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EFFECTS OF CHILLING STRESS ON CHLOROPHYLL FLUORESCENCE, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF *PHOTINIA GLOMERATA* SEEDINGS

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Abstract

The half-year-old *Photinia glomerata* seedlings were exposed to low temperatures (15, 5 and 0°C) and a control temperature (25°C) for 15 days. The results showed that the maximal photochemical efficiency of PSII (*Fv/Fm*), the photochemical quenching (*qP*) and the actual quantum yield efficiency of PSII photochemistry (*Y(II)*) of plants significantly decreased at 0°C treatment, but not at 15 and 5°C treatments. On the other hand, the Chl *a* and Chl *b* contents decreased while the Chl *a/b* ratio increased during low temperature stress. Furthermore, the malondialdehyde (MDA) content progressively increased at low temperature. Additionally, the proline content and the activities of antioxidant enzymes including the superoxide dismutase (SOD), the ascorbate peroxidase (APX) and the peroxidase (GPX) were significantly improved by low temperature. These results suggested that *P. glomerata* seedlings were less affected by 15 and 5°C chilling while inhibited by 0°C chilling treatment.

Introduction

Among the environmental factors, temperature plays an important role in the distribution of woody plants and the productivity of crops. Chilling stress is very common in the subtropical and temperate regions and can greatly inhibit the growth and the development of most plants. Chilling stress can induce plant via chloroplasts molecular redox signaling transduction mechanisms, ultimately leading to the acclimation of the photosynthetic apparatus (Lin *et al*. 2007), and that the photosynthetic apparatus easily suffer from oxidative damages with the increase of chilling stress due to energetic and metabolic imbalance. In addition, the photosynthetic CO_2 fixation was inhibited at low temperature. Many studies have suggested that the maximum photochemical efficiency of PSII (*Fv/Fm*) was used to examine the inhibition of the photosystem caused by chilling stress (Hou *et al*. 2016, Oustrica *et al*. 2017). Therefore, the chlorophyll fluorescence has been proposed as a convenient, sensitive, non-intrusive and easy-to-use method of determining the chilling stress effects on photosynthesis.

There are many literatures and reviews on chilling stress published every year. However, the mechanisms of the chilling stress were often studied in herb and crops, such as maize (Foyer *et al*. 2002), rice (Bonnecarrere *et al*. 2011) and potato (Lin *et al*. 2007). A few researches about chilling stress were explored in the woody plants, except of *Populus* (Zhang *et al*. 2011, 2012). In the present study, a good basis for studying the response to abiotic stress in woody plants through measuring chlorophyll fluorescence and some physiological characteristics on *Photinia glomerata* responding to chilling stress may be achieved. The objectives of this study are: (1) to explore the physiological and biochemical changes of plant cells under chilling stress, and (2) to assess the defensive mechanisms of *P. glomerata* seedlings to chilling stress.

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Materials and Methods

Half-year-old and healthy *Photinia glomerata* seedlings of a uniform height (15 - 20 cm) were grown in the Southwest Forestry University with a similar climate condition with their original site. The seedlings were transplanted into 5.0 litre plastic pots containing a medium consisting of red soil, perlite, and humus soil in a ratio of 3 : 3 : 2 on 26 June 2015. The seedlings were randomly divided into four groups. Each group included 10 pots. Plants were allowed to grow for a month before the chilling stress was imposed. Each pot contained one seedling. Next, the seedlings were placed in four versatile environmental text chambers (MLR-351H, SANYO) from 26 July 2015. One was the control (25°C) and the others were treated with different chilling temperatures (15, 5 and 0°C), respectively. In the chambers, the seedlings were exposed to chilling stress for 15 days with a 13,000 lux photo radiations, a 10 hrs photo period, and 55 - 75% relative humidity.

Various chlorophyll fluorescence, physiological, and biochemical parameters were measured at the end of the experiment. The third or fifth completely unfolded leaves were selected to measure the fluorescence parameters, physiological and biochemical indexes, and at least five seedlings of each repetition were used in each treatment.

Chlorophyll fluorescence parameters were performed using the Imaging-PAM M-series (Walz, Effeltrich, Germany) as described by Brugnoli and Björkman (1992). After full dark adaption, leaves were used to determine the *F*o (the minimal fluorescence after the dark adaptation), the maximum efficiency of *PSII* and *F*v/*F*m. Non-photochemical quenching coefficients *(qN)* and photochemical quenching *(qP)* coefficients were calculated as described by van Kooten and Snel (1990). The concentrations of chlorophyll (*a*, *b*) were calculated using adjusted extinction coefficients (Inskeep and Bloom 1985). The absorbance of the free proline concentration was measured at 520 nm. Free proline was measured as described by Bates *et al*. (1973). The proline content was expressed as μg per gram of fresh weight. The guaiacol peroxidase (EC 1.11.1.7, GPX) activity of leaves was measured by the methods as described by Chance and Maehly (1955). The ascorbate peroxidase (EC 1.11.1.11, APX) activity was measured using a modification of the procedure of Nakano and Asada (1981). The total superoxide dismutase (EC 1.15.1.1, SOD) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitrobluetetrazolium (NBT) (Beauchamp and Fridovich 1971). The malondialdehyde (MDA) contents were measured following the methods of Hodges *et al*. (1999). Statistical analyses were performed with the statistical software package for social science (SPSS), version 19.0. One-way analyze of variance (ANOVA) were conducted to evaluate the significance of the heavy metal effects. Among all treatments, the means were compared by Duncan's tests at the significance level ($p < 0.05$).

Results and Discussion

Chlorophyll fluorescence parameters have been suggested to reflect the plant tolerance to adverse environmental stress (Li *et al*. 2013). The changes of the chlorophyll fluorescence observed are presented in Table 1. With the decrease of treatment temperature, the minimal fluorescence (*F*o) increased initially and then decreased, compared with the control, the *F*o values significantly increased by 19.3 and 26.4% under the 15 and 5°C stress, respectively, while declined sharply under the 0°C stress (Table 1). Usually, under the adverse environmental conditions, the PSII reaction centers are damaged or reversible inactivated, which resulted an increased *F*o and a decreased *F*v/*F*m. The change of minimal fluorescence (*F*o) suggested that the energy transport abilities of PSII antenna pigments was partially suppressed by low temperature. *F*v/*F*m showed the maximum photochemical efficiency of PSII indicating all the reaction centers

are opened, and it was more sensitive to temperature in plants (Salvucci and Crafto-Brandner 2004). This experiment showed that *F*v/*F*m had a slight improvement at 15°C stress, but a significantly decline at 0°C stress compared with the control treatment (Table 1), showing that photoinhibiton of the *P. glomerata* seedlings occurred at 0°C but not at 15°C. The improved tendency of *F*o and the declined tendency of *F*v/*F*m in the *P. glomerata* leaves were consistent with the previous report (Lin *et al*. 2007), indicating the occurrence of the photo-inhibitory damage in PSII.

Table 1. Effects of chilling stress on chlorophyll fluorescence of *P. glomerata.*

Treatment	*Fo*	*Fv/Fm*	*Y(II)*	*qN*	*qP*
25°C	0.064 ± 0.0048ab	0.753 ± 0.0173b	0.349 ± 0.0635b	0.677 ± 0.0989a	0.608 ± 0.1150b
15°C	0.077 ± 0.011bc	0.772 ± 0.0060b	0.403 ± 0.0690b	0.795 ± 0.0554b	0.769 ± 0.0833b
5°C	0.081 ± 0.0092c	0.712 ± 0.0419b	0.368 ± 0.0392b	0.765 ± 0.0372ab	0.756 ± 0.1024b
0°C	0.062 ± 0.0045a	0.576 ± 0.0748a	0.148 ± 0.0479a	0.724 ± 0.0550ab	0.395 ± 0.1137a
p	0.013*	0.000***	0.000***	0.119ns	0.002**

Values followed by the same letter within a column indicate nonsignificant differences at $p < 0.05$ (Duncan's multiple range test). Each value represents the mean ± SE of five replicates.

In addition, the non-photochemical quenching (*qN*) reflected the proportion of energy, which was absorbed by the PSII antenna pigments; the excess energy was dissipated effectively by the increased values of *qN* in order to avoid the damage of PSII reaction centers (Sağlam *et al*. 2011). In the experiment, the *qN* value increased under the low temperature (Table 1), suggesting that the excess energy in leaves was dissipated in the form of heat, which indicated some protection on the photochemical apparatus. Besides, the photochemical quenching (*qP*) parameters reflected the proportion of energy absorbed by the PSII antenna pigments (van Kooten and Snel 1990). In this study, the *qP* values were severely decreased at 0°C chilling stress (by 35.0%) (Table 1), which suggest that the open proportion of PSII reaction centers was significantly influenced by 0°C but little injured by 15 and 5°C. This might be due to be some protective mechanisms activation to defense the chilling stress in some degree. The actual quantum yield of PSII (*Y(II)*) is correlated well with the activities of PSII and the activation of some enzymes. In the present study, the *Y(II)* significantly decreased at 0°C stress but not markedly affected by 15 and 5°C stress (Table 1), indicating that both the activation of the key RuBPcase enzyme and the activation of PSII reaction centers were decreased only at 0°C stress.

Compared with the control (25°C) group, the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total chlorophyll (Chl *a*+*b*) significantly decreased under the low temperature treatments (Table 2). These results are in agreement with the observations made by Garstka's *et al*. (2007) and Kudoh and Sonoike (2002), indicating that the occurrence of damage on CP complexes in chloroplasts. As the decrease of the chlorophyll content, there may be an inhibition of chlorophyll-synthesizing enzymes and/or the chilling stress might improve the chlorophyllase activity, resulting the decomposition of the chlorophyll accelerated under the chilling stress, which resulted the decreases of the chlorophyll content.

Compared with the control group, the increase percentage of proline content was 120.9 after exposing to 0°C, while there was no significant difference of proline content between the 15 and 25°C treatment (Fig. 1). The present results reveal that proline accumulation improved the self-protection ability of the *P. glomerata* seedlings. More or less similar result was observed in pea seedlings, which accumulated more proline under chilling stress, and finally showed lower plant injury rate (Kuznetsov and Shevyakova 1999).

Table 2. Effects of different chilling stress on chlorophyll contents of *P. glomerata*

Treatment	Chl *a* (μg/cm^2)	Chl *b* (μg/cm^2)	Chl *a*+*b* (μg/cm^2)	Chl *a/b*
25°C	5.90 ± 0.52b	1.66 ± 0.14b	7.56 ± 0.65b	3.55 ± 0.007a
15°C	4.19 ± 0.11a	1.14 ± 0.03a	5.33 ± 0.15a	3.67 ± 0.01ab
5°C	4.29 ± 0.49a	1.12 ± 0.16a	5.41 ± 0.65a	3.83 ± 0.12b
0°C	4.26 ± 1.07a	1.14 ± 0.22a	5.40 ± 1.30a	3.71 ± 0.24ab
p	0.006*	0.001**	0.004**	0.083ns

Values followed by the same letter within a column indicate nonsignificant differences at $p < 0.05$ (DMRT). Each value represents the mean ± SE of five replicates.

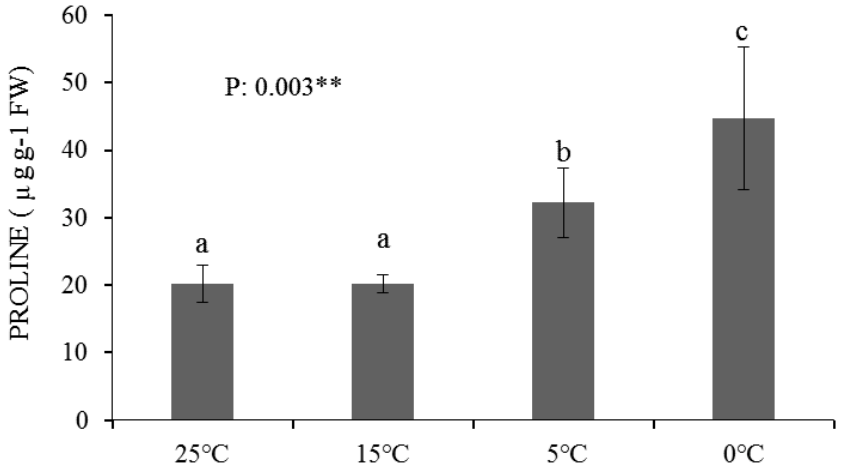

Fig.1. Effect of chilling stress on proline content. Each value represents the mean ± SE of five replicates. ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (DMRT).

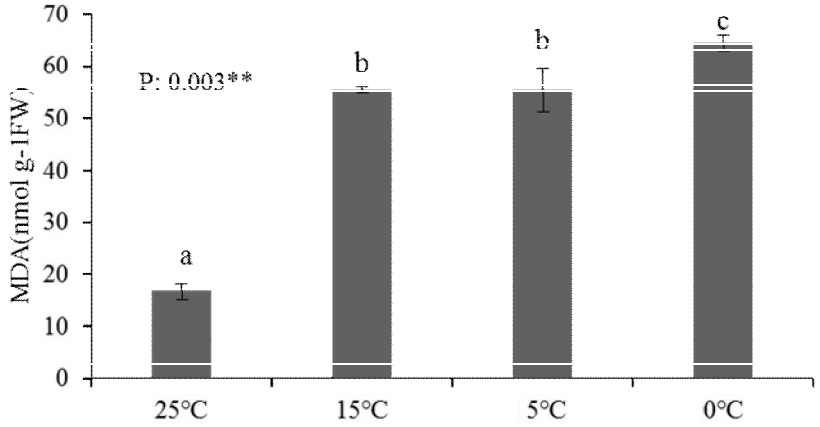

Fig. 2. Effect of chilling stress on MDA content. Each value represents the mean ± SE of five replicates. ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ (DMRT).

Chilling stress also caused changes in activities of the antioxidant enzymes, such as SOD, APX and GPX. and their activities in the plant cells could show the ability of scavenging the damages of reactive oxygen species (ROS). In this study, for the low temperature treatments, the SOD, APX and GPX activities of seedlings were more pronounced by 0°C stress (Table 3). These results suggested that a more serious oxidative damage was occurred under 0°C than that under 15 and 5°C. These enzymes were involved in plant responses to cold stress (Prasad 1997, Asada 1999, Santini *et al*. 2013). On the other hand, the concentration of MDA was an indicator of the injury by chilling temperature in plant cells, the higher content of MDA under chilling stress means more serious damage of the seedlings caused by low temperature (Zhao *et al.* 2003, Zhang *et al.* 2011). In the present study, the data showed that the leaves of *P. glomerata* seedlings accumulated the peroxidation products under chilling stress and the levels of MDA was more under 0°C chilling stress (Fig. 2). In general, the activities of antioxidant enzymes (SOD, APX and GPX) were significantly increased under 0°C chilling stress, which suggested that the antioxidant enzymes can scavenge the ROS and alleviate the chilling injury of the *P. glomerata* seedlings in some degree.

Table 3. Effects of chilling stress on antioxidant enzyme activities of *P. glomerata.*

Treatment	SOD (Unit/mg FW)	APX (μmol H_2O_2 /min/mg/protein)	GPX (μmol guaiacol/ min/mg/protein)
25°C	33.47 ± 0.28a	301.02 ± 49.62a	1.92 ± 0.54a
15°C	36.56 ± 0.42b	293.43 ± 23.79a	1.85 ± 0.62a
5°C	37.76 ± 0.33b	419.90 ± 32.38a	7.44 ± 1.24b
0°C	58.91 ± 1.29c	503.90 ± 31.71b	17.27 ± 2.63c
p	0.000***	0.000***	0.000***

Values followed by the same letter within a column indicate nonsignificant differences at $p < 0.05$ (DMRT). Each value represents the mean ± SE of five replicates.

Chilling stress that can cause complex changes of biochemical and physiological in the same species has often been investigated under controlled conditions (Bonnecarrere *et al.* 2011). In the present study, among the 16 measured physiological and biochemical indices, 14 were significantly influenced by chilling stress, which could reflect the damage degree and the defense capacity of *P. glomerata* seedlings affected by low temperature. On the one hand, the chilling can cause photoinhibition and oxidative damages in the *P. glomerata* seedlings, especially at 0°C. The increase of proline accumulation and activities of SOD, GPX and APX, suggesting that the defense mechanism can be activated to protect the seedlings from damage by the chilling stress in some degree.

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