Pak. J. Bot., 53(6): 2025-2031, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-6(15)

EXOGENOUS SALICYLIC ACID IMPROVES CHILLING TOLERANCE OF EDIBLE LILY BULBS IN COLD-STORAGE

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Abstract

In the experiment, we examined the effects of SA on post-harvest physiology and storage quality of edible Lily (*Lilium lancifolium* Thunb). The bulbs were stored under cold temperature (0°C /-1°C) after being treated with SA (0.1 mmol/L, 0.5 mmol/L and 1.0 mmol/L) for 30 min, respectively. During storage, we measured the SOD activity, POD activity, CAT activity, PPO activity soluble protein content, soluble sugar content and other indicators of lilies at a 30-day interval. The results showed that 0.5 mmol/L SA pretreatment could significantly inhibit the lily bulbs SOD, POD, CAT activity of metabolic rate, and inhibit the osmotic regulation substances, such as soluble sugar, soluble protein decomposition speed to improve the low temperature adaptability of lily bulbs. In conclusion, SA liquid could effectively inhibit the post-harvest physiology of lily bulbs and maintain their storage quality.

Key words: Exogenous SA; *Lilium lancifolium* Thunb; Antioxidant activities; Low temperature storage.

Abbreviations: SOD– superoxide dismutase, POD– peroxidase, SA– salicylic acid, PPO–polyphenoloxidase, CAT– catalase.

Introduction

Edible lily was a multi-functional lily variety with Chinese characteristics of flowers, medicine and food. At present, it mainly includes several varieties such as *Lilium David* II var. *unicol*, *Lilium Brown* II var. *viridulum* and Yixing lily (Galli *et al.*, 2009). Its bulbs were nutritious, had health care and ornamental functions for a wide application prospect. The edible lily was a perennial herbaceous bulbous flower, and the bulbous bulb had the characteristic of natural dormancy (Mao *et al.*, 2007). The traditional 'sandy soil layer method' was usually used for its natural storage, but the quality of the bulbous preservation was low and it was easy to cause the bulbous scale to rot (*Yordanova et al.*, 2007; González-Aguilar *et al.*, 2004). In recent years, the cold storage method instead of the original traditional storage method to ensure the quality of edible lily bulbs was gradually adopted (Imahori *et al*., 2008), but the low-temperature storage method had accelerated the dormancy release of lily bulbs. Previous studies had shown that lily bulbs had vigorous physiological metabolism after dormancy-releasing and were prone to freezing damage after low-temperature stimulation, resulting in poor storability and great loss, making it difficult to achieve the goal of annual production for long-term storage of lily bulbs (Tang *et al.*, 2020). Therefore, the storage quality of edible lily bulbs for long-term low-temperature storage was an urgent problem to be solved.

SA was a phenolic compound commonly found in plants (Raimbault *et al.*, 2011). As a natural signaling molecule, SA played an important role in inducing plant defense to produce corresponding resistance (Lafuente *et al.*, 2004; Castillo *et al.*, 2015). At present, the study of using exogenous SA to improve plant defense response, antioxidant response and abiotic stress resistance had attracted much attention. The physiological and biochemical effects of SA solution on fresh-cut apples after storage for a period of time (Supapvanich *et al.*, 2013) were discussed. It was found that 0.25 mmol/L SA could effectively inhibit the activities of GR and APX, improve the antioxidant activity of fresh-cut apples, protect nutrients and improve the internal quality. Ding *et al.*, (2018) studied the proteomic effects of different concentrations of SA on different ripening stages of cherries and found that SA could stimulate the transcriptional level of oxidoreductase to increase to improve the resistance of cherry to pathogen invasion, thus reducing the decay rate of cherry after harvest. Although SA had been reported in postharvest storage of many plants, it was rarely used in lily bulbs, especially in low temperature storage of edible lilies.

In view of above reasons, edible lilies were used as the research material in our study and the pretreatment method of exogenous SA was adopted to study the physiological function and mechanism of exogenous SA in low-temperature storage of edible lily bulbs from the perspective of soluble protein, soluble sugar, antioxidant enzyme system and other active substances, in order to provide a theoretical basis for physiological research and application of edible lily bulbs in low-temperature storage and to seek for exogenous regulation and control in long-term storage of edible lily bulbs.

Materials and Methods

Plant materials and treatment: Yixing lily variety with 16-18 cm bulbs was harvested in Ludong Unversity in August 2017, cleaned and disinfected, and then treated by

natural air drying. Before cold storage for 48 h, bulbs were soaked in SA solution for a total of 0.1 mmol/L, 0.5 mmol/L and 1.0 mmol/L concentration gradients, with clear water as control (CK). Each treatment was soaked for 30 min with 10 bulbs and three times were repeated. After being soaked, bulbs were naturally air-dried and then were packed into boxes. The treatment experiment was conducted in August 2017 at Ludong Unversity. Bulbs with healthy scales and consistent sizes were selected for treatment. The pretreated bulb boxes were placed in a cold storage with a temperature of 0°C / - 1°C (day / night) and a relative humidity of 30%.

Division of development period for dormancy release: The dormancy-releasing process of lily was a change from vegetative growth to reproductive growth. A series of physiological and metabolic activities occurred inside the bulb, and its growing point also underwent certain morphological changes. According to the results of the pre-experiment in the early stage, the time interval was sampled as six development stages, from the initial stage to the end of cold storage (Ⅰ ~ VI) after storage, about 30 days per period (Table 1).

Determination of physiological indexes: The total soluble sugar content was determined by colorimetry (Nanjing built kit, No. A145) and the soluble protein content was determined by Coomassie brilliant blue G-250 (Nanjing built kit, No.A045-2). SOD, POD, CAT and PPO enzyme activities were also selected from Nanjing built kit (Serial numbers A001 - 4, A084 - 3, A007 - 1, A136).

Data analysis

The experimental data obtained from the measurement were preliminarily processed by WPS table, and further variance analysis, principal effect analysis and interaction effect analysis were carried out by SAS software and R language software.

Results and analysis

Changes of soluble sugar content in lily bulbs during cryogenic storage: During the whole cryogenic storage process, the total soluble sugar content in bulbs increased first and then decreased, and the total soluble sugar content in bulbs after SA pretreatment was consistent with the overall trend of 'parabola' (Fig. 1). However, as the cold storage continues, the total soluble sugar content pretreated by SA stablely rised and got to a small peak at stage IV of late induction, that was different from the peak period of the control group (stage III). After the peak, the soluble sugar content of the control group decreased significantly, but the soluble sugar content after SA pretreatment decreased slightly. Tendency for 0.1 mol/L of SA pretreatment concentration, in addition to the stage V with obvious difference from the control group, the rest showed extremely significant differences in the concent of souble sugar.When SA pretreatment concentration were 0.5 mmol/L and 1.0 mmol/L, the soluble sugar content in phase III were significantly different from that in the control, and the other phases were also extremely significant.

Changes of soluble protein content in lily bulbs during low temperature storage: During the cold storage period, the soluble protein content of lily bulbs increased first and then decreased, while the change trend of bulbs treated with SA was basically consistent with the control (Fig. 2).There was no significant difference in soluble protein content between the control and SA pretreatment during the first and second stages of cold storage.When the concentration of SA pretreatment was 0.1 mmol/L, the content of soluble protein in the IV period was significantly different from that of control, while the remaining stages were no obvious difference. When SA pretreatment concentration was 0.5 mmol/L, the III period was significantly different from the control group, and the IV period was significantly different from that of control.When the SA treatment concentration was 1.0 mmol/L, the V period was significantly different from the control, and the IV period group appeared remarkably different. It showed that the temperature sensitivity of lily bulbs after SA pretreatment was reduced, and the cold resistance of lily bulb was improved.

Changes of antioxidant enzyme activity of edible lily during low temperature storage: The SOD activities of lily bulbs with different concentrations of SA were increased first and then decreased (Fig. 3). The SOD activities of SA pretreatment in refrigerated I and II period were consistent with that of soluble protein content, and there was no obvious difference compared with the control. When the concentration of SA pretreatment was 0.1 mmol/L, except for the IV period, the SOD activity was significantly different from the control, and the remaining stages were not significantly different. When the SA pretreatment concentration was 0.5 mmol/l, the III period and the IV phase were significantly different than those of the control group, and there were no obvious differences in the remaining stages. When the SA treatment concentration was 1.0 mmol/l, the V period and the control showed significant difference, and the III, IV and VI periods were significantly different from the control. It was shown that the effect of SOD activity on lily bulb was higher when the concentration of SA pretreatment was 1.0 mmol/L.

Table 1 Periods of sampling for the experiments.

No.	I	II	III	IV	V	VI
Sampling time	Aug. 6th	Sep. 6th	Oct. 6th	Nov. 6th	Dec. 6th	Jan. 6th
Sampling periods	The early period of cold storags	The period of cold storage	The middle period of cold storage	The middle and later period of cold storage	The later period of cold storage	The end period of cold storage

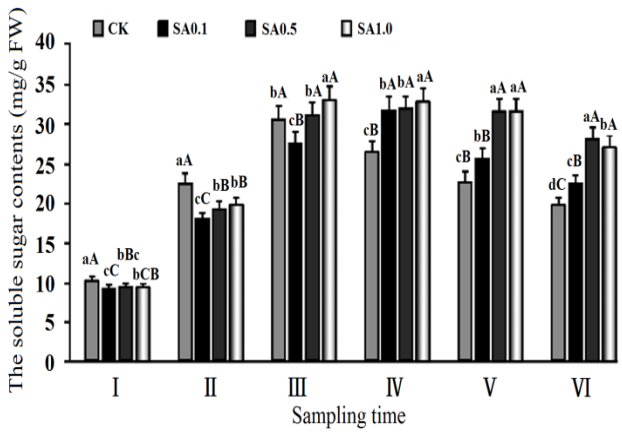

Fig. 1. Effects of exogenous SA on soluble sugar contents in lily bulbs under low temperature for storage.

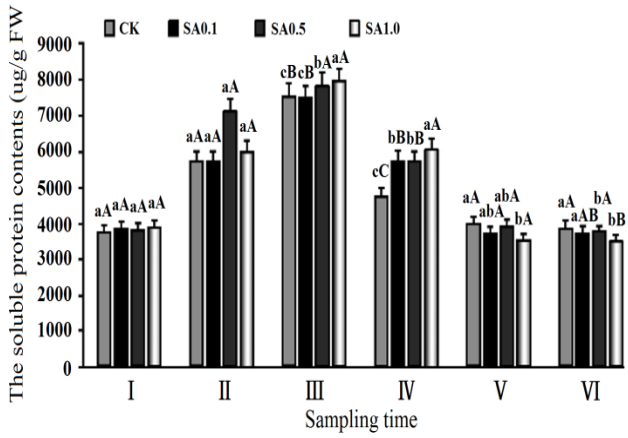

Fig. 2. Effects of exogenous SA on soluble protein contents in lily bulbs under low temperature for long-term storage.

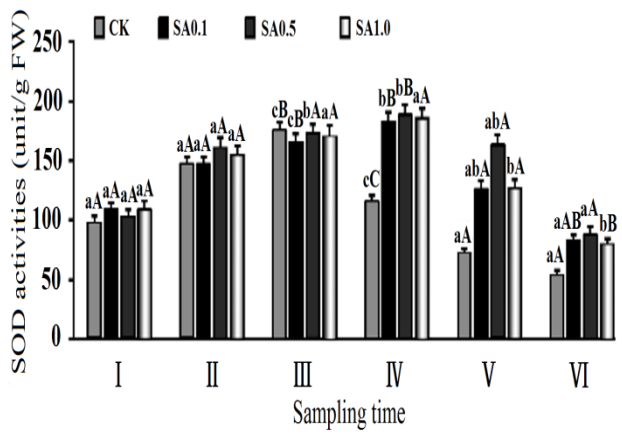

Fig. 3. Effects of exogenous SA on SOD activities in lily bulbs under low temperature for storage.

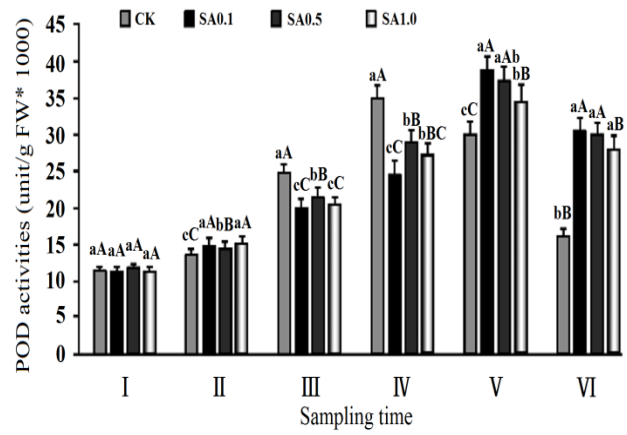

Fig. 4. Effects of exogenous SA on POD activities in lily bulbs under low temperature for storage.

With the increasing of refrigeration time, the POD activity of lily bulbs showed a tendency to rise first and then decreased, and the bulbs of the control group reached the highest active value in the IV period, and then began to show a significant decrease (Fig. 4). The peak of POD activity reated with different concentrations of SA appeared in the V period, and then also began to decline. The peaks of POD activity in three different treatments: SA 0.1> SA 0.5> SA 1.0; When SA concentrations were 0.1 mmol/L and 0.5 mmol/L, there were no significant differences between I period and control, but the remaining stages were significantly different; There were significant differences between 0.1 mmol/l and 0.5mmol/l in II, III and IV periods, and there was no significant difference between the other two phases. When SA pretreatment concentration was 1.0 mmol/L, POD activity in I period was not significantly different from control, while the remaining stages were not significantly different. When SA concentration was 1.0mmol/l, there were significant differences between the control and the II to the V periods.

CAT was one of the most important respiratory enzymes in plants, and the change of CAT activity showed a tendency to increase and decrease after different SA concentrations for lily bulbs during cold storage (Fig. 5). The POD activity was the highest in the IV stage of lily bulbs with different concentrations of SA, but the peak of CAT activity was highest in control.When SA concentration was 0.1 mmol/L, there was significant difference between CAT enzyme activity and the control in I, II and III period, and there was significant difference between IV and VI period and the control group, but no significant difference in V period. When SA concentration was 0.5 mmol/L, there were also significant differences between the first three stages and the control. There was significant difference in V period, but there was no significant difference between IV and VI period and control. while SA pretreatment was 1.0 mmol/L, the I, II and VI periods were significantly different from those of control, and there was no significant difference in the remaining stages. When the concentration of SA was 0.5 mmol/L among the three different treatments, the increasing and decreasing of CAT activity was slow, which showed that concentration pretreatment had some delaying effect on the low temperature stimulation of lily bulbs.

The PPO activity of lily bulbs in control group was continuously enhanced during refrigeration (Fig. 6); The PPO activities of lily bulbs in 0.1 mmol/L and 0.5 mmol/L concentration were consistent with the contrast, and there was a continuous enhancement trend in different concentrations of SA pretreatment. When the concentration of SA pretreatment was 1.0 mmol/L, the concentration showed a decreasing trend after the peak of IV period. When the concentration of SA pretreatment was 0.1 mmol/L, the

PPO activity of lily bulbs was only significant difference in III and V periods, and there was significant difference in IV period, and there was no significant difference between the remaining stages and control. When the SA pretreatment concentration was 0.5 mmol/l, the I and VI periods were not significantly different from the control, however, there was significant difference between the middle stage of refrigeration and the control.When the concentration of SA pretreatment was 1.0 mmol/L, except for the III period and the control, there were significant differences in the remaining stages. The results showed that SA pretreatment had a certain effect on PPO activity of lily bulbs.

Main Effects and interaction effects of edible lily in low temperature storage: The soluble sugar content of lily bulbs fluctuated obviously with the change of storage time and SA treatment concentration (Fig. 7A). For the untreated lily bulbs, the content peak appeared in the third stage. For lily bulbs with SA treatment concentration of 0.1 mmol/L, there was an obvious peak content in phase IV. For lily bulbs with SA treatment concentration of 0.5 mmol/L, the content of soluble sugar also peaked in phase IV, and was higher in phases III, VI and IV. The peak of soluble sugar content in lily bulbs with SA treatment of 1.0 mmol/L also appeared in phase III compared with the control, but the difference between phase III and phase IV was not obvious. All lily bulbs treated with SA were the same as the control, the lowest soluble sugar content appeared in the first stage, indicating that lily bulbs maintained a high metabolic activity in the frozen environment.

The soluble protein content of lily bulbs showed an obvious peak value in phase III with the different cold storage stages and SA treatment concentrations (Fig. 7B), indicating that phase III was the main stage of soluble protein content effect in lily bulbs, while the effect of different SA concentrations on soluble protein content was more balanced. The soluble protein content was significantly different from that of the control when SA treatment concentration was 0.5 mmol/L in the second stage. The soluble protein content of lily bulbs treated with SA in phase IV was higher than that of the control. However, the soluble protein of lily bulbs treated with SA was basically the same as that of control in the remaining several periods.

The SOD activity of lily bulbs showed obvious changes with different chilling stages and SA concentrations (Fig. 8A). The SOD activity of the control lily bulbs showed a peak in phase III, and there was a significant change in each phase After treated with SA, all lily bulbs showed obvious peak activity in phase IV. For lily bulbs with SA concentration of 0.5 mmol/L, the peak also appeared in phase IV, but SOD activity was similar in phase II and VI. The SOD activity of lily bulbs with SA of 0.5 was significantly different from that of the control. SOD enzyme was easy to inactivate, but this experiment was carried out under the environment below zero, so SOD enzyme maintained a high activity.

The POD activity of lily bulbs changed significantly with different chilling stage and SA concentrations (Fig. 8B). The POD activity peak of the control appeared in phase IV. After treated with SA, all lily bulbs showed obvious peak activity in phase V; After six cold storage periods, POD activity of lily bulbs with SA increased a lot compared with the control.

The CAT activity of lily bulbs changed significantly with different chilling stage and sa treatment concentration (Fig. 8C). The CAT activity of control showed a peak in phase IV, and there was a significant change in each phase. The lily bulbs with SA of 0.1 mmol/L also showed obvious peak activity in phase IV, compared with control. Lily bulbs with SA of 0.5 mmol/L also showed peak content in phase IV, but CAT activity in phases II and VI showed the lowest value in this time phase. The CAT activity of lily bulbs treated with SA at the concentration of 1.0 mmol/L in phase IV was consistent with that of control.

The PPO activity of lily bulbs changed significantly with different chilling stage and SA concentrations (Fig. 8D). The PPO activity of control showed a peak value in phase IV and V, and a lowest value in phase I; When the SA concentrations were 0.1 mmol/L and 0.5 mmol/L, the lily bulbs showed an obvious peak activity in phase V; When the SA concentration was 1.0 mmol/L, the PPO activity of lily bulb changed greatly in phase V. Through visual investigation, it was found that large area of mold appeared in phase V, presumably due to the bulbs decay, which greatly reduced the activity of PPO enzyme.

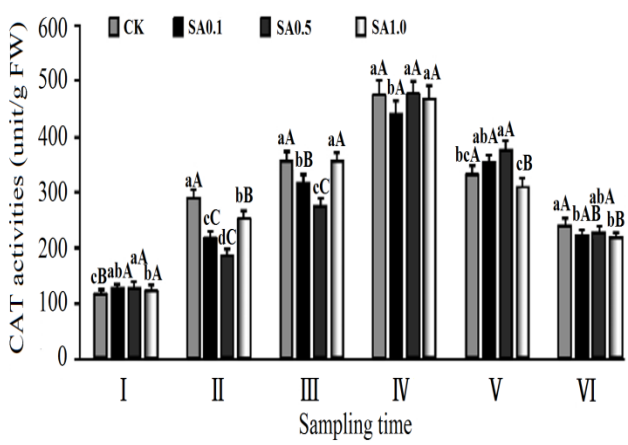

Fig. 5. Effects of exogenous SA on CAT activities in lily bulbs under low temperature for storage.

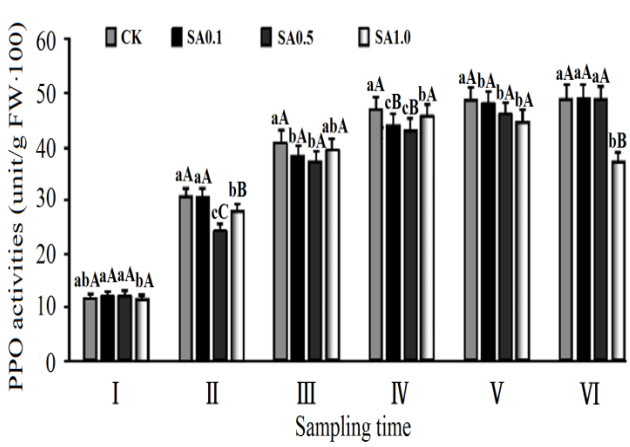

Fig. 6. Effects of exogenous SA on PPO activities in lily bulbs under low temperature for storage.

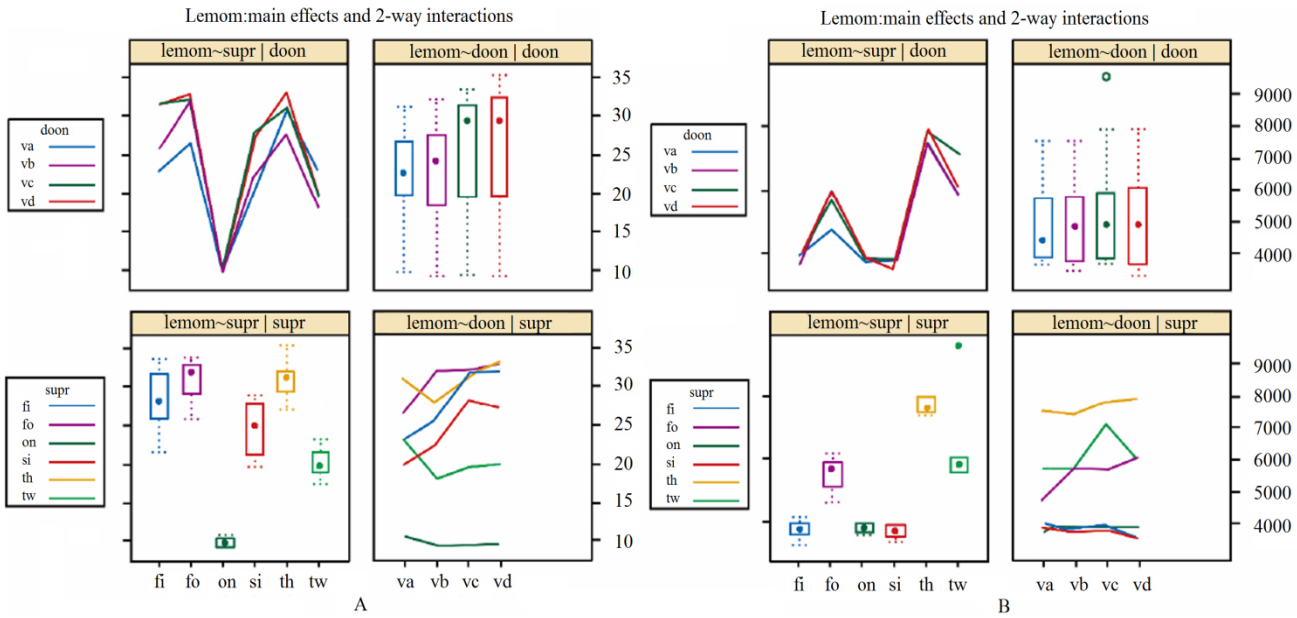

Fig. 7. Interaction effect of osmotic substances contents and different treatments under low temperature for storage.
A: Soluble sugar; B: Soluble protein.

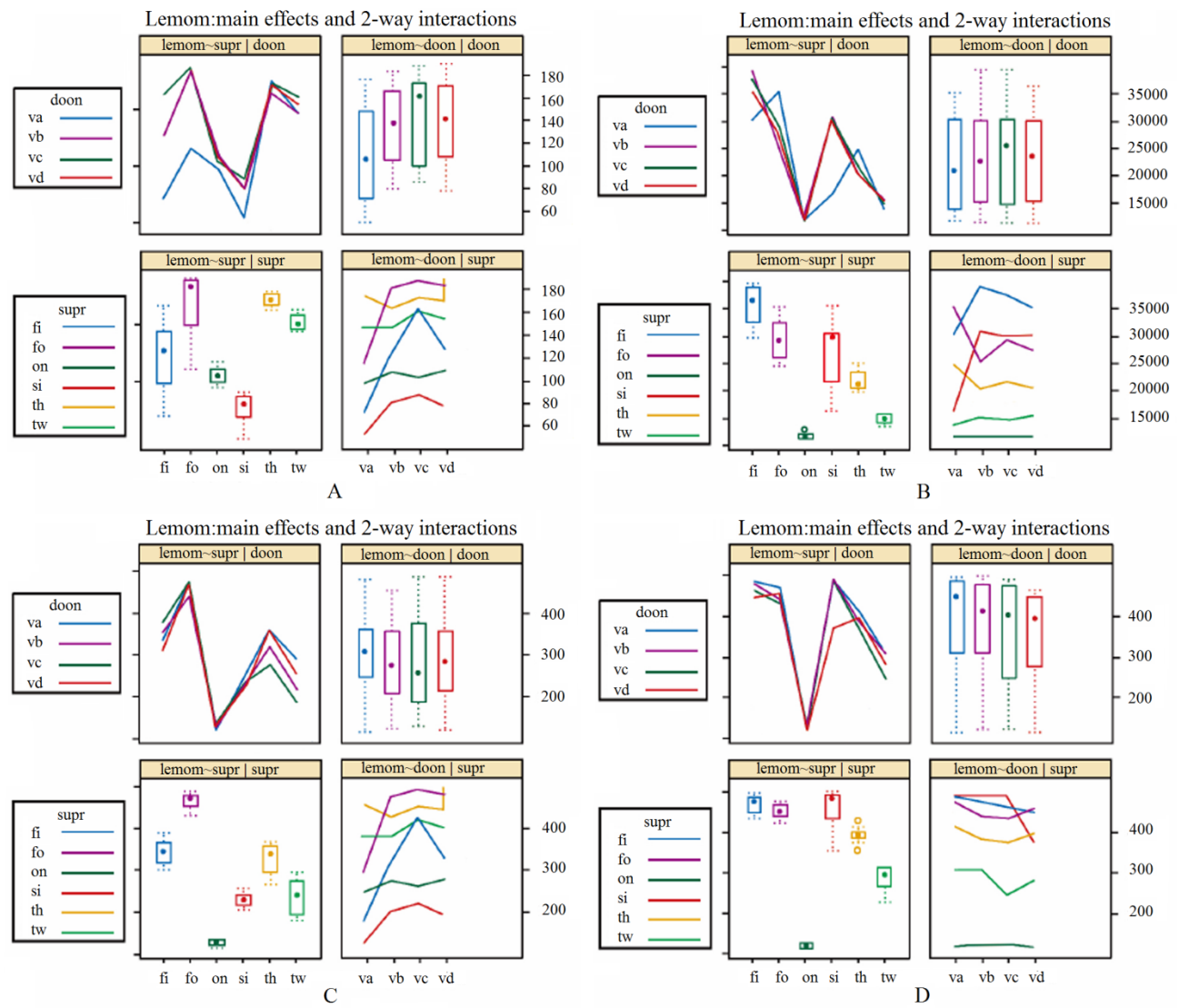

Fig. 8. Interaction effect of antioxidant enzyme activity and different treatments under low temperature for long-term storage.
A: SOD; B: POD; C: CAT; D: PPO

Discussion

The change of physiological index in storage period was usually regarded as an important criterion for plant storage performance and preservation effect. The purposes of this experiment was to prolong the storage time of lilies by delaying metabolic process to affect the physiological changes of storage (Fujii *et al.*, 2007; Ke *et al.*, 2007). The level of physiological metabolism of lily in the low temperature storage process would gradually increase with dormancy releasing and scale hardness declining, texture softening were the most significant changes in the loss of edible lily aging, but were alsod the important index of t storage resistance and commodity value (Radojicic *et al.*, 2018; Xu *et al.*, 2008) Siboza *et al*., (2013) found that the combined treatment of 10μmol/L acid methyl ester and 2μmol/L SA could reduce the membrane permeability caused by chilling injury and lipid peroxidation of cell membrane, inhibited PPO activity by increasing phenolic content and PAL activity to increase the cold tolerance of lemon effectively. Zhao *et al*., (2011) studied the effect of SA and on fresh-cut broccoli, and found that SA treatment increased the antioxidant enzymes (SOD, APX and POD) activity, inhibited the early storage of CAT activity, improved the level of endogenous H_2O_2, and SA's freshness effect was better than H_2O_2. The results of our study showed that SA pretreatment inhibited the activity of antioxidant enzymes (SOD and POD) of lily bulbs, and the peak of SA pretreatment was later than that of control, and the reduction rate declined less later. After 6 months of refrigeration, it was still keeping high activity. Antioxidant enzyme activity decreased the sensitivity of lily bulbs to low temperature, and delayed the aging process of edible lily. Compared with the control, the CAT activity of SA pretreatment was increased and decreased slowly, indicating that the pretreatment of the concentration delayed the low temperature stimulation of lily bulbs, slowed the respiration of bulbs and prolonged the low temperature storage time of bulbs. The growth of PPO activity was accelerated when the bulbs were induced by hypothermia and dormancy, but the bulbs adapted to low temperature induction and relieved dormancy late with the PPO activity tending to be stable. The PPO activity of lily bulbs treated by 1.0 mmol/l SA concentration was decreased after dormancy releasing, which showed that the concentration treatment slowed the aging of bulbs, and kept high activity in the low temperature environment.

The changes of physiological indexes after postharvest could affect the storage effect and commodity value, so the changes of physiological indexes such as soluble sugar and soluble protein couold directly indicate the quality of plants in the process of storage (Meng *et al.*, 2009) Our study found that the soluble sugar content appeared to be a 'parabolic' trend of edible lily in the long-term low-temperature storage process, speculating that starch transformed into soluble sugar in the early stage of cold storage to accumulate a large number of sugars to release the accumulation of nutrients for bulbs dormancy. The increased sugar concentration increased the temperature of bulbs freezing point at the same time to reduce the damage caused by chilling injury for bulbs in low temperature storage. The soluble sugar content decreased when the dormancy was relieved, but in our experiment that the SA treatment was less than that of soluble sugar content, and it was speculated that SA treatment the increased concentration of edible lily bulbs' sugar reduced the cold damage caused by low temperature and prolonged the storage time of edible lily bulbs. Similarly, the increased content of soluble protein in the early stage of cold storage accumulated the storage nutrient during the dormancy releasing process of lily bulbs, while the soluble protein showed a significant decrease after dormancy releasing, and an amount of protein was consumed in the process of dormancy stage. Compared with the control, after the SA pretreatment, the content of soluble protein was high and the decreasing amplitude was small, it was inferred that the SA pretreatment alleviated the decomposition of a variety of protease and increased the solubility of bulbs to delay the preservation time. In addition, the change of protein content in lilies, which was also a sign of plant activity, showed different levels of protein content during lily refrigeration, which indicated that lily bulbs had a strong vitality of life metabolism.

The application of exogenous SA solution slowed down the hardness of edible lily bulbs, inhibited the respiration intensity, delayed the aging, enhanced the storage resistance, and kept the quality of edible lily bulbs for a long time (Wang *et al.*, 2010; Huanga *et al.*, 2008). The suitable SA pretreatment improved the content of soluble protein and soluble sugar in a long-term storage of lily bulbs, retained a high activity of antioxidant enzymes, had no influence on bulb quality, and kept the activity quality and commodity value of bulbs. Comprehensive analysis of the test results, 0.5 mmol/l concentration of external SA solution was the best for a long-term low-temperature refrigeration preservation of Yixing lily.

Acknowledgements

This work has been jointly supported by the following grants: the National Key R&D Program Project (Grand No. 2019YFD1000503); the Shandong Provincial Natural Science Foundation (ZR2018PH041); the Key Research and Development Program of Shandong Province of China (2019GSF108154); the Agricultural Variety Improvement Project of Shandong Province (2020LZGC007); the Shandong Provincial Natural Science Foundation (ZR2020MC138).

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(Received for publication 3 March 2020)