Effects of Abscisic Acid on Cold Resistance in *Digitaria sanguinalis* (L.) Scop.

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Abstract [**Objectives**] This study was conducted to detect the protective effect of abscisic acid on chilling injury of *Digitaria sanguinalis* (L.) Scop, and whether this effect is related to antioxidant enzymes and osmotic adjustment. [**Methods**] *D. sanguinalis* plants were sprayed with abscisic acid solution, and exposed to cold stress at 15 ℃ for 3 d after one day and then at 5 ℃ for 25 to 30 d in a growth chamber. The changes of plant osmotic potential under this treatment were detected. [**Results**] Under low temperature stress, the osmotic potential of plants in the abscisic acid treatment and the control increased, but the osmotic potential level of the abscisic acid treatment plants was lower. The SOD activity of plants in the ABA treatment and the control decreased under low temperature stress. Under low temperature stress, the activity of catalase and peroxidase in ABA-treated plants was higher than that in control plants. [**Conclusions**] This study provides a theoretical basis for the impact of abscisic acid on the physiological response of *D. sanguinalis* to cold injury.

Key words *Digitaria sanguinalis*; Abscisic acid; CAT; POD; ROS; SOD

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Reactive oxygen species (ROS) are normal metabolites of plants[1-2]. Higher plants have a defense system for clearing reactive oxygen species, consisting of several defense enzymes such as superoxide dismutase (SOD), ascorbic acid peroxidase, glutathione reductase and catalase (CAT), as well as antioxidants such as ascorbic acid and reduced glutathione. The balance between ROS production and clearance is disrupted under stress conditions (such as refrigeration), leading to the accumulation of ROS and lipid peroxidation of cell membranes[3]. Low temperature-induced oxidative stress has been also observed in corn and coffee[4-5]. Oxidative stress is considered an important factor related to cold damage in *Arabidopsis thaliana* and rice. The activity of reactive oxygen species-scavenging enzymes under low temperature stress is related to their tolerance to stress. Higher levels of defense enzymes are associated with higher cold resistance in cucumber roots.

Abscisic acid (ABA) plays an important role in the response to low temperatures and is associated with enhanced cold resistance in certain plant species[6]. Exogenous ABA treatment improves the cold resistance of maize (*Zea mays* L.) and *Stylosanthes guianensis* seedlings. ABA induces the antioxidant system in maize and *Cynodon dactylon* (L.) Persoon.

Digitaria sanguinalis (L.) Scop. is a weed distributed worldwide and can also be used as a lawn grass and pasture. However, its growth is limited by low temperatures. Experiments were conducted under controlled conditions to determine the protective effect of ABA treatment on cold damage and its effects on the antioxidant system and osmotic regulation in *D. sanguinalis*. We determined the osmotic potential and SOD, CAT and POD activity in leaves of *D. sanguinalis* treated with ABA under low temperature stress.

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Materials and Methods

Materials

D. sanguinalis seeds were sown in plastic pots (20 cm in diameter, 15 cm in depth) filled with a mixture of topsoil (3 parts) and coarse sand (1 part). In the late summer and autumn of 2020, plants grew in a greenhouse under natural light for two months, with temperatures between 30 and 35 ℃. They were irrigated every 3 d and then refrigerated.

ABA treatment and cold treatment

Plant leaves were evenly sprayed with 20 mg/L (S)-ABA prepared with ABA powder (purity 80%) until the leaves were completely moist, with clean water as a control. One day later, the plants were transferred to a growth chamber at 15 ℃ for 2 d, and finally grew at 5 ℃ for 25 – 30 d with a photoperiod of 12 h and a photosynthetic photon flux density of 220 μmol/(m^2 · s). The flower pots were placed completely randomly in the growth chamber. Samples were collected from each pot of plant for measurement. Each treatment consisted of five flower pots for sampling and analysis. Each measurement was completed through three separate sampling.

Determination of osmotic potential

Five leaves were rinsed with distilled water and immersed in 25 ml of distilled water overnight. The conductivity of solution (R1) was determined using a conductivity meter. Next, the sample was heated in boiling water for 15 min and cooled to room temperature. The electrical conductivity (R2) of the killed tissue was

determined again.

Determination of SOD, POD and CAT activity

Fresh leaves (0.5 g) were ground in a mortar and pestle in 5 ml of 50 mM cold phosphate buffer (pH 7.8). Centrifugation was performed at 4 ℃ and 10 000 × g for 20 min. The supernatant was determined for enzyme activity. The activity of superoxide dismutase was determined according to the method of Giannopitis and Ries[7]. A 3 ml of reaction solution contained methionine 13 μM, ρ-nitro-blue tetrazolium chloride (NBT) 63 μM, riboflavin 1.3 μM, phosphate buffer (pH 7.8) 50 mM, and enzyme extract 50 μl. The reaction solution was incubated under the light intensity of 80 μmol/(m^2 · s) for 10 min. The absorbance was measured at 560 nm by a spectrophotometer. One unit of SOD activity is defined as the amount of enzyme needed to inhibit the photochemical reduction of NBT by 50%.

According to the method of Change and Maehly, the activity of catalase was determined by spectrophotometry by tracking the decrease of absorbance of H_2O_2 at 240 nm within 1 min[8]. A 3 ml of reaction solution contained H_2O_2 15 mM, phosphate buffer (pH 7.0) 50 mM and enzyme extract 100 μl. The reaction was initiated by adding enzyme extract.

Peroxidase activity was determined by tracking the decrease of absorbance at 470 nm. The reaction mixture contained phosphate buffer (pH 7.0) 50 mM, guaiacol 5mM and enzyme extract 50 μl. The reaction was initiated by adding 10 μl of 30% H_2O_2.

Results and Analysis

Changes in osmotic potential of *D. sanguinalis*

The osmotic potential in plants of the control and ABA treatment hardly changed after 5 d of cold stress, but increased with cold treatment from 10 to 20 d (Fig. 1), indicating that plants were injured after 10 d of cold stress. Plants treated with ABA had lower osmotic potential than plants in the control. Therefore, 20 mg/L of ABA was used in the following experiments.

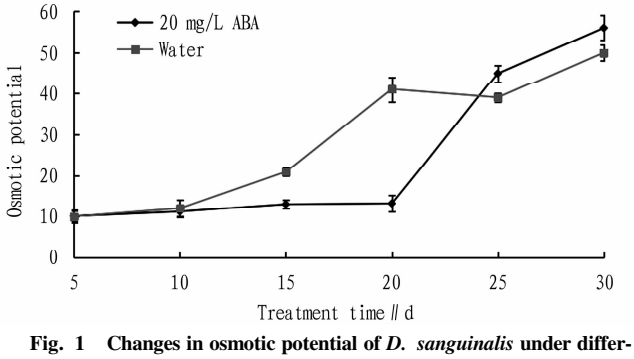

Fig. 1 Changes in osmotic potential of *D. sanguinalis* under different time of treatment

Changes in the activity of protective enzymes

The SOD activity of *D. sanguinalis* was easily affected by low temperature stress and showed a continuous decreasing trend during the process of low temperature stress. The ABA treatment had little effect on the decrease of SOD activity under low temperature stress (Fig. 2).

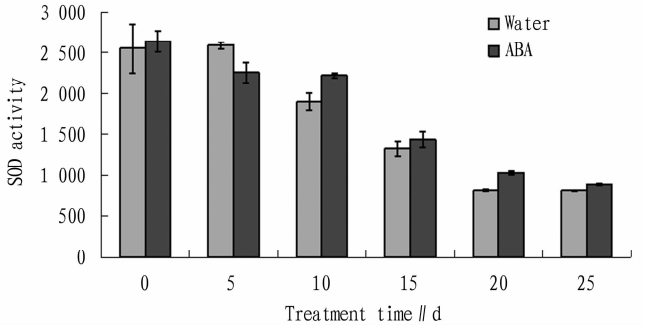

Fig. 2 Changes in SOD activity of *D. sanguinalis* under different time of treatment

The activity of CAT and POD showed similar patterns. The activity in plants of the control and ABA treatment peaked at day 10, and then decreased greatly with low temperature treatment (Fig. 3 and Fig. 4). Plants of the ABA treatment maintained higher activity than plants of the control, and at the end of low temperature treatment, the activity decreased to a similar low level.

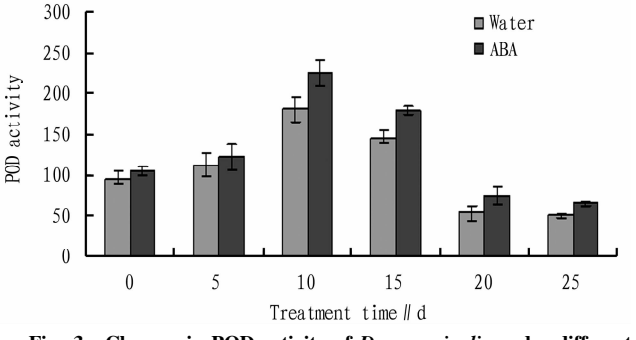

Fig. 3 Changes in POD activity of *D. sanguinalis* under different time of treatment

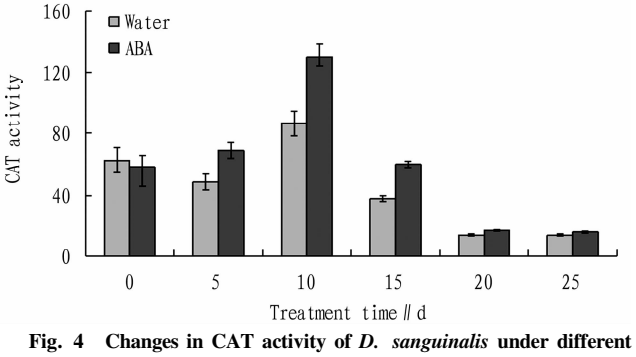

Fig. 4 Changes in CAT activity of *D. sanguinalis* under different time of treatment

Conclusions and Discussions

Osmotic potential is closely related to cell membrane damage. On day 5 after cold storage, the osmotic potential of *D. sanguinalis* did not change, indicating that the plants were not harmed. After 10 d at low temperature, the osmotic potential increased, indicating that the membrane system of *D. sanguinalis* was destroyed under low temperature stress.

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orchard was relatively rich in trace elements.

② Fertilization suggestions and measures: Appropriate amounts of grey shell and organic fertilizer can be applied to adjust soil pH value, accelerate soil mineral weathering, and promote the release and activation of trace elements such as B and Cu. Or the supplemented content of organic matter can be increased to increase the content of humus. It is also necessary to reasonably apply nitrogen, phosphorus and potassium compound fertilizers and maintain Se levels. Due to the lack of available B in the soil, B-containing microelement fertilizers should be applied in an appropriate amount, and it is recommended to mix B fertilizer with calcium phosphate or organic fertilizer for supplementary application. The soil in peach orchards is deficient in available Zn, and appropriate supplementation of Zn-containing microelement fertilizers is necessary. The mixed application of Zn, phosphorus fertilizer and urea has a more effective fertilizer efficiency. Efforts in soil and water conservation should be strengthened to prevent the loss of trace elements.

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Environmental stress induces oxidative damage and changes the balance of ROS production and clearance. The damage of drought and heat stress to turfgrass in cold season is related to the decrease of antioxidant enzyme activity and lipid peroxidation induced by oxidative stress[9]. In *C. dactylon* under drought stress and *S. guianensis* in warm season under cold stress, the antioxidant enzyme activity decreased[10]. During the whole stress period, the SOD activity of *D. sanguinalis* decreased easily due to the influence of low temperature. After 5 and 10 d of cold storage, the activity of CAT and POD increased temporarily, while plants were not or rarely damaged by cold storage stress. After 10 d of cold storage, the activity of CAT and POD decreased with the increase of cold storage days, and was related to the increase of osmotic potential, indicating that the decrease of antioxidant enzyme activity was related to 10 d of cold storage.

ABA has a positive effect on the cold resistance of maize[10]. *D. sanguinalis* treated with ABA had higher CAT and POD activity than untreated plants, especially after 10 d of low temperature stress. Our results are consistent with previous investigations on other plants. ABA increased the activity of CAT and POD, and ABA triggered the increase of ROS production, which further led to the induction of the antioxidant system. The results showed that ABA improved the cold resistance of *D. sanguinalis*, which was related to the induction of the antioxidant system.

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