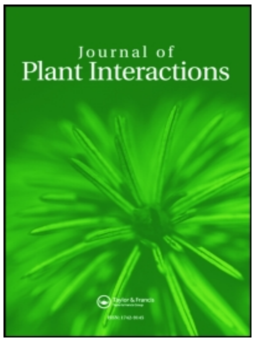

Journal of Plant Interactions

ISSN: 1742-9145 (Print) 1742-9153 (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

Evaluation of physiological responses and tolerance to low-temperature stress of four Iceland poppy (*Papaver nudicaule*) varieties

Zhuo Huang, Jiao He, Dong Xia, Xiao-Juan Zhong, Xi Li, Ling-Xia Sun & Shi-Zhen Cai

To cite this article: Zhuo Huang, Jiao He, Dong Xia, Xiao-Juan Zhong, Xi Li, Ling-Xia Sun & Shi-Zhen Cai (2016) Evaluation of physiological responses and tolerance to low-temperature stress of four Iceland poppy (*Papaver nudicaule*) varieties, Journal of Plant Interactions, 11:1, 117-123, DOI: 10.1080/17429145.2016.1217433

To link to this article: https://doi.org/10.1080/17429145.2016.1217433

JOURNAL OF PLANT INTERACTIONS, 2016
VOL. 11, NO. 1, 117–123
http://dx.doi.org/10.1080/17429145.2016.1217433

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS

Evaluation of physiological responses and tolerance to low-temperature stress of four Iceland poppy (*Papaver nudicaule*) varieties

Zhuo Huang[†], Jiao He[†], Dong Xia, Xiao-Juan Zhong, Xi Li, Ling-Xia Sun and Shi-Zhen Cai

College of Landscape Architecture, Sichuan Agricultural University, Wenjiang, Sichuan, People's Republic of China

ABSTRACT

Iceland poppy (*Papaver nudicaule*) is a boreal flowering plant and native to subpolar regions of Europe, Asia and North America, and the mountains of Central Asia. To investigate response and tolerance to low-temperature stress, the four varieties of Iceland poppy, Champagne Bubbles (CB), Domestic Variety (DV), Wonderland (WL), and Garden Gnome (GG), were subjected to low-temperature treatment from 0°C to −9°C. The relative electrical conductivities in four varieties increased along with the decrease in temperature. Treated by −3°C, the morphological changes of seedlings at different time points were investigated. Four varieties were differentially injured by freezing temperature. The dynamic accumulations of malondialdehyde (MDA), free proline (FP), soluble proteins (SP), and activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) during low-temperature treatment were measured and compared. A minor change in SP content during treatment was found, whereas MDA and FP were significantly induced. Complex patterns of activity alternations of SOD, POD, and CAT were found. Based on the calculation of semi-lethal temperature and comprehensive physiological parameters investigation, the tolerance of four varieties was evaluated as CB > GG and DV > WL.

ARTICLE HISTORY
Received 28 March 2016
Accepted 19 July 2016

KEYWORDS
Iceland poppy (*Papaver nudicaule*); freezing tolerance; antioxidant enzymes; osmolytics; morphological response

Introduction

As sessile organisms plants have evolved a wide spectrum of adaptations to cope with the inevitable challenges of environmental stress. Many aspects of these adaptation processes originate from developmental, physiological, and biochemical changes (Qin et al. 2011). Low-temperature stress, as one of the most serious environmental stresses, adversely affects the growth and development of plants and significantly constraints the spatial distribution of plants and agricultural productivity (Chinnusamy et al. 2007). Under low temperature, plasma membrane is thought to be the primary site of injury because of its central role in regulation of various cellular processes (Takahashi et al. 2013). Low-temperature stress will initiate peroxidation of the membrane lipids and cause the accumulation of malondialdehyde (MDA), which will bind with some proteins and destructs the membrane system (Chen 1991; John 1993), and therefore has been considered as an important indicator of membrane system injuries (Fan et al. 2012). Some studies showed that more MDA will be generated in plants with less tolerance to stress (Zhai et al. 2013).

Low-temperature stress will also induce the accumulation of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyradicals (Suzuki & Mittler 2006). Plants under abiotic stress have evolved a defense system against oxidative stress by increasing the activities of ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Miller et al. 2010). SOD plays a crucial role in antioxidant defense because it catalyzes the dismutation of O_{2-} into H_2O_2 and CAT and POD can reduce the H_2O_2 to H_2O and protect SOD from oxidation (Tian et al. 2012). Therefore, the activities of these enzymes may be connected with plant tolerance to stress.

The accumulation of osmolytes is also related to the low-temperature tolerance. The free proline (FP), soluble proteins (SP), and soluble sugar are among the major osmolytes. Accumulation of proline was reported in many plant species under diverse abiotic stress conditions and its role in plant stress tolerance was widely investigated and discussed (Delauney & Verma 1993). The amount of SP increased under low-temperature conditions (Bravo et al. 1999) and their abundance is correlated to the degree of freezing tolerance (Karimzadeh et al. 2006).

The Iceland poppy (*Papaver nudicaule*) is native to subpolar regions of Europe, Asia and North America, and the mountains of Central Asia. As a boreal flowering plant, it is adaptive to low-temperature environment and has been widely utilized as ornamental plant. Previous studies mainly focused on extraction and analyses of its alkaloid (Istatkova et al. 2008). However, no attention had been paid on its adaptation to low temperature. To facilitate understanding the adaptive responses and tolerance to low temperature, four varieties of Iceland poppy were collected and treated under low temperatures. Their morphological and physiological responses to low-temperature stress were measured and their tolerance to freezing temperature was also investigated. This study provided the basis to clarify the adaptation mechanism of Iceland poppy, which is valuable for identification of low-temperature-stress-related genes and their application in ornamental plant improvement in the future.

CONTACT Zhuo Huang duoduo609@sina.com

[†]These authors contributed equally to this paper.

Supplemental data for this article can be accessed at 10.1080/17429145.2016.1217433

Materials and methods

Plant materials

Four elite varieties of Iceland poppy (*P. nudicaule*), Champagne Bubbles (the United States, CB), Domestic Variety (China, DV), Wonderland (Japan, WL), and Garden Gnome (Germany, GG), were used for analyses and bought from Beijing Linda Forestry Sciences and Technologies Inc. Seeds were surface sterilized with 30% (w/v) hydrogen peroxide solutions and germinated on plates containing the mixture of local soil and humus soil (with a ratio of 1:1). The seedlings were maintained in a greenhouse with a relative humidity of 50–70%, 12 h/12 h at 15°C/10°C, and 12 h/12 h (day/night) photoperiod with light at 500 lmol m^{-2} s^{-1}. After three or four leaves emerged, the plants were transferred to plastic pots with a diameter of 15 cM (one plant per pot). The four-month-old plants with uniform growing status were subjected to experiments.

Evaluation of the low semi-lethal temperature (LT50)

Twenty-five individuals of each variety were divided to five groups and treated in a light incubator at the temperatures of 3°C, 0°C, −3°C, −6°C, and −9°C, respectively. Under each temperature, the plants were treated for 24 h and their leaves were sampled and thawed for 2 h at 4°C. Three replicates were performed for each treatment. The treated leaves were washed by distilled water for 2 min and surface dried by filter paper. Leaf discs were made by a puncher. Ten leaf discs packed by gauze were put into a 15 ml centrifugal tube containing 10 ml distilled water and maintained at room temperature for 24 h. Then the electrical conductivity (S1) was measured by a Leici-DDS-11A conductivity meter (Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China). After that, the tubes were incubated in boiling water for 15 min and cooled to room temperature. Then, the electrical conductivity was measured again (S2). The ratio of S1 and S2 is the relative electrical conductivity (REC). Triplicates for each variety under a treatment were analyzed. The low LT50 was calculated according to the method by Gai (2000). REC fitting Logistic Equation is $y = K/(1 + ae^{-bx})$, in which y indicates injured cells rate, x indicates temperature of treatment, K indicates the saturation capacity of injured cells rate, and a and b are equation parameters. The equation is linearized to $\ln((K{-}y)/y) = \ln a^{-bx}$, assign $y1 = \ln((K{-}y)/y)$. a, b, and correlation coefficient (R) could be calculated by the linear regression method. Then the low LT50 was calculated by the equation $LT_{50} = \ln((l/a)/b)$.

Morphological characterization and freezing injury index (FII)

Twenty individuals with uniform growth status of each variety were grown in incubator at −3°C with light/dark of 12 h/12 h. At 0, 24, 48, and 72 h, the treated plants were photographed and the injury degrees were measured in 0–5 scales according to the following standards: 0, no injury; 1, leaves drooping, softening, edge of leaf curling, slight wilting, and no frozen stains observed; 2 (mild injury), leaves drooping, softening, and visible frozen stains on 20–30% of leaf area; 3 (moderate injury), leaves drooping, softening, and visible frozen stains on 30–50% of leaf area; 4 (moderate injury), leaves drooping, softening, and visible frozen stains on 50–70% of leaf area; 5 (severe injury), visible frozen stains on whole leaf area. The FII is calculated by the following formula by Liu et al. (2015): FII = $\sum$injury degree × corresponding leaf no./total leaf no. investigated

Measurement of contents of MDA, FP, and SP under low temperature

The low-temperature-treated plants as described above were sampled at each time point for MDA content according to the method by Heath and Packer (1968). Approximately 0.1 g fresh weight (FW) leaf sample for each variety was homogenized in 8.0 ml (Vt) of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged (4000 × g for 10 min). 2.0 ml (V1) of the supernatant was mixed with 2.0 ml of 0.6% (w/v) thiobarbituric acid (TBA) and heated for 15 min at 100°C. The cooled mixture was centrifuged again and the absorbance of the supernatant was measured at 450 nm (A450), 532 nm (A532), and 600 nm (A600) in a spectrophotometer. The MDA content was calculated by the following formula: MDA (μmol/g FW) = (6.45 × (A532−A600)−0.56 × A450) × Vt/(V1 × FW). Three samples for each variety at each time point were measured and the means were used for result analysis.

About 0.1 g of leaf samples was put into a 15 ml centrifugal tube containing 5 ml 3% (w/v) aqueous sulfosalicylic acid and estimated by using ninhydrin reagent according to the method of Bates et al. (1973). The absorbance of the fraction with toluene aspired from the liquid phase was read at 520 nm. The Proline content was determined using the calibration curve and expressed as μg proline/g FW.

Contents of SP were measured following the method by Wang (2006). About 0.1 g of fresh leaf was ground and homogenized in 6 ml sodium phosphate buffer (pH = 7.0) and then the homogenate was subjected to centrifuge at 15,000 × g for 20 min at 4°C. The SP content in the supernatant was determined according to the method reported by Bradford (1976) with bovine serum albumin as protein standard.

Measurements of antioxidant enzyme activities

SOD (EC 1.15.1) activity was measured spectrophotometrically at 560 nm based on inhibition of the photochemical reduction of nitroblue tetrazolium (Beauchamp & Fridovich 1971). CAT (EC 1.11.1.6) activity was measured by following the decomposition of H_2O_2 at 240 nm according to the method described by Aebi (1984). POD (EC 1.11.1.7) activity was recorded by measuring the increase in absorbance at 470 nm due to guaiacol oxidation, as described by Curtis (1971).

Statistical analyses

All experiments in this study were repeated three times. Statistical analysis (mean ± standard error) was performed and a chart was created using Microsoft Excel 2007. All data were analyzed by one-way analysis of variance (ANOVA) and the mean differences were compared by the least significant difference (LSD) test. ANOVA, correlation coefficients, principal component analysis (PCA), and the logistic equation were performed by using SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA).

Integrated evaluation of tolerance

The PCA and subordinate function were used to comprehensively evaluate the freezing tolerance of Iceland poppy. The main formulas used were as follows:

(1) Change rate $= \dfrac{\text{value at } 72\,\text{h} - \text{value at } 0\,\text{h}}{\text{value at } 0\,\text{h}}$.

(2) Weight: $w_j = \dfrac{p_j}{\sum_{j=1}^{n} p_j}, \quad j = 1, 2 \ldots, n.$

Wj indicates that the weight of contribution of component j to total variance percentage.

(3) Degree of subordinate: $u(X_j) = 1 - \frac{X_j - X_{\min}}{X_{\max} - X_{\min}}$, $j = 1, 2 \ldots, n.$

X_j indicates the no. j of comprehensive index for a variety, $X_{\min}$ and $X_{\max}$ indicate the minimum and maximum values among the no. j of comprehensive indices, respectively.

(4) Comprehensive evaluation value for variety i (D_i)

$$D_i = \sum_{j=1}^{n} [u(X_j) \times w_j], \quad j = 1, 2 \ldots, n.$$

Results

Changes of REC under low temperatures and LT50

The changes in REC under 3°C to −9°C were investigated. From 3 to 0°C, the REC in four varieties slightly decreased. When the temperature falls below 0°C, they rapidly and continuously increased and reached the maximum value at −9°C (Figure 1). Below −6°C, the REC in WL increased faster than other three varieties, whereas from −6°C to −9°C, it reduced and its value was not significantly different from that at −6°C. By logistic equation of the REC, the low semi-lethal temperatures (LT50) of CB, DV, WL, and GG were calculated as −7.7°C, −5.2°C, −1.9°C, and −4.5°C, respectively (Table S1).

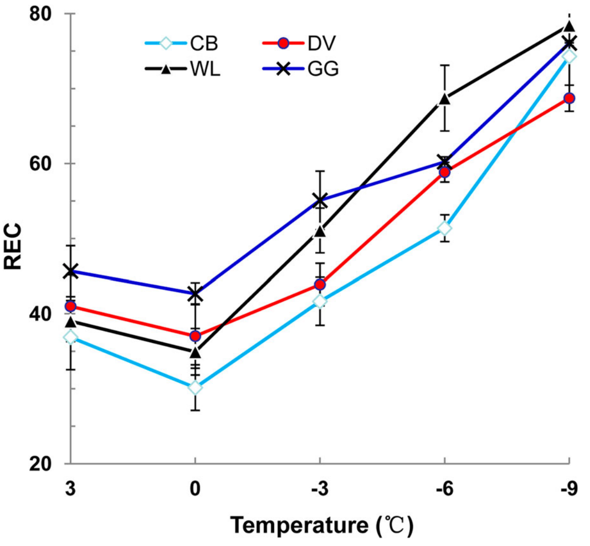

Figure 1. Dynamics of REC of four varieties under different temperatures. CB, DV, WL, and GG indicate the varieties CB, DV, WL, and GG, respectively.

Morphological response to low temperature

Under −3°C, symptoms of freezing injury, such as leaf drooping, softening, edge of leaf curling, wilting, leaf and petiole browning, were found in all four varieties of Iceland poppy. However, the degree of injury and symptoms occurrence time were different (Figure 2). CB suffered the mildest injury. Slight injury appeared after 48 h, very few leaves wilted with edge browning. After 72 h, more leaves wilted, some turned to yellow slightly. Leaf drooping initiated after 24 h in DV, moderate injury appeared after 48 h, petioles were heavily browned, a majority of leaves drooped; 72 h later, petioles, bases, and surfaces of leaves were largely browned. Slight browning appeared on petiole of GG after 24 h. With the extent of treatment time, the browning got worse, liquid stains appeared on the edge and back of leaves; 72 h later, browning and wilting were found on most of leaves. WL was most seriously injured by low temperature. Moderate injury was noticed at 24 h, ∼30% portion of some plants turned brown, petioles were almost brown; 72 h later, WL was severely injured, ∼75% portion of plants were browned and wilted. The FII for CB, DV, GG, and WL were 0.30, 1.46, 1.67, and 2.34, respectively.

Changes of MDA content under low temperature

Treated by low temperature, the MDA content in four varieties exhibited similar pattern, which was slightly decreased from 0 to 48 h, but significantly increased after 48 h (Figure 3(a)). Comparing to those at 0 h, the MDA content in WL was increased by ∼29% till 72 h, which is higher than those of CB (9.7%), DV (14.4%), and GG (22%).

Changes of osmotic substances under low temperature

Contents of SP of CB were decreased first and then increased during the low-temperature treatment, whereas other three varieties exhibited a similar pattern which increased along the initiation of low-temperature stress and decreased 24 h later. In CB, contents of SP maintained at stable levels during 0–24 h; at 48 h, it was significantly decreased and recovered to normal level at 72 h. In GG, DV, and WL, SP reached to the highest level at 24 h, which were 17.5%, 11.9%, and 10.2% higher than those at 0 h, respectively (Figure 3(b)). At 72 h, only GG had slightly higher value than that at 0 h.

In CB, DV, and WL, the contents of FP were increased after 0 h and reached maximum levels at 48 h (Figure 3(c)). Comparing to 0 h, they increased by 65.8%, 53.9%, and 58.9%, respectively. From 0 to 24 h, FP levels in CB were not remarkably different, but were significantly increased from 24 to 48 h. FP of DV, WL, and GG were significantly increased from 0 to 24 h. Differently, FP of GG kept increasing after 48 h.

Changes in antioxidant system under low temperature

The four varieties exhibited different pattern of SOD activities under low-temperature treatment (Figure 4(a)). SOD in CB kept increasing from 0 to 72 h; in DV, it increased from 0 to 48 h and deceased after 48 h; the SOD activities in WL and GG decreased after treatment, however, they stayed at

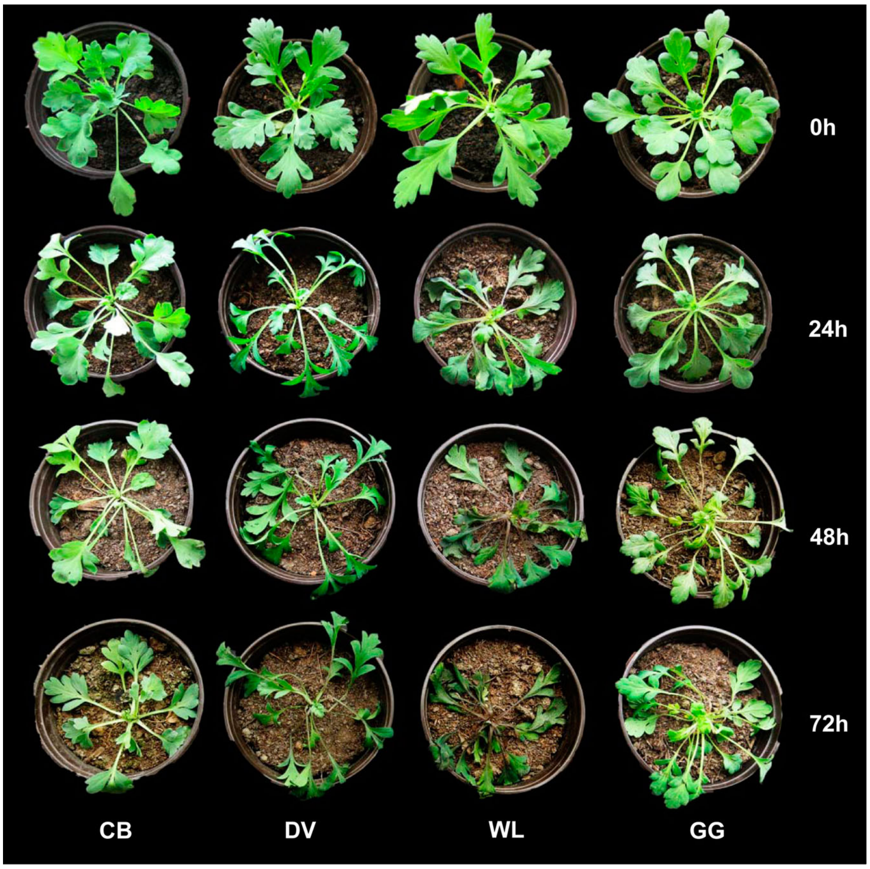

Figure 2. Morphological changes of seedlings under low-temperature treatments. CB, DV, WL, and GG indicate the varieties CB, DV, WL, and GG, respectively.

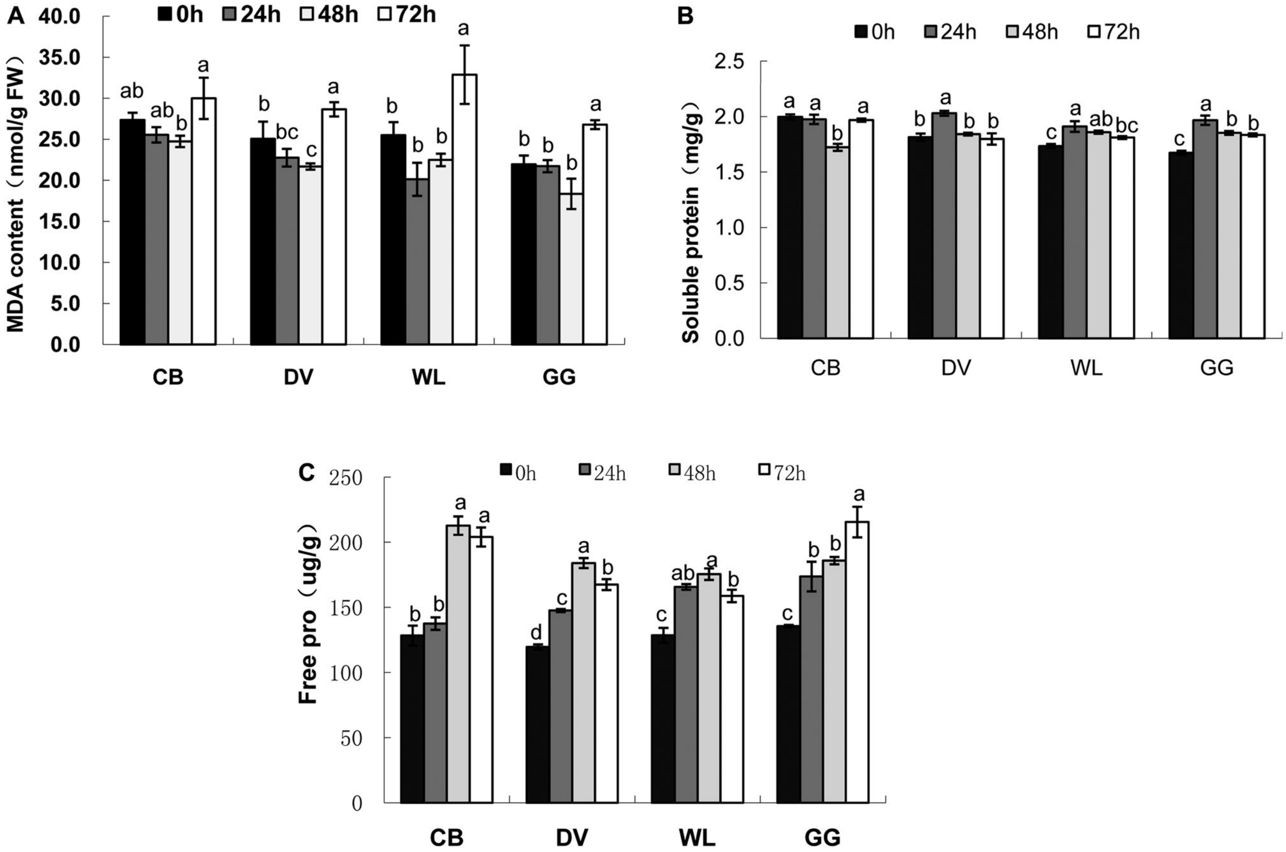

Figure 3. Dynamic changes of contents of MDA, SP, and FP under −3°C treatments. CB, DV, WL, and GG indicate the varieties CB, DV, WL, and GG, respectively. Letters above the data columns indicate whether the means are significantly different or not ($P < .05$).

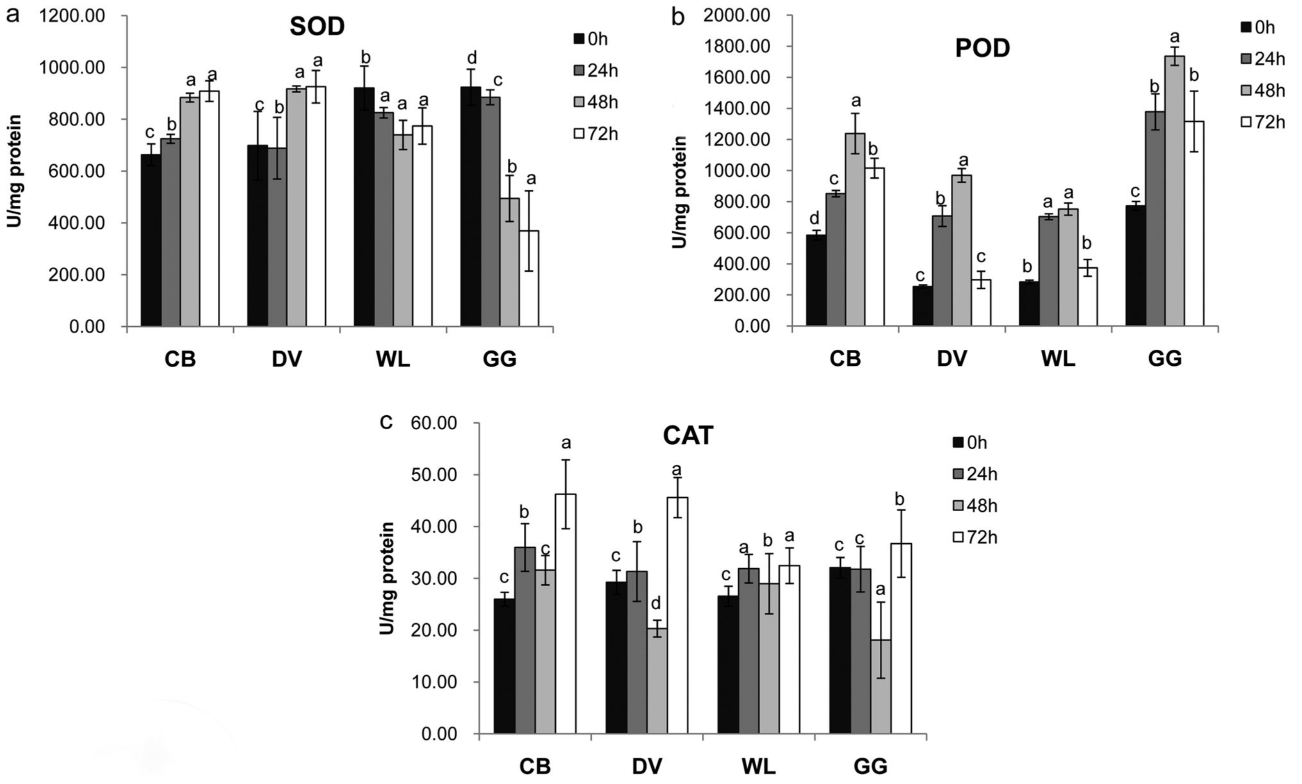

Figure 4. Dynamic changes of specific activities of SOD, POD, and CAT under −3°C treatments. CB, DV, WL, and GG indicate the varieties CB, DV, WL, and GG, respectively. Letters above the data columns indicate whether the means are significantly different or not ($P < .05$).

a stable level since 24 h in WL, whereas kept decreasing during the treatment in GG. SOD activities in CB and DV reached their maximum values around 48 h after treatment, which increased by ∼45% and 33%, respectively.

The POD activities in the four varieties increased from 0 to 48 h and then decreased (Figure 4(b)). They are higher in CB and GG than those in other two varieties at all time points. GG reached its max value at 48 h, which was higher than those in other varieties. However, the max percentage of increase appeared in DV.

The CAT activities in four varieties showed double-wave-like patterns during the low-temperature stress, which increased from 0 to 24 h, decreased from 24 to 48 h, and then increased again during 48–72 h (Figure 4(c)). At 72 h, CAT activities of CB, DV, WL, and GG reached their maximum levels, which increased by 77.2%, 55.6%, 24.8%, 13.4% comparing to those at 0 h, respectively.

Change rates and correlation coefficients

Table S2 presents the change rates of MDA, SP, FP, SOD, POD, and CAT from 0 to 72 h of low-temperature treatment in four varieties. It indicated that in all varieties investigated, the contents of MDA and FP, and activities of POD and CAT increased during low-temperature treatment, whereas SP content and SOD activity exhibited varied tendencies. Correlation analyses indicated that SP showed significant negative correlation with SOD, and the remaining indices also had certain correlations (Table S3).

Principal component analysis (PCA)

To further reveal the relationship among investigated indices and freezing tolerance, the PCA was performed that reduced six indices down to two components (Table 1). The first component explained 55.54% of the variation with MDA, SP, SOD, and CAT, and the second component explained 35.77% the variation with Pro and POD, which cumulatively contributed to 91.31% of total variation.

Based on the coefficient of comprehensive indices (Table 1) and normalized change rates of the six physiological traits (data not shown), the comprehensive indices of four varieties were calculated (Table 2). For the first component, the comprehensive indices for CB, DV, WL, and GG were 1.89,1.04, −0.76, and −2.18, and 1.22, −0.82, −1.65, and 1.24 for the second component, respectively. We further calculated the weight (W_j), the degree of subordinate ($u(X_j)$), and subsequently the comprehensive evaluation value (D_i). The D_i of CB, DV, WL, and GG was 0.89, 0.51, 0.25, ad 0.46, respectively.

Discussion

In this study, the responses of four varieties of Iceland poppy to the low temperature were investigated at morphological and physiological levels. We first calculated the LT50 based on the dynamic changes of relative electrical conductivities under low temperatures and indicated that the tolerance of four investigated varieties to low temperature was CB > DV and GG > WL. This was also confirmed by the morphological observation under −3°C treatment. These results indicated that the four varieties exhibit significantly different capacity to low temperature.

The MDA content is a reflection of lipid peroxidation and was usually used to measure stress-induced damage at the cellular level. In this study, the most tolerant variety CB showed minimum change rate of MDA, and inversely in WL which is the most sensitive to low-temperature stress than others. This results are similar to those obtained from Rose (Deng et al.

Table 1. The PCA for drought tolerance indices.

Component	Eigen value	% of variance	Cumulative %	MDA	SP	Pro	SOD	POD	CAT
1	3.33	55.54	55.54	0.43	−0.53	0.04	0.55	−0.07	0.48
2	2.15	35.77	91.31	0.36	0.16	0.67	0.00	0.61	−0.12

Table 2. The value of each cultivar's comprehensive index, index weight, $u(X_j)$, and D_i.

Varieties	C1	C2	U(x1)	U(x2)	D_i
CB	1.89	1.22	**1.00**	0.99	1.00
DV	1.04	−0.82	**0.79**	0.29	0.59
WL	−0.76	−1.65	**0.35**	0.00	0.21
GG	−2.18	1.24	**0.00**	1.00	0.40
Weight (W_j)			0.60	0.40	

Note: u(X1) and U(X2) indicated the degree of subordinate, which were used to calculate D value.

2012), indicating that the MDA content is a good indicator of low-temperature tolerance.

The accumulation of SP has been reported to be correlated to low-temperature tolerance (Bravo et al. 1999, Karimzadeh et al. 2006). However, our results indicated that the SP contents in all four varieties were not remarkably changed. After 72 h of treatment, only GG had slightly higher content of SP than control level. This result indicated that SP contents in Iceland poppy may not be significantly induced by low temperature.

Some studies suggested that FP accumulation contributes to increasing osmotic stress tolerance (Kavi-Kishor et al. 1995; Zhu et al. 1998). However, some other reports argued that Pro accumulation is only an adaptive response to stress and is not associated with stress tolerance (Bawa & Sen 1993; Deng et al. 2013). In this study, the FP content in CB, DV, and WL changed significantly during treatment and reached the highest levels at 48 h. From 24 to 48 h, more Pro was accumulated in CB than in other varieties, which is also higher than those in other three varieties at 48 h. However, unlike CB, DV, and WL whose Pro levels slightly decreased after 48 h, Pro accumulation in GG kept increasing at 72 h. Although our results indicated that the accumulation degrees (Table S2) seem higher in more tolerant varieties from 24 to 48 h, the total accumulation levels of FP are not in accordance with their tolerance to low temperature.

The dynamic changes of activities of SOD, POD, and CAT under stress have been widely investigated in many plant species (Liu et al. 2013, 2015). Cold-tolerant lilies kept high SOD activities 15 days after low temperature (Wang 2012); wheat varieties with higher tolerance to low temperature showed higher increase in SOD activities (Wang et al. 2011); under chilling stress, the CAT activities of *Anthurium andraenum* kept increasing, whereas those of POD decreased first and then increased (Tian et al. 2011); POD activities of maize increased under low temperature, whereas CAT activities decreased (Wu et al. 2004). In our study, we found that the SOD activity in CB kept increasing till 72 h, indicating that SOD kept continuously higher activity in CB, which may be helpful to maintain the ability of ROS scavenging. The dynamics of CAT activities in four varieties exhibited same patterns. In CB, CAT activity increased to significantly higher level at 72 h than other three varieties. However, the dynamics of POD activities in four varieties seem not in accordance with their respective freezing tolerance. These results suggest that the antioxidant enzymes participating in oxidation scavenging may coordinate differently under different backgrounds. For example, the change rates of POD and CAT are both higher in CB than in other varieties, which are consistent with higher change rate of SOD; GG has higher change rate of POD, and lower rate of CAT, whereas inverse conditions appear in DV and WL.

The combined results of physiological parameters investigated showed that although some parameters reflect, to a certain extent, the tolerance of a variety, it is hard to employ a single physiological parameter to accurately evaluate freezing tolerance of Iceland poppy. We then employed the PCA and subordinate function to integrate obtained parameters to comprehensively evaluate freezing tolerance in Iceland poppy. These methods have been used in stress tolerance evaluations on different plant species and generated reliable results (Xu et al. 2005; Xu & Chen 2008). By PCA analyses, six parameters were integrated to two independent components, which could explain more than 90% of total variance. Based on this, coefficient of comprehensive indices, comprehensive indices for individual variety, degree of subordinate, index weight, and comprehensive evaluation D_i were calculated. These results showed that the freezing tolerance of four varieties evaluated by physiological parameters was CB > DV and GG > WL. This is in accordance with LT50 and morphological evaluation , indicating that the PCA and subordinate function are sound methods and are fit to comprehensively evaluate the freezing tolerance in Iceland poppy.

Generally, this study indicates that different varieties of Iceland poppy exhibit different morphological and physiological responses to low temperature. The accumulation degrees of MDA and FP could partly reflect the tolerance to low temperature. The activities of antioxidant enzymes in four varieties are low temperature inducible with different patterns. Their coordination in ROS scavenging deserved further investigation. The variety CB is highly tolerant to freezing temperature and could be considered as a valuable resource for identification of freezing-tolerant genes and genetic improvement of freezing tolerance in ornamental plant.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work is supported by Scientific Research Project (15ZA0014) founded by Education Department of Sichuan Province, China.

ORCiD

Jiao He http://orcid.org/0000-0002-1649-3650
Dong Xia http://orcid.org/0000-0002-6235-9815
Xiao-Juan Zhong http://orcid.org/0000-0002-7542-4241
Xi Li http://orcid.org/0000-0002-5956-7680

References

Aebi H. 1984. Catalase in vitro. Methods Enzymol. 105:121–126.
Bates LS, Waldren RP, Teare JD. 1973. Rapid determination of free proline for water-stress studies. Plant Soil. 39:205–207.

Bawa AK, Sen DN. 1993. Moisture stress-induced proline accumulation in ephemeral grasses from the arid zone of India. J Arid Environ. 24:135–137.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 44:276–287.

Bradford MM. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248–254.

Bravo LA, Close TJ, Corcuera LJ, Guy CL. 1999. Characterization of an 80kDa dehydrin-like protein in barley responsive to cold acclimation. Physiol Plant. 106:177–183.

Chen S. 1991. Injury of membrane lipid peroxidation to plant cell. Plant Physiol Commu. 27: 84–90.

Chinnusamy V, Zhu JH, Zhu JK. 2007. Cold stress regulation of gene expression in plants. Trends Plant Sci. 12:444–451.

Curtis CR. 1971. Disc electrophoretic comparisons of protein and peroxidases from *Phaseolus vulgaris* leaves infected with *Agrobacterium tumefaciens*. Can J Bot. 49:333–337.

Delauney A, Verma DPS. 1993. Proline biosynthesis and osmo regulation in plants. Plant J. 4:215–223.

Deng G, Liang J, Xu D, Long H, Pan Z, Yu M. 2013. The relationship between proline content, the expression level of *P5CS* (Δ1-pyrroline-5-carboxylate synthetase), and drought tolerance in Tibetan Hulless barley (*Hordeum vulgare* var. *nudum*). J Sou China Norm Univ (Nat Sci Edit). 60:733–740.

Deng J, Jian, H, Li S, Wang Q, Guo Y, Zhang H. 2012. Comprehensive evaluation of five wild Rosa species in cold tolerance ability. J Sou China Norm Univ (Nat Sci Edit). 37:70–75.

Fan WJ, Zhang M, Zhang HX, Zhang P. 2012. Improved tolerance to various abiotic stresses in transgenic sweet potato (*Ipomoea batatas*) expressing spinach betaine aldehyde dehydrogenase. PLoS One. 7(5): e37344.

Gai J, editor. 2000. Trial statistical methods. Beijing: Chinese Agriculture Press.

Heath RL, Packer L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 125:189–198.

Istatkova R, Philipov S, Yadamsurenghiin GO, Samdan J, Dangaa S. 2008. Alkaloids from *Papaver nudicaule* L. Nat Prod Res. 22:607–11.

John GS. 1993. Oxygen stress and superoxide dismutase. Plant Physiol. 101:7–12.

Karimzadeh G, Sharifi-Sirchi GR, Jalali-Javaran M, Dehghani H, Francis D. 2006. Soluble proteins induced by low temperature treatment in the leaves of spring and winter wheat cultivars. Pak J Bot. 38:1015–1026.

Kavi-Kishor PB, Hong ZL, Miao GH, Hu CAM, Verma DPS. 1995. Overexpression of delta-pyrroline-5-car-boxylate synthetase increases proline production and confers osmo tolerance in transgenic plants. Plant Physiol. 108:1387–1394.

Liu R-L, Zhang Y-T, Pan L-JP, Yu, C, Ai J-G, Zhu Z-J, Wang H-S. 2015. Effect of low temperature stress on the physiological mechanism of kidney bean varieties with different chilling tolerance. Acta Agri Zhej. 27:189–193.

Liu W, Yu K, He T, Li F, Zhang D, Liu J. 2013. The low temperature induced physiological responses of *Avena nuda* L., a cold-tolerant plant species. The Sci World J. 2013: Article ID 658793.

Miller G, Suzuki N, Citci-Yilmaz S, Mittler R. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Envir. 33(4):453–467.

Qin F, Shinozaki K, Yamaguchi-Shinozaki K. 2011. Achievements and challenges in understanding plant abiotic stress responses and tolerance. Plant Cell Physiol. 52:1569–82.

Suzuki N, Mittler R. 2006. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiol Plant. 126:45–51.

Takahashi D, Li B, Nakayama T, Kawamura Y, Uemura M. 2013. Plant plasma membrane proteomics for improving cold tolerance. Front Plant Sci. 4: Article ID 90.

Tian D, Ge Y, Pan G, Shen X, He Y, Zhang Z, Liu X, Zhu Z. 2011. Morphological and physiological characteristics of different cultivars of *Anthurium andraenum* under chilling stress. Acta Hort Sin. 38:1173–1179.

Tian Z, Wang F, Zhang W, Liu C, Zhao X. 2012. Antioxidant mechanism and lipid peroxidation patterns in leaves and petals of marigold in response to drought stress. Hort Environ Biotechnol. 53 (3):183–192.

Wang K. 2006. The principle and technology of plant physiology and biochemistry experiments. Beijing: Higher Education Press.

Wang L. 2012. Cold resistant physiology of different lilies under low temperature stress [dissertation]. Wuhan: Huazhong Agri. Univ.

Wang S, Wang Z, Wang P, Wang H, Li F, Huang W, Wu Y, Yin Y. 2011. Evaluation of wheat freezing resistance based on the responses of physiological indices to low temperature stress. Acta Ecol Sin. 31:1064–1072.

Wu J, Yang L, Sun G. 2004. Generation of activated oxygen and change of cell defense enzyme activity in leaves of maize seedling under the stress of low temperature. Bul Bot Res. 24:456–459.

Xu K, Xia Y, Xu B, Lin T, Yang X. 2005. Measurement of cold tolerance based on REC and the logistic equation in *Camellia hiemalis* ‘Shishi Ga shira’. Acta Hort Sin. 32:148–150.

Xu Y, Chen F. 2008. The LT50 and cold tolerance adaptability of *Chrysanthemum* during a natural drop in temperature. Acta Hort Sin. 35:559–564.

Zhai F-F, Han L, Liu J-X, Qian Y-Q, Ju G-S, Li W, Zhang S-W, Sun Z-Y. 2013. Assessing cold resistance of mutagenic strains of perennial ryegrass under artificial low-temperature stress. Acta Prata Sin. 22:268–279.

Zhu B, Su J, Chang M, Verma DPS, Fan YL, Wu R. 1998. Overexpression of a Δ^1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water and salt-stress in transgenic rice. Plant Sci. 139(4):1–4.