

EVALUATION OF LOW-TEMPERATURE ADAPTABILITY OF DIFFERENT LILY VARIETIES

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Abstract. Individuals of 13 lily varieties were treated in artificial climate chambers at a day/night temperature of 25/15 °C as control, and low-temperature at 15/5 °C for 1 day (D₁), 3 days (D₂), 5 days (D₃) respectively, and rewarmed under the control temperature for 2 days (D₄) after D₃ treatment. The physiological indexes including electrical conductivity (Rec), osmotic adjustment substance contents of proline (Pro), soluble sugar (SS) and soluble protein (SP), and activity of superoxide dismutase (SOD) were measured. The results showed that the Rec of most varieties increased significantly under 1 day low-temperature stress and then showed a decreasing trend with the extension of stress time. The Pro of all varieties increased significantly under low-temperature stress, but the SP of most varieties decreased significantly. The SS content of more than half of the varieties increased significantly with the extension of low-temperature stress. The SOD activities of all varieties increased and most of them increased significantly under stress. After rewarming, the Rec of 54% of the varieties increased, and 31% increased significantly compared with D₃. SS contents of about 62% of the varieties and SP contents of about 46% of the varieties increased significantly. Pro contents of 77% of the varieties decreased. The SOD activity of 77% of the varieties increased and 31% of the varieties increased significantly. After comprehensive analysis by the membership function method the cold adaptability of lily varieties showed a descending order as Concad'Or > Tiber > Asopus > Golden Matrnx > Red twin > Willmottiae > Orange Matrnx > Maximowiczii > Viviana Zantriana > Dauricum > Maldano > Robina > Eyeliner. The results of this study provided a theoretical basis for screening suitable lily varieties for gardens in the northeast of China.

Keywords: *lily varieties, low-temperature stress, rewarming treatment, physiological indicators, osmoregulatory substances, SOD activity, membership function analysis*

Introduction

To improve the landscape effect of gardens and enrich plant diversity, ornamental plants are often introduced to other places all over the world, and the adaptability of the introduced plants to the new environment is the key to the success of the introduction. In the northeast of China, early spring heats up quickly, but the climate is unpredictable and the temperature fluctuation is usually large due to the exchange of cold and warm air currents (Ren, 2021). Introduced plants are often susceptible to cold damage due to their inability to adapt to this environment, which seriously influences the introduction and cultivation of ornamental garden plants. Evaluating the cold adaptability of introduced plants is very important for the screening and application of introduced garden plants.

The effects of low temperature on plants have been a hot research topic in related fields (Dingy et al., 2019). Temperature fluctuations lead to changes in plant cell membrane fluidity and cytoskeletal rearrangement, triggering the cytoplasmic flow of Ca²⁺ (Pu et al., 2021) and subsequently triggering a low-temperature response, resulting in low-temperature tolerance (Chan et al., 2007). Thus, cell membrane fluidity with altered cytoskeleton confirmation is considered a potential low-temperature receptor (Potaczek

and Elżbieta, 2000). The disruption of the cell membrane system increases membrane permeability and results in a large amount of extravasation of electrolytes and certain small organic molecules, thus causing an increase in relative conductivity (Xie et al., 2022). Thus, for cold-tolerant varieties we expect to see a large increase in relative conductivity after cold treatment.

Excessive ROS production leads to oxidative damage to cellular proteins, lipids, nucleic acids and plasma membranes (Li, 2018; Xu and Cai, 2014; Paredes and Quiles, 2017). Antioxidant enzyme systems such as superoxide dismutase SOD can scavenge reactive oxygen species, thus reducing the damage caused by low temperature (Zhang et al., 2019). At low temperatures, plants also initiate osmoregulatory systems, with changes in the content of osmoregulatory substances such as soluble sugars, soluble proteins and free proline (Parviz et al., 2014). It has been demonstrated that the content of osmoregulatory substances is positively correlated with plant cold resistance 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 (Finca et al., 2012). When plants are subjected to low-temperature stress and then encounter suitable temperatures, they will initiate their repair mechanisms to eliminate the adverse effects of low temperature. To a certain extent, rewarming can alleviate the damage caused by cold damage to plants, and the alleviation effect of rewarming is also related to factors such as species differences and the degree of low-temperature stress. Li et al. (2007) measured the changes in reactive oxygen species (ROS) production and protective enzyme activities in leaves of new iron gun lily (*Lilium sayaka*) seedlings under different low-temperature stress. The study of recovery by rewarming found that rewarming for some time could restore the SOD activities and the rate of ROS production in lily leaves after low-temperature stress. Ge (2020b) treated Sorbonne lilies (*Lilium oriental* Hybrids 'Sorbonne') at different periods of low temperature for 10 days and found that the content of osmoregulatory substances for cold resistance all increased significantly and all physiological indexes were equal to the control after rewarming treatment. 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 controlling the cold resistance, rather than determined by a single factor alone (Rana et al., 2021).

Lily (*Lilium* spp.) is a perennial bulbous flower of the genus *Lilium* under the Liliaceae family. Lilies in China are mainly produced in the provinces of Hebei, Shanxi, Henan, Shanxi, Hubei, Hunan, Jiangxi, Anhui and Zhejiang (O'Neill, 1965). A great varieties of ornamental lily garden species are bred in the world and they have great ornamental, edible and medicinal value (Zhang et al., 2020). The huge demand for more lily varieties might be underlined by the economical value of the lily varieties already in cultivation. It is very important for screening varieties to adapt the dramatic temperature fluctuation in spring of northeast, China by researching the change of physiological indexes under cold stress and their performance under rewarming after low-temperature stress. In the meantime, the ability of cold resist difference and diversity of adaptive mechanism to low-temperature stress of different lily varieties could be revealed.

Materials and methods

Plant material

Thirteen lily varieties introduced to Shenyang, Liaoning province of China by Shenyang Academy of Landscape-gardening (*Table 1*) were used for low-temperature stress studies.

Table 1. Lily varieties and symbol code of experimental materials

Ethnography	Cultivar or species name	Symbol
Lilium Asiatic hybrids	Orange MatrIx	B
	Golden MatrIx	D
	Dauricum Ker-Gawl	L
OT	Conca d'Or	H
	Robina	I
	Maldano	G
<i>L. casablanca</i>	Viviana Zantriana	K
LA	Eyelinex	M
	Red twin	C
	Asopus	A
Oriental hybrids	Tiber	J
Others	<i>L. leichtlinii</i> var. <i>maximowiczii</i> (Regel) Baker	E
	<i>L. davidii</i> var. <i>willmottiae</i> (E. H. Wilson) Raffill	F

OT: Oriental Hybrids × Trumprt Hybrids; LA: Longiflorum Hybrids × Asiatic Hybrids

Experimental design and low-temperature treatment

Three individuals of each variety were planted in 25 cm diameter pots respectively in the greenhouse for one week and then put in the artificial climate chamber (RXZ-0288) for one week at 25 °C/15 °C (day/night), 12h/12h light (1200lx)/dark cycle and 50%-60% relative humidity. Then the low-temperature treatment began under 15 °C/5 °C (day/night) with the same other conditions as above, and the time gradient of the stress was 1 day (D₁), 3 days (D₂) and 5 days (D₃). Then it was put under the rewarming treatment for 2 days (D₄) after the D₃ treatment and the condition of rewarming was 25 °C/15 °C (day/night), and other conditions were not changed. The control group (CK) was incubated under 25 °C/15 °C (day/night) and other conditions were the same as above.

The leaves were collected from three individuals of each variety respectively to measure the physiological indexes of D₁, D₂, D₃, D₄ and CK, and each index was repeated three times.

Estimate electrolyte leakage (Rec)

One leaf at the fourth leaf position was taken from each treated individual. 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 and Tobita, 1998):

$$Rec = \left(\frac{EC_1}{EC_2} \right) \times 100\% \quad (\text{Eq.1})$$

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 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 (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 (Xiu et al., 2019), which was calculated as follows.

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

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

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

substances and 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 Rec

The Rec of each variety showed different patterns of change under low temperatures (*Fig. 1*). Compared with CK, except B, C, D, F and G the Rec of the other 62% of the varieties increased significantly ($P < 0.05$) under D₁. Under D₂ treatment, varieties of H, I and M increased significantly ($P < 0.05$). Variety C decreased significantly ($P < 0.05$). The other 69% of the varieties had no significant difference compared with CK. Under D₃ treatment, the Rec of varieties of A, E, F, K and M increased significantly ($P < 0.05$). Others had no significant difference compared with CK. The Rec differences of variety B under all cold treatments were not significant compared with CK, but variety M showed a significant increase under all cold treatments ($P < 0.05$). Variety E showed a maximum increase by 243.7% under D₃ compared with CK.

Under D₂ treatment, except Rec of I and M increased slightly the other about 77% of the varieties decreased and B, D, F, I, M decreased significantly ($P < 0.05$) compared with D₁. Under D₃ treatment, the Rec of 69% of the varieties increased. A, C, E, F and K increased significantly ($P < 0.05$) and B, G, J, L and M increased with no significant difference compared with D₂. Varieties of H and I decreased significantly ($P < 0.05$) and D decreased with no significant difference compared with D₂.

After rewarming, 46% of the varieties including A, E, F, G, K and M decreased in Rec under D₄ compared with D₃ and A, E decreased significantly ($P < 0.05$) with down of 41.8% and 70.9%, respectively. The Rec of the other 7 varieties under D₄ increased and C, D, H, I increased significantly ($P < 0.05$) by 57.9%, 110.4%, 61.7% and 8.1% respectively. Compared with CK, the Rec of about 85% of the varieties increased and C, D, H, I, K and M increased significantly ($P < 0.05$). Only E and G decreased compared with CK.

The change of Rec showed that cell membranes of most varieties were damaged under the first day (D₁) of low temperature stress, but their adaptive mechanisms were activated at the third day (D₂) because the Rec of most varieties decreased. As the stress time continue to extend to 5 days (D₃) the increase of Rec showed that the cell membranes of most varieties were seriously damaged again and they could not tolerant long time cold stress. Only very few varieties could adapt to long time cold stress accompanied by their values of Rec decreased than those under D₂ treatment. The cell membrane of more than half varieties were further damaged under rewarming (D₄)

because their Rec increased compared with D₃ treatment. But other varieties could repair the damage under rewarming condition. This result showed that lily varieties had multi-performance and different mechanisms under different low temperature stress and rewarming condition.

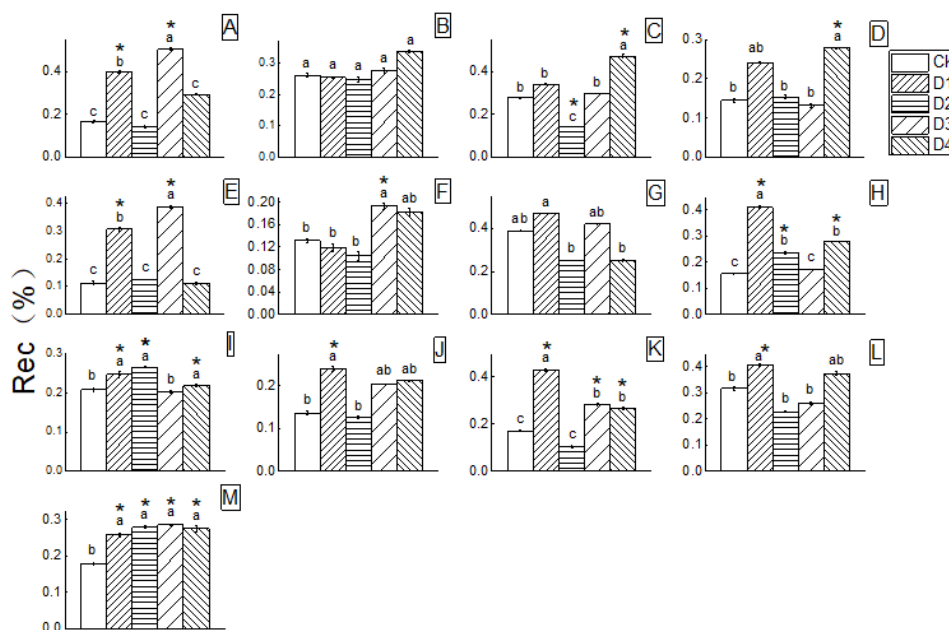


Figure 1. The influence of different treatments on the Rec of the lily varieties

Variations of proline content (Pro)

The changes of Pro content under low-temperature stress were shown in *Figure 2*. Most varieties showed a significant increase ($P < 0.05$) with the extension of stressing time. Compared with CK, the Pro content of I and K decreased significantly ($P < 0.05$) under D₁. The other about 85% of the varieties increased and A, B, C, D, G, H, J, L, M increased significantly ($P < 0.05$). Under D₂ treatment, about 92% of the varieties increased significantly ($P < 0.05$) and A, E, H increased significantly at the 0.01 level ($P < 0.01$). Only I decreased with no significance. Under D₃ treatment, 100% of the varieties increased significantly compared with CK ($P < 0.05$) and A, C, G, H, J, L increased significantly at the 0.01 level ($p < 0.01$). Variety J showed the greatest increase (3450%) under D₃.

Under D₂, the Pro of about 92% of the varieties increased and A, B, E, F, G, H, I, L, M increased significantly ($P < 0.05$) compared with D₁. Only C decreased and had no significant difference compared with D₁. Under D₃, only 3 varieties including E, F and M decreased significantly compared with D₂ ($P < 0.05$). The other 77% of the varieties increased significantly ($P < 0.05$).

After rewarming, Pro contents of 77% of the varieties were still higher than CK. A, B, C, H, I, J and M increased significantly at the 0.05 level and D, E and L increased significantly at the 0.01 level. Only K was lower significantly than CK ($P < 0.05$). Pro contents of D, E and I increased highly significantly ($P < 0.05$) under D₄ compared with D₃. The other 10 varieties (77%) decreased and 9 varieties except M decreased significantly ($P < 0.05$). The biggest drop was variety B by 88.7%.

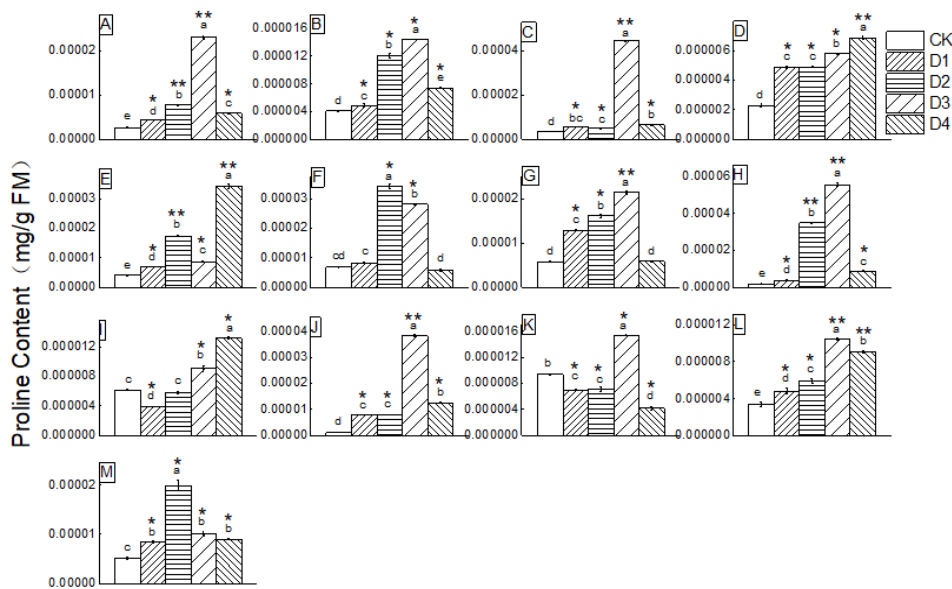


Figure 2. The influence of different treatments on the Pro content of the lily varieties

Variations of soluble sugars content (SS)

The dynamics of SS content under low-temperature stress differed significantly among varieties (Fig. 3). Compared with CK, SS content of about 46% of the varieties including C, F, G, I, J and L increased significantly ($P < 0.05$) under D₁ treatment. Others decreased and varieties of E, K decreased significantly ($P < 0.05$). Under D₂ treatment, SS content of about 69% of the varieties including A, C, E, F, I, J, K, M and L increased and the increases were significant ($P < 0.05$) except variety L. Variety I increased significantly ($P < 0.01$). Others decreased and B, G decreased significantly ($P < 0.05$). Under D₃ treatment, SS content of 69% of the varieties including A, C, F, H, I, J, K, L and M increased and the increases were significant ($P < 0.05$) except F and H. Varieties of C and I increased significantly ($P < 0.01$). Others decreased and D, G decreased significantly ($P < 0.05$).

Under D₂ treatment, SS content of about 62% of the varieties including A, E, F, H, I, J, K and M increased and A, E, I, J, K, M increased significantly ($P < 0.05$). Others decreased and C, G and L decreased significantly ($P < 0.05$) compared with D₁. Under D₃ treatment, SS content of about 62% of the varieties including A, B, C, G, H, I, J and L increased and the increases were significant except varieties of B, F, H and J ($P < 0.05$). Others decreased and D, E and K decreased significantly ($P < 0.05$) compared with D₂.

SS contents of C, F, I and J increased under all low-temperature treatments and the increases of C, I and J were significant ($P < 0.05$). Variety C showed the greatest increase under D₃ (701.2%). SS contents of H had no significant change under all low-temperature treatments.

After rewarming, the SS contents of 8 varieties (about 62%) including A, C, D, G, H, J, K and L under D₄ were significant ($P < 0.05$) higher than D₃. B and M changed with no significant difference. E, F and I decreased significantly ($P < 0.05$) by 51.3%, 19.1% and 43.9%, respectively. SS contents of about 77% of the varieties were higher than CK. D, G, H, I, J, K, L and M increased significantly ($P < 0.05$). A and C increased significantly ($P < 0.01$).

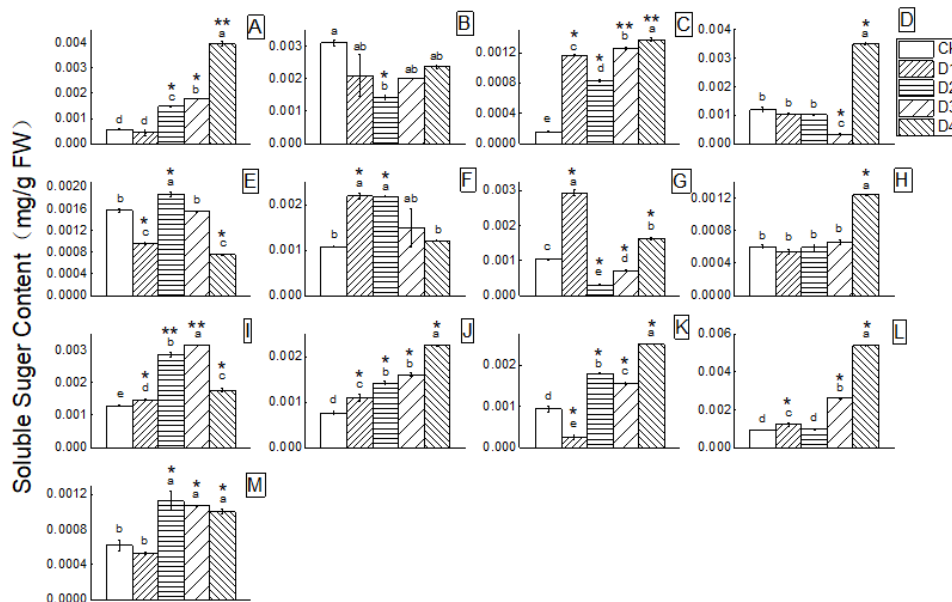


Figure 3. The influence of different treatments on the SS content of the lily varieties

Variations of SP content

The dynamics of SP content under low-temperature stress differed significantly among varieties (Fig. 4). Compared with CK, SP content of 6 varieties including A, C, F, G, J, L decreased significantly ($P < 0.05$) and B, D, E, I, K, M decreased significantly ($P < 0.01$) under D₁ treatment. Only H increased significantly ($P < 0.05$). The change tendency of SP contents was the same under D₂ and D₃ treatment, about 77% of the varieties including B, C, D, E, F, G, I, K, L and M decreased significantly ($P < 0.05$). Varieties of C, E under D₃ and variety M under D₂ decreased significantly ($P < 0.01$). Only 3 varieties increased and H, J increased significantly ($P < 0.05$).

Under D₂ treatment, about 85% of the varieties including A, B, C, D, E, F, H, I, J, K and L increased significantly compared with D₁ treatment ($P < 0.05$). Variety G increased with no significance and M decreased significantly ($P < 0.05$). Under D₃ treatment, about 54% of the varieties increased and I, M increased significantly ($P < 0.05$) compared with D₂. Others including B, C, D, E, G and L decreased and the decrease was significant ($P < 0.05$) except variety D.

Except A and H, the SP contents of 85% of the varieties were still significantly lower than their CK ($P < 0.05$) after rewarming. Varieties D, F, J and L showed a significant decrease ($P < 0.05$) compared with D₃ and the declines were 15.5%, 13.6%, 6.6% and 34.6%, respectively. Varieties A, B and G had no significant difference and varieties C, E, H, I, K and M showed a significant increase ($P < 0.05$) compared with D₃. Variety C showed the maximum increase of 36.9%.

The SP contents of variety H increased significantly ($P < 0.05$) under all low-temperature treatments and it showed a maximum increase of 116.3% under D₃. The SP contents of variety A had no significant change under stress treatments except decreased significantly under D₁ ($P < 0.05$). The other 11 varieties (about 85%) decreased significantly under all treatments compared with CK and C showed a maximum drop of 48.3% under D₃.

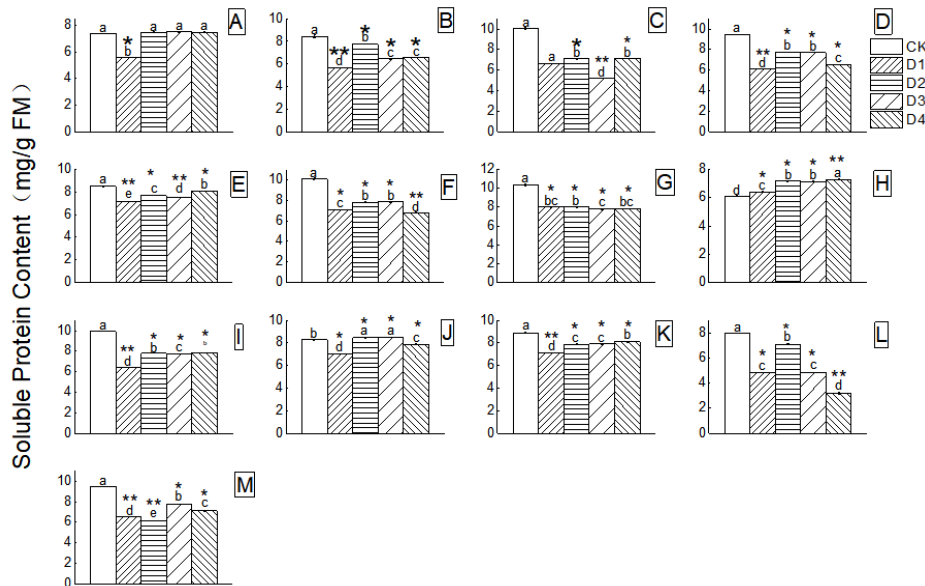


Figure 4. The influence of different treatments on the SP content of the lily varieties

Variations in the activities of superoxide dismutase (SOD)

All varieties showed an increasing trend of SOD activity under low-temperature treatments (Fig. 5). E and M increased significantly ($P < 0.05$) and F, J and L increased with no significant difference under all low-temperature stresses. Other varieties including A, B, C, D, G, H, I and K showed a significant increase under D₃ ($P < 0.05$) and H showed the maximum increase (428.8%).

After rewarming, the SOD activities of all varieties were significantly higher than their CK ($P < 0.05$). Compared with D₃, B, J and M decreased and B decreased significantly ($P < 0.05$). The other 10 varieties (77%) increased compared with D₃ and A, H, I and K increasing significantly ($P < 0.05$) by 100.4%, 37.1%, 116.5% and 77.1%, respectively.

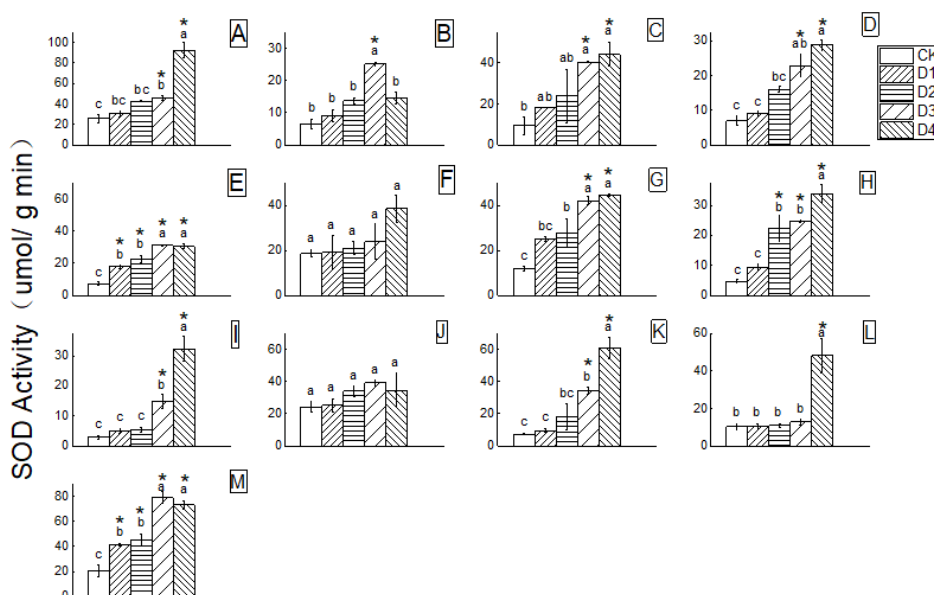


Figure 5. The influence of different treatments on the SOD activity of the lily varieties

Pro, SOD, SP and SS were resisting indexes for plant and their increases could represent the resistance ability under stress condition. According the results above, most lily varieties could enhance the contents of Pro, SS and the activity of SOD to resist cold stress.

Comprehensive analysis of lily cold resistant ability

Therefore, the affiliation function values of each index were calculated based on the physiological indexes under low-temperature treatment. The cold resistance of different lily varieties was ranked by the average value of affiliation function (*Table 2*). The higher the value of cold resistance membership function, the stronger the cold resistance. The cold resistance ability of the 13 varieties according to the comprehensive analysis was in the order of H (Concad'Or) > J (Tiber) > A (Asopus) > D (Golden Matrlx) > C (Red twin) > F (Willmottiae) > B (Orange Matrlx) > E (Maximowiczii) > K (Viviana Zantriana) > L (Dauricum) > G (Maldano) > I (Robina) > M (Eyeliner). Variety H could raise its contents of SP and Pro, activity of SOD significantly under the condition of low temperature to improve its cold resistant ability and its Rec decreased with the prolongation of stress time which meant that its damage of cell membrane was repaired. The Rec of variety M increased with stressing time, its SP decreased significantly under low temperature and Pro decreased significantly at the 5 days of cold stress. The physiological changes of H and M represented the different performance and mechanism to resist cold stress of the different varieties tested in this study.

Table 2. Subordinate function values and comprehensive sorting of cold resistance of 13 lily varieties

Varieties	Rec	Pro	SS	SP	SOD	Average	Cold resistance ranking
H	0.540	0.535	0.429	0.743	0.592	0.568	1
J	0.437	0.451	0.692	0.614	0.614	0.562	2
A	0.430	0.435	0.621	0.652	0.661	0.560	3
D	0.700	0.824	0.463	0.315	0.486	0.558	4
C	0.640	0.363	0.815	0.223	0.525	0.513	5
F	0.610	0.595	0.706	0.175	0.426	0.503	6
B	0.883	0.405	0.264	0.322	0.533	0.482	7
E	0.417	0.512	0.530	0.225	0.705	0.478	8
L	0.604	0.534	0.398	0.250	0.470	0.451	9
K	0.483	0.371	0.623	0.284	0.467	0.445	10
G	0.410	0.674	0.370	0.067	0.604	0.425	11
I	0.429	0.401	0.648	0.252	0.368	0.419	12
M	0.102	0.475	0.540	0.198	0.594	0.382	13

Discussion

Rec is an important indicator of cell membrane permeability under low-temperature stress, and the larger the value, the more leakage of electrolytes and the more severe the cell membrane damage (Li et al., 2014; Zhong et al., 2018). Li (2017) found that the Rec of the vast majority of lily species increased significantly with decreasing temperature and the Rec fitting curve showed a growth by subjecting 30 species of lilies

to low-temperature treatment from 0 °C to -15 °C. In this study, the relative conductivity of some lilies showed a significant increase with the extension of the low-temperature stress time. In terms of Rec changes, the differences between the low-temperature treatment gradients of individual varieties were not significant, indicating that the damage was not further aggravated with the extension of the low-temperature stress time and they were resistant to low temperature. Some varieties showed a highly significant increase only at 5 days of the stress, indicating that they could withstand the low temperature for a certain period. Some species showed a significant increase in Rec after the first day of stress, followed by a continuous decrease, indicating that the repair mechanism after the damage has been functioning. Some species showed an increase on the first day of stress, a decrease on the third day, and another increase on the fifth day, probably because the repair mechanism emerged after the damage to the cell membrane system, but the damage intensified again with the extension of time; after the warming treatment, the Rec of some lily species had a decreasing trend, indicating that these varieties could repair the damage after rewarming. But some varieties were still higher than the control, indicating that the low-temperature injury persisted and the repair mechanism of these species did not work. The trend of Rec changes in different varieties under low-temperature stress reflected the differences in low-temperature resistance and resistance mechanisms among varieties.

Osmoregulation plays an important role in plant resistance to cold, and plants under low-temperature stress can improve their cold tolerance by increasing the content of the main osmoregulatory substances (Aslam et al., 2022; Zhang et al., 2017). Increasing soluble sugar content can improve the osmoregulatory capacity of plant cells and reduce the freezing point of cellular protoplasm, enhance cold resistance, and maintain normal cell membrane function under low-temperature conditions (Kitamura et al., 2018; Levitt, 1980). By studying the effects of low-temperature refrigeration at 5 °C on soluble sugar content and sugar synthase in bulbs of wild species of fine leaf lily (*Lilium pumilum*), Liu et al. (2016) found that the soluble sugar content in lily increased with the prolongation of low-temperature stress. In this study, the soluble sugar content of most lilies showed an increasing trend with the increase of stress time, while some lilies showed a decreasing and then increasing trend. These different changes reflected the differences in cold resistance among lily species. The increase in soluble protein content SP was also able to reduce the stress injury to the plant (Kinnersley and Turano, 2000). In this study, a decrease in SP was found in most varieties, which showed that they did not have a mechanism to resist low-temperature stress by elevating SP. He (2021) found that some gramineous grasses resisted the cold damage by increasing the Pro content and the Pro content of *Elymus sibiricus* 'Chingmu No. 1' showed a significant increase with decreasing temperature. In this study, the Pro of most lily varieties showed an increasing trend under low-temperature stress. The lily varieties in this study showed different trends in the contents of SS, SP and Pro when subjected to low-temperature stress reflecting the differences in cold resistance among lily species.

Ge (2020a) treated Robina lilies (Robina) at different periods of low temperature for 10 days and found that the content of osmoregulatory substances for cold resistance all increased significantly. After 10 days of rewarming treatment, the physiological indexes were equal to the control and this result reflected that the plants themselves had certain ability to repair cold damage under rewarming. In this study, the osmoregulatory substance content of some lilies increased and the other lilies decreased under 2 days of rewarming treatment, which indicated the difference in their response mechanisms.

Superoxide dismutase (SOD) is an important antioxidant enzyme in living organisms, which is widely distributed in various organisms and is the primary substance for scavenging free radicals (ROS) in living organisms (Kim et al., 2021), which can reduce the damage of ROS on cells. Wei (2014) compared the cold resistance among maize varieties by studying the indices of protective enzymes and hormones of different varieties of maize under low-temperature stress and found that the activity of protective enzymes under low-temperature stress was significantly higher compared with the control, and the enzyme activity was enhanced with the decrease of stress temperature and the increase of stress time. In this study, SOD activity of all lily varieties was enhanced with increasing time of low-temperature stress and most varieties showed significant increase. The SOD activities of some varieties were significantly lower than those of the low-temperature treatment group after the rewarming treatment, while some varieties continued to increase. This is also a reflection of the repair ability of the plant itself and the complexity of the physiological changes as well as the strength of the cold resistance of the plant. This result was later contradicted by Wu et al. (2004).

Plant resistance is a complex quantitative trait affected by many factors, and the evaluation of plant resistance with a single index is incomplete. A comprehensive evaluation with multiple indexes can reflect the plant resistance more accurately (Meng et al., 2022; Zhu et al., 2022; Zhang et al., 2016; Tao et al., 2018).

The affiliation function analysis provides a way to comprehensively evaluate the cold resistance of different lily varieties based on the determination of multiple indicators, avoiding the one-sidedness of a single indicator. Different lilies may have different cold resistance mechanisms, therefore, a comprehensive evaluation of their cold resistance using multiple indicators can better reveal the adaptation mechanism of lilies to low-temperature stress and improve the accuracy of cold resistance identification (Li et al., 2021). Wang (2014) used the affiliation function method to obtain the cold resistance among different lily strains. In this study, some varieties with high cold resistance were also screened by the affiliation function method, reflecting the objectivity of the comprehensive evaluation method.

Conclusion

The Rec of most varieties increased significantly under 1 day low-temperature stress compared with CK. The Rec of most varieties under 3 days stress and half varieties under 5 days stress had no significant difference compared with CK. This result showed the function of the damage repair mechanism on cell membrane of some lily varieties worked.

The Pro content and SOD activity of all varieties increased under low-temperature stress, but the SP content of most varieties decreased. The SS content of more than half of varieties increased with the extension of low-temperature stress. These results showed that enhancing the contents of Pro, SS and the activity of SOD were the common way of lilies to resist cold stress.

After rewarming, the Rec of 54% of the varieties increased compared with 5 days stress. The Pro and SP contents of more than half varieties decreased. But the SOD activity and SS contents of most varieties continued to increase. These results showed that there were different responses of physiological indexes under rewarming condition after cold stress.

The cold resistance ability of lilies was evaluated by the comprehensive analysis of the affiliation function method and the order was Concad'Or > Tiber > Asopus > Golden MatrIx > Red twin > Willmottiae > Orange MatrIx > Maximowiczii > Viviana Zantriana > Dauricum > Maldano > Robina > Eyeliner.

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