# STUDY ON LOW TEMPERATURE PHYSIOLOGY AND EVALUATION OF COLD RESISTANCE OF DIFFERENT PEONY VARIETIES

KE, J. H.[1] – WANG, J. X.[1] – WU, S. L.[1] – ZHOU, K. H.[1] – DAI, B. Q.[2] – XUE, S. Y.[2] – ZHOU, Y.[2] – XIE, Y.[2] – WANG, Y.[1*]

[1]*College of Life Science, Shenyang Normal University, Shenyang, Liaoning, China*

[2]*Shengyang Academy of Landscape-Gardening, Shenyang, Liaoning, China*

**Corresponding author*
*e-mail: wyancn2002@aliyun.com*

(Received 10th Oct 2022; accepted 7th Dec 2022)

**Abstract.** The cold resistance of 14 cultivars of tree peony introduced in Shenyang, China was studied. For each cultivar of peony, 3 branches with the length of 15 to 20 cm growing in the middle section of each cultivar were cut and put into a sealed plastic bag, and then they were put into a low-temperature incubator with 400 (lux) light intensity for low-temperature stress. The treatment gradients were 5 °C (CK), 0 °C, -5 °C and -10 °C. After the branches were treated for 24 h, the physiological indexes including relative conductivity (Rec), osmotic adjustment of Soluble Sugar (SS), Soluble Protein (SP), and Superoxide dismutase (SOD) of leaves were measured. Increasing the contents of SS and Pro and the activity of SOD were the effective way to improve their resistance according to the changes of the measured indexes. Using the membership function to analyze the cold resistance of different cultivars of peony, the order of cold resistance was as follows: Juanyehong > Juhesanbian > Huihe > Daojin > Xinshiji > Murun Juelun > Taohuayushuang > Fenjinyu > Huawang > Jinge > Guijiucui > Caozhouhong > Panda > Heilongtan. This study provided a theoretical basis for the selection of peony cultivars for overwintering in the open field in northern China.
**Keywords:** *peony, low temperature stress, physiological indicators, membership function, cold resistance evaluation*

## Introduction

Peony is one of the most famous flowering plant and a candidate for the national flower of China. Peony is introduced to many regions including Liaoning Province, China as an ornamental plant. However, the low temperature in winter of Liaoning Province, China may be an important limitation factor for the success of introduction, so it is important to conduct research on the cold resistance ability of the introduced cultivars of peonies.

The effects of low temperature stress on plants are manifested in many ways. Dynamic changes in the structure of biological membranes are an important basis for the study of plant cold tolerance and cold resistance mechanisms (Ding et al., 2019). The fluctuations in temperature lead to changes in the fluidity of plant cell membranes and rearrangement of the cytoskeleton, triggering the cytoplasmic flow of $Ca^{2+}$, which subsequently triggers a low-temperature response, resulting in low-temperature tolerance. Thus, cell membrane fluidity with altered cytoskeleton conformation is considered as a potential low-temperature receptor (Masouleh et al., 2019). The cell membrane is sensitive to adversity response and is the main target of plant adversity attack. When plants are under adversity stress, the cell membrane system is injured, resulting in loss of selectivity and massive exocytosis of electrolytes and certain small molecules of organic matter, which causes disruption of the cell membrane system,


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

exhibiting increased membrane permeability and malondialdehyde (MDA) production from membrane oxidation, leading to an increase in relative conductivity (Zhao, 2008). It is an important indicator of cell membrane permeability under low temperature stress, and the larger the value, the greater the leakage of electrolytes and the more severe the damage to the cell membrane (Liu et al., 2017; Li et al., 2014; Shiming et al., 2009). Osmoregulation plays an important role in plant resistance to cold, and plants under low temperature stress can improve their cold adaptation by increasing the content of major osmotic substances in their bodies (Xue and Jian, 2020). At low temperatures, plants activate the osmoregulatory system and the content of osmoregulatory substances such as soluble sugars, soluble proteins and free proline changes (Li et al., 2019). The increased content of soluble sugars improves the osmoregulatory capacity of plant cells and also reduces the freezing point of cell protoplasm, enhances cold resistance, and plays an important role in maintaining normal cell membrane function under low temperature conditions (Li, 2020). The increased content of soluble proteins is also an effective mechanism to reduce the stress damage to plants (Wang, 2010; Tian, 2020). It has been demonstrated that the content of osmoregulatory substances is positively correlated with the cold resistance of plants and that the increase in the content of osmoregulatory substances plays an important role in maintaining the normal function of cell membranes under low temperature conditions (Yve, 2012). On the other hand, the activity of antioxidant enzymes in plants is also changed. When subjected to low temperature stress, plant cells are accompanied by the production of reactive oxygen species (ROS) in the form of free radicals or non-free radicals, and the excessive production of ROS can lead to oxidative damage to cellular proteins, lipids, nucleic acids and plasma membranes (Yang, 2015; Jiang, 2009), antioxidant enzyme systems such as superoxide dismutase and peroxidase can scavenge reactive oxygen species and thus reduce the damage caused by low temperature. Superoxide dismutase (SOD) is an important antioxidant enzyme that is widely distributed in various organisms and is the primary substance for scavenging free radicals in living organisms (Wang, 2017). Plants enhance their resistance to adversity through antioxidant enzymes, thus preventing free radical poisoning (Han, 2006). When plants are stressed, not only defense mechanisms are activated, but also self-repair mechanisms, and increasing the temperature to a suitable level can accelerate self-repair. The strength of cold resistance of different plants is related to their own heredity, their own nutritional development, the degree of external low temperature and cultivation conditions, among which heredity plays a dominant role and external factors work through the internal factor of heredity. Therefore, the cold resistance mechanism of plants is a complex physiological and biochemical process, and the strength of their cold resistance is the result of a variety of complex factors, not just a single factor (Rana et al., 2021).

In this study, we compared the physiological changes of different peony cultivars under low-temperature stress to reveal their difference of cold resistance ability and lay a foundation for the introduction of peony cultivars in northern China by screened out well cold-resistant cultivars from physiological level.

## Materials and methods

### *Test materials*

A total of 14 cultivars introduced by the Shenyang Institute of Garden Science were tested. 8 cultivars of *Paeonia rockii* (S. G. Haw & Lauener) T. Hong & J. J. Li ex D. Y.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

Hong were introduced from Gansu province, China, 4 cultivars of *Paeonia suffruticosa* Andr. were introduced from Shandong province, China and other 2 cultivars of *Paeonia jishanensis* T. Hong & W. Z. Zhao were introduced from Japa (*Table 1*).

***Table 1.*** *Cultivars and codes of peony*

| Code | Cultivars | Species | Source of introduction |
|---|---|---|---|
| G1-1 | Heilongtan | *Paeonia rockii* | Gansu |
| G1-2 | Huihe | *Paeonia rockii* | Gansu |
| G1-3 | Xiongmao | *Paeonia rockii* | Gansu |
| G1-4 | Guifeichachui | *Paeonia rockii* | Gansu |
| G1-5 | Xinshiji | *Paeonia rockii* | Gansu |
| G1-6 | Juhesanbian | *Paeonia rockii* | Gansu |
| G1-7 | Taohuayushuang | *Paeonia rockii* | Gansu |
| G1-8 | Fenjinyu | *Paeonia rockii* | Gansu |
| J1-1 | Jinge | *Paeonia jishanensis* | Japan |
| J1-2 | Huwang | *Paeonia jishanensis* | Japan |
| S1-1 | Murun Juelun | *Paeonia suffruticosa* Andr. | Shandong |
| S1-2 | Daojin | *Paeonia suffruticosa* Andr. | Shandong |
| S1-3 | Caozhouhong | *Paeonia suffruticosa* Andr. | Shandong |
| S1-4 | Juanyehong | *Paeonia suffruticosa* Andr. | Shandong |

### *Experimental design and low-temperature treatment*

Three branches with 15 ~ 20 cm long growing on the middle part of each variety plant were cut and sealed in plastic bags and then placed in a low temperature incubator (LRX-580 B-led) with a light level of 400 (Lux) and under temperature of 5 °C (CK) for 24 h. Then the temperature dropped to 0 °C, -5 °C and -10 °C for 24 h respectively. The leaves from the 3 branches of each variety were taken respectively to measure the physiological indexes, namely each index of a variety for the same treatment was repeated three times.

### *Estimate electrolyte leakage (Rec)*

The leaf at the fourth leaf position was taken from each treated branch. After being washed with deionized water, each leaf was put into a marked vial filled with 20 ml of deionized water respectively and incubated at room temperature in the dark for 6 h. The electrolytic conductivity (EC1) of the solution was measured using a conductivity meter (SA29-DDB11A, Midwest Group, Beijing, China). The solution was heated to 100 °C, then cooled to room temperature and the electrolytic conductivity (EC2) was measured once again. The percentage electrolyte leakage (Rec) of the leaf discs was calculated as follows (Dionisio-Sese et al., 1998):

$$\mathrm{Rec} = \left({}^{\mathrm{EC}_1}/_{\mathrm{EC}_2}\right) \times 100\% \qquad \text{(Eq.1)}$$

### *Estimation of proline (Pro) content*

Leaf samples (50 mg) were blended in 3% sulfosalicylic acid (10 mL) followed by filtration to determine the Pro contents. Taking 2 mL supernatant and acid ninhydrin


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

reagent (2 mL) along with CH3COOH (2 mL) were reacted in glass vials, subsequently cooled in ice and the resulting amalgam was extracted with toluene (4 mL) using a vortex shaker for 15-20 s. The change in color was measured at 520 nm using a spectrophotometer (D-16C) at room temperature with toluene as blank (Bates et al., 1973). A calibration curve based on proline standard was developed to assess the proline concentrations.

### *Estimation of soluble sugars (SS) content*

Fresh leaves (0.5 g) were homogenized in deionized water, heated to 100 °C for 30 min and then cooled to room temperature, and this process was repeated twice. The extract was moved into 50 mL volumetric flasks and volume was completed to scale. The anthrone-sulfuric acid method was used to quantify the total soluble sugars. The absorbance at 630 nm was measured using a UV-5900 spectrophotometer using sucrose as standard (Chen et al., 2007).

### *Estimation of soluble protein (SP) content*

For the contents of soluble proteins, 0.2 g of fresh leaves were extracted in 2 mL buffer phosphate (0.1 M and pH = 7.8). The extract was then centrifuged at 3,000 *g* for 10 min at 4 °C and supernatant was collected. Soluble protein contents were determined according to the method of Bradford (1976), using the reagent Coomassie Brilliant Blue G-250, followed by absorbance readings at 595 nm using bovine serum albumin as standard.

### *Determination of activities of superoxide dismutase (SOD)*

Fresh leaf materials (0.2 g) were ground into homogenate in ice-bath using a mortar and pestle. 0.1 mol/L phosphate-buffered saline (pH 7.8) was added during grinding. The homogenate was centrifuged at 10,000 r/min for 20 min at 4 °C. The supernatant was collected to determine. Superoxide dismutase (SOD) activity, was assayed using the photochemical nitroblue tetrazolium (NBT) method (Beyer and Fridovich, 1987).

### *Comprehensive analysis of the cold resistance of lilies*

The physiological indices measured above of lily varieties under low-temperature treatment were analyzed comprehensively using the membership function method. The measured values of each physiological index were converted quantitatively using the fuzzy mathematical affiliation formula, and the affiliation of each measured index was calculated separately according to the following equation (Wang et al., 2019), which was calculated as follows.
Membership function value:

$$R(X_i) = (X_i - X_{min}) / (X_{max} - X_{min}) \quad \text{(Eq.2)}$$

Anti-membership function value:

$$R(X_i) = 1 - (X_i - X_{min}) / (X_{max} - X_{min}) \quad \text{(Eq.3)}$$

where $X_i$ is the measured value of the index, $X_{min}$ and $X_{max}$ are the minima and maximum values of a certain index for each of the materials tested. Osmoregulatory substances and


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

antioxidant defense system enzymes play a positive role in plant resistance and are calculated using the membership function equation. Rec plays a negative role in measuring cold resistance and is calculated using the anti-membership function equation.

### *Statistical methods*

We performed our statistical analysis using version 26.0 of the SPSS statistics software. One-way ANOVA followed by LSD's multiple-range test for multiple comparisons was used to detect differences among treatments. We defined significance at $P < 0.05$ and 0.01. We used version 9.0 of the Origin Pro software (https://www.originlab.com/) to prepare the graphs.

## Results

### *Variations of estimate electrolyte leakage*

Compared with CK, under 0 °C stress, only Rec of cultivar G1-5 increased significantly ($P < 0.01$), while those of G1-8, S1-1, S1-4, S1-2, J1-2 and J1-1 did not change significantly, while those of G1-1, G1-2, G1-3, G1-4, S1-3, G1-6 and G1-7 decreased significantly ($P < 0.01$) (*Fig. 1*), indicating that the cells of most cultivars were not significantly damaged at 0 °C. Rec of G1-5, S1-2, G1-8 and J1-2 increased by 27.89%, 4.24%, 1.24% and 13.02% respectively. Under the stress of -5 °C, the Rec of all cultivars were significantly higher than that of CK ($P < 0.01$), S1-2, J1-2 and G1-7 increased 652. 25%, 673. 27% and 890. 03% compared with CK. Except G1-1 and G1-2 the Rec of the rest cultivars increased significantly ($P < 0.01$) compared with CK under -10 °C stress and the increase ranges of some cultivars were big as follows: S1-4 (709. 60%), J1-2 (740. 68%), J1-1 (628. 42%), G1-7 (980. 04), S1-1 (704. 36%) and S1-2 (652. 25%).

Rec of all cultivars increased significantly ($P < 0.01$) at -5 °C compared with 0 °C and G1-7 increased 890.03% and S1-2 increased 621.62%. Rec of all cultivars increased significantly ($P < 0.01$) at -10 °C compared with 0 °C and S1-4, S1-1, G1-7, J1-2 and J1-1 increased 709. 60%, 704. 36%, 645. 14%, 643. 88% and 628. 42% respectively. Rec of G1-3, S1-1, S1-4, J1-2, G1-6 and J1-1 6 cultivars increased significantly ($P < 0.01$) at -10 °C compared with at -5 °C and S1-4 increased 114.02%, followed by S1-1 (90.59%).

### *Variations of soluble sugars content (SS)*

The change of SS content under low temperature stress was shown in *Figure 2*.

Low temperature stress effectively induced the increase of SS in all peony cultivars, and the SS contents of all cultivars under all low temperatures were significantly higher than that of CK ($P < 0.01$). At 0 °C, the SS content of 6 cultivars (G1-2, G1-4, G1-5, S1-4, G1-8 and J1-2) were more than twice as many as CK and the increase of G1-5 was 569.03%. Under the stress of -5 °C, some cultivars showed a larger increase of SS contents including G1-5 (540.43%), G1-4 (351.31%), G1-2 (265.25%), J1-1 (131.20%) and G1-8 (123.64%). At -10 °C, cultivars of G1-5, S1-2, G1-2, G1-3, S1-4, J1-1 and G1-1 increased by 645.96%, 281.56%, 274.68%, 161.46%, 146.75%, 106.60% and 105.36% respectively.

The SS content of cultivars S1-3, G1-5, S1-4, G1-8, J1-2 and G1-6 decreased significantly ($P < 0.01$) at -5 °C compared with at 0 °C, and S1-3 had a larger decrease (38.36%). The SS content of cultivars G1-1, G1-2, G1-4, S1-1, S1-2, J1-1 and G1-7 at -5 °C was significantly higher than that at 0 °C ($P < 0.01$) and G1-2 (69.30%) and G1-4


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

(62.57%) had a relatively larger increase. Compared with 0 °C, the SS content of S1-3, G1-8, J1-2 and G1-6 decreased significantly ($P < 0.01$), while the SS content of the other 11 cultivars increased significantly ($P < 0.01$) and G1-1 (94. 72%), G1-2 (73.67%) and G1-7 (68.63%) had larger increase. Under -10 °C stress, the SS content of G1-4, S1-1, J1-2 and J1-1 decreased significantly ($P < 0.01$). The SS content of G1-1 (68.36%), G1-3 (39.45%) and S1-3 (37.02%) increased significantly ($P < 0.01$) and G1-8 increased but no significant difference.

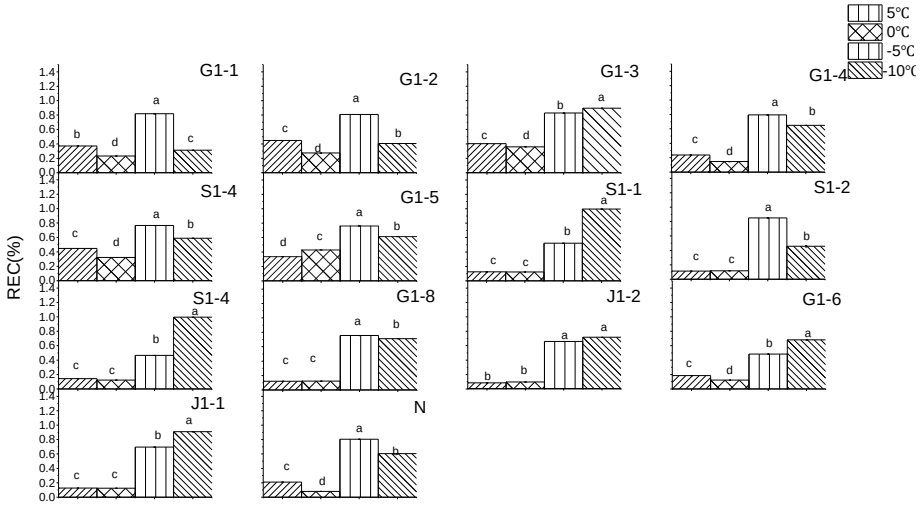


***Figure 1.*** *Rec change of 14 Peony cultivars under different temperature treatments*

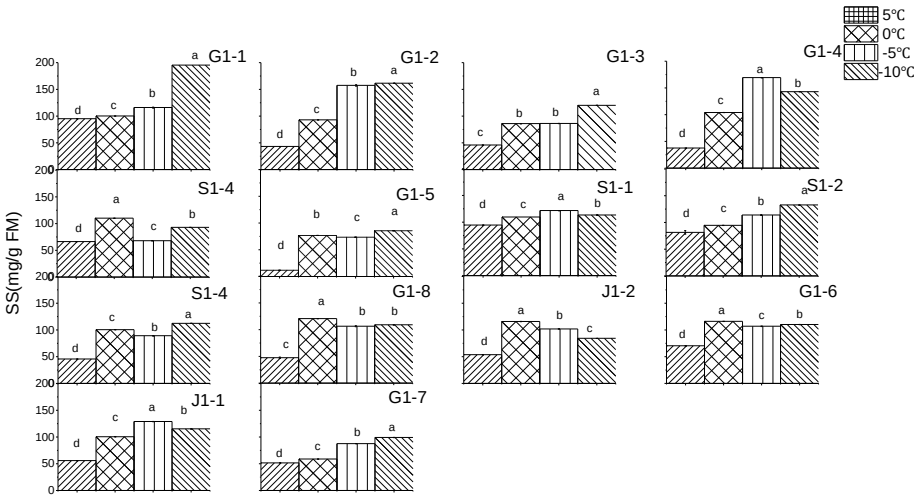


***Figure 2.*** *SS contents of 14 peony cultivars under different temperature treatments*

It can be seen that the SS content of each variety showed different characteristics at different low temperatures. G1-1, G1-2, G1-3, S1-2 and G1-7 showed an obvious tendency that SS content increased gradually with the decrease of temperature, and there were significant differences among the treatments ($P < 0.01$). The SS content of S1-3, G1-8, G1-5, S1-4 and G1-6 decreased at -5 °C and increased at -10 °C, while only J1-2 decreased at -5 °C and -10 °C.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

### *Variations of soluble protein content (SP)*

Compared with CK, the content of SP was significantly decreased ($P < 0.01$) at 0 °C, except for variety G1-6, which was significantly increased ($P < 0.01$). Under the stress of -5 °C, the SP content of cultivars G1-4, S1-3, G1-5 and G1-8 was significantly higher than that of CK ($P < 0.01$) and S1-3 (55. 26%) and G1-5 (40. 99%) had a larger increase. Under the stress of -10 °C, except G1-4 the SP contents of all other cultivars were significantly lower than that of CK ($P < 0.01$).

Compared with 0 °C, the content of SP in 13 cultivars increased significantly ($P < 0.01$) except G1-6, and the cultivars G1-1 (397.50%), S1-4 (383.15%) and S1-3 (178.21%) increased significantly at -5 °C. The SP content of G1-1, G1-3, G1-4, S1-3, S1-1, S1-2 and S1-4 increased significantly at -10 °C compared with 0 °C and G1-1, G1-3, G1-4, S1-3 (about 36%) increased significantly ($P < 0.01$). S1-4 (584.80%) and G1-1 (197.41%) had larger increase. The content of the other seven cultivars decreased significantly ($P < 0.01$).

Compared with -5 °C, the SP of S1-4, G1-3 and G1-4 increased significantly ($P < 0.01$), while the SP of the other 11 cultivars decreased significantly ($P < 0.01$) at -10 °C. Among them, G1-8 (-80.25%), G1-2 (-70.49%) and G1-6 (-61.74%) showed lager decreases (*Fig. 3*).

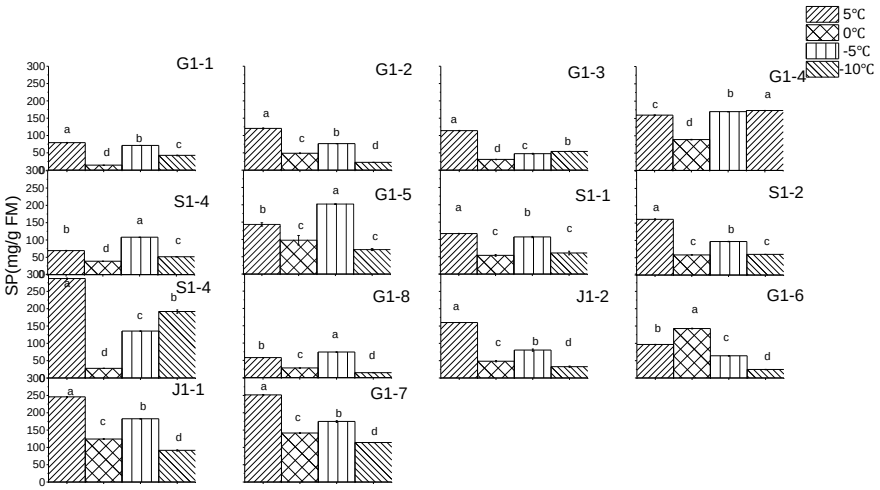


***Figure 3.*** *SP contents of 14 peony cultivars under different temperature treatments*

### *Variations of proline content (Pro)*

The Pro content of G1-4 increased gradually with the decrease of temperature with significant difference among treatments ($P < 0.01$) except between -5 °C and -10 °C treatments (*Fig. 4*). Although the Pro contents of G1-1 and G1-3 were lower than that of the control under low temperature, they also showed an increasing trend with the decrease of stress temperature.

Compared with CK, most of the Pro contents increased significantly ($P < 0.01$) at 0 °C except cultivars G1-1, G1-2, G1-3, G1-4 and J1-1. Under the stress of -5 °C, only G1-4 and S1-4 was significantly higher than that of CK ($P < 0.01$). Under the stress of -10 °C, the Pro content of cultivars G1-3, G1-4, G1-5 and S1-4 increased significantly ($P < 0.01$) compared with CK and the largest increase occurred in G1-4 (83. 25%).


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

Compared with 0 °C, only the Pro content of G1-1, G1-3 and G1-4 did not increase significantly at -5 °C. At -10 °C, the Pro content of G1-1, G1-2, G1-3 and G1-4 increased and G1-2 (64. 71%) and G1-4 (47. 51%) increased significantly compared with 0 °C ($P < 0.01$).

Compared with -5 °C stress, except S1-3, S1-4, J1-2 and G1-6, the Pro content of other 10 cultivars increased under -10 °C stress and G1-2 (145.95%) and G1-5 (76.00%) increased significantly ($P < 0.01$).

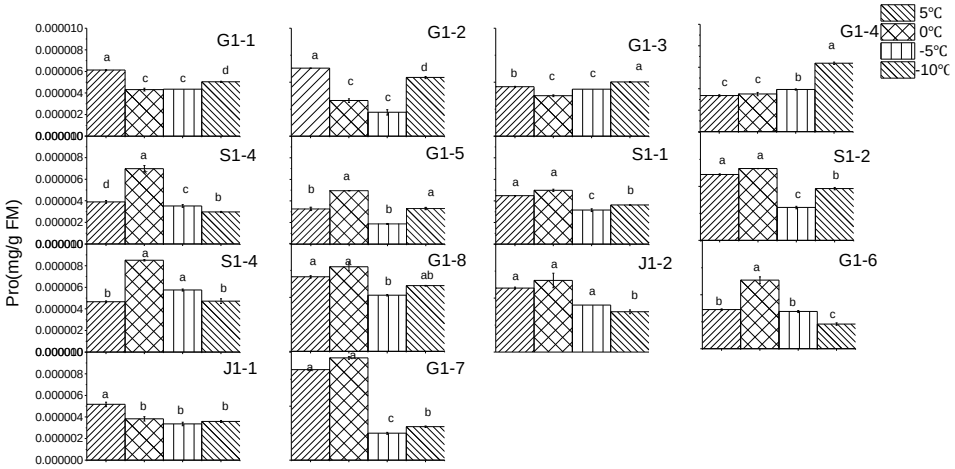


***Figure 4.*** *Pro of 14 peony cultivars under different treatments*

### *Variations in the activities of superoxide dismutase (SOD)*

Compared with CK, the SOD activity of almost all cultivars except G1-1 and J1-2 increased significantly ($P < 0.01$) under 0 °C stress, among which 9 cultivars G1-3, S1-3, S1-1, S1-2, S1-4, G1-8, G1-6, J1-1 and G1-7 increased by more than one time (*Fig. 5*). At -5 °C, the SOD activities of all cultivars were significantly higher than that of CK ($P < 0.01$), among which S1-2 (2923.97%), S1-1 (1776.48%) and G1-4 (1145.79%) had greater increases. Under -10 °C stress, the SOD activities of cultivars G1-3, G1-4, S1-1, S1-2, S1-4, G1-8 and G1-7 increased significantly ($P < 0.01$) compared with the control, but cultivar G1-2 decreased significantly ($P < 0.01$).

Compared with at 0 °C, the SOD activities of G1-8, J1-1 and G1-7 decreased significantly at -5 °C ($P < 0.01$). The SOD activities of the other cultivars increased significantly ($P < 0.01$) except S1-3 and the cultivars with larger increase were G1-4 (617.52%) and J1-2 (771.79%). At -10 °C, the SOD activity of all peony cultivars decreased compared with 0 °C and -5 °C stress, and the cultivars of G1-2, S1-3, S1-1, S1-4, G1-6, J1-1 and G1-7 decreased significantly ($P < 0.01$). S1-3 (95.93%), J1-2 (95.54) and S1-2 (93.64) had larger decreases.

### *Comparison of subordinate functions of physiological indexes of peony*

According to the subordinate function values calculated from the physiological indexes of different peony cultivars, the cold resistance of different peony cultivars was ranked and the results are shown in *Table 2*. Comprehensive analysis showed that the order of cold resistance of cultivars was S1-4 > G1-6 > G1-2 > S1-2 > G1-5 > S1-1 > G1-7 > G1-8 > J1-2 > J1-1 > G1-4 > S1-3 > G1-3 > G1-1.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

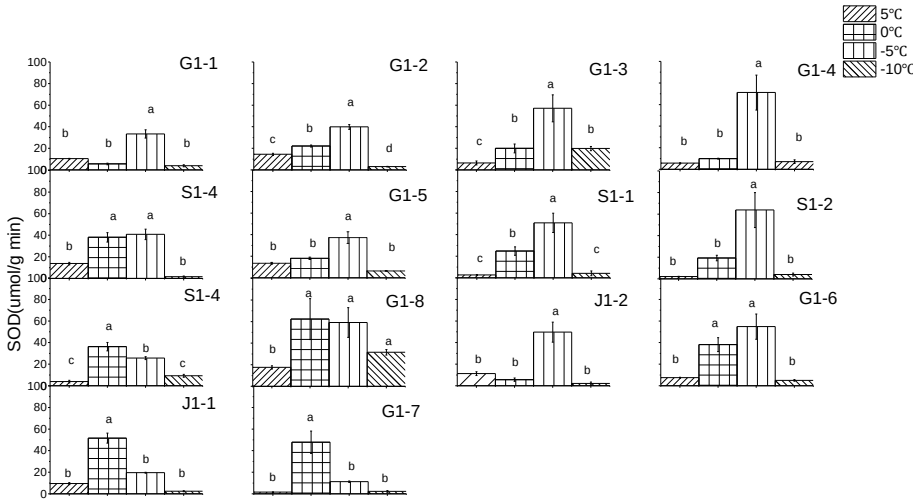


***Figure 5.*** *Pro of 14 peony cultivars under different treatments*

***Table 2.*** *Membership function values of 14 Peony cultivars under low temperature stress*

| Code | PRO membership function value | REC membership function value | SOD membership function value | SP membership function value | SS membership function value | Average membership function value | Cold-resistant ability ranking |
|---|---|---|---|---|---|---|---|
| S1-4 | 0.6584 | 0.6176 | 0.5059 | 0.5179 | 0.4054 | 0.5410 | 1 |
| G1-6 | 0.7380 | 0.6630 | 0.4817 | 0.5369 | 0.2140 | 0.5267 | 2 |
| G1-2 | 0.3839 | 0.5959 | 0.7517 | 0.4630 | 0.3828 | 0.5155 | 3 |
| S1-2 | 0.6623 | 0.5036 | 0.3458 | 0.7004 | 0.2957 | 0.5016 | 4 |
| G1-5 | 0.4959 | 0.6810 | 0.4526 | 0.5704 | 0.3072 | 0.5014 | 5 |
| S1-1 | 0.5196 | 0.5649 | 0.5139 | 0.5810 | 0.3110 | 0.4981 | 6 |
| G1-7 | 0.6550 | 0.4740 | 0.4075 | 0.6943 | 0.2492 | 0.4960 | 7 |
| G1-8 | 0.6398 | 0.6665 | 0.4814 | 0.4500 | 0.2171 | 0.4910 | 8 |
| J1-2 | 0.6895 | 0.5680 | 0.3905 | 0.5244 | 0.2794 | 0.4903 | 9 |
| J1-1 | 0.6164 | 0.6034 | 0.4568 | 0.3372 | 0.3200 | 0.4668 | 10 |
| G1-4 | 0.5169 | 0.5760 | 0.7045 | 0.2987 | 0.2036 | 0.4600 | 11 |
| S1-3 | 0.4931 | 0.4122 | 0.4103 | 0.5217 | 0.4012 | 0.4477 | 12 |
| G1-3 | 0.5408 | 0.5202 | 0.3693 | 0.4369 | 0.3515 | 0.4437 | 13 |
| G1-1 | 0.4321 | 0.3159 | 0.5767 | 0.3870 | 0.2691 | 0.3962 | 14 |

## Discussion

When plants are subjected to low temperature stress, the biomembrane system of plants is first damaged, resulting in increased cell membrane permeability and electrolyte extravasation. The change of cell membrane permeability can directly reflect the degree of low temperature damage to plants (Xiong et al., 2004; Liu and Zhong, 2018). Studies have shown that when plants are injured by low temperature, the electrical conductivity of leaves increases (Zhang et al., 2010). The results of this study showed that the Rec of peony leaves increased with the decrease of temperature. However, when the temperature is -10 °C, the REC of cultivars decreases, which may be due to their low temperature defense response during the cooling process, and the cell membrane is repaired to a certain extent (Wang et al., 2021).

Studies have shown that the content of SS is positively correlated with the cold resistance of plants (Hou et al., 2019). After low temperature stress, the activity of hydrolases is generally higher than that of synthetases. The enzymatic reaction is out of balance, the decomposition of substances is accelerated, the content of starch is reduced,


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

and the content of SS is increased (Liu et al., 2018), thus improving the osmotic adjustment ability of plant cells and reducing the freezing point of cell protoplasm. Enhance cold resistance and maintain the normal function of cell membrane under low temperature conditions (Rana et al., 2021). Hironakakazunori et al. showed that the invertase activity and reducing sugar content of three potato varieties increased under low temperature storage conditions (Hironakakazunori et al., 2005). Ren and Yve found that with the decrease of treatment temperature, the SS content of peony leaves increased gradually, and the increase of cold-tolerant varieties was greater than that of cold-intolerant varieties (Ren and Yve, 2010). In this study, most of the peony cultivars were showed an upward trend of SS content after subjected to low temperature stress and some showed a trend of SS content first increased and then decreased. These changes reflected the differences in cold resistance among peony cultivars.

Xiong Y Q found that in the low temperature environment, the SP content of peony leaves gradually increased with the decrease of temperature (Xiong et al., 2014). The SP content of peony varieties with strong cold resistance were higher, and with the decrease of temperature, the SP content increased quickly. Some varieties with weak cold resistance have lower SP content and with the decline of temperature, the increases of SP content were slower. In this study, the SP content of most peony cultivars decreased at 0 °C compared with CK, while the SP content of most peony cultivars increased at -5 °C compared with 0 °C, but when the temperature dropped to -10 °C the SP contents of most cultivars decreased compared with at -5 °C.

Low temperature can increase the content of active oxygen free radicals such as $O^{2-}$ in plants, and enhance the peroxidation of membrane lipids. Organisms reduce the level of free radicals by enhancing antioxidant defense system enzymes and other protective systems, thereby reducing the damage of free radicals (Xiong et al., 2014). Studies have shown that SOD activity is closely related to plant cold resistance and plays an important protective role in the process of plant cold hardening (Wang, 2021). This study found that the effect of low temperature stress on the SOD activity of most peony cultivars showed a trend of increasing under 0 °C and -5 °C stresses and then decreasing sharply under -10 °C, which may be due to the sudden increase of active oxygen content of $O^{2-}$ in plants in a short time under lower temperature stress and the decrease of SOD activity (Wang and Liu, 1989) or may be due to the frozen damage.

Under low temperature stress, Pro content gradually accumulated, with the extension of low temperature treatment time, Pro content gradually increased. In this study, the result that Pro of more than half of peony cultivars significantly at 0 °C may be due to the stress response of peony after entering the low temperature treatment. It plays a role in balancing cell metabolism when plants resist low temperature stress to maintain the relative stability of intracellular environment (Shi and Zhang, 2014).

## Conclusion

In this study, it was found that the Rec of most peony cultivars did not increase significantly at 0 °C, but increased significantly with the further aggravation of low temperature stress, which indicating that peony did not suffer significant damage at 0 °C. The content of osmotic adjustment substances and the activity of SOD of different cultivars showed different characteristics under low temperature stress. The SS content of all cultivars was significantly higher than that of CK under all low temperature treatments. Pro content of most cultivars increased significantly at 0 °C. The activities


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

of superoxide dismutase in most of the tree peony cultivars were significantly increased under low temperature. Although the SP content decreased under all low temperature stress compared with CK, but the -5 °C stress could induce the increase of SP content of most peony cultivars compared with at 0 °C. The above results indicated that increasing the contents of SS and Pro and the activity of SOD were effective ways for peony cultivars to resist low temperature damage, but SP was not the mechanism of enhancing resistance. It was found that the cold resistance of *Paeonia suffruticosa* Andr was better than that of others. The cold resistance of different cultivars was ranked according to the comprehensive analysis results of the membership function converted from the physiological indexes, which provided a basis for further study and evaluation of the introduction of peony cultivars in northern China.

## REFERENCES

[1] Bates, L. S., Waldren, R. P., Teare, I. D. (1973): Rapid determination of free proline for water-stress studies. – Plant and Soil 39: 205-207.
[2] Beyer, W. F., Fridovich, I. (1987): Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. – Analytical Biochemistry 161: 559-566.
[3] Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Analytical Biochemistry 7: 248-54.
[4] Chen, P., Chen, X., Wang. H. (2007): Determination of polysaccharide from Panax japonicus of Hubei by anthrone-sulfuric acid Method. – Chinese Journal of Hospital Pharmacy 27: 1654-1656.
[5] Ding, Y., Shi, Y., Yang, S. (2019): Advances and challenges in uncovering cold tolerance regulatory mechanisms in plants. – New Phytologist 222: 1690-1704.
[6] Dionisio-Sese, M. L., Tobita, S. (1998): Antioxidant responses of rice seedlings to salinity stress. – Plant Science 135: 1-9.
[7] Han, R. H., Lu, X. S. (2006): Analysis of the principal components and the subordinate function of alfalfa drought resistance. – Acta Agrestia Sinica 14(02): 142-146 (in Chinese).
[8] Hironaka, K., Ishibashi, K., Koaze (2005): Effect of storage temperature on invertase, sucrose-6-phosphate synthase and UDP-glucose pyrophosphorylase activities of Japanese processing potatoes. – Journal of the Japan Society of Food Protection Sciences 31(2): 67-74.
[9] Hou, Z. D., Qu, Y., Huang, H. (2019): Effects of low temperature stress on physiological and biochemical indexes of three kinds of Meconopsis. – Southwest China Journal of Agricultural Sciences 32(3): 522-526.
[10] Jiang, N., Zhu, P. F., Yan, F. Y. (2009): Studies on cold resistance of 6 cultivars of bulb in lily. – Chinese Agricultural Science Bulletin 25(04): 157-161 (in Chinese).
[11] Li, H. S. (2000): Principles and Techniques of Plant Physiology and Biochemistry Experiment. – Higher Education Press, Beijing (in Chinese).
[12] Li, S., Leng, Y., Feng, L. (2014): Involvement of abscisic acid in regulating antioxidative defense systems and IAA-oxidase activity and improving adventitious rooting in mung bean [Vignaradiata (L.) Wilczek] seedlings under cadmium stress. – SciPollut Res 21(1): 525-537.
[13] Li, R. X., Jin, X. L., Hu, X. J., Wang, J. M., Luo, F., Zhang, F. J. (2019): Physiological responses and differential expression of cold resistance-related genes of six cultivars of Magnoliaceae under low temperature stress. – Acta Ecologica Sinica 39(8): 2883-2898.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

[14] Liu, Z. Q., Zhang, S. C. (1994): Physiology of Plant Resistance. – China Agriculture Press, Beijing (in Chinese).
[15] Liu, B. B., Chen, L. N., Li, H. S. (2017): Research progress on molecular mechanism of fruit trees in response to low temperature stress and identification methods of cold resistance. – Northern Horticulture 15: 174-179 (in Chinese).
[16] Liu, T., Hu, X., Zhang, J., Zhang, J., Du, Q., Li, J. (2018): $H_2O_2$ mediates ALA-induced glutathione and ascorbate accumulation in the perception and resistance to oxidative stress in Solanum lycopersicum at low temperatures. – BMC Plant Biol. 18(1): 34 (in Chinese).
[17] Masouleh, S. S. S., Aldine, N. J., Sassine, Y. N. (2019): The role of organic solutes in the osmotic adjustment of chilling-stressed plants (vegetable, ornamental and crop plants). – Ornamental Horticulture 25(4): 434-442.
[18] Rana, N. U. R., Sajid, A., Mahmood, U. H., et al. (2021): Low temperature and hypoxic conditions induce flavonoids biosynthesis and enhances antioxidant potential of crabapple (*Malus profusion)* fruits. – Acta Physiologiae Plantarum 43: 131.
[19] Ren, L., Yve, H. (2010): Cold resistance of 4 types of central plains tree peony cultivars in Harbin area. – Bulletin of Botanical Research 30(02): 228-237 (in Chinese).
[20] Shi, H. M., Zhang, G. X. (2014) Influence of low temperature stress on proline accumulation in leaves of sorghum seedling. – Journal of Shanxi Agricultural Sciences 42(09): 945-947 (in Chinese).
[21] Shiming, H. E., Ping, F., Guo, L. (2009): Study on antioxidant ponent of houttuynia. – Southwest China Journal of Agricultural Sciences 22(3): 625-631.
[22] Tian, H. X. (2020) Transcriptome sequencing reveals cold resistance pathway and related genes in Lilium davidii Franch under sudden low temperature. – Jiangsu Agricultural Sciences 48(22): 28-36.
[23] Wang, X. X. (2021): Effects of exogenous SOD on physiological characteristics of Petunia hybrida under low temperature stress. – Seed 40(09): 108-111 + 117 (in Chinese).
[24] Wang, J. H., Liu, H. X. (1989): The role of superoxide dismutase (SOD) in stress physiology and senescence physiology of plant. – Plant Physiology Communications 3(1): 1-7 (in Chinese).
[25] Wang, D., Xuan, J. P., Zhu, X. C. (2010): Relationships of freezing tolerance and the contents of carbohydrates, proline, protein in centipede grass (Eremochloa ophiuroides (Munro.) Hack.). – Acta Agrestia Sinica 15(11): 7893-906 (in Chinese).
[26] Wang, Y. W., Xu, C., Zhang, B. B. (2017): Physiological and proteomic analysis of rice (Oryza sativa L.) in flag leaf during flowering stage and milk stage under drought stress. – Plant Growth Regul 82(2): 201-218.
[27] Wang, Y., Xiu, Y. F., Zhu, Chen, B. H. (2019): Effects of paclobutrazol on the physiological characteristics of Malus halliana Koehne Seedlings under drought stress via principal component analysis and membership function analysis. – Arid Land Research and Management 33: 93-113.
[28] Xiong, Y. Q., Li, C. T., Liu, X. H. (2004): A study of the cold resistance of Euonymus japonicus and its application. – Chinese Landscape Architecture 20(04): 39-41 (in Chinese).
[29] Xue, H., Tian, J. M. (2020): Physiological response of different edible lily cultivars to low temperature stress. – Guizhou Agricultural Sciences 48(6): 89-93.
[30] Yang, W., Cui, G. F., Ma, L. L. (2015): Effects of low temperature stress on photosynthetic characteristics of cut lily 'Robinna'. – Agricultural Science and Technology and Information 12(04): 291-292 (in Chinese).
[31] Yve, L. (2012): Research progress on resistance of Lilium. – Horticulture and Seedlings 6: 119-122.
[32] Zhang, J. Y., Guo, H., Zhang, L. (2012): Effects of low temperature stress on morphological and electrical conductivity of ten species of afforestation trees along road sides in Nanjing. – Journal of Northeast Forestry University 40(05): 25-28 (in Chinese).


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005

[33] Zhao, X. Y., Wang, W. H. (2008): Degeneration causes of lily cultivars and rejuvenation techniques of bulbs made in China. – China Flower & Horticulture 6: 14-17 (in Chinese).
[34] Zhong, P., Liu, J., et al. (2018): Physiological response and cold resistance evaluation of peanut to low-temperature stress. – Henongxue Bao (Journal of Nuclear Agricultural Sciences) 32: 1195-1202.


http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2102_9931005