leaves.

Real-time PCR Assay

The results of the semi-quantitative PCR showed that the gene expressions of the different groups had significant differences. Thus, real-time PCR was conducted to validate the results. The primers were similar to those used in the semi-quantitative PCR. The melting curve did not contain a hybrid peak, indicating the absence of pollution or impurities in the amplification. As such, the gene expression was stable and the results of the real-time PCR were reliable.

The CAT2 relative gene expression in leaves turned to be extremely low when plants were sprayed with 5.0 mg L^{-1} ABA at 25ºC for 3 days. Under the condition of 4ºC, from 0 to 5.0 mg L^{-1}, as the concentration of ABA increased, the CAT2 relative gene expression in plant leaves gradually increased. But, beyond 5.0 mg L^{-1}ABA, the value of CAT2 relative gene expression in 7.5 mg L^{-1} and 10.0 mg L^{-1} ABA treated groups decreased compared with that in 5.0 mg L^{-1} ABA treated plants (Figure 4). However, in chilling condition, ABA could help *CAT2* gene get more expression. At 4ºC, the CAT2 relative gene expression had a high degree of positive correlation with the CAT activity (Figures 2 and 4).

DISCUSSION

MDA is an indicator of plant lipid peroxidation (Lu *et al*., 2009). Hung and Kao (2003; 2004) stated that at room temperature, ABA increases MDA content in rice leaves. This result is in line with the results of the current study, wherein at 25ºC, the *Trichosanthes kirilowii* leaves treated with ABA had higher MDA content than the untreated groups (Figure 1). In this study, the MDA content was expected to increase when subjected to a cold environment or when treated with ABA only. However, the chilling and ABA treatments were not

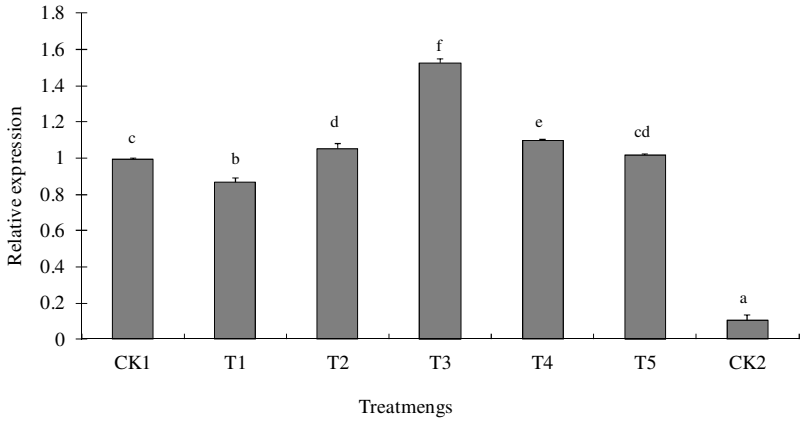

Figure 4. Relative expression of CAT2 mRNA in leaves sprayed with different concentrations of ABA. Value with the same letter are not significantly different at P 0.05 level, according to Duncan's multiple range test. Data are Means±SD (n= 3). CK1: Sprayed no ABA at 25ºC; T1: Sprayed no ABA at 4ºC; T2: Sprayed 2.5 mg L^{-1} ABA at 4ºC; T3: Sprayed 5.0 mg L^{-1} ABA at 4ºC; T4: Sprayed 7.5 mg L^{-1} ABA at 4ºC; T5: Sprayed 10.0 mg L^{-1} ABA at 4ºC, CK2: Sprayed 5 mg L^{-1} ABA at 25ºC.

synergistic when applied together. Lee and Lee (2000) stated that the chilling stress in cucumber leaves increased SOD activity, indicating a positive effect on the removal of reactive oxygen species. This result is consistent with the results of the current study, wherein the MDA content in the *Trichosanthes kirilowii* leaves was lower at 4ºC than at 25ºC, despite both groups having been treated with 5.0 mg L^{-1} ABA (Figure 1). Therefore, exogenous ABA can obviously affect the chilling tolerance of *Trichosanthes kirilowii*.

Cold tolerance and the activity of CAT do not always show a positive correlation. In some research, chilling decreases the CAT activity, but increase the tolerance of plants (Marc *et al.*, 1994). However, in most condition, they show a positive correlation. Hung and Kao (2004) stated that the addition of ABA increased the CAT and SOD activities of rice leaves. The current study found a high positive correlation between CAT and SOD at 4ºC. Also, both CAT and SOD had a significant negative correlation with MDA content, which is a typical indicator of chilling tolerance (Figures 1-3). Therefore, the exogenous ABA can enhance the chilling tolerance of *Trichosanthes kirilowii* by raising the CAT and SOD enzyme activities in its leaves. This result is in line with the results of the current study, wherein the CAT activity in *Trichosanthes kirilowii* leaves was higher at 25ºC than at 4ºC.

Hu *et al*. (2010) stated that CAT2 may play an important role in the scavenging of H_2O_2 produced under stressful conditions. Real-time quantitative PCR was conducted to further investigate the role of CAT2 in the chilling tolerance of *Trichosanthes kirilowii*. At 4ºC, the CAT2 relative gene expression showed a positive correlation with CAT enzyme activity, consequently increasing the chilling tolerance (Figures 2 and 4). *Trichosanthes kirilowii* leaves sprayed with ABA at 25ºC were highly damaged. Ling *et al*. (2000) stated that only the CAT1 and CAT3 transcripts in young maize leaves increased in response to wounding. Scandalios *et al*. (2000) stated that the *Car1* gene product can become inhibited at elevated temperatures when the trans-acting gene locus Car1 regulates the *CAT2* gene activity levels in maize leaves. The current study showed that at 25ºC, the *Trichosanthes kirilowii* sprayed with ABA had a low *CAT2* gene expression, but high enzyme activity (Figures 2 and 4). This result can be attributed to the fact that the *CAT2* gene no longer was the main form of the *CAT* gene expression. However, at 4ºC, the ABA not

only assisted the chilling in damaging the cells, but also alleviated the damage caused by chilling. Thus, at 4ºC, the gene expression of *CAT2* gene induced by ABA plays an important role in enhancing the chilling tolerance of *Trichosanthes kirilowii.*

At 25ºC, no significant differences in CAT enzyme activities were observed between the leaves treated with ABA and the untreated leaves (Figure 2). However, the untreated *Trichosanthes kirilowii* leaves showed visibly lower SOD enzyme activity than the treated ones at 25ºC (Figure 3). Thus, at 25ºC, ABA treatment had no obvious effect on CAT activity, but had significant effect on SOD, probably because the CAT activity was not able to clear up the H_2O_2 that led to membrane damage and intracellular substance leak under the condition of the ABA damaging the reactive oxygen species. Other mechanisms, such as the APX catalysis, may play important roles in the process of clearing up the H_2O_2. In the chloroplast, H_2O_2 mainly depended on APX and on its corresponding circulatory system, which was called ascorbic acid-glutathione (AsA-GSH), when the CAT was insufficient (Esfandiari *et al.*, 2007).

In summary, exogenous ABA was shown able to significantly enhance the chilling tolerance of *Trichosanthes kirilowii*. Exogenous ABA can significantly increase the CAT and SOD enzyme activities of *Trichosanthes kirilowii* leaves under cold environments. The *CAT2* gene plays an important role in the enhancement of the chilling tolerance induced by ABA. The mechanism of the transformation of the three kinds of *CAT* genes during chilling stress requires further research.

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ابسیزیک اسید تنش اکسایشی ناشی از سرما را در گیاهچه *Trichosanthes kirilowii* Maxim بهبود می بخشد

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چکیده

گیاه *Trichosanthes kirilowii* که از گیاهان اقتصادی مهم درچین است به سرما حساس میباشد. به منظور بررسی اثر ابسایسیک اسید(ABA) روی تحمل سرما در *Trichosanthes kirilowii* Maxim، غلظت های مختلفی از آن روی برگهای این گیاه پاشیده شد و سپس مقدار کاتالاز(CAT) سوپر اکسید دیسموتاز(SOD) و مالون دی الدهاید (MDA) در برگها اندازه گیری شد. نتایج نشان داد که در درجه حرارت ۴ درجه سانتی گراد فعالیت کاتالاز و سوپر اکسید دیسموتاز نخست زیاد شد و بعد با افزایش غلظت ABA کاهش یافت. بر خلاف این ، مقدار MDA ابتدا کم شد و سپس رو به فزونی گذاشت. همچنین، در تیمارهای ۵ و هفت و نیم میلی گرم در لیتر ABA ،هر دو آنها به حد بیشینه رسیدند. به منظور بررسی اثر ابسایسیک اسید خارجی روی بیان ژن CAT در برگ *Trichosanthes kirilowii* ، یک واکنش زنجیره ای پلیمراز همزمان(در زمان واقع) انجام شد. بر اساس نتایج ، ، بیان نسبی ژن CAT در ۴ درجه سانتی گراد همبستگی مثبت بالایی با فعالیت آنزیمی CATو SOD نشان میداد ولی با مقدارMDA همبستگی منفی داشت. این یافته ها سه نتیجه گیری داشت. نخست اینکه ابسایسیک اسید خارجی اثر معنی داری روی تحمل سرمای *kirilowiiTrichosanthes* نشان می دهد. دوم آنکه ابسایسیک اسید خارجی به طور معنی داری فعالیت آنزیمی CATو SODرا در محیط سرد افزایش می دهد. نتیجه سوم اینکه در محیط سرد و تحت تاثیر ABA ، بیان نسبی ژن کاتالاز ۲(CAT2) نقش مهمی در افزایش تحمل به سرما در برگ *Trichosanthes kirilowii* دارد.