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Comparison of leaves and stems of *Paederia scandens* (Lour.) Merr. in tolerance to low temperature

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Abstract

Low temperature is an important environmental factor that affects plant growth and development. To determine the adaptability of different organs of *Paederia scandens* (Lour.) Merr. to low temperature, the chlorophyll (Chl) fluorescence parameters as well as the Chl, malondialdehyde (MDA), soluble sugar, and anthocyanin contents of leaves and stems were measured under low temperature. The results confirmed that the Chl fluorescence parameters and Chl content of the leaves and stems of *P. scandens* tended to decrease consistently, while the MDA content increased, and the change range of the stems was much lower than that of the leaves. The soluble sugar and anthocyanin contents rapidly increased in the leaves and stems to cope with low temperature. Our results suggest that stems are more tolerant than leaves during winter and may continue to grow during that time, which could provide theoretical guidance for clonal propagation of plants to study stem tolerance in the future.

Additional key words: cold; non-leaf photosynthetic organs; osmoprotectants; photosynthesis.

Introduction

Plants compose a large class of organisms in nature, and their growth and development are closely related to environmental changes. In particular, temperature is a primary factor that affects plant growth, development, and yield (Hatfield and Prueger 2015).

As an important abiotic stress, low temperature limits plant growth, reduces crop yields, and even results in plant death (Sanghera *et al.* 2011, Sharma *et al*. 2015). To adapt to adverse environmental conditions, plants have evolved a series of adaptive and self-protection strategies that involve many biochemical and physiological changes (Bressan *et al*. 2009, Liu *et al*. 2012). Many changes including alterations to membrane permeability, increase of the abundance of osmotic regulators and antioxidants (Wu *et al*. 2008, Korn *et al*. 2010), changes in photosynthesis capabilities (Cui *et al*. 2019), and regulation of endogenous hormone contents, occur in response to low temperatures (Sharma *et al*. 2015, Zwack and Rashotte 2015, de Zelicourt *et al*. 2016).

Under low temperature, osmoprotectants, which include soluble sugars, proline, and other substances, are considered crucial materials involved in plant adaptations to the various stresses. Increases in these substances can effectively promote the ability of osmotic regulation and prevent excessive dehydration of protoplasts (Vágújfalvi *et al*. 1999, Lu *et al*. 2006). Malondialdehyde (MDA), a product of membrane lipid peroxidation, can aggravate membrane lipid peroxidation. MDA accumulation indicates the degree of plant damage caused by stress and reflects the strength of membrane peroxidation (Sun *et al*. 2004). In addition, low temperature can also cause oxidative stress, such as the accumulation of reactive oxygen species (ROS) in cells. The accumulation of ROS can result in membrane lipid peroxidation and the destruction of membrane structure, leading to leakage of intracellular components (Chen *et al*. 2000, Shahandashti *et al*. 2013).

Leaves are the main photosynthetic organ for plants. Many studies have focused primarily on the response mechanism of leaves (Kuk *et al*. 2006). Some adverse environments, such as insect bites, drought, and other conditions can lead to plant leaf wilting and litter while other plant parts can retain (Vanderklein and Reich 2000, Chen *et al*. 2001). At present, some non-leaf organs, including stems, bracts, and ears, have also actual or

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Abbreviations: Chl *a*(*b*) – chlorophyll *a*(*b*); Chl_{tot} – total chlorophyll; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; FM – fresh mass; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; MDA – malondialdehyde; ROS – reactive oxygen species; TBA – thiobarbituric acid; TCA – trichloroacetic acid; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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potential photosynthetic capacity (Aschan and Pfanz 2003). In wheat, photosynthesis of leaves was inhibited; the main contribution factor of grain yield may be photosynthesis from ear under drought (Tambussi *et al*. 2007). Zhan *et al* (2014) found that photosynthesis of non-leaf organs made an important contribution to the formation of seed yield when cotton fruit was under water stress in the developmental stage. Studies have shown that non-leaf organ stems accounted for a large proportion of vine and could form or contain Chl, which plays an important role in the growth and development of plants (Yang *et al*. 2006). However, few studies have investigated the possible response mechanism in stems of vine under stress of adversity.

Paederia scandens (Lour.) Merr., a climbing vine, is a good source of nutrients and has medicinal function. This species contains an abundance of ascorbic acid, rich mineral elements, and complete amino acids (Zhang 2006). Whether planted from seeds or propagated *via* cuttings, this species has strong vitality and reproductive capabilities. In addition, *P. scandens* exhibits abnormal tolerance to environmental conditions, such as cold resistance and shade resistance, and this species is widely distributed in Guangdong, Guangxi, and other provinces in China. Many studies have focused mainly on analyzing the pharmacological and chemical components of *P. scandens*, such as flavonoids (Nariyuki *et al*. 1990), and identifying its volatile oil compounds (Ma *et al*. 2000, Yin *et al*. 2009). However, information on the morphological, physiological, and ecological characteristics of this species is limited. Therefore, the purpose of this study was to explore the physiological responses of different organs of *P. scandens* to low temperature and whether the trend and range of change are consistent. We analyzed several physiological indexes, including chlorophyll fluorescence parameters, osmoprotectants, and chlorophyll contents. The results would provide theoretical guidance for clonal propagation of plants to study stem tolerance in the future.

Materials and methods

Plant materials, cultivation, and low-temperature treatment: Plant material was collected from South China Agricultural University, Guangzhou, China. Collected plant material was cut into 8–10-cm lengths with two internodes, and cultivated using hydroponics in an incubator that had a light intensity of 100 μmol(photon) m^{-2} s^{-1}, a light period of 16 h, a dark period of 8 h, a humidity of approximately 65%, and a temperature of 25°C. After the seedlings were cultivated for half a month, they were transplanted into pots, after which they grew for one month. Similarly appearing seedlings, which were approximately 45 cm in length, were subjected to low-temperature treatment at 16°C. Relevant physiological indexes were measured by collecting leaf and stem materials at 0 and 10 d after treatment, with each material replicated three times.

Analysis of Chl fluorescence: Chl fluorescence was tested using a *PAM-2100* device (*Walz*, Germany) with a PPFD of 800 μmol m^{-2} s^{-1}. The plants were placed in dark conditions for 20 min. The maximal quantum yield of PSII photochemistry (F_v/F_m) was calculated by the instrument as $F_v/F_m = (F_m - F_0)/F_m$ (Oxborough and Baker 1997), where F_0 is the minimal fluorescence yield of the dark-adapted state, and F_m is the maximal fluorescence yield of the dark-adapted state. The steady-state Chl fluorescence (F_s), maximal fluorescence yield of the light-adapted state (F_m'), and effective quantum yield of PSII photochemistry (Φ_{PSII}) of the leaves and stems were measured. The Φ_{PSII} was calculated as $\Phi_{PSII} = \triangle F/F_m' = (F_m' - F)/F_m'$ (Genty *et al*. 1989), and the electron transport rate (ETR) was calculated as $ETR = \Phi_{PSII} \times PPFD \times 0.85 \times 0.5$, where PPFD is the actinic light intensity of 800 μmol m^{-2} s^{-1}, 0.85 is the effective leaf absorption, and 0.5 is the fraction of absorbed quanta used by PSII (Melis *et al*. 1987).

Chl content: Leaves (0.05 g) and stems (0.05 g) from the 3rd to 5th nodes (starting at the top) were placed into 10-mL centrifuge tubes and then crushed into a powder in liquid nitrogen. Afterward, 4 mL of 80% acetone was added, and the mixture was subsequently incubated in the dark at 4°C for 24 h. The supernatant was measured at wavelengths of 663 nm and 645 nm, with 80% acetone used as a blank control (*Shimadzu*, Tokyo, Japan). The contents of Chl *a*, Chl *b*, and the total Chl were calculated by a UV-Vis *2450* spectrophotometer (*Shimadzu*, Tokyo, Japan) in accordance with the methods of Wellburn (1994):

$$\text{Chl } a \text{ [μg mL}^{-1}\text{]} = 12.21 \times A_{663} - 2.81 \times A_{645}$$

$$\text{Chl } b \text{ [μg mL}^{-1}\text{]} = 20.13 \times A_{645} - 5.03 \times A_{663}$$

$$\text{Total Chl [μg mL}^{-1}\text{]} = \text{Chl } a + \text{Chl } b$$

MDA and soluble sugar contents: MDA and soluble sugar contents were determined by the thiobarbituric acid (TBA) method (Zhao *et al*. 1994). Leaves (0.05 g) and stems (0.05 g) were crushed into a homogenate in conjunction with a small amount of quartz sand and 2 mL of precooled 10% trichloroacetic acid (TCA) in a mortar. The homogenate was then centrifuged at 10,000 rpm for 10 min to obtain the supernatant, after which 1 mL of the supernatant and 1 mL of 0.6% TBA solution were mixed together. The solution was heated in boiling water for 30 min and then quickly cooled in an ice bath, after which its absorbance at wavelength of 600, 532, and 450 nm was measured by a UV-Vis *2450* spectrophotometer. The following equations were subsequently used:

$$\text{MDA [μmol L}^{-1}\text{]} = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

$$\text{soluble sugar [mmol L}^{-1}\text{]} = 11.71 \times A_{450}$$

The anthocyanin content was analyzed according to the methods of a previous report (Zhang *et al*. 2016). Leaves (0.05 g) and stems (0.05 g) were soaked in 2 mL of 1% methanol: HCl (99:1, v/v) at 4°C for 6–12 h in the dark. Afterward, 1 mL of chloroform and 0.5 mL of pure water were added to the solution, after which mixture was fully blended and allowed to separate into layers. The upper layer comprised anthocyanins, and the lower layer comprised Chl. The absorption spectrum of the upper layer

was scanned with a UV-Vis *2450* spectrophotometer at wavelengths of 400–700 nm, after which the absorbance of the anthocyanins at 530 nm was determined. The anthocyanin content was calculated *via* the standard curve of cyanidin-3-O-glucose.

Statistical analysis: The data represent the means ± standard deviations (SD) of three samples in the experiment. The statistical analysis and multiple comparisons of the data were performed by *IBM SPSS Statistics 19.0* software (*SPSS*, Chicago, IL, USA). *Duncan*'s multiple comparison methods were used to test the significance of the different physiological indexes of *P. scandens* leaves and stems under low temperature, and differences were considered significant at $p<0.05$. Figures were constructed by *SigmaPlot 12.5* software (*Systat Software*, San Jose, CA, USA).

Results

Phenotypic changes of leaves and stems: After 10 d of treatment, the leaves of *P. scandens* displayed a yellowing phenomenon. Leaf margins and petioles showed reddening, but it was not obvious on the whole leaves (Fig. 1*A*). The reddening phenomenon of the stems was obvious after low-temperature treatment, and the reddening of the stems was much greater than that of the leaves (Fig. 1*B*).

Chl fluorescence parameters: When plants are under optimal conditions, the value of F_v/F_m generally ranges from 0.75–0.85, but it obviously decreases under stress or injury (He *et al*. 2005). We found that the F_v/F_m of the stems and leaves of *P. scandens* tended to decrease after 10 d of treatment, which was most significant in the leaves (18.3%) and was approximately 9 times that of the stems (2.2%) (Fig. 2*A*). Here, Φ_{PSII} represents the actual light energy conversion efficiency of PSII and is also positively correlated with the activity of PSII. Under low temperatures, the decreasing trend of the Φ_{PSII} of the stems and leaves was the same as that of F_v/F_m, and the range of decrease was much greater in the leaves than that in the stems (Fig. 2*B*). The ETR reflects the activity of PSII and is directly related to the photosynthetic rate of plants. The ETR of both the stems and leaves tended to decrease under low temperature, which was consistent with the trend of the Φ_{PSII} (Fig. 2*C*).

Chl content: As one of the important indexes of photosynthesis, the Chl content can reflect certain physiological

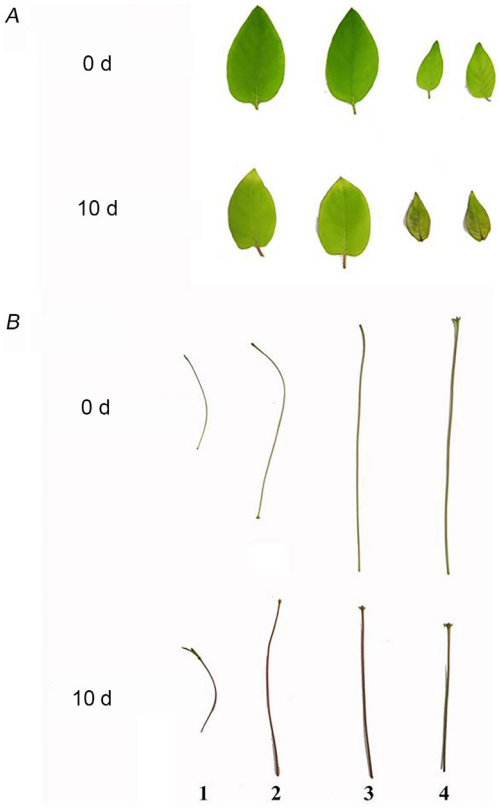

Fig. 1. Apparent changes in leaves and stems of *Paederia scandens* under low temperature for 0 and 10 d (T = 16°C). (*A*) Changes in the leaves of plants. (*B*) Changes (from left to right) of the first internode, second internode, third internode and fourth internode of the stems.

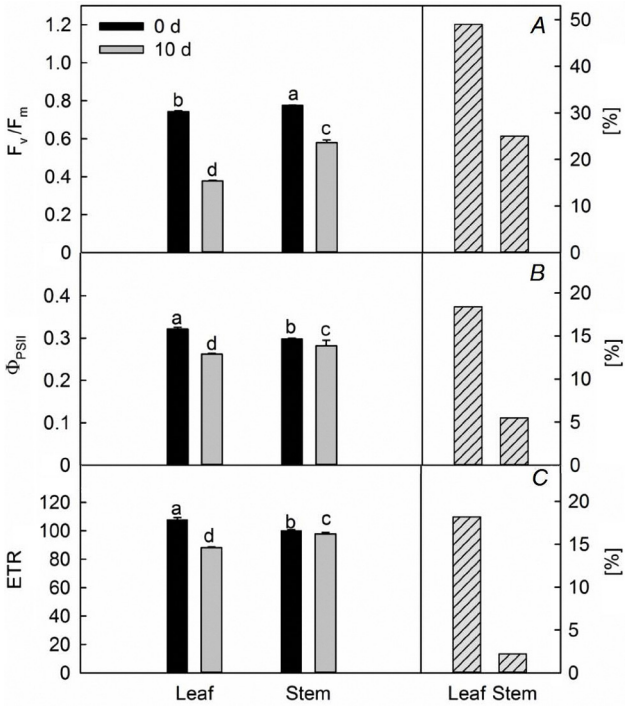

Fig. 2. Chlorophyll fluorescence parameters of leaves and stems of *Paederia scandens* under low temperature for 0 and 10 d (T = 16°C) (*left*). (*A*) F_v/F_m – maximal quantum yield of PSII photochemistry. (*B*) Φ_{PSII} – PSII maximum photochemical efficiency. (*C*) ETR – electron transport rate. The comparison of the increased percentage in chlorophyll fluorescence parameters of the leaves and stems under low-temperature treatment for 10 d (*right*).Values are means ± SD (n = 3). Statistical significance was determined using one-way *ANOVA* with *Duncan*'s multiple comparison test ($p<0.05$).

responses to the external environment (drought, low temperature, light) and functions in light absorption and light energy conversion (Xu *et al*. 2000). The Chl contents in the leaves and stems of *P. scandens* tended to decrease under low temperature. The degree of reduction in Chl *b* and total Chl contents was lesser in the stems than that in the leaves (Fig. 3*B*,*C*), while the Chl *a* content was similar in the stems and leaves (Fig. 3*A*), which indicates that the Chl synthesis in the leaves under low temperature stress was greater than that in the stems. Moreover, the Chl *a*/*b* radio of the stem decreased while that of the leaves increased (Fig. 3*D*).

MDA content: When plants are under stress, large amounts of ROS are produced and accumulate within the plant. MDA is a degradation product formed from the attack of ROS on membrane lipids and is an important manifestation of the degree of membrane structure and loss of function. We measured the changes in MDA contents in the leaves and stems for 10 d and found that the MDA contents significantly increased. The content of MDA in the stems increased by 25%, the content in the leaves increased by 84%, and that in the leaves was nearly 4 times that in the stems (Fig. 4).

Anthocyanin content: Under low-temperature stress, anthocyanins accumulated in the leaves and stems, and the anthocyanin content in the stems was much greater than that in the leaves, the contents significantly increased by 50 and 43%, respectively (Fig. 5). This finding is consistent with the phenotype observed after 10 d of treatment, the stems clearly turned red, while the leaves did not.

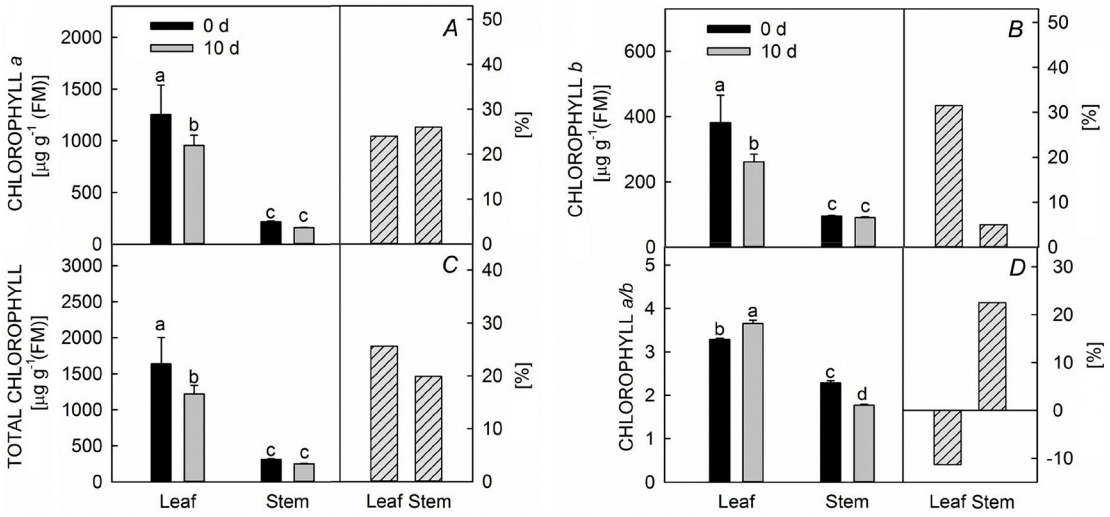

Fig. 3. Changes in the chlorophyll *a* (*A*), chlorophyll *b* (*B*), total chlorophylls (*C*) content as well as the chlorophyll *a*/*b* ratio (*D*) of leaves and stems of *Paederia scandens* under low temperature for 0 and 10 d (T = 16°C) (*left*), and comparison of the increased percentage in chlorophyll contents of the leaves and stems under low-temperature treatment for 10 d (*right*). Values are means ± SD (n = 3). Statistical significance was determined using one-way *ANOVA* with *Duncan*'s multiple comparison test (p<0.05).

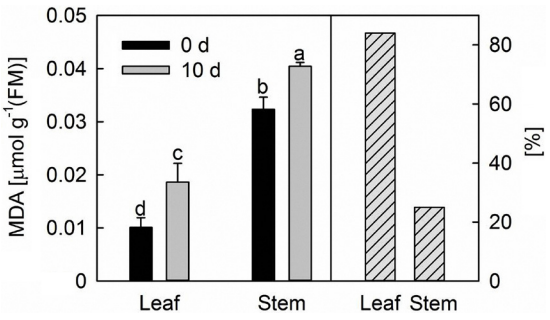

Fig. 4. The malondialdehyde (MDA) content in the leaves and stems of *Paederia scandens* under low temperature for 0 and 10 d (T = 16°C) (*left*), and comparison of the increased percentage in MDA contents of the leaves and stems under low-temperature treatment for 10 d (*right*). Values are means ± SD (n = 3). Statistical significance was determined using one-way *ANOVA* with *Duncan*'s multiple comparison test (p<0.05).

Soluble sugar content: As osmoprotectants, soluble sugar is a protective molecule in cells under temperature stress (Awasthi *et al*. 2015). The results showed that the contents of soluble sugar in the leaves and stems of *P. scandens* significantly increased after 10 d; the content in the stems significantly increased by 118%, which was nearly 10 times of that in the leaves (16%) (Fig. 6).

Discussion

Temperature is a primary factor that affects plant growth and development. Plants have several adaptive mechanisms in response to certain adversities, such as low temperature and drought stress (Popov *et al*. 2010). Different organs of plants (stems and leaves) may also exhibit different physiological responses to cope with stress.

Low temperature can affect the photosynthesis of plants and reduce the utilization of light energy mainly because the

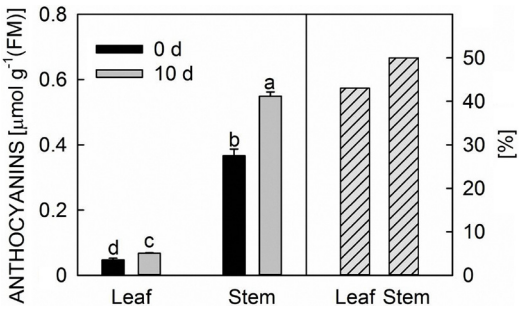

Fig. 5. Content of anthocyanins in the leaves and stems of *Paederia scandens* under low temperature for 0 and 10 d (T = 16°C) (*left*) and a comparison of the increased percentage of anthocyanins contents of in the leaves and stems of plants under low temperature treatment for 10 d (*right*). Values are means ± SD (n = 3). Statistical significance was determined using one-way *ANOVA* with *Duncan*'s multiple comparison test (p<0.05).

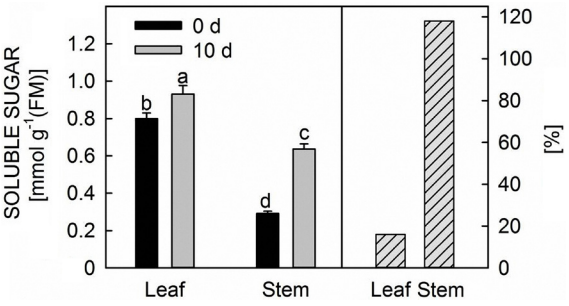

Fig. 6. The content of soluble sugars in the leaves and stems of *Paederia scandens* under low temperature for 0 and 10 d (T = 16°C) (*left*) and a comparison of the increased percentage of soluble sugar contents in the leaves and stems of plants under low-temperature treatment for 10 d (*right*). Values are means ± SD (n = 3). Statistical significance was determined using one-way *ANOVA* with *Duncan*'s multiple comparison test (p<0.05).

Chl content is affected. The Chl-related indexes (including the Chl *a*, Chl *b*, total Chl content) of the leaves and stems tended to decrease under low temperature, and the degree of decrease was much lower in the stems than that in the leaves (Fig. 3), indicating that the leaves were significantly more stressed than were the stems. Previous reports have also confirmed that the Chl content in plants decreases under low temperature (Borowski and Blamowski 2009). The results indicated that low temperature influences the synthesis of Chl by affecting the activity of enzymes, resulting in increased degradation of Chl (Glaszmann *et al*. 1990).

In plants, photosynthesis is a process that is highly sensitive to adversity. Changes in Chl fluorescence kinetic parameters can reflect all aspects of the effects of stress on photosynthesis (Fu *et al*. 2012). In our research, the Φ_{PSII} and ETR of the stems and leaves exhibited the same decreasing trend as did the F_v/F_m under low temperature, but the decreases were stronger in the leaves than that in the stems (Fig. 2). These results suggested that low temperature inhibits PSII electron transfer, damaging the photosynthetic structure and blocking photosynthetic electron flow, resulting in a decrease in photosynthesis capability. Some studies have shown that the decrease in photosynthesis capability under low temperature may be due to the phase transition of thylakoid membrane lipids, leading to a decrease in thylakoid membrane fluidity (Öquist *et al*. 1983). Thus, overall, these phenomena inhibit the function of PSII by affecting the reaction center and light energy capture ability of thylakoid membrane, and the inhibition of photosynthesis in the leaves is more severe than that in stems.

The ability of plants to assimilate carbon for photosynthesis is weak under low temperature (Kubien and Sage 2004). Although Chl absorbs a small amount of light energy, photochemical processes can easily cause photoinhibition and increase the content of active oxygen, resulting in damage to the membrane system which can be assessed by MDA production (Hodges *et al*. 1999). In this study, we found that the MDA content in the leaves and stems after treatment increased significantly (Fig. 4), which indicated that both the leaves and stems suffered membrane lipid peroxidation, ion extravasation, and cell membrane system damage to a certain extent. These results were consistent with Zhang and Yang (2010) who reported that, compared with that under normal temperature, the MDA content in *Microsorium pteropus* under low temperature increased significantly. Therefore, compared to the leaves, the stems maintained relatively much stable and lower contents of Chl under low temperature, resulting in less active oxygen production and lower membrane peroxidation levels.

The production of ROS affects the normal growth of plants under low temperature. Plants can generally improve their resistance to adversity by inducing metabolic activities in their cells to adapt to low temperature and increase cold resistance at certain temperatures (Liu *et al*. 2013). Studies have shown that low temperature can induce anthocyanin accumulation (Chang *et al*. 1989, Carmona *et al*. 2017, Zhang *et al*. 2019). The results showed that the stems could accumulate more anthocyanins than the leaves (Fig. 5); the former of which could decrease light absorption and better remove ROS generated in plants (Zhu *et al*. 2018). These results were consistent with the findings by which the cold resistance of *Begonia semperflorens* obviously improved when the anthocyanin content increased (Zhang *et al*. 2019). In addition, the contents of soluble sugars in leaves and stems increased significantly under low temperature. Specifically, the increase of soluble sugar content in the stem was much greater than that in leaves (Fig. 6), which improved the low temperature tolerance of the stem. The increase of soluble sugar can increase the concentration of cell solution, decrease the water potential, and increase the water absorption capacity of plants, which helps alleviate the degree of cell damage at low temperature (O'Neill 1983, Sasaki *et al*. 1996). Several studies have shown that sugar has antioxidant effects in plants, especially as a scavenger of ROS at high concentrations (Couée *et al*. 2006, Awasthi *et al*. 2015). However, it is still necessary to

study further its internal mechanism.

As an important economic and medicinal plant species, *P. scandens* exhibited similar trends in response to low temperature stress in its leaves and stems, although the stress was more severe in the leaves than that in the stems (Fig. 1). Both the stems and leaves have evolved several mechanisms to adapt the low temperature *via* enhanced contents of osmoprotectants and anthocyanins. In addition, the self-protection abilities were greater in the stems than that in the leaves. These results suggested that *P. scandens* is a kind of plant that can be propagated asexually by stem cuttings. Moreover, the stems were more tolerant than the leaves during winter and continued to grow normally (Fig. 1S, *supplement*). These findings could provide theoretical guidance for the study of stem tolerance of clonal propagation plants in the future, especially for invasive plants, which could effectively prevent and control plant invasion.

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