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Effect of Exogenous Salicylic Acid on Induction of Cold Tolerance in Young Seedlings of Pomegranate (*Punica granatum* L.)

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Abstract. **To investigate the regulatory mechanism of exogenous salicylic acid (SA) on the low-temperature resistance of pomegranate seedlings, 'Fuanyihao' pomegranate seedlings were selected as experimental materials and placed in a −20 °C incubator to simulate low-temperature stress environment. The treatment groups were treated with 0 mmol/L SA + 2-h cold treatment (A0), 1 mmol/L SA + 2-h cold treatment (A1), and 1 mmol/L SA + 4-h cold treatment (A2); a control group (CK, 0 mmol/L SA + 0-h cold treatment) was also included. After processing, the pomegranate seedlings were cut from the roots, stems, and leaves of each group for paraffin sectioning to compare the differences in anatomic structure. The experimental results showed that after cold treatment, the chlorophyll content of pomegranate seedlings decreased, peroxidase activity increased, and catalase activity decreased. Pomegranate seedlings treated with exogenous salicylic acid showed some relief in various indicators. Therefore, spraying exogenous salicylic acid can maintain cell membrane permeability and enhance the cold resistance of pomegranate seedlings. By comparing the anatomic structures of each treatment group, it could be clearly seen that, compared with the control group, the cell membrane of pomegranate seedlings under cold stress is damaged, the tissue is loose, the formation of secondary xylem and phloem is reduced, the mechanical support of the stem decreases, and the structure of leaf mesophyll cells is destroyed. After spraying exogenous salicylic acid, the symptoms resolved, indicating that exogenous SA can alleviate the stress of low temperature on pomegranate seedlings and restore their normal growth state.**

Pomegranate (*Punica granatum* L.), as a common fruit tree, is cultivated in both the north and south of China and is native to Central Asia, with Iran as a primary site of origin (Luo et al. 2025; Teixeira da Silva et al. 2013; Yuan et al. 2018). Its flowers, leaves, skin, seeds, and juice contain abundant active ingredients such as sugars, polyphenols, flavonoids, and alkaloids, which have multiple effects such as anti-inflammatory, antioxidant, antitumor, hypoglycemic, and insect resistance. As a healthy fruit, pomegranate not only has significant economic potential but is also rich in various nutrients and has demonstrated unique value in the field of medicine. Its high economic value, rich nutritional characteristics, and extensive medical applications have led to the gradual rise of the pomegranate industry in the global economic market (Qin et al. 2017; Yuan et al. 2018). Plants are often planted in parks and scenic areas to beautify the environment.

Temperature is the primary factor restricting plant growth and development, as well as the main environmental factor affecting crop geographic distribution, yield, and quality. Throughout the growing season, crops are often subjected to various types of environmental stresses. As one of the main abiotic stresses, temperature stress mainly includes high temperature stress and cold stress (Zhou et al. 2025). Cold stress is an important environmental factor that limits plant growth, development, and distribution. Cold stress can interfere with the physiological activities of plants, such as water status, photosynthesis, and nitrogen metabolism. At the same time, it can slow down the absorption and transportation of water and mineral elements in the plant. In addition, low temperature can cause damage to the membrane system, decreased enzyme function, delayed physiological responses, and, in severe cases, cell freezing, leading to cold and frost damage. Cold damage usually causes yellow spots, dryness, or local necrosis in plant leaves, whereas freezing damage, due to its destruction of cell structure, causes the leaves to lose elasticity, ultimately leading to irreversible damage. Low temperature often leads to a slowdown in plant growth, leaf senescence, and an increase in intracellular reactive oxygen species, inducing enhanced antioxidant enzyme activity (Feng et al. 2025). Therefore, we must explore effective scientific methods through a series of experimental studies and theoretical reasoning to alleviate the adverse effects of cold stress on plant growth. Through systematic experimental verification and scientific analysis, effective strategies for coping with cold stress are explored, providing scientific basis for improving plant cold resistance and adaptability. In addition, it will provide important theoretical support for breeding cold-resistant varieties and optimizing cultivation techniques, helping plants better cope with the challenges brought by low-temperature environments.

Salicylic acid (SA), as an endogenous signaling molecule in plants, is essentially a small molecule phenolic substance that is widely distributed in the plant and plays a key regulatory role in physiological activities (Salinas et al. 2025). It not only plays an important role in the regulation of plant growth and development, absorption and transport of mineral elements, and metabolic activities such as photosynthesis, thereby significantly improving crop yield and quality, but also serves as an important signaling molecule for plant response to adversity (Decsi et al. 2025). Under adverse conditions, plants can enhance stress adaptability by regulating their antioxidant defense system, reducing damage caused by membrane lipid peroxidation. Research has shown that spraying exogenous SA can effectively improve the physiological response of plants to cold stress (Li and Wang 2021). Specifically, this substance significantly enhances the activity of superoxide dismutase (SOD) and catalase (CAT), while inhibiting the biosynthesis of malondialdehyde (MDA), thereby maintaining the stability of cell membrane structure. This dual regulatory effect not only reduces lipid peroxidation levels, but also significantly improves plant cold tolerance, providing effective protection against metabolic disorders caused by low temperatures (Ortega et al. 2024). The study by Du et al. (2022) confirmed that exogenous SA can enhance the cold resistance of wheat seedlings. Meanwhile, studies have shown that using SA to treat blackberry fruits after harvesting can effectively alleviate the negative effects of cold damage and help maintain the quality and nutritional value of the fruit (Sakaldaş et al. 2024).

Currently, research on the physiological indicators of pomegranate under abiotic stress mainly focuses on fields such as drought stress and salt stress. However, there are few reports on the screening of cold-resistance-related indicators of pomegranate seedlings under low-temperature stress. This study takes 'Fuanyihao' pomegranate seedlings as the research object, adopts branch cutting cultivation, and cultivates them in a −20 °C refrigerated room to simulate a cold-stress environment. The 'Fuanyihao' pomegranate seedlings are treated with exogenous SA solution at a concentration of 1 mmol/L and cold stress at different times. The cold resistance of pomegranate seedlings treated with exogenous SA solution at a concentration of 1 mmol/L is studied by measuring various morphological and physiological indicators of each treatment group. By comparing the anatomic structures of nutrient organs in each treatment group, the effect of exogenous SA on pomegranate seedlings is analyzed, providing theoretical basis for the improvement of cold resistance of 'Fuanyihao' pomegranate seedlings by exogenous salicylic acid treatment.

Materials and Methods

Plant materials and treatments. Punica granatum 'Fuanyihao' cuttings were hydroponically cultivated (in Huaibei Fu'an Family Farm, Huaibei, China) at 20 to 27 °C in a greenhouse. This variety was developed in 2010 using the Tunisian soft seed pomegranate as the maternal parent through natural pollination (the paternal pollen includes 'Huaibei Soft Seed 1', 'Huaibei Soft Seed 2', 'Huaibei Soft Seed 3', 'Wanhei No. 1', 'Mount Taishan Red', 'Tashan Acid Pomegranate', etc.) of a single plant.This variety has a semiopen tree shape, fast growth, early fruiting, and good fertility. Fruit close round, the young fruit has green skin, and the mature fruit has a yellow green color with a red halo.The fruit shape and grains are large, with high juice yield, softcore, and a pure sweet taste (Liu and Li 2025). The sample materials used for the experiment were micro annual cuttings.

Select pomegranate cuttings with good and robust growth for treatment. In the experiment, 0 mmol/L SA + 2-h cold treatment (A0), 1 mmol/L SA + 2-h cold treatment (A1), and 1 mmol/L SA + 4-h cold treatment (A2) were used as treatment groups, and a blank control group (CK, 0 mmol/L SA + 0-h cold treatment) was included; they were evenly sprayed with 0 mmol/L (for CK) and 1 mmol/L SA solutions (treatment seedlings). During the spraying process, the front and back of the leaves were sprayed evenly, and the formation of a layer of small droplets on the surface of the leaves was ensured for each treatment. Plants were sprayed once every 72 h, 48 h, and 24 h before low-temperature stress at 25°C as the control temperature and −20 °C for 2 h and 4 h, respectively, in treatment conditions. Each group of plants was allowed to warm to room temperature gradually, and leaves were taken for physiological index measurement, with three biological replicates performed for each treatment.

Determination of the physiological index. The treated leaves from each group were measured to determine their growth indicators as well as their physiological and biochemical indicators. The grinding method was used to measure chlorophyll content (Nagata and Yamashita 1992). MDA content was determined by using thiobarbituric acid (TBA) method (Lin et al. 2024). Peroxidase (POD) activity was measured using the guaiacol method (Li and Wu 2016), and catalase (CAT) activity was determined by ultraviolet absorption method (Zhang et al. 2023). Tissues such as roots, stems, and leaves were selected from each group for paraffin sectioning, and the structural characteristics of each group were compared after dissection. The middle of the leaf containing the main vein, was cut to a size of 0.6 mm × 35 mm. Approximately 3 to 5 cm of stem and 4 to 5 cm of root tissue were cut and place them in 50% FAA fixative for 24 h. Paraffin sections were used to dissect the material, with each section maintained at a thickness of 12 μm. The sections were then stained with safranin solid green solution for 10 min, and neutral gum was used as the sealing material. The structure of various tissues was observed through an optical microscope, and photos were taken using Image-Pro Plus 5.0 for measurement and statistics (Li et al. 2018).

Statistical analysis. Data are presented as the means of replications (plants), data were analyzed with IBM SPSS 25.0 and Excel 2010. All statistically significant differences were tested at the $P \leq 5\%$ level (Liu et al. 2024).

Results

Growth status of pomegranate seedlings under different treatments. As shown in Fig. 1, compared with the CK group treated with distilled water, the pomegranate seedlings in the low-temperature stress group (A0) exhibited typical cold-damage symptoms, specifically manifested as curled leaf edges. After spraying exogenous SA solution, the symptoms of pomegranate seedlings were alleviated to varying degrees. By comparing the growth status of leaves in each treatment group, the A1 treatment group showed a significant advantage in alleviating seedling cold damage.

The effect of treatments on the growth indicators of pomegranate seedlings. According to Table 1, under cold stress, pomegranate seedlings were induced to lose leaf water due to low temperature, resulting in a reduction in leaf area and a decrease in aboveground weight. The growth indicators of Group A1 were second only to the CK group. This study confirms that treatment with 1 mmol/L exogenous SA can significantly enhance the low-temperature resistance of pomegranate seedlings.

The effect of treatments on chlorophyll, MDA, POD, and CAT content in pomegranate seedlings. From Fig. 2, it can be seen that the chlorophyll content of the CK group is the highest—significantly higher than other treatment groups. Among them, the chlorophyll content of the A1 group is second only to the blank control CK group. Due to cold stress, the cooperative mechanism of pomegranate seedlings in the A0 group is severely damaged, and the membrane phase and permeability of plant biofilms change, damaging the plant enzyme system and affecting enzyme activity. After spraying exogenous SA, the chlorophyll content of the CK group increased by 32.39% and 42.17%, respectively, compared with the A0 and A2 groups, with no significant difference between the A0 and A2 treatment groups. Experimental results have shown that 1 mmol/L exogenous SA treatment can effectively alleviate the degree of chlorophyll content decline in pomegranate seedlings under cold stress.

As shown in Fig. 3, compared with the CK group, the MDA content in the leaves of pomegranate seedlings in group A0 increased by 76.09% ($P < 0.05$), whereas the MDA content in the leaves of seedlings in group A1 decreased by 26.75% ($P < 0.05$). When plants are subjected to cold stress, their cell membrane lipid peroxidation reaction intensifies, leading to an increase in the accumulation of MDA in tissues. Spraying exogenous SA can effectively alleviate membrane system damage, control MDA content at a lower level, and improve stress resistance.

As shown in Fig. 4, the peroxidase (POD) activity in the leaves of A0 group pomegranate seedlings after treatment showed an increasing trend compared with the control group (CK), with an increase of 91.29% in enzyme activity ($P < 0.05$). In the A1 treatment group regulated by SA, POD activity in seedling leaves showed a decreasing trend of 26.28% compared with the A0 group ($P < 0.05$). After cold treatment, the POD activity of pomegranate seedlings is enhanced, and the spraying of exogenous SA effectively alleviated the damage of low temperature stress to pomegranate seedlings. At the same

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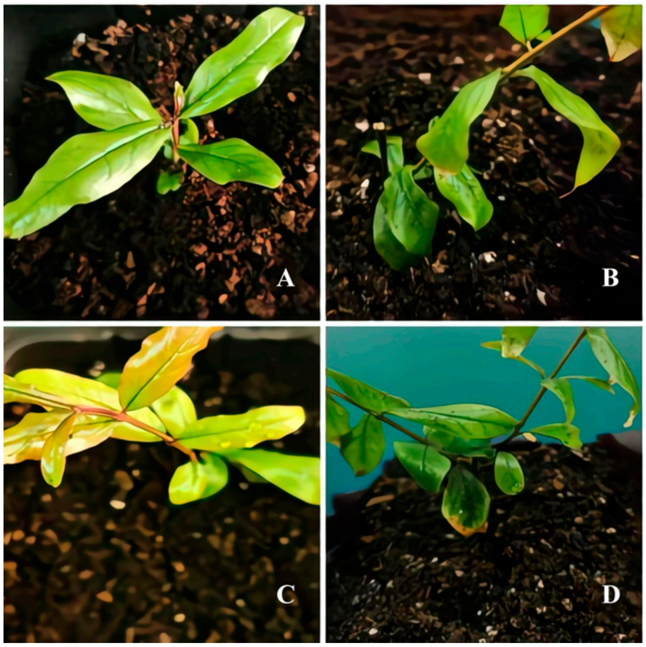

Fig. 1. Growth of pomegranate seedlings under different treatments. (**A**) Control group (CK) seedlings: 0 mmol/L SA + 0-h cold treatment. (**B**) A0 group seedlings: 0 mmol/L SA + 2-h cold treatment. (**C**) A1 group seedlings: 1 mmol/L SA + 2-h cold treatment. (**D**) A2 group seedlings: 1 mmol/L SA + 4-h cold treatment.

Table 1. Effects of exogenous salicylic acid treatment on the growth indicators of pomegranate seedings.

Group	Leaf area (cm^2)	Root length (cm)	Aboveground mass (g)	Underground mass (g)
CK	8.02 a	8.31 a	0.878 a	0.643 a
A0	6.05 b	5.23 c	0.653 c	0.442 c
A1	6.80 b	7.32 b	0.716 b	0.535 b
A2	5.82 bc	6.54 b	0.668 c	0.476 c

Different letters meant significant difference among different treatments at 0.05 level.

time, by synergistically regulating the activity of antioxidant enzymes, the cold resistance of seedlings is significantly enhanced.

As can be seen in Fig. 5, compared with the CK group, the CAT activity of group A0 was inhibited under cold stress. The A1 and A2 groups sprayed with exogenous SA showed an increase in CAT activity of 44.97% and 26.34%, respectively, compared with group A0 ($P < 0.05$). The CAT activity of group A1 sprayed with exogenous SA was higher than that of the CK group. This indicates that the application of SA can protect the biofilm by enhancing antioxidant enzyme activity, effectively reducing the damage of low temperature to the membrane system and further improving the cold resistance of pomegranate seedlings.

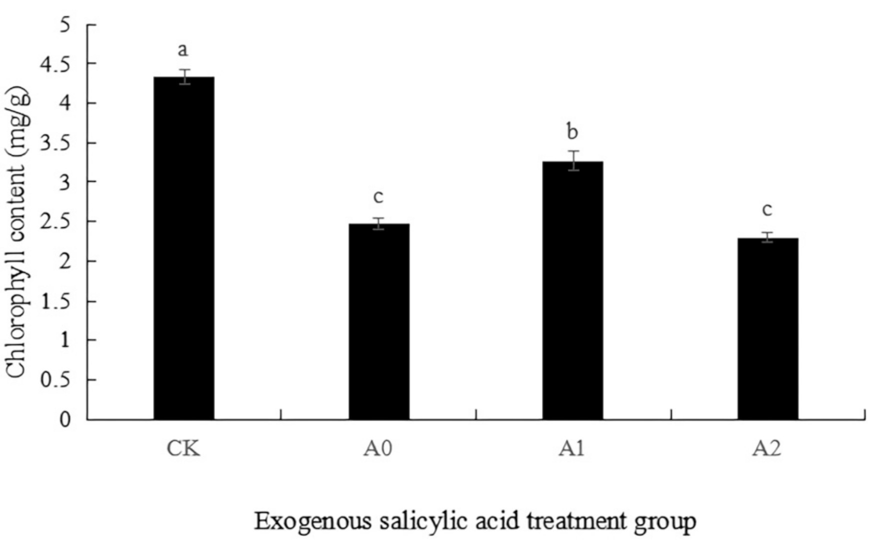

Fig. 2. Effect of exogenous salicylic acid (SA) treatment on chlorophyll content in leaves of pomegranate cuttings. Different letters meant significant difference among different treatments at 0.05 level. The following are the same in Figs. 3–5. CK = 0 mmol/L SA + 0-h cold treatment; A0 = 0 mmol/L SA + 2-h cold treatment; A1 = 1 mmol/L SA + 2-h cold treatment; A2 = 1 mmol/L SA + 4-h cold treatment.

Correlation analysis between physiological and morphological indicators of pomegranate seedlings under different treatments. Table 2 shows the correlation analysis between physiological and biochemical indicators (including MDA content, chlorophyll content, CAT activity, and POD activity) and morphological indicators (seedling leaf area and average root length) of pomegranate seedlings under different treatment conditions. Bivariate Pearson test data showed that the physiological and biochemical indicators of pomegranate seedlings were significantly correlated with the morphological indicators of the seedlings in each treatment group of exogenous SA. The chlorophyll content of pomegranate seedling leaves is significantly positively correlated with morphological indicators, whereas the MDA content is significantly negatively correlated with morphological indicators. Research data show that under cold stress conditions, the growth and development of pomegranate seedlings are inhibited, and their chlorophyll synthesis ability and CAT activity decrease, whereas MDA content and POD activity increase. After spraying exogenous SA treatment, various detection parameters in the experimental group were significantly improved, the inhibitory effect of morphological indicators was weakened, chlorophyll content, and CAT activity were increased, whereas MDA content and POD activity were relatively reduced. The correlation analysis results further confirm that the higher the chlorophyll content and CAT content of pomegranate seedlings, and the lower the MDA content, the better the growth condition of the seedlings and the stronger their cold resistance.

Comparison of anatomic structures of plant tissues processed by different groups. There were differences in the anatomic characteristics of roots, stems, and leaves among the groups treated (Fig. 6). After cold treatment, pomegranate seedlings (Fig. 6D) showed damage to the cell membrane of their roots. Low temperature caused lipid peroxidation of the cell membrane, increased membrane permeability, and partial separation of the cytoplasmic wall of some roots; damage to cortical structure, decreased activity of root meristematic tissue, loose arrangement of cortical cells, increased intercellular space, and obstruction of water and nutrient transport; and abnormal vascular system, underdeveloped xylem vessels, and reduced formation of secondary xylem. The epidermal cells of the stem (Fig. 6E) ruptured due to freeze-thaw alternation, causing separation of cortical cytoplasmic walls, breakage of intercellular filaments, and enlargement of intercellular spaces. Ice crystal damage occurs in the xylem ducts, leading to a decrease in water transport

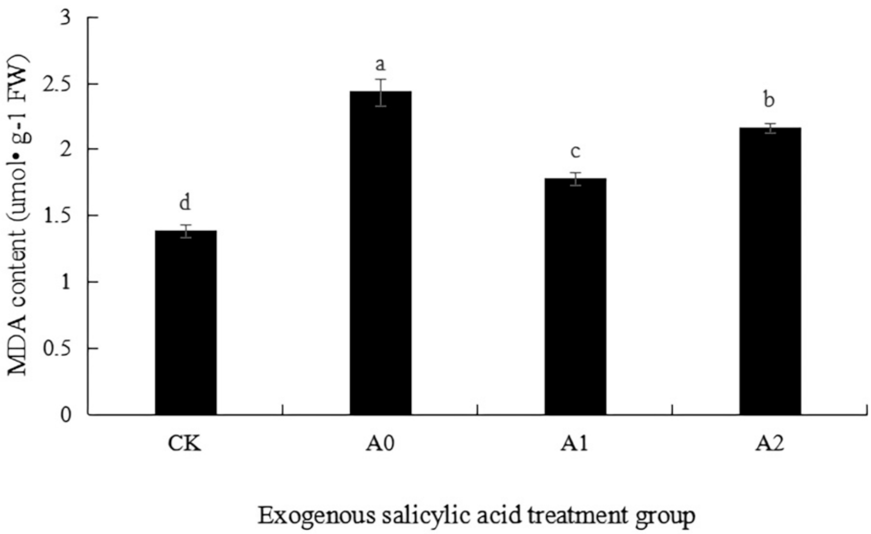

Fig. 3. Effect of exogenous salicylic acid treatment on malondialdehyde content in leaves of pomegranate cuttings. CK = 0 mmol/L SA + 0-h cold treatment; A0 = 0 mmol/L SA + 2-h cold treatment; A1 = 1 mmol/L SA + 2-h cold treatment; A2 = 1 mmol/L SA + 4-h cold treatment.

efficiency. The activity of cell division in the cambium is weakened, the formation of secondary xylem and phloem is reduced, and the mechanical support of the stem decreases. This is similar to the damage pattern of the nutritional organ tissue structure of black walnuts in eastern China under salt stress (Tang et al. 2024). In the anatomic structure of the leaves (Fig. 6F), the mesophyll cells disintegrate, the structure of the mesophyll cells is disrupted, the chloroplast membrane system disintegrates, the thylakoids disintegrate, and the photosynthetic capacity significantly decreases. Abnormal regulation of stomata leads to the inactivation of guard cells, causing uncontrolled opening and closing of stomata, imbalance of transpiration, exacerbating water loss and cell dehydration. By spraying exogenous SA, it can be seen from Fig. 6G that the disintegration of cortical cells in the root was reduced, and the normal development of vascular bundles was maintained. The activity of the cambium cells in the stem Fig. 6H is restored, the secondary xylem thickens, and the mechanical support is enhanced. The decrease in secondary xylem formation indicates that low temperature inhibits vascular tissue differentiation, whereas SA treatment promotes the recovery of cambium cell activity, which is consistent with the mechanism of exogenous substance induced vascular regeneration in tung oil rootstock research (Zhang et al. 2025). The palisade tissue of the leaves (Fig. 6I) is tightly arranged, the chloroplast structure is intact, and the ability to regulate stomatal opening is enhanced. The improvement in the compactness of leaf palisade tissue arrangement (Fig. 6I) further confirms the ability of SA to maintain the integrity of leaf mesophyll cells, which is consistent with the structural adaptability changes in water stress studies of alfalfa (Yao et al. 2025). Therefore, spraying exogenous SA can alleviate the relief effect of pomegranate seedlings under cold stress and improve their stress resistance.

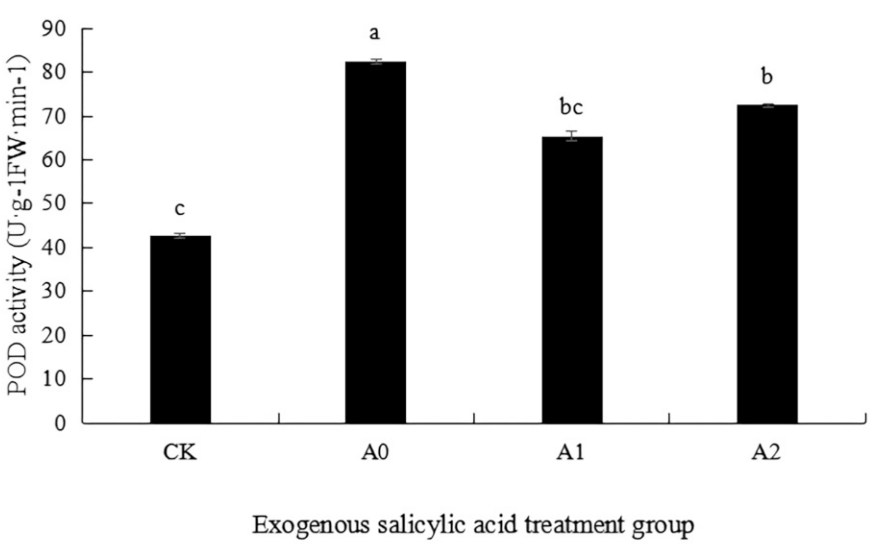

Fig. 4. Effect of exogenous salicylic acid treatment on peroxidase activity of pomegranate cuttings. CK = 0 mmol/L SA + 0-h cold treatment; A0 = 0 mmol/L SA + 2-h cold treatment; A1 = 1 mmol/L SA + 2-h cold treatment; A2 = 1 mmol/L SA + 4-h cold treatment.

Discussion

Cold stress, as a key environmental factor restricting crop growth and development, restricts the normal growth and development of crops. The precise regulation of endogenous hormone balance through the application of plant growth regulators has become an effective agronomic measure to enhance crop low-temperature tolerance (Waititu et al. 2021). Cold stress can cause physiological and metabolic disorders in plants, manifested mainly as a significant decrease in photosynthetic efficiency under low temperature conditions, and the negative impact on plants becomes more severe with the decrease of temperature and the prolongation of stress time (Jiang et al. 2020). Cold stress can cause damage to the function of photosynthetic institutions, resulting in hindered root development and significantly limited mineral element absorption capacity (Xu et al. 2023). At the same time, the osmotic regulation disorder caused by membrane lipid phase transition can induce cytoplasmic flow obstruction and electrolyte leakage, leading to an imbalance of transmembrane ion gradient and triggering conformational changes of key enzymes, thereby inhibiting their catalytic activity(Lu et al. 2023). Plant hormones are important substances that regulate plant growth and development. After being subjected to cold stress, the endogenous hormone content of plants will undergo significant changes, thereby affecting the plant's ability to adapt to low temperatures (Zhang et al. 2022).

This study showed that the growth of ‘Fuanyihao’ pomegranate seedlings was affected when cultured in a −20 °C incubator. The vitality level of the seedlings decreased, and various morphological and physiological indicators significantly decreased, resulting in wilted leaves. Multiple studies have shown that plants suffer from various forms of damage in cold stress environments, leading to abnormal growth and development (Jin et al. 2023; Lantzouni et al. 2020). Meanwhile, studies have shown that exogenous SA plays an important role in regulating plant growth, development, and abiotic stress (Decsi et al. 2025). The multiple physiological and biochemical indicators of ‘Fuanyihao’ pomegranate seedlings treated with exogenous SA were significantly improved, which is similar to the research conducted by many researchers on the ability of exogenous SA to alleviate plant cold stress damage. Jalili et al. (2023) found that use of SA can enhance the cold resistance of several grape varieties by strengthening the antioxidant defense system of plant cells, stabilizing cellular conditions, and thereby reducing low-temperature damage. The application of SA increased the tolerance of cucumber

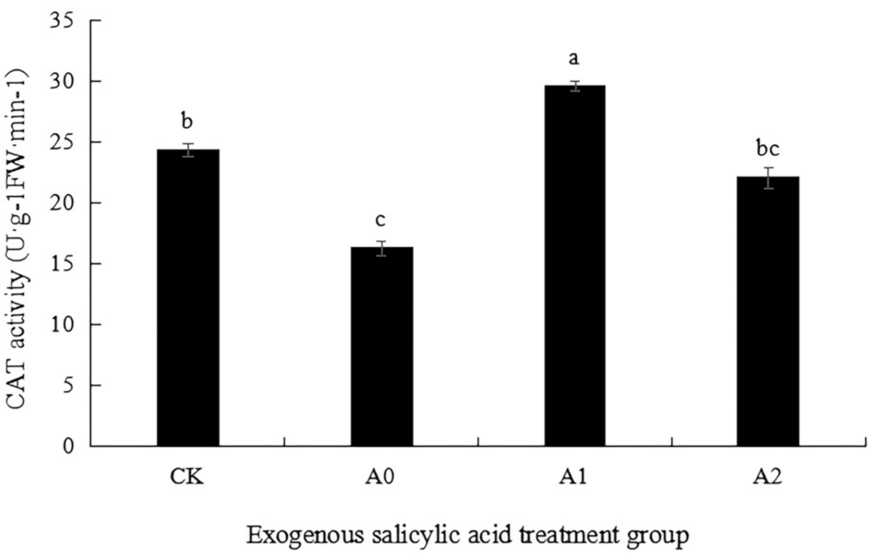

Fig. 5. Effect of exogenous salicylic acid treatment on catalase activity of pomegranate cuttings. CK = 0 mmol/L SA + 0-h cold treatment; A0 = 0 mmol/L SA + 2-h cold treatment; A1 = 1 mmol/L SA + 2-h cold treatment; A2 = 1 mmol/L SA + 4-h cold treatment.

seedlings to low-temperature stress (Sayyari et al. 2013). Pretreatment of frost-sensitive banana plants with a 0.5 mM SA solution increased frost tolerance under frost stress of 5 °C (Kanget al. 2003). In this study, cold stress had a negative impact on the leaves and roots of pomegranate cutting seedlings, leading to accelerated production of harmful metabolites. External application of SA can alleviate the damage of cold stress to seedlings.

At the same time, cold stress also affects plant physiological metabolism by hindering the biosynthesis of chlorophyll, resulting in a decrease in leaf chlorophyll content and interfering with the normal operation of the plant photosynthetic systemchlorophyll loss and impaired photosynthetic performance (Garstka et al. 2007; Maciej et al. 2007; Mattila et al. 2020). The weakening of photosynthesis will affect the growth and development of 'Fuanyihao' pomegranate seedlings. In this experiment, the chlorophyll content in the leaves of group A0 pomegranate seedlings treated with cold stress decreased by 32.39%, whereas the MDA content in group A0 increased by 76.09%, which is consistent with the trend of low-temperature stress research in roses (Ma et al. 2023) and kumquats (Liu et al. 2024), confirming the correlation between membrane lipid peroxidation and photosynthetic damage. However, SA treatment reduced chlorophyll loss to 12.7% (Group A1), which was significantly better than the control and directly related to the protective effect of SA on chloroplast membrane structure (Ding et al. 2021; Li et al. 2021) reported that the foliar application of different concentrations of SA greatly increased antioxidant enzyme activities, soluble sugars, proline, and chlorophyll content of grapes under frost stress. In this experiment, the application of SA can significantly alleviate the damage of cold stress to pomegranate seedlings. Its mechanism of action is mainly reflected in increasing the chlorophyll content in leaves to improve photosynthetic efficiency, while activating the plant's antioxidant defense system and enhancing the cells' cold resistance. This is consistent with previous research findings.

When plants are exposed to cold stress, the antioxidant system plays a crucial role. The main ROS removal enzymes in plants are the first to resist the harmful effects of cold stress. POD and CAT degrade excess reactive oxygen species (ROS), such as H_2O_2, through enzymatic action to prevent plant damage from peroxidation. Improving antioxidant capacity is one of the methods for plants to enhance their tolerance to cold stress (Karimi et al. 2016). Aazami et al. (2015) found that SA treatment improved the activity of the CAT enzyme in grape and increased the activity of APX, SOD, and GR in both cultivars under cold stress. In this study, after cold treatment of pomegranate seedlings, compared with the control group, the activity of POD enzyme in the leaves of plants treated with A0, A1, and A2 groups showed an increasing trend, indicating that plants resist the damage of cold stress by increasing the activity of POD enzyme. In addition, the POD activity of the A2 treatment group was significantly higher than that of the A1 treatment group, indicating that with the prolongation of cold stress time, spraying SA can further increase the POD activity of plants to enhance their resistance to cold stress damage.

When pomegranate seedlings were subjected to cold stress, the CAT enzyme activity in the leaves of plants in the A0 treatment group showed a decreasing trend, compared with CK. The CAT activity in the leaves of plants in groups A1 and A2 treated with SA was significantly increased compared with group A0, indicating that SA treatment can enhance CAT activity in plants to cope with cold stress damage. Compared with CK, the CAT activity of plants in groups A0 and A2 showed a decreasing trend compared with POD activity under the same conditions. This is because the activity of CAT enzyme is more sensitive to cold stress, whereas POD enzyme is less sensitive to low temperature (Wang et al. 2019). Meanwhile, this study found that POD activity significantly increased (91.29%) under cold stress, whereas CAT activity decreased, which is consistent with the results of X. Zhang et al. (2020) in seaside barnyard grass. Given that a key enzyme for clearing H_2O_2, the rapid increase in POD activity may be the primary defense response of plants to oxidative stress. The decrease in CAT activity may be related to low-temperature induced enzyme protein denaturation (Mittler et al. 2011). After exogenous SA treatment, POD activity decreased by 26.28%, whereas CAT activity increased by 44.97%, indicating that SA avoids excessive consumption of POD by coordinating the antioxidant enzyme system (Liu et al. 2024). The reverse changes in POD and CAT activities under low-temperature stress reveal the hierarchical response of the antioxidant system: POD has a lower affinity for H_2O_2 [higher Michaelis-Menten constant (Km) value] and can quickly activate in high concentration H_2O_2 environments. CAT has a high affinity for H_2O_2 (low Km value), but low temperatures can easily cause its tetramer structure to dissociate and become inactive (Gill and Tuteja 2010). In this study, the POD of group A0 increased by 91.29% whereas CAT decreased, indicating that the short-term accumulation of H_2O_2 triggered the POD dominated clearance mechanism. Exogenous SA can restore antioxidant balance by upregulating CAT gene expression and stabilizing enzyme structure, which is consistent with the 40% increase in CAT activity by SA in wheat seedlings (Du et al. 2022).

The plant cell membrane is usually the first site of cold-stress damage, and MDA is often used as an important indicator of the degree of cell membrane damage (Karimi et al. 2016). MDA is a product of lipid peroxidation in plant cell membranes, and excessive accumulation of its content under stress can cause serious damage to plant cells, thereby

Table 2. Correlation analysis of physiological and biochemical indicators and morphological indicators of pomegranate seedlings under different treatments.

Dimension	*M* ± *SD*	Leaf area	Avg root length
MDA content	1.93 ± 0.50	−0.857[i]	−0.824[i]
Chlorophyll content	3.09 ± 0.85	0.882[i]	0.796[i]
CAT activity	23.05 ± 4.97	0.902[i]	0.848[i]
POD activity	65.76 ± 14.92	−0.916[i]	−0.893[i]

[i] At the 0.05 level (double tail), the correlation is significant; at the 0.01 level (double tail), the correlation is significant.

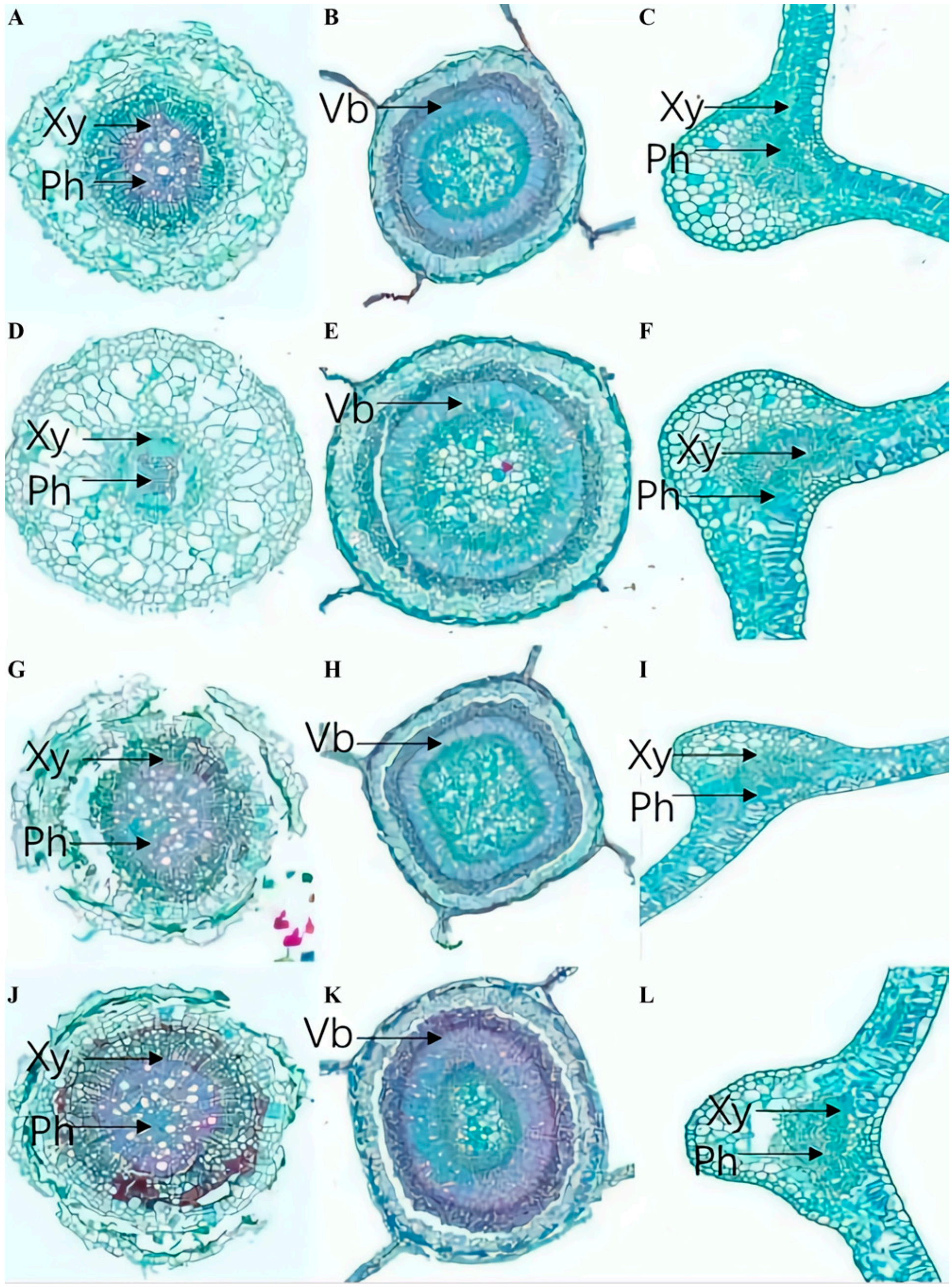

Fig. 6. Anatomic structure comparison of each group of treated vegetative organs. Xy = xylem; Ph = phloem; Vb = vascular bundle. (**A**) Control group (CK) group root; (**B**) CK group stems; (**C**) CK group leaf midvein; (**D**) A0 group root; (**E**) A0 group stems; (**F**) A0 group leaf midvein; (**G**) A1 group roots; (**H**) A1 group stems; (**I**) A1 group leaf midvein; (**J**) A2 group root; (**K**) A2 group stems; (**L**) A2 group leaf midvein. CK = 0 mmol/L SA + 0-h cold treatment; A0 = 0 mmol/L SA + 2-h cold treatment; A1 = 1 mmol/L SA + 2-h cold treatment; A2 = 1 mmol/L SA + 4-h cold treatment.

exacerbating stress damage (Ouyang et al. 2025). The experimental data show that low-temperature treatment significantly increased the content of MDA in 'Fuanyihao' pomegranate seedlings, which was significantly negatively correlated with membrane stability (Gill et al. 2021), indicating that cold stress caused certain damage to the membrane system of the plant. The results of this experiment showed that, compared with the CK group, the MDA content in the leaves of pomegranate seedlings in group A0 increased by 76.09% ($P < 0.05$), and the MDA content in the leaves of plants in the cold-injury treatment group increased significantly. However, the MDA content in the leaves of plants in groups A1 and A2 treated with SA showed a decreasing trend compared with group A0. The MDA content in the leaves of seedlings in group A1 decreased by 26.75% compared with group A0 ($P < 0.05$), indicating that the use of SA treatment can reduce the MDA content in plants and alleviate the damage caused by membrane lipid peroxidation. Exogenous SA treatment can effectively maintain the integrity of cell membrane function and play an important role in maintaining the stability of cell structure.

The root system is the main organ for plants to absorb water and various nutrients from the soil. The spatial distribution of plant roots in the soil is regulated by both their genetic characteristics and habitat conditions. As the primary sensing organ of plants in response to environmental stress, leaves play a key role in mediating water transpiration and gas exchange by regulating stomatal opening and closing mechanisms (Bharath et al. 2021; Hasanuzzaman et al. 2023). The structural characteristics of leaves can reflect the adaptability of plants to environmental stress (Haworth et al. 2021), and the anatomic features of leaves can also serve as the core characterization of plant environmental adaptability (R. Zhang et al. 2020), playing a crucial role in maintaining the stability of terrestrial ecosystem functions (Zubair et al. 2019).

Under low-temperature stress, plants adapt to unfavorable environments by changing their own morphology, manifested as thicker leaves, reduced stomatal density, thicker leaf cuticle, increased leaf tissue structure tightness, increased number of leaf vein conduits, thickened xylem, and decreased phloem thickness (Yang et al. 2008). Through study of the differentiation, boundary clarity, and cell wall thickness of thin-walled cells around the vascular bundles in the main veins of *Brassica napus* leaves against cold and cold sensitive types, He et al. (2017) found that these indicators can be used to evaluate the strength of cold resistance in *Brassica napus.* Lai et al. (2015) studied the microstructure of young loquat (*Eriobotrya japonica*) seeds at low temperatures and found that as the temperature decreased, the cell structure was more severely damaged. The seedcoat was the first to be affected, followed by the true leaves, and finally the cells, which ruptured. This indicates that the plants changed their internal cell structure to adapt to low temperatures, exacerbating the degree of cell damage. In this experiment, paraffin sectioning was performed on the roots, stems, and leaves treated in each group, and their anatomical structures were compared; it can be seen that under cold stress, the cell membrane of pomegranate seedlings' roots was damaged, membrane permeability was increased, and some root cytoplasm walls were separated. Damage to cortical structure, decreased activity of root meristematic tissue, loose arrangement of cortical cells, increased intercellular space, and obstruction of water and nutrient transport were also observed, as was abnormal vascular system, underdeveloped xylem vessels, and reduced formation of secondary xylem. The epidermal cells of the stem ruptured due to freeze–thaw alternation, the cytoplasmic wall of the cortex separated, the intercellular filaments broke, the intercellular space increased, and ice crystal damage occurred in the xylem ducts. The activity of cell division in the cambium was weakened, the formation of secondary xylem and phloem was reduced, and the mechanical support of the stem decreased. In the anatomic structure of leaves, mesophyll cells disintegrated, and the structure of mesophyll cells was disrupted. Low temperature stress not only changed the microstructure of plants themselves, but also affected their submicroscopic structure, and different organelles responded differently to low temperature (Jalili et al. 2023).

In summary, by placing pomegranate cuttings in a $-20\,^{\circ}$C incubator to simulate a cold-stress environment and comparing the physiological and biochemical indicators and anatomic structures of seedlings in each of the study's treatment group, the physiological mechanism of exogenous SA regulating resistance to low temperatures in pomegranate seedlings was explored. The research results confirmed that, compared with the simple cold-stress treatment group, the treatment group sprayed with exogenous SA had significantly increased CAT activity in the pomegranate cutting seedlings, while promoting the increase of chlorophyll content in seedling leaves and enhancing POD activity. This treatment reduced MDA content, indicating that the stability of the pomegranate membrane system was effectively improved, thereby enhancing the seedlings' resistance to adversity. This work confirms that spraying exogenous SA on pomegranate seedlings can effectively alleviate physiological damage under cold stress and significantly improve their adaptability to low temperatures.

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