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Effects of Exogenous SA and H_2O_2 on Wheat-Seedling's Antioxidant Enzymes in Different Temperatures

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Abstract: *In order to clarify the compensation effect of exogenous salicylic acid (SA) and H_2O_2 on wheat growth, this paper, by setting different growth temperatures in the artificial climate chamber, has studied the effects of antioxidant enzymes and proline content of wheat seedlings with spraying SA, hydrogen peroxide (H_2O_2) and SA+H_2O_2. The results show that catalase (CAT) activity, in 0 and 5 °C is lower than 10 and 15 °C, while the peroxidase (POD) activity is opposite. The activity of ascorbate peroxidase (APX) increases gradually with temperature increasing. Exogenous SA, H_2O_2, and SA+H_2O_2 could effectively improve the activity of wheat antioxidant enzymes and the content of proline (Pro), while the SA+H_2O_2 treatment improves obviously.*

Keywords: *wheat; salicylic acid(SA); hydrogen peroxide(H_2O_2); antioxidant enzyme*

1. Introduction

Wheat is one of the main food crops in China. The production has been affected by extreme weather in recent years, especially in northern of China. In winter, such as warm and sudden cold often occur, which have great negative effects on wheat production. Salicylic acid (SA) and hydrogen peroxide (H_2O_2) can effectively improve plant resistance [1]. The research about tobacco, which was treated with SA in 4℃ for 4 days, shows that the activity of SOD, CAT, POD and APX increased [2, 3]. In addition, after soaking melon fruits with salicylic acid, it was found that the content of H_2O_2, SOD activity and malondialdehyde content significantly increased, but the POD activity was inhibited on the 10th day. Vigliocco Ana et al. [4] studied the relationship between SA and H_2O_2 during sunflower seed germination, and pointed out that the decrease of SA was related to the increase of H_2O_2 content, which was attributed to the decrease of CAT activity. Meanwhile, Anna Ignatenko et al. [5] treated winter wheat with exogenous SA, and found that, in 4 ℃, exogenous SA could increase the activity of SOD, CAT, POD and the content of Pro, but the content of MDA and H_2O_2 firstly increased and then decreased. Lv et al. [6] pointed out that the accumulation of H_2O_2, in the injured agarwood stems, would induce the accumulation of Jasmonic acid and SA. And Liu et al. [7] treated oat seedlings with H_2O_2 and found that the activity of CAT, POD and APX increased after 2 days. Moreover, Du et al. [8] showed that salicylic acid could improve the resistance of maize for the high and low temperature stress. Furthermore, SA and H_2O_2 are important components of plant disease resistance mechanism. Previous studies has shown that, whether SA and H_2O_2 quickly enter into cell and play a synergistic role in promoting plant disease resistance are influenced by the plants' own ion channels [9]. A. al et Al. [10] pointed out that the barley with strong affinity for SA and H_2O_2 had higher synergistic effect. However, earlier studies showed that the increase of H_2O_2 could not be detected or the production of H_2O_2 in apple leaves treated by the increase of exogenous SA [11] during the formation of SA receptor protein complex (SAR) in plants. In addition, by the exogenous SA on the melon, it improved the production rate of O^{2-} and the content of H_2O_2 in the fruit, while Yang et al. [13] found that the content of H_2O_2 decreased in water stress, by spraying salicylic acid on maize seedlings. Moreover, the study of Anna Ignatenko etc. [5] showed that the H_2O_2 content of wheat, treated with exogenous SA in 4 °C, showed a trend of rising, firstly, and then reducing. On the other

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hand, the research of Zhang Man, Wan Lin etc. [14, 15] shows that exogenous H_2O_2 could improve rape seed germination rate, promote CAT POD enzyme activity, so as to enhance its cold resistance, while Liu [16] pointed out that H_2O_2 might be located in the upstream of ABA, by controlling its expression, which could alleviate cell membrane damage in low 5 °C. However, in different studies, there are few studies on wheat, especial the combined effect of SA and H_2O_2 on the wheat fewer.

In order to improve the wheat resistance to the abnormal temperature, this paper has studied the main antioxidant enzymes and osmotic regulation substances changing of wheat seedlings, by exogenous SA, H_2O_2 and SA+ H_2O_2, in different temperatures, hoping to provide theoretical basis and reference to the interaction mechanism of SA and H_2O_2, and also provides technical guidance for its wide application in wheat cultivation and production.

2. Materials and Methods

2.1. Test Materials and Design

Zhoumai 18 was selected as the test variety and treated with exogenous salicylic acid (SA) hydrogen peroxide (H_2O_2) and its compound. After germinating, the better and similar seedlings were planted in the 10*10 cm plastic bowl and putted on the cultivate frame, which growing on the conditions of 25 °C/18 °C and day and night (12/12 h). In the trifoliate stage, clear water was sprayed on the leaf surface as the contrast (the leaf surface was uniformly covered with a layer of water film). Treatments (0.3 g/L SA,150 μL/L H_2O_2, 0.3 g/L SA+150 μL/L H_2O_2), which the water (CK) was repeated three times in the artificial climate chamber of 15, 10, 5, 0 ℃ for 12, 24, and 48 hours. Taking the Leaf and putting in -80 ℃ refrigerator, which was used to test the CAT, APX, POD activity and PRO content.

2.2. Determine items and methods

The test methods are as follows [17], enzyme extraction buffer (PH 7.0 phosphate buffer, containing 20% glycerin, 1 mmol/L EDTA, 1 mmol/L ASA, 1 mmol/L DTT, 5 mmol/L $MgCl_2$, and 1 mmol/L GSH). Leaf blade 0.5 g, add 5mL extract buffer, Ice bath grinding, 12000 g 4 ℃ centrifugation 20min. The supernatant is the enzyme extract, which can be determined by POD, CAT and APX at one time.

(1) CAT test methods, the reaction solution (for immediate use and preparation): 10mM 30% H_2O_2 is diluted to 250mL with PH 7.0 phosphate buffer, and 0.1 ml enzyme extract which is added to 2.9 ml CAT reaction solution for coloration at 240 nm.

(2) APX test methods, the reaction solution (for immediate use and preparation): 2 mM 30% H_2O_2+0.5 mM ASA（0.5 mM），phosphate buffer (pH 7.0) has a constant volume of 500 mL, 0.1 mL of the enzyme extract and 2.9 mL of the APX reaction solution are colorimetric at 290nm.

(3) POD test methods, the reaction solution (for immediate use and preparation): 0.05% guaiacol + 10mM 30% H_2O_2, dilute with PH7.0 phosphate buffer to 250mL, the enzyme extract of 20μL plus 3mL of the reaction solution is colorimetric at 470 nm.

(4) PRO test methods: the enzyme extract of 1mL plus 5 mL of the Coomassie Brilliant Blue G-250, react 2min, 595 nm colorimetric.

2.3. Data processing and statistical analysis

Data were statistically analyzed by software such as Excel and origin.

3. Results and discussions

3.1. Effects of exogenous SA and H_2O_2 on CAT activity in wheat seedlings

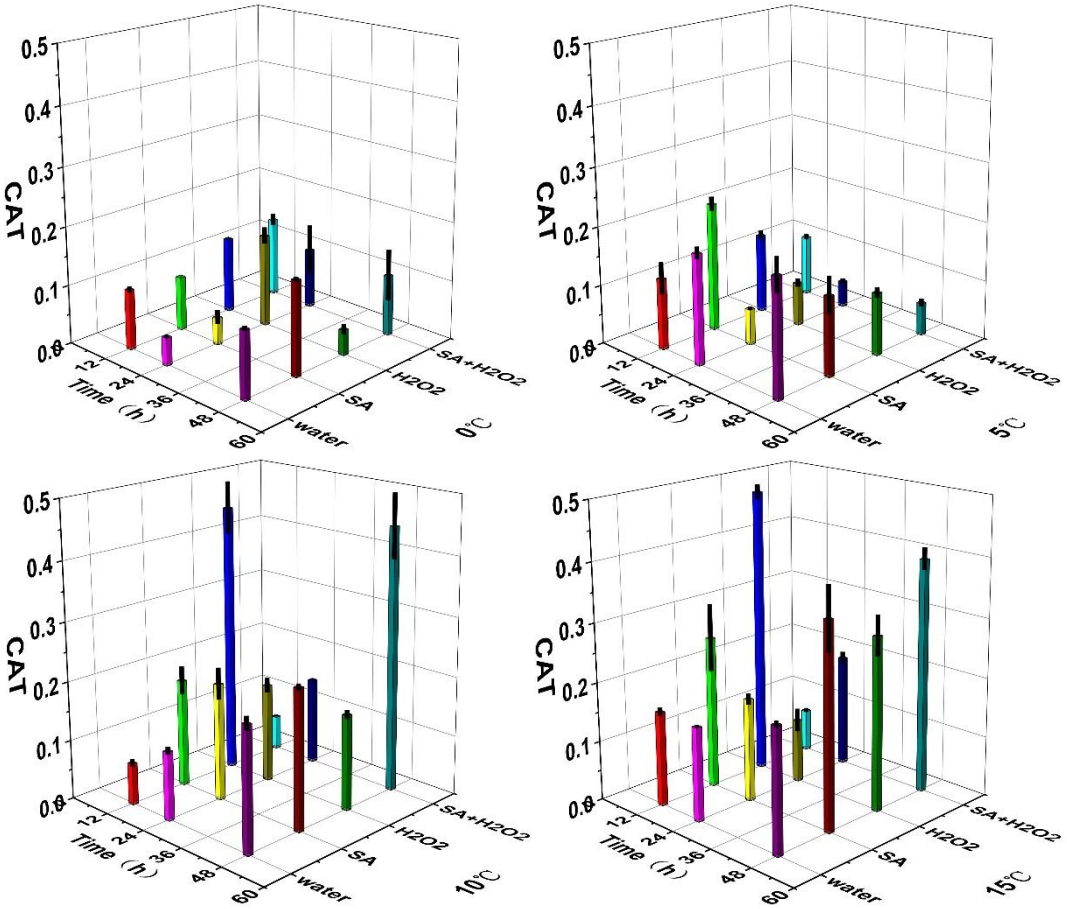


Figure 1. The CAT activity of different treatment (Enzyme Unit: u/g min)

As shown in Figure 1, at the time of 12 h, compared with CK, the SA and H_2O_2 treatments, in 10 and 15℃, are significantly higher than CK, but the improvement are not obvious in 0 and 5 °C. Besides compared with CK, the treatment effect of SA+H_2O_2 does not show significant differences. At the time of 24 h, compared with CK, the enzymatic activity of SA, H_2O_2 and SA+ H_2O, in different temperatures, are similar to or higher than CK, except in 5 °C. The enzyme activity of SA and H_2O_2 treatments show a trend of similar degree comparing with that of treatments for 12 h, in which, the activity of SA+H_2O_2 decreases in 0 and 5 °C, but increases in 10 and 15 °C.

At the time of 48 h, except that SA in 0 °C is slightly higher than CK, other treatments in 0 and 5 °C are lower than CK, and the enzyme activity of SA+H_2O_2 significantly reduce in 5 °C. Under the conditions of 10 and 15 °C, except that the 10°C H_2O_2 treatment is slightly lower than CK, the other treatments are all higher than CK, and the enzyme activity of SA+H_2O_2 treatment increase significantly. Compared to treatments at 24 hours, the enzyme activity of SA and H_2O_2 treatments at 48 h is lower than those at 24 h, except the 0 °C treatment of H_2O_2. Under 0 and 5 °C, SA+H_2O_2 treatment shows a trend of gradually decreasing with the advance of time. However, it shows a trend of gradual increase, obviously at 48 h comparing to the CK.

In conclusion, SA, H_2O_2 and SA+H_2O_2 can improve the CAT activity of wheat seedlings and the temperature is one important factor. When the temperature greater than 10 °C, the effects of SA and H_2O_2 gradually enhance, while the treatment of SA+H_2O_2 is easy to increase the enzyme activity at higher temperature.

3.2. Effects of exogenous SA and H_2O_2 on POD activity in wheat seedlings

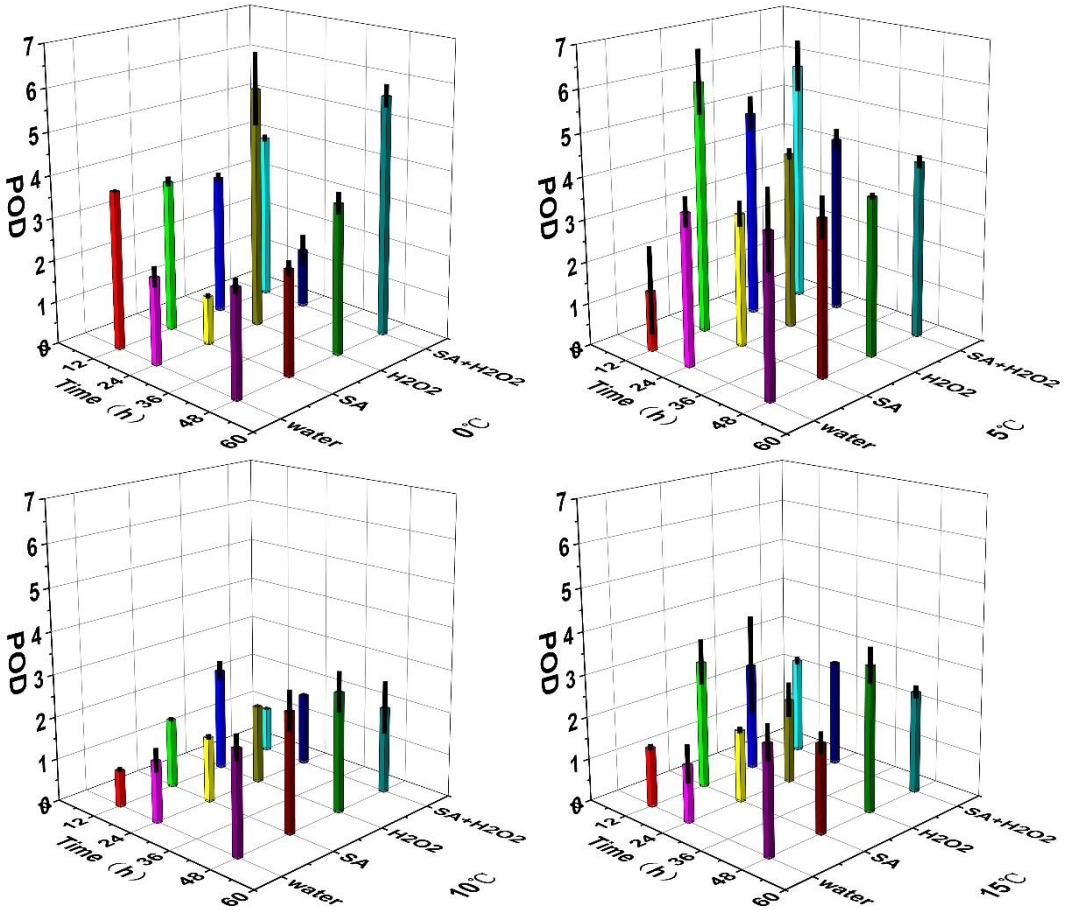


Figure 2. The POD activity of different treatment (Enzyme Unit: u/g min)

As shown in Figure 2, at the time of 12 h, compared with treatments at 0 and 5 °C, POD activity decreases at 10 °C, but they are higher than CK by the treat of SA, H_2O_2 and SA+H_2O_2. For 24h, at different temperatures, except for SA and SA+H_2O_2 at 0 °C and SA treatment at 5 °C, other treatments at different temperatures are higher than CK, especially H_2O_2 at 0 °C, which is significantly higher than CK. For 48h, at different temperatures, SA+H_2O_2 under 10°C and SA+H_2O_2 under 15 °C are slightly lower than CK, while other treatments are higher than CK. In particular, SA+H_2O_2 in 0 °C are more significant. In different temperature conditions, the POD activity of seedlings with SA, H_2O_2 and SA+H_2O_2 vary. SA, H_2O_2 (10 and 15 °C) and SA+H_2O_2 (0 °C) firstly decrease and then increase, while H_2O_2(0°C) firstly increase and then decrease, H_2O_2 (5 °C) and SA+H_2O_2 (5 °C) gradually decrease, but SA+H_2O_2 (10 and 15 °C) gradually increase. Visibly, under low temperature conditions, the treatment of SA, H_2O_2 and SA+ H_2O_2 can improve the POD activity of wheat seedlings, and the compensation temperature has a negative effect on the seedlings.

3.3. Effects of exogenous SA and H_2O_2 on APX activity in wheat seedlings

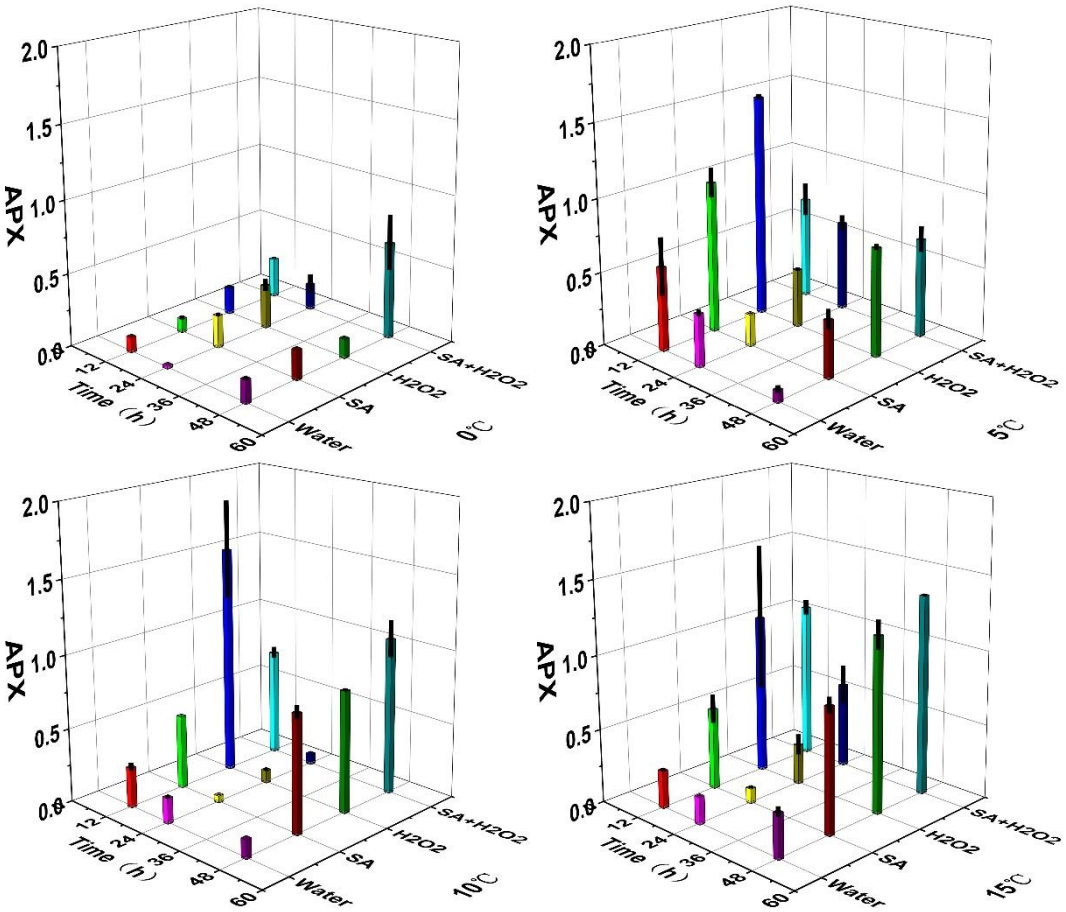
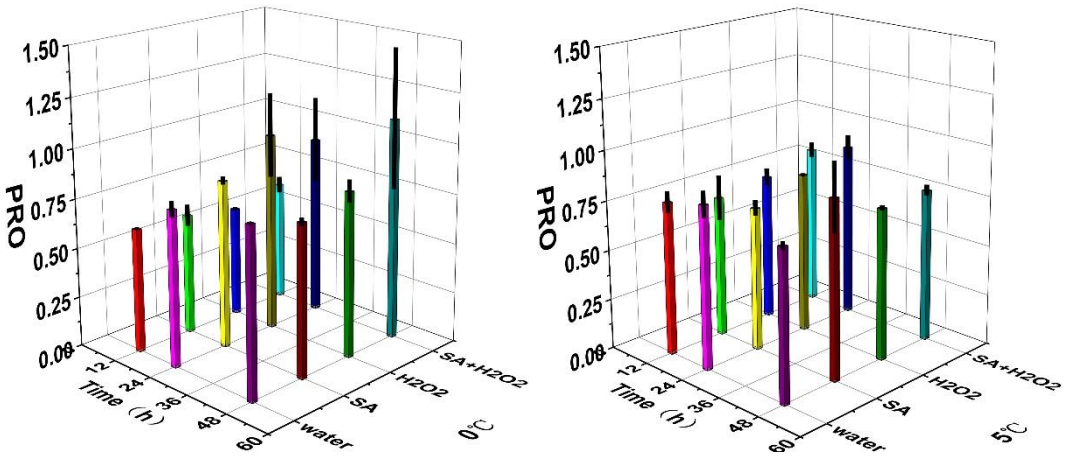


Figure 3. The APX activity of different treatment (Enzyme Unit: u/g min)

As shown in Figure 3，the activity of APX is lower in different treatments at 0 °C. But compared with CK, the enzyme activity of SA+H_2O_2 treatment increased significantly at 48 h. Under the conditions of 5, 10, and 15 °C, the change trend of enzyme activity of different treatments was basically the same. Among them, H_2O_2 (12 h) and SA+H_2O_2 (48 h) were significantly higher than CK. The enhancement of APX enzyme activity by SA, H_2O_2 and SA + H_2O_2 mainly occurred at 12 h and 48 h, and different treatments were significantly higher than CK at 48 h. It can be seen that SA, H_2O_2 and SA + H_2O_2 can increase APX activity of wheat seedlings.

3.4. Effects of exogenous SA and H_2O_2 on PRO content in wheat seedlings



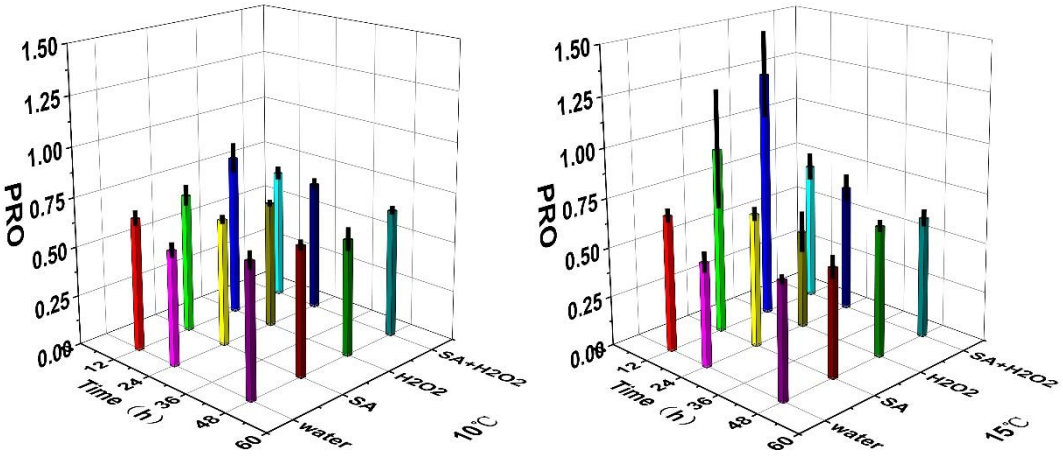


Figure 4. The PRO content of different treatment (Content unit: μg/g)

As shown in Figure 4, the change trend of Pro content in different treatments is basically the same under different temperature conditions. At 0 °C, PRO content in different treatments showed an upward trend, SA+H_2O_2 treatment improved significantly compared with CK. At 10 and 15 °C, the PRO content of different treatments showed a downward trend, the content of SA and H_2O_2 increased significantly at 15 °C compared with CK. Finally, the trend of each treatment was not obvious at 5 °C. It can be seen that SA, H_2O_2 and SA+H_2O_2 can increase the PRO content of wheat seedlings, and the effect is different at different time points

4. Conclusions

Antioxidant enzyme is an important protective system to maintain cell physiological function and structural stability under stress. In this study, CAT & POD, APX (antioxidant protection in chloroplast) and PRO (cytoplasmic colloid stability), which are directly related to H_2O_2 metabolism, were selected as the main research indicators to explore the effect of SA and H_2O_2 on wheat growth at different temperatures.

According to Figures 1-3, although compared with 10 and 15 °C, the overall CAT activity of SA, H_2O_2 and SA + H_2O_2 is lower at 0 and 5 °C. However, POD is on the contrary, except at 0°C, the variation trend of APX at 5, 10, and 15°C is basically the same. Among them, the treatment with exogenous SA, H_2O_2 and SA + H_2O_2 was equal to or higher than the control for 48h, and these studies were consistent with the seed germination of *Platycladus orientalis* [18] and *Artemisia sphaerocephala* [19], the root [20] development of wheat and the heat resistance of Rhododendron [21], indicating that it can increase the compensation effect of wheat on the impact of different temperatures by increasing the activity of antioxidant enzymes and the like.

As shown in Figure 4, SA + H_2O_2 treatment at 0 °C for 12 to 48 h and at 10 and 15 °C for 12 h could effectively increase the proline content of cells, which indicated that SA + H_2O_2 treatment could increase the permeation level of cells to enhance the stress resistance of cells. However, for different enzymes, the enzymatic activities of exogenous SA, H_2O_2 and SA+ H_2O_2 treated at 0-5°C and 10-15°C were significantly different especially CAT and POD as shown in Figure 1 and 2. POD activity was higher and CAT was lower at 0-5 °C, but the opposite was true at 10-15 °C. These results indicate that temperature has a certain influence on the induction effect of exogenous SA, H_2O_2 and SA + H_2O_2 treatment, and the specific mechanism needs to be further studied. The APX activity is generally lower except at 0 °C. As the temperature rises, the induction effect of SA and H_2O_2 is enhanced, especially the treatment of SA + H_2O_2.

Based on the above, it can be seen that exogenous SA and H_2O_2 and their synergistic application can improve the compensation effect of wheat on different temperatures, but affected by temperature factors, the induction effect of SA + H_2O_2 treatment is obvious especially at lower temperatures.

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