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Full Length Article

Effects of Low Temperature on Photosynthesis and Antioxidant Enzyme Activities of *Panax notoginseng* during Seeding Stage

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Abstract

In order to find out the low temperature (LT) meteorological disaster index of *Panax notoginseng*. One–year–old *P. notoginseng* seedlings were used as experimental materials. The LT treatments were 9, 6, 3 and 0°C, with 25°C as control. The experiments lasted for 7 d, and then all plants were moved to 25°C for 5 d of recovery. The leaves of *P. notoginseng* showed different degrees of chlorosis after the LT treatment. With the extension of low temperature stress (LTS) time, the contents of Chlorophyll a (*Chl a*), Chlorophyll b (*Chl b*), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in the leaves of *P. notoginseng* showed a tendency to increase first and then decrease. At 9 and 6°C, all maxima appeared at 5d, but at 3 and 0°C, appeared at 3d. Non–photochemical quenching (NPQ) at 9 and 6°C increased with the prolongation of LT stress time, while at 3 and 0°C, it increased first and then decreased, and both maxima appeared at 3d. Carotenoid, *Chl a*/b, maximum photosynthetic rate (P_{max}), apparent quantum efficiency (AQE), electron transport rate (ETR) and light saturation point (LSP) decreased under LTS. At 3°C for 3d, these values were significantly lower than the control. Malondialdehyde (MDA) and light compensation point (LCP) were contrary to the P_{max}. After 5 d of recovery, the values of Net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci), and stomatal limitation value (Ls) at 9 and 6°C were recovered to normal levels, while 3 and 0°C failed to recover. The results suggested that the key low temperature threshold point for *P. notoginseng* seedlings was 3°C for 3d. © 2019 Friends Science Publishers

Keywords: Chlorophyll content; Chlorophyll fluorescence; Malondialdehyde; Meteorological disaster; Photoinhibition

Introduction

Panax notoginseng (Burk.) F.H. Chen, also called "sanchi ginseng", has a long history as a traditional Chinese herbal medicine and is one of the most characteristic medicinal biological resource in Yunnan Province (Wang *et al*., 2006; Tung and Hai, 2016). Yunnan Province is located in the low–latitude plateau mountainous areas, and the terrain conditions are complex and variable, affected by the monsoon climate. Extreme or abnormal weather frequently occurs, which has a great impact on the growth of *P. notoginseng*. In addition, the *P. notoginseng* continuous cropping obstacle is serious, and the rotation needs to be separated by 10–20 years, which leads to the reduction of planting sites in the main producing areas, and then the gradual northward movement of the planting belt (Zuo *et al*., 2017), but its growth after the northward movement is susceptible to the continuous chilling temperature in winter and spring.

A large number of studies have revealed that low temperature (LT) strongly affects a number of plant physiological processes, being photosynthesis one of the processes most sensitive to this stress (Xin and Browse, 2000; Karimzadeh *et al*., 2005; Bilska and Sowinski, 2010). Photosynthesis is substantially reduced after plants exposure to chilling stress (Kingston–Smith *et al*., 1997; Huner *et al*., 1998). It also has been proven that LT can damage essentially all major apparatus and processes of photosynthesis including Chlorophyll content, photosynthetic enzyme activity, transport of thylakoid electron, cycle of carbon reduction and control of stomatal conductance (Farquhar and Sharkey, 1982; Jahnke *et al*., 1991; Perera *et al*., 1995; Allen and Ort, 2001; Yamori and Shikanai, 2016; Farooq *et al*., 2017). The decline in photosynthesis may lead to excessive accumulation of energy, especially under high light, resulting in photodamage or chronic photoinhibition of PSII. There are several protective ways for plants against the impact of

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photodamage, such as the rapidly reversible inactivation of PSII reaction centers involving the mutual conversion of xanthophyll pigments and the development of electrochemical potential difference of thylakoid protons (Allen and Ort, 2001; Feng and Cao, 2005). In addition, Plants will also produce reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxide radicals (OH^-) and superoxide radical (O^{-2}) to dissipate the surplus thermal energy, but excess radical production will in turn induce membrane lipid peroxidation and photosynthetic components damage (Foyer *et al*., 1994; Singh *et al*., 2016). Antioxidative enzymes such as superoxide dismatase (SOD), catalase (CAT) and peroxidases (POD) play important roles in the scavenging system of ROS (Gulen *et al*., 2008). Malondialdehyde (MDA) is the main product of the peroxidation of membrane lipid, which inhibits the activity of antioxidant enzymes and ultimately exacerbates membrane lipid peroxidation.

Chlorophyll (Chl) fluorescence analysis is an effective and non–destructive method to evaluate plant susceptibility index to LTS (Rizza *et al*., 2001; Ehlert and Hincha, 2008). This method reflects the functional changes of the PSII in the photosynthesis electron transport chains (Maxwell and Johnson, 2000). Moreover, the maximum photochemical efficiency of PSII (F_v/F_m), the actual photochemical efficiency of PSII (ΦPSII), the relative electron transport rate (rETR), the photochemical quenching coefficient (qP) and non–photochemical quenching (NPQ) can be determined, which are usually the most important parameters for evaluating the effects of chilling stress (Maxwell and Johnson, 2000; Feng and Cao, 2005; Dai *et al*., 2007).

Previous researches concerns were usually on the chemical components of *P. notoginseng* (Wan, *et al*., 2006; Zhou, *et al*., 2012), while the effects of low temperature on physiological characteristics and antioxidant enzyme activity have not been reported. In this study, the photosynthetic physiological characteristics of *P. notoginseng* seedlings in different levels of LT were investigated. The purpose of our research is to find the threshold of low temperature disasters for the growth of *P. notoginseng* seedlings. Two hypotheses were tested: (1) the maximum photosynthetic rate would decrease with the increase of LTS time increases based on the fact that photosynthesis was hampered in LT conditions; and (2) *P. notoginseng* which underwent different levels of LT have different photosynthetic physiological response.

Materials and Methods

Experimental Materials and Treatments

Experimental material: The experiments were conducted from October 2018 to December 2018 at the agricultural meteorological experiment station, located in Nanjing, Jiangsu Province, China. The variety for experiments was One–year–old Wenshan Sanchi seedlings (*Panax notoginseng* (Burk.) F.H. Chen), provided by farmers in Qiubei County, Wenshan Prefecture, Yunnan Province. *P. notoginseng* seedlings with identical size were singly planted into plastic pots (18 cm×11 cm×10.5 cm). The pots were filled with Humus–rich red loam soil. The seedlings were grown in a greenhouse for 2 weeks with 25/15°C (day/night) temperatures and were watered when necessary. The photoperiod, relative humidity and illumination of greenhouse were set at 10/14 h (day/night, day from 07:00 to 17:00), 65±5% and 400±10 μmoL m^{-2} s^{-1}, respectively, which were the most suitable environment for growth of *P. notoginseng* seedlings.

Treatments: LT treatment was carried out as follows: Similarly, sized seedlings were transferred to the intelligent climate chamber (A1000, Conviron, Canada). The seedlings were stressed at temperatures of 0, 3, 6, and 9°C for 1, 3, 5 and 7 d, respectively, with 25°C as control. During the LT treatments, the photoperiod, relative humidity and light irradiation intensity were set at 10/14 h (day/night, day from 07:00 to 17:00), 65±5% and 400±10 μmoL$m^{-2}s^{-1}$, respectively. Before the beginning of dark period, the most suitable soil water moisture (30–40% of soil volume water content) for *P. notoginseng* growth was obtained by artificial watering according to the measured soil water content. After 7 d of treatment at each low temperature, the plants were moved to greenhouse conditions and recovered for 5 d, and the corresponding photosynthetic parameters were determined.

Determination of Chlorophyll and Carotenoid Content

The fully expanded healthy leaves of *P. notoginseng* in each treatment were selected. The fresh leaves of about 0.2 g were weighed and placed into 50 mL centrifugal tube, then added 25 mL 95% ethanol into the centrifugal tube and sealed. After 48 h of extraction in darkness, chlorophyll was completely extracted. The extracts were colored at 665, 649, and 470 nm, respectively, by using spectrophotometry (Cary 50 Conc UV–VIS, Varian, Victoria, Australia). The concentrations of *Chl a*, *Chl b* and carotenoids were calculated according to the OD values of different wavelengths by the method of Antal and Rubin (2008).

Measurement of Gas Exchange Parameters

The 5^{th}–7^{th} true leaves of the plant were selected and labeled. From 9:00 to 11:00 a.m., the photosynthetic system LI–6400 (LI–COR Inc., USA) was used to automatically record the important photosynthetic parameters such as net photosynthetic rate (Pn), stomatal conductance (Gs) and intercellular carbon dioxide concentration (Ci).The stomatal limitation value (Ls) was calculated by formula Ls =1–Ci/Ca, in which Ca is the atmospheric CO_2 concentration. During the measurement, the light intensity, the temperature of the leaf chamber, and the carbon dioxide

concentration were set to 400 μmoL m^{-2} s^{-1}, 25°C, and 390 μmoL mol^{-1}, respectively. The leaves were measured after induction for 15 min under illumination of 800 μmoL m^{-2} s^{-1}.

Determination of Photosynthetic Light Response Curve

From 09:00 to 11:00 a.m., the photosynthetic response curve was measured by using LI–6400, a photosynthetic measurement system of LI–COR Company in the United States. The 5^{th}–7^{th} healthy leaves were selected for determination. During the measurement, the temperature of the leaf chamber was set at 25°C, the concentration of CO_2 was maintained at 390 μmoL^{-1}, and the photosynthetic active radiation (PAR) was 1200 000 800 400 200 150 80 50 30 and 0 μmoL m^{-2} s^{-1}, respectively.

The light response curve of Pn to PPFD was simulated by a non–orthogonal hyperbolic model as described by Farquhar *et al*. (2001):

$$P_N = \frac{\alpha PPFD + P_{max} - \left[(\alpha PPFD + P_{max})^2 - 4k\alpha PPFDP_{max}\right]^{0.5}}{2k}$$

Where, P_N is the net photosynthetic rate, α is the initial quantum efficiency, PPFD is the photosynthetic photon flux density [μmoL m^{-2} s^{-1}], P_{max} is the maximum photosynthetic rate [μmoL(CO_2) m^{-2} s^{-1}], and κ is the curve angle of the light response curve($0 \le \kappa \le 1$). Model parameter estimation using nonlinear regression fitting method in SPSS 17.0 statistical analysis software.

Measurement of Chlorophyll Fluorescence Parameters

The 5^{th}–7^{th} healthy leaves of the plant was subjected to a portable fluorimeter (FMS2, Hansatech, U.K.) to measure chlorophyll fluorescence parameters. The light–adaptation fluorescence parameters (F_m', F_0'and F_s) were measured under light intensity of 600 μmoL m^{-2} s^{-1}, then the dark adaptation fluorescence parameters (F_m and F_0) of *P. notoginseng* leaves after dark adaptation for 20 min were measured. Based on the measured parameters, four main fluorescence indices are calculated by using the following formulas. (1) $F_v/F_m = (F_m–F_0)/F_m$, (2) Φ PSII = $(F_m'–F_s)/F_m'$, (3) rETR = ΦPSII×PAR×0.84×0.5, (4) NPQ = $(F_m–F_m')/F_m'$, and (5) qP = $(F_m'–F_s)/(F_m'–F_0')$ (Maxwell and Johnson, 2000).

Lipid Peroxidation and Antioxidant Enzyme Activity Assay

From 09:00 to 11:00 a.m., healthy leaves were taken from *P. notoginseng* seedlings, then frozen in liquid nitrogen immediately, and then stored in a –40°C ultra–low temperature freezer. Estimation of lipid peroxidation based on MDA content. The activity of SOD was determined byprevious methods (Rabinowitch and Sklan, 1980). POD activity was determined using the guaiacol method Ghanati *et al*. (2002). CAT activity was measured by UV absorption Rabinowitch and Sklan (1980). The determination of MDA content was based on the method of Zhao *et al*. (1994).

Statistical Analysis

The collection of experimental samples was completely random. The Sigma plot 14.0 was used to draw the graph and the variance test for all data. All the data in the paper were expressed by the mean ± standard error of three replicates.

Results

Photosynthetic Physiological Traits of *P. notoginseng* under Low Temperature

Chlorophyll content: From Table 1, it can be seen that the *Chl a* content in the leaves of Panax not ginseng first decreased gradually, and then decreased with the increase of stress time. Compared with the control group (25°C, 7 d), the *Chl a* content at 9°C and 6°C reached the maximum at 5d, but decreased by 8.29% and 6.17%, respectively. *Chl a* reached its maximum at 3d at 3°C and 0°C, but the content of *Chl a* decreased by 19.45% and 18.67%, respectively compared with the control group (25°C, 7 d). The change trend of *Chl b* content under LT was similar to that of CLLA. With the prolongation of LTS time, carotenoid content showed an upward trend, while *Chl a*/*b* ratio decreased gradually under LTS, ranging from 1.65 to 3.12.

Characteristic parameters of light response curve: Table 2 showed the variation of Characteristic parameters of light response curve in *P. notoginseng* leaves at LTS for 1, 3, 5 and 7 days. The lower the temperature, the longer is the stress time, the lower the values of P_{max}, AEQ and LSP. At 3°C for 3 d, P_{max}, AEQ, and LSP were significantly reduced, compared with controls of the same stress time, by 3%, 4%, and 6%, respectively. The trend of LCP is opposite to that of P_{max}, and increased with decreasing temperature and prolonged stress time. At 3°C, the value of LCP at 3 d was 30% higher than at 1 d. At 9 and 6°C, NPQ increased with the prolongation of stress time, while at 3 and 0°C, NPQ increased first and then decreased, and both maximum values appeared at 3d.

Chlorophyll fluorescence parameters: The F_v/F_m, ΦPSII, ETR, qP, and NPQ of *P. notoginseng* leaves subjected to LTS for 1, 3, 5and 7d were determined (Table 3). With the extension of stress days, F_v/F_m continued to decline, similar to F_v/F_m, ΦPSII, ETR and qP also showed a decreasing trend. At 0 and 3°C, the values of F_v/F_m, ΦPSII, ETR, and qP were always at a low level since the third day. At 9 and 6°C, NPQ always showed an upward trend with the extension of stress time, but it increased first and then decreased at 3 and 0°C. The maximum NPQ values appeared at 3 d at 3°C and

Table 1: Effects of low temperature on chlorophyll contents in *P. notoginseng* leaves

Temperature (°C)	Stress day (d)	Chl a content [mg g^{-1}(FM)]	Chl b content [mg g^{-1}(FM)]	Car content [mg g^{-1}(FM)]	*Chl* (*a*/*b*)
25 (CK)	1	3.88±0.02b	1.59±0.01b	1.71±0.01b	2.32±0.02a
	3	4.08±0.09a	1.72±0.06b	1.86±0.02a	2.34±0.03a
	5	4.46±0.03a	1.95±0.04a	1.82±0.03a	2.24±0.03a
	7	4.69±0.07a	2.02±0.06a	1.91±0.02a	2.13±0.04a
9	1	3.98±0.06bc	1.75±0.03c	1.85±0.02a	2.27±0.05a
	3	4.02±0.04b	1.84±0.06b	1.97±0.03a	2.18±0.01b
	5	4.31±0.04a	1.85±0.03a	1.99±0.02a	2.15±0.07b
	7	3.88±0.07c	1.97±0.02b	2.02±0.07a	2.10±0.05c
6	1	3.89±0.06b	1.60±0.03c	1.96±0.05b	2.43±0.04a
	3	3.89±0.07a	1.60±0.06b	2.01±0.01b	2.43±0.06a
	5	4.13±0.03a	1.83±0.02a	2.25±0.04a	2.38±0.03b
	7	3.78±0.07c	1.61±0.02b	2.34±0.08a	2.27±0.04c
3	1	3.65±0.08b	1.43±0.08b	2.52±0.03b	2.55±0.05a
	3	4.36±0.03a	1.61±0.01a	3.61±0.02a	2.38±0.01a
	5	3.89±0.05c	1.67±0.06b	3.72±0.03a	2.32±0.02b
	7	2.47±0.07c	1.50±0.05b	4.12±0.04b	1.65±0.04c
0	1	3.75±0.01a	1.20±0.03a	3.01±0.05b	3.12±0.09a
	3	4.45±0.02a	1.51±0.04a	3.42±0.02a	2.94±0.02a
	5	3.29±0.03a	1.32±0.02a	4.01±0.01a	2.49±0.06b
	7	2.98±0.02b	1.24±0.02a	4.52±0.04a	2.40±0.03c

Each value represents the mean ± standard error of three replicates. At the same temperature, Different letters represent significant differences at P<0.05 by Duncan's test

Table 2: Effects of low temperature on Photosynthetic parameters in *P. notoginseng* leaves

Temperature (ºC)	Stress day (d)	Pmax (μmol $m^{-2}s^{-1}$)	AEQ	LSP (μmol $m^{-2}s^{-1}$)	LCP (μmol $m^{-2}s^{-1}$)
25(CK)	1	4.81±0.03a	0.52±0.01a	182.23±15b	4.45±0.04a
	3	4.51±0.05a	0.51±0.02a	193.75±13a	4.04±0.04a
	5	4.79±0.06a	0.50±0.04a	184.36±10b	4.05±0.06a
	7	5.02±0.07a	0.51±0.03a	177.43±10c	4.35±0.08a
9	1	4.59±0.05a	0.49±0.04a	18032±11a	4.39±0.02a
	3	4.43±0.03b	0.45±0.01b	153.56±9b	5.30±0.07b
	5	4.12±0.05c	0.42±0.06c	132.12±6c	5.39±0.03bc
	7	3.95±0.06d	0.39±0.04d	127.47±10d	5.47±0.04c
6	1	4.32±0.04a	0.46±0.01a	139.11±16a	4.99±0.06a
	3	4.05±0.04b	0.42±0.02b	125.98±14b	5.46±0.05b
	5	3.86±0.07c	0.39±0.03c	100.23±13c	5.89±0.04c
	7	3.52±0.05d	0.33±0.04c	98.21±6c	6.01±0.06c
3	1	3.98±0.06a	0.44±0.02a	110.09±10a	5.23±0.03a
	3	3.21±0.07c	0.40±0.03a	98.75±5a	5.51±0.07b
	5	2.09±0.06c	0.33±0.05b	89.64±6c	5.96±0.04c
	7	1.98±0.05c	0.27±0.04c	74.13±9d	6.01±0.06d
0	1	2.77±0.06a	0.40±0.02a	85.11±4a	5.50±0.05a
	3	2.42±0.09c	0.30±0.02b	73.17±3b	5.96±0.06b
	5	1.58±0.07c	0.25±0.03c	54.13±4c	6.22±0.02c
	7	1.47±0.03c	0.14±0.01d	27.55±7d	6.54±0.05d

Each value represents the mean ± standard error of three replicates. At the same temperature, Different letters represent significant differences at P<0.05 by Duncan's test

0°C, which were 71.88% and 81.25% higher than the maximum value of the control (25°C, 7 d), respectively.

Antioxidant enzyme: Antioxidant enzyme activities (CAT, POD and SOD) at LT were significantly greater than that of the control (25°C) (Fig. 1). CAT, POD and SOD at LT all showed a trend of rising at first and then decreasing. The maximum values of CAT, POD and SOD appeared at 3d at 3 and 0°Cand 5d at 9 and 6°C, respectively. At 9, 6, 3 and 0°C, the maximum value of CAT was 20%, 30%, 40% and 5% higher than the maximum of the control group, POD was 20, 30, 40 and 50% higher, respectively and SOD was 20, 30, 40 and 5% higher, respectively.

Malondialdehyde (MDA): MDA increased significantly under LTS, and the lower the temperature, the longer the stress time, the more pronounced the increase (Fig. 2). As can be seen from Fig. 2, at 7d, the MDA increased significantly at 3 and 0°C, and its values were 2.57 and 2.38 times higher than that of control (25°C), respectively. At 3 and 0°C, the values of MDA at 7d were 2.61 times and 2.40 times higher than at 1 d, respectively.

Light Response Curve and Photosynthetic Parameters after 5 d of Recovery

The seedlings of *P. notoginseng* were recovered for 5 d after 7 d of low temperature stress, and their photosynthetic light

Table 3: Effects of low temperature on Chlorophyll fluorescence parameters in *P. notoginseng* leaves

Temperature (ºC)	Stress day（d）	F_v/F_m	ΦPSII	ETR	qP	NPQ
25(CK)	1	0.81±0.02a	0.52±0.01a	45.62±5.27a	0.64±0.02a	0.29±0.03a
	3	0.80±0.09a	0.51±0.02a	44.83±4.13a	0.63±0.04a	0.30±0.06a
	5	0.79±0.03a	0.50±0.04a	46.32±4.75a	0.63±0.07a	0.32±0.04a
	7	0.80±0.07a	0.51±0.03a	44.19±3.19a	0.63±0.08a	0.32±0.07a
9	1	0.78±0.06a	0.49±0.04a	40.45±2.56a	0.62±0.02a	0.33±0.02a
	3	0.75±0.04b	0.45±0.01b	39.17±6.31a	0.60±0.03b	0.35±0.06b
	5	0.70±0.04c	0.42±0.06c	37.85±2.13a	0.60±0.02b	0.42±0.07c
	7	0.66±0.07d	0.39±0.04d	32.14±3.56b	0.59±0.04b	0.45±0.01d
6	1	0.76±0.06a	0.46±0.01a	37.23±5.12a	0.60±0.06a	0.37±0.03a
	3	0.72±0.07b	0.42±0.02b	34.26±4.14b	0.58±0.02b	0.45±0.02b
	5	0.68±0.03c	0.39±0.03c	30.16±3.78c	0.57±0.03b	0.47±0.04c
	7	0.65±0.07d	0.33±0.04c	29.46±6.21c	0.50±0.04c	0.48±0.09d
3	1	0.70±0.08a	0.44±0.02a	36.21±2.31a	0.62±0.05a	0. 40±0.08a
	3	0.65±0.03c	0.40±0.03a	28.46±2.55b	0.61±0.04a	0.55±0.04c
	5	0.60±0.05c	0.33±0.05b	26.47±2.46b	0.55±0.05c	0.38±0.05c
	7	0.58±0.07c	0.27±0.04c	25.16±1.28b	0.46±0.03d	0.35±0.06c
0	1	0.66±0.01a	0.40±0.02a	32.56±3.11a	0.60±0.04a	0.52±0.05a
	3	0.58±0.02c	0.30±0.02b	26.12±4.21b	0.51±0.01b	0.58±0.04c
	5	0.58±0.03c	0.25±0.03c	24.35±3.56b	0.43±0.02c	0.45±0.033c
	7	0.57±0.02c	0.14±0.01d	22.39±2.74b	0.24±0.01d	0.39±0.07c

Each value represents the mean ± standard error of three replicates. At the same temperature, Different letters represent significant differences at P<0.05 by Duncan's test

response curves were measured. The non–orthogonal hyperbolic model was used to nonlinearly fit the photosynthetic process of photosynthesis. The results were shown in Fig. 3. The coefficient of determination of the equation is above 0.97, indicating that the model better reflects the response of P_n to PAR in *P. notoginseng* seedling leaves after low temperature stress. At each temperature level, P_n increased with PAR. At low PAR, Pn increased linearly, but when PAR reached a certain value, the growth of P_n gradually becomes flat. After 5 d of recovery, the light response curves at 6 and 9°C did not differ from the control, but the light response curves at 3 and 0°C were significantly lower than the control.

Linear fitting of the initial part of the P_n–PAR curve (PAR<150 μmoL m^{-2} s^{-1}) and the fitting degree of each equation reached a significant level (p<0.05) (Table 4).

As shown in Fig. 4, after 5d of recovery, the P_n, Ci, Gs and Ls at 9 and 6°C were not different from the control, while the values at 3 and 0°C were significantly different from the control. At 3°C, P_n, G_s, and Ls were 65%, 52% and 40% lower than the control, respectively, while Ci was 35% higher.

Discussion

Chlorophyll is the main pigments of plant photosynthesis, which plays a central role in the light absorption of photosynthesis and also very sensitive to LTS (Yamori *et al.*, 2011). Therefore, chlorophyll content can be used as the most direct indicator of the degree of chilling damage in plant leaves. Xu *et al.* (2016) showed that the chlorophyll content of *P. notoginseng* leaves continued to decrease with the prolongation of LTS. But, in our study, the results demonstrated that the contents of *Chl a* and *Chl b* in the

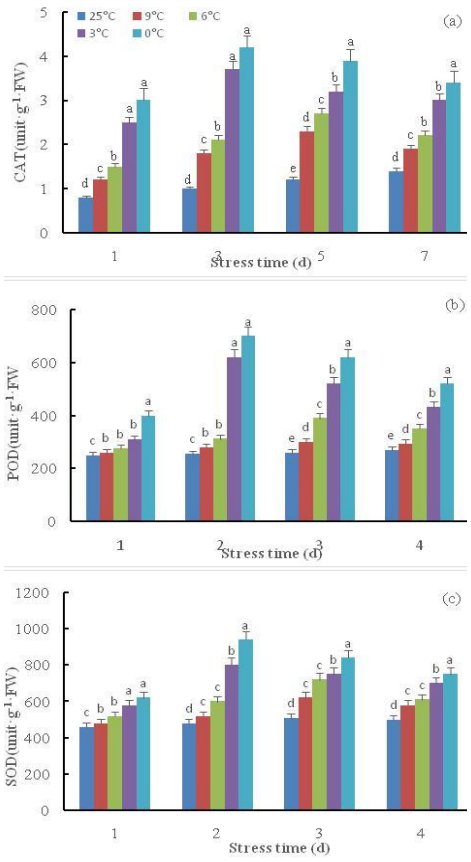

Fig. 1: Effects of low temperature on CAT, POD and SOD under different stress times

At the same time point, different small letters represent significant differences at P<0.05 by Duncan's test

leaves of *P. notoginseng* increased first and then decreased, the content of carotenoids increased, and the

Table 4: The fitting equation of the initial part of the light response curve (PAR<150 μmol m^{-2} s^{-1})

Temperature (°C)	Recovery days(d)	Linear fitting equation	R^2
25(CK)	5	y= 0.7150x-0.4372	0.9483*
9	5	y= 0.6760x-0.4812	0.9642*
6	5	y=0.6590x-0.6810	0.9720*
3	5	y=0.3250x-0.2810	0.9375*
0	5	y=0.3506x-0.3364	0.9486*

* represents significant at P<0.05 by Duncan's test

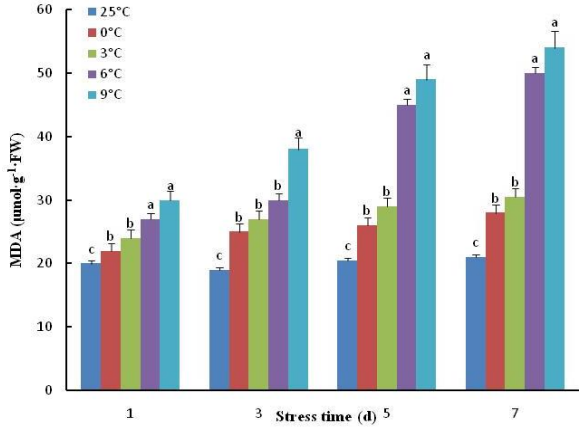

Fig. 2: Effects of low temperature on MDA under different stress times

At the same time point, different small letters represent significant differences at P<0.05 by Duncan's test

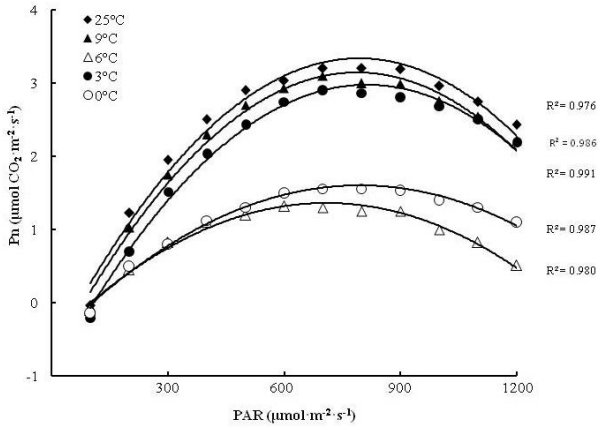

Fig. 3: Light response curves of *P. notoginseng* seedlings under 5 d recovery treatment after 7 d of low temperature stress

ratio of Chl *a/b* decreased after different LT treatments. The primary reasons for the changes of Chl content under LTS are complicated, mainly because active oxygen produced under low temperature conditions causes oxidative damage to photosynthetic apparatus and other biomacromolecules, leading to yellowing, browning and necrosis of leaves (Sharma *et al.*, 2005).

P_{max}, similar to ETR, decreased under LTS. At the same time, AQY and LSP decreased rapidly, and LCP

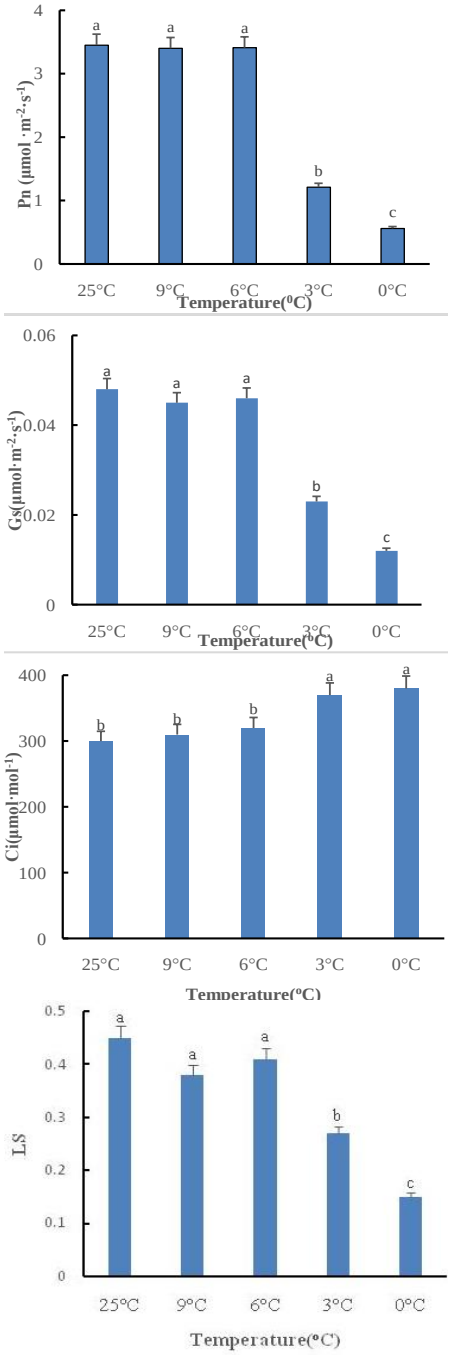

Fig. 4: Photosynthetic parameters of *P. notoginseng* seedlings under 5 d recovery treatments after 7 d of low temperature stress

Different small letters represent significant differences at P<0.05 by Duncan's test

increased rapidly, indicating that low temperature leads to increased consumption of photosynthetic products, and the ability to absorb, convert and utilize weak light is severely inhibited, but under strong light, photoinhibition occurs, light energy cannot be effectively converted into available chemical energy, and even damage to photosynthetic structure, resulting in decreased photosynthetic capacity of leaves (Verhoeven, 2013). After 5 d of recovery, the

values of Pn, Gs, Ci and Ls at 9 and 6°C were recovered to normal levels, while 3 and 0°C failed to recover (Fig. 4). These results indicated that at 6 and 9°C, the injury degree of leaf tissue was relatively low, especially by slowing down Gs to regulate Pn and ultimately improving the ability of the leaf to transport photosynthetic raw materials and products. However, at 0 and 3°C, the damage of leaf tissue was aggravated, the stability of photosynthetic apparatus in mesophyll cells decreased, the supply of ATP and NADPH was insufficient during dark reaction, and the decrease of key enzyme activities hindered the fixation of carbon dioxide (Yu *et al*., 2002), resulting in the accumulation of carbon dioxide in intercellular space (Allen and Ort, 2001), which led to the decrease of photosynthetic activity in mesophyll cells.

Fv/Fm is the maximum photochemical efficiency when the PSII reaction center is completely open under dark adaptation, reflecting the maximum light energy conversion efficiency of the PSII reaction center, and is also an indicator sensitive to LTS (Maxwell and Johnson, 2000; Cramer *et al*., 2011). The results of our study demonstrated that Fv/Fm gradually decreased with increasing LT strength and duration. Similar to Fv/Fm, the ΦPSII, ETR and qP also showed a decreasing trend, which indicated the occurrence of photoinhibition. NPQ is a non–photochemical quenching coefficient that reflects the ability of plants to dissipate excess light energy into heat. Increased NPQ are found in many plants exposed to low temperature (Feng and Cao, 2005; Dai *et al*., 2007). At 9 and 6°C, NPQ showed an upward trend with the extension of stress time. The reason is that most of the energy of plants is dissipated as heat energy, so the energy for photochemical quenching is reduced, which can regulate the contradiction of inhibiting the reduction of energy consumption caused by carbon assimilation and form photoprotection for plants. But at 5d of 3 and 0°C, NPQ decreased, indicating that excessive energy and electrons accumulate in the chloroplasts beyond the tolerance of the plant, leading to long–term photoinhibition or photodamage.

Studies have found that under adverse environmental conditions, the dynamic balance of reactive oxygen species (ROS) in plants is broken, leading to imbalance of active oxygen metabolism and accumulation of free radicals, thereby further causing or increasing lipid peroxidation resulting in plant membrane system damage and physiological metabolic disorders (Li *et al*., 2017). In vivo, free radicals act on lipid peroxidation, and the end product of oxidation is MDA, which can cause cross–linking polymerization of proteins, nucleic acids and other living macromolecules, and has cytotoxicity (Janero, 1990). There is a consensus that the MDA content is negatively correlated with low temperature stress of plants (Li *et al*., 2017). The higher the content, the greater the damage of plant tissue, and the weaker the cold resistance of plants. Our results showed that membrane lipid peroxidation gradually increased with the prolongation of stress time (Fig. 2). This may be due to the accumulation of MDA triggers non–specific hydrogen extraction, which induces the polymerization and cross–linking of structural proteins and enzymes in biofilms, leading to the loss of original structure and catalytic functions, the inhibition of protective enzyme activity and the reduction of antioxidant content, thereby accelerating membrane lipid peroxidation. ROS are known to be generated by chilling plants. SOD, POD and CAT are important protective enzymes in enzyme defense systems for anti–membrane lipid peroxidation in plants. Zuo *et al*. (2017) showed that low temperature caused the CAT activity of *P. notoginseng* leaves to decrease significantly, and the activity of POD and SOD increased significantly. In our study, SOD, POD, and CAT activities increased in LTS within a short period of time, mainly because the plants initiated stress response and self–protection mechanisms, enabling them to adaptto the corresponding LT condition and reconstruct the balance between the production and scavenging of ROS by regulating the activity of antioxidant enzymes, so that the plants could eliminate the accumulation of damage and improve cold resistance. However, as the stress time increases, the activity of antioxidant enzymes in *P. notoginseng* leaves decreased. It is likely that the dynamic balance of reactive oxygen and defense systems were destroyed, leading to excessive accumulation of H_2O_2 and O^{-2} in plants, which in turn inhibits the activity of antioxidant enzymes.

Conclusion

Low temperature may damage the photosynthesis of *P. notoginseng* through complex physiological processes such as photoinhibition, accumulations of MAD and ROS. At 3°C for 3d, the *Chl a*, *Chl b*, NPQ, SOD, POD and CAT of *P. notoginseng* reached the maximum, while P_{max}, AQE, LSP, F_v/F_m, ΦPSII, ETR and qP still remained at a relatively low level. After 5 d of recovery, the values of P_n, G_s, C_i and Ls at 9 and 6°C were recovered to normal levels, while 3 and 0°C failed to recover. The result showed that 3d at 3°C is the key meteorological disaster indicator of *P. notoginseng*.

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References

Allen, D.J. and D.R. Ort, 2001. Impacts of chilling temperatures on photosynthesis in warm–climate plants. *Trends Plant Sci*., 6: 36–42

Antal, T. and A. Rubin, 2008. In vivo analysis of chlorophyll a fluorescence induction. *Photosynth. Res*., 96: 217

Bilska, A. and P.L. Sowinski, 2010. Closure of plasmodesmata in maize (Zea mays) at low temperature: a new mechanism for inhibition of photosynthesis. *Ann. Bot. London*., 106: 675–686

Cramer, G.R., K. Urano, S. Delrot, M. Pezzotti and K. Shinozaki, 2011. Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biol*., 11: 163

Dai, F., M. Zhou and G. Zhang, 2007. The change of chlorophyll fluorescence parameters in winter barley during recovery after freezing shock and as affected by cold acclimation and irradiance. *Plant Physiol. Bioch.*, 45: 915–921

Ehlert, B. and D.K. Hincha, 2008. Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in Arabidopsis leaves. *Plant Methods.*, 4: 12

Farooq, M., M. Hussain, A. Nawaz, D.J. Lee, S.S. Alghamdi and K. Siddique, 2017. Seed priming improves chilling tolerance in chickpea by modulating germination metabolism, trehalose accumulation and carbon assimilation. *Plant Physiol. Biochem.*, 111: 274–283

Farquhar, G.D., S. von Caemmerer and J.A. Berry, 2001. Models of photosynthesis. *Plant Physiol.*, 125: 42–45

Farquhar, G.D. and T.D. Sharkey, 1982. Stomatal conductance and photosynthesis. *Ann. Rev. Plant Physiol.*, 33: 317–345

Feng, Y. and K. Cao, 2005. Photosynthesis and photoinhibition after night chilling in seedlings of two tropical tree species grown under three irradiances. *Photosynthetica*, 43: 567–574

Foyer, C.H., M. Lelandais and K.J. Kunert, 1994. Photooxidative stress in plants. *Physiol. Plant.*, 92: 696–717

Gulen, H., C. Çetinkaya, M. Kadıoğlu, M. Kesici, A. Cansev and A. Eriş, 2008. Peroxidase activity and lipid peroxidation in strawberry (Fragaria X ananassa) plants under low temperature. *J. Biol. Environ. Sci.*, 2: 95–100

Ghanati, F., A. Morita and H. Yokota, 2002. Induction of suberin and increase of lignin content by excess boron in tobacco cells. *Soil Sci. Plant Nutr.*, 48: 357–364

Huner, N.P., G, Öquist and F. Sarhan, 1998. Energy balance and acclimation to light and cold. *Trends Plant Sci.*, 3: 224–230

Jahnke, L.S., M.R. Hull and S.P. Long, 1991. Chilling stress and oxygen metabolizing enzymes in Zea mays and Zea diploperennis. *Plant, Cell Environ.*, 14: 97–104

Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid–reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Bio. Med*., 9: 515–540

Karimzadeh, G., R. Darvishzadeh, M. Jalali–Javaran and H. Dehghani, 2005. Cold–induced accumulation of protein in the leaves of spring and winter barley cultivars. *Acta Biol. Hung.*, 56: 83–96

Kingston–Smith, A.H., J. Harbinson, J. Williams and C.H. Foyer, 1997. Effect of chilling on carbon assimilation, enzyme activation, and photosynthetic electron transport in the absence of photoinhibition in maize leaves. *Plant Physiol.*, 114: 1039–1046

Li, M.A., G. Tingsong, C. Xuesen, Z. Dekui, G. Xiaojun, S. Jingjing, Q. Yanfen and W. Yanling, 2017. Physiological responses of actinidia arguta (Seib. et. Zucc.) to low temperature stress. *Agric. Sci. Technol*., 18: 767–776

Maxwell, K. and G.N. Johnson, 2000. Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.*, 51: 659–668

Perera, N.H., E. Hartmann and A.S. Holaday, 1995. Regulation of cotton photosynthesis during moderate chilling. *Plant Sci.*, 111: 133–143

Rabinowitch, H.D. and D. Sklan, 1980. Sunscald tolerance in tomatoes: role of superoxide dismutase. *Planta*, 148: 162–167

Rizza, F., D. Pagani, A.M. Stanca and L. Cattivelli, 2001. Use of chlorophyll fluorescence to evaluate the cold acclimation and freezing tolerance of winter and spring oats. *Plant Breed.*, 120: 389–396

Sharma, P., N. Sharma and R. Deswa, 2005. The molecular biology of the low–temperature response in plants. *Bioessays*, 27: 1048–1059

Singh, R., S. Singh, P. Parihar, R.K. Mishra, D.K. Tripathi, V.P. Singh, D.K. Chauhan and S.M. Prasad, 2016. Reactive oxygen species (ROS): beneficial companions of plants developmental processes. *Front. Plant Sci.*, 7: 1299

Tung, B.T. and N.T. Hai, 2016. Phytochemical and pharmacology effect of *Panax notoginseng*. *J. Appl. Pharm. Sci.*, 6: 174–178

Verhoeven, A., 2013. Sustained energy dissipation in winter evergreens. *New Phytol*., 201: 57–65

Wan, J.B., F.Q Yang, S.P Li, Y.T Wang and X.M Cui, 2006. Chemical characteristics for different parts of Panax notoginseng using pressurized liquid extraction and HPLC–ELSD. *J. Pharm. Biomed. Anal.*, 41: 1596–1601

Wang, C., E. McEntee, S. Wicks, J. Wu and C. Yuan, 2006. Phytochemical and analytical studies of Panax not ginseng (Burk.) FH Chen. *J. Nat. Med. Tokyo*, 60: 97–106

Xin, Z. and J. Browse, 2000. Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant, Cell Environ.*, 23: 893–902

Xu, D.M., Z.Q. He, Y.P. Zhao, Y.M. Wang, and S.J. Long, 2016. Effects of arbuscular mycorrhizal fungi on growth and physiological characteristics of sedum aizoon under low temperature stress. *Pratacultural Sci.*, 33: 2452–2464

Yamori, W. and T. Shikanai, 2016. Physiological functions of cyclic electron transport around photosystem I in sustaining photosynthesis and plant growth. *Annu. Rev. Plant Biol.*, 67: 81–106

Yamori, W., N. Sakata, Y. Suzuki, T. Shikanai and A. Makino, 2011. Cyclic electron flow around photosystem I via chloroplast NAD (P) H dehydrogenase (NDH) complex performs a significant physiological role during photosynthesis and plant growth at low temperature in rice. *The Plant J*., 68: 966–976

Yu, J., Y. Zhou, L. Huang and D.J. Allen, 2002. Chill–induced inhibition of photosynthesis: genotypic variation within Cucumis sativus. *Plant Cell Physiol.*, 43: 1182–1188

Zhao, S.J., C.C. Xu, Q. Zou and Q.W. Meng, 1994. Improvements of method for measurement of malondialdehvdein plant tissues. *Plant Physiol. Commun*., 30: 207–210

Zhou, J., M.G. Kulkarni, L.Q Huang, L.P Guo and J.V Staden, 2012. Effects of temperature, light, nutrients and smoke–water on seed germination and seedling growth of Astragalus membranaceus, Panax notoginseng and Magnolia officinalis-Highly traded Chinese medicinal plants. *S. Afr. J. Bot.*,79: 62–70

Zuo, Y., J. Zhang, T. Yang, S. Yang, W. Yang, J. Li, M. Yang, Z. Xu, B. Jian and Others, 2017. Effects of temperature stress on photosynthetic characteristics and physiological indexes of *Panax notoginseng* (Burk.) FH Chen. *J. South. Agric.*, 48: 2145–2151

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