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EFFECTS OF CALCIUM ON PHYSIOLOGY AND PHOTOSYNTHESIS OF *PARIS POLYPHYLLA* SM. UNDER HIGH TEMPERATURE AND STRONG LIGHT STRESS

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Abstract

The present study aimed to explore the feasibility of application of exogenous Ca^{2+} to alleviate the damage caused by high temperature and strong light stress of *Paris polyphylla* by analyzing the effects of different Ca^{2+} concentrations on photosynthesis, stress resistance, and several physiological parameters of *P. polyphylla* under high temperature and strong light stress. The multi-flowered *P. polyphylla* plants with shading treatment were used as the experimental material and cultured with a modified Hoagland solution. The Ca^{2+} concentrations in the culture medium were set as 10, 14, 18, 22, and 26 (mmol/L), respectively. The shading shed was removed and the stress treatment was carried out before the approaching of high temperature and strong light stress. After 10 days of stress treatment, the photosynthesis rate and physiological parameters of *P. polyphylla* were measured. After the death seedling of *P. polyphylla*, the root and stem/shoot yield and the content of active ingredient were determined. In the study, two normal controls were set, one is shading at normal temperature (25℃) and other is spraying Hoagland solution under high temperature and strong light stress. The results showed that Ca^{2+} treatment with the appropriate concentration significantly enhanced the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in the leaves of *P. polyphylla*, reduce the contents of $O_2^{-\bullet}$ and H_2O_2 in cells, decreased the content of malondialdehyde (MDA) and the relative conductivity, and effectively alleviated the damage caused by high temperature and strong light stress. At the same time, Ca^{2+} treatment also increased chlorophyll content, stomatal conductance, contents of soluble protein and proline, and accelerated photosynthetic electron transport, reduced non-photochemical quenching (NPQ), and improved the actual photochemical efficiency, electron transport rate (ETR) and photochemical quenching index (qP) of photosystem II (PSII), making *P. polyphylla* to maintain a high net photosynthetic rate (Pn) under high temperature and strong light stress. However, as the concentration of Ca^{2+} increased, the capacity of *P. polyphylla* to resist stress was decreased. We found that 18mmol L-1 Ca^{2+} treatment could significantly reduce the damage caused by high temperature and strong light stress to *P. polyphylla*, and increased its root and stem/shoot yield and the content of saponin.

Key words: *Paris polyphylla;* High temperature and strong light; Physiological parameters; Photosynthesis.

Introduction

As the second messenger of cell signaling, Ca^{2+} is involved in the regulation of plant's responses to environmental stresses (Bhattacharjee, 2008). After the membrane of plant cells senses the environmental stress signal, Ca^{2+} can quickly transmit the signal to the cells, and triggers the expression of corresponding gene(s) and the downstream physiological and biochemical reactions, and reduce the damage caused by environmental stress to the plants (Medvedev, 2005). It has been shown that the application of exogenous Ca^{2+} can effectively enhance the plant's capacity of stress resistance and reduce the damage caused by high temperature and strong light stress to plants (Gang *et al*., 1998). Gong *et al.,* (1997) reported that the application of exogenous Ca^{2+} could increase the activities of several antioxidant enzymes of plants under high temperature and strong light stress, and reduce the peroxidation damage to cell membrane. Ca^{2+} can also alleviate the degradation of photosynthetic pigments in plant leaves under high temperature and strong light stress (Wise *et al*., 2004), and enable the reaction center of photosystem II (PSII) to maintain a large degree of openness, and thus, increase the maximum photochemical efficiency (Fv/Fm) of PSII, and maintain a high net photosynthetic rate (Pn) of plants (Tan *et al*., 2011). Based on previous observations, the present study aimed to further explore the feasibility of Ca^{2+} alleviation of the damage of caused by high temperature and strong light stress to *P. polyphylla* in order to provide a theoretical basis for delaying the seedlings of *P. polyphylla* happening in the actual production process and for improving the yield and quality of *P. polyphylla* according to the actual problems encountered in the cultivation process of *P. polyphylla*. The effects of application of exogenous Ca^{2+} on the active oxygen scavenging system and photosynthesis rate of *P. polyphylla* were studied by applying different concentrations of $CaCl_2$ solution to culture medium for *P. polyphylla*.

Material and Methods

Experimental materials: *P. polyphylla* was provided by the Dong people Medicinal Botanical Garden of Huaihua College of Hunan Province, China, and identified as the perennial herb plant, belonging to Genus Paris in the family lily by Professor Han-Yuan Zeng of the Huaihua College. The root and stem/shoot of *P. polyphylla* plants with substantially identical size, one complete buds was selected as the experimental material for asexual reproduction.

Experimental design: The experiment was conducted in the Experimental Greenhouse of Huaihua College, Human Province, China, from October 2016 to November

2017. The root system and stems/shoot of *P. polyphylla* were cultivated on a plastic pot with a height of 26 cm and a diameter of 29 cm on October 22, 2016. The cultivation medium was a mixed matrix of vermiculite and perlite with a ratio of 5:1. The volume of cultivation matrix was about 80% of the pot volume. One root system and one stem/shoot were cultivated in one pot and the covering soil depth was 6-7 cm. After planting, Hoagland nutrient solution was applied to the pot every 5 days with 500 mL per pot. Exogenous Ca^{2+} was continuously applied until death seedling. High temperature and strong light stress treatment was performed in July, 2017.

On March 18, 2017, the unleashed *P. polyphylla* was selected and planted in a greenhouse with a layer of shade net. The temperature in the shed was 20-28°C, the relative humidity was 50%-70%, the shade shelter of the light transmittance was 70% and the Ca^{2+} solution (supplied as $CaCl_2$) was applied. Ca^{2+} concentrations were set as 10, 14, 18, 22, and 26 (mmol/L), respectively. The treatment solution was configured by Hoagland nutrient solution, repeated 15 times for each concentration. Ca^{2+} solution was applied every 5 days with 500 mL per pot. Ca^{2+} treatment continued until *P. polyphylla* fell. The high temperature and strong light stress treatment was performed in July, 2017. On July 20, 2017, the shaded greenhouse and surrounding obstructions were removed for treatment of high temperature and intense light stress. The highest temperature during the high temperature and strong light treatment period was shown in Figure 1. The light intensity was 132400-145900 lx, and the highest light intensity was 20150-36450 lx. In this experiment, two groups of controls were set up, control 1 (CK1) was applied with Hoagland nutrient solution plus shading at 25 °C; CK2 was applied with Hoagland nutrient solution and treated with high temperature and intense light.

Measurement of parameters: After 10 days of stress treatment with high temperature and strong light, the relevant physiological and photosynthetic parameters in the middle and upper mature leaves were measured on July 30, 2017. After the above-ground parts of seedlings of *P. polyphylla* were death seedling, the roots and stem/shoot of *P. polyphylla* were harvested on November 6, 2017, and the fresh weight, dry weight and active ingredients of the roots and stem/shoot were measured.

Determination of physiological parameters: H_2O_2 and O_2^- were determined according to the method of Li *et al*., (2013); Malondialdehyde (MDA) content was determined by thiobarbituric acid (TBA) method. Relative conductivity was measured with conductivity meter (Yang *et al*., 2017); Protein content was determined by Bradford Bright Blue G-250 staining; Proline content was determined by ninhydrin method; Superoxide dismutase (SOD) was determined by nitroblue tetrazolium (NBT) method; Peroxidase (POD) activity was assayed by guaiacol method; and catalase (CAT) activity was assayed by potassium permanganate titration.

Determination of the contents of chloroplast pigments and photosynthetic parameters: The chlorophyll content was determined according to the method described by Wang (2000). The photosynthesis parameters were measured using a Li-6400 portable photosynthetic apparatus obtained from LI-COR Biosciences. The photosynthetic parameters were measured under natural conditions from 09:00 to -11:00 AM. Net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and other parameters were directly measured by the instrument. The open gas path and red/blue light source were selected. The photosynthetically active radiation inside leaf chamber was 800 Mmol•m-2•s^{-1}. Sample chamber airflow rate was 500 μmol•s^{-1}; reference chamber CO_2 concentration was 380-410 μmol•mol^{-1}; and relative humidity (RHS) inside sample chamber was 25%-40%. At the time of measurement, 5 healthy plants with good growth and basically the same size were selected. From each plant, 2-4 leaflets with the same size and strong growth were selected from the top to the bottom 15 cm, and the middle part of the leaves was determined. For each measurement, the fixed-labeled leaves were selected, and each with the three replicates was repeated, and five observations were repeatedly recorded, and the average value was taken as the measured value.

Determination of chlorophyll fluorescence parameters: The same leaves that were selected for the photosynthesis assay were used for assays and the chlorophyll fluorescence parameters were measured using a portable modulation chlorophyll fluorescence meter (PAM2100, WALZ, Germany). Before the assay, the leaves of *P. polyphylla* were firstly dark-adapted for 30 min, and the initial fluorescence (Fo) was measured with weak light, followed by with a saturated and pulsed light to measure the maximum fluorescence (Fm). When the fluorescence yield rapidly decreased from Fm and returned to Fo, the active light (530 μmol•m^{-2}•s^{-1}) was turned on and the saturation pulse was continuously turned on every 20 second to measure. When the leaf photochemical reaction reached a steady state (about 6 min), the steady-state fluorescence (Fs) and the maximum fluorescence (Fm') under the light were measured. At this time, the far red light was turned on, and Fo' was measured. The following parameters were calculated by the above parameters: PSII maximum photochemical efficiency Fv/Fm=(Fm-Fo)/Fm, PSII actual photon quantum efficiency Yield=(Fm'-Fs)/Fm', relative photosynthetic electron transport rate ETR=ΦPSII×PAR×0.5×0.84, photochemical quenching coefficient qP=(Fm'-Fs)/(Fm'-Fo'), and non-photochemical quenching coefficient NPQ=(Fm-Fm')/Fm'.

Determination of fresh weight, dry weights and contents of active ingredients of root and stem/shoot: The roots and shoot of that year were cut and washed. Their surface moisture was absorbed, the fresh weight was firstly measured and then dried at 105°C for 15 min and then dried at 65°C to constant weight. The dry weight was measured. The contents of active ingredients of *P. polyphylla* were determined by vanillin-glacial acetic acid-perchloric acid with UV colorimetry.

Data analysis

Statistics and figure-drawing were performed using Excel. The significance in difference between groups was tested by the Duncan method and the LSD method in SPSS Statistics 13 software. All the analysis results were expressed as mean ± standard deviation (SD).

Results and Analysis

Effects of application of $CaCl_2$ on $O_2^{-\bullet}$ content, H_2O_2 content, malondialdehyde (MAD) content and relative conductivity of *P. polyphylla*: It can be seen from Table 1 that compared with the normal temperature plus shading treatment (CK1), the $O_2^{-\bullet}$ production rate and H_2O_2 content in the leaves of *P. polyphylla* in high temperature and intense light stress group (CK2) were significantly increased by 67.27% and 166.56%, indicating that high temperature leads to an increase in reactive oxygen species (ROS) in the leaves of *P. polyphylla*. After treatment with different concentrations of $CaCl_2$, $O_2^{-\bullet}$ production rate and H_2O_2 content in leaves of *P. polyphylla* were significantly lower than those in CK2, and the $O_2^{-\bullet}$ production rate and H_2O_2 content in leaves of 18 mmol•L^{-1} $CaCl_2$–treated group were the lowest among all $CaCl_2$ treatment groups, which were 37.32% and 55.53% (P<0.05), respectively, as compared with those of CK2. When $CaCl_2$ concentration reached 26 mmol/L, and $O_2^{-\bullet}$ production rate in leaves of *Paris polyphylla* was significantly higher than that in CK2, but there was no significant difference in H_2O_2 content as compared with that of CK2.

Under high temperature and strong light stress, the changes in MDA content and relative conductivity in leaves of *P. polyphylla* were consistent with the changes of $O_2^{-\bullet}$ production rate and H_2O_2 content. MDA content and relative conductivity were significantly increased as compared with those of CK1. Application of exogenous $CaCl_2$ could significantly affect the MDA content and relative conductivity of *P. polyphylla*. With the increase in $CaCl_2$ concentration, MDA content and relative conductivity of the leaves of *P. polyphylla* were decreased first and then increased. When the concentration of $CaCl_2$ was 18 mmol•L^{-1}, the MDA content and relative conductivity of the leaves reached the lowest levels, which were 53.2% and 62.4% lower as compared to those of CK2 (p<0.05). The MDA content in the leaves of *P. polyphylla* in the 24ml•L-1 $CaCl_2$ treatment was significantly higher than that of CK2, but the relative conductivity was not significantly different from that of CK2.

Effect of $CaCl_2$ on contents of soluble protein and proline in *P. polyphylla*: The soluble protein and proline content in the leaves of *P. polyphylla* were the lowest under normal temperature plus shading treatment. Under high temperature and intense light stress, the contents of soluble protein and proline in leaves of *P. polyphylla* were significantly increased by 12.61% and 26.49%, respectively (p<0.05), as compared with those of CK1. The application of $CaCl_2$ solution could further improve the contents of soluble protein and proline in leaves of *P. polyphylla* under high temperature and strong light stress, and they were increased first and then decreased with the increase in $CaCl_2$ concentration. When concentration of $CaCl_2$ was 18 mmol•L-1, Ca^{2+} had the optimal effect on *P. polyphylla*. The contents of soluble protein and proline in leaves were significantly higher than those in other $CaCl_2$ treatment groups. The contents of soluble protein and proline of 18 mmol•L-1CaCl2-treated group were significantly increased by 53.61% and 33.63%, respectively (p<0.05), as compared with those of CK2 (Table 2).

Table 1. Effects of $CaCl_2$ concentrations on relative conductivity and the contents of $O_2^{-\bullet}$, H_2O_2 and MDA of *P. polyphylla*.

$CaCl_2$ (mmol·L^{-1})	$O_2^{-\bullet}$ (nmol·g^{-1}·min^{-1})	H_2O_2 (μmol·g^{-1})	MDA (μmol·g^{-1})	Relative conductance (%)
CK1	4.43 ± 0.56d	0.28 ± 0.03f	0.18 ± 0.01f	22.35 ± 1.25f
CK2	6.86 ± 0.67b	0.69 ± 0.05a	0.50 ± 0.03b	65.32 ± 1.24a
10	6.34 ± 0.86c	0.52 ± 0.04c	0.42 ± 0.02e	48.24 ± 1.65c
14	4.85 ± 0.63d	0.42 ± 0.04e	0.27 ± 0.01f	34.95 ± 1.34e
18	4.26 ± 0.48c	0.33 ± 0.04d	0.22 ± 0.01d	29.56 ± 1.34d
22	5.65 ± 0.48b	0.52 ± 0.01b	0.39 ± 0.04c	52.96 ± 1.45b
26	6.43 ± 0.78a	0.60 ± 0.02a	0.47 ± 0.02a	68.95 ± 1.45a

Table 2. Effects of $CaCl_2$ on contents of soluble protein and proline of *P. polyphylla* Sm.

$CaCl_2$ (mmol·L^{-1})	Soluble protein (mg·g-1)	Proline (g·g-1)
CK1	6.85 ± 0.25a	435.65 ± 8.56a
CK2	10.84 ± 0.36f	524.56 ± 9.78f
10	11.68 ± 0.35d	584.56 ± 9.78d
14	13.25 ± 0.45c	619.65 ± 7.85c
18	15.58 ± 0.47b	652.35 ± 8.42b
22	14.56 ± 0.28e	613.34 ± 5.78e
26	10.68 ± 0.28g	665.32 ± 4.56g

Effect of $CaCl_2$ on the activities of SOD, POD and CATs of *P. polyphylla*: The activities of SOD, POD and CAT in the leaves of *P. polyphylla* in high temperature/strong light stress group were lower than those of CK1, which were significantly decreased by 54.16%, 36.73% and 38.52%, respectively (p<0.05). After being treated with $CaCl_2$, the activities of SOD, POD and CAT in leaves of *P. polyphylla* were significantly changed as compared with those of CK2, indicating that application of appropriate concentration of $CaCl_2$ causes the change in the capacity of *P. polyphylla* to resist high temperature and strong light stress. After being treated with $CaCl_2$ at different concentrations, the activities

of SOD, POD and CAT in leaves of *P. polyphylla* were significantly higher than those in CK2. The activities of SOD, POD and CAT in leaves of *P. polyphylla* treated with 18 mmol•L-1$CaCl_2$ were most significantly increased, as compared with those of CK2, which were significantly increased by 114.78%, 49.16%, and 57.77%, respectively (p<0.05). The SOD activity in leaves of *P. polyphylla* treated with 22mmol•L-1 $CaCl_2$ was significantly higher than that of CK2, but the activities of POD and CAT were not significantly different from those of CK2. When the concentration of CaCl2 reached 26mmol•L-1, the activities SOD, POD and CAT were not significantly different from those of CK2 (Table 3).

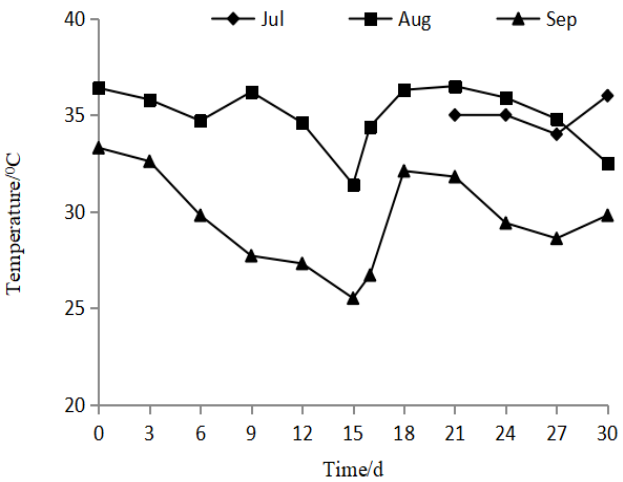

Fig. 1. Air temperature change during the treatment.

Effect of $CaCl_2$ on content of photosynthetic pigments in the leaves of *P. polyphylla*: Under high temperature and strong light stress, the contents of chlorophyll a, chlorophyll b and chlorophyll (a+b) in leaves of *P. polyphylla* were lower than those of CK1 at room temperature. The contents of chlorophyll a, chlorophyll b and chlorophyll (a+b) were decreased by 35.52%, 35.38% and 35.48%, respectively (p<0.05). Compared with those of CK2, different concentrations of $CaCl_2$ could significantly increase the chlorophyll content in leaves of *P. polyphylla*, and the chlorophyll content was increased first and then decreased with the increase in $CaCl_2$ concentration, and the effect of treatment with $CaCl_2$ at 18mmol•L-1 was observed. Preferably, the levels of chlorophyll a, chlorophyll b and chlorophyll (a+b) were significantly increased by 71.94%, 47.62% and 50.00%, as compared to those of CK2. When $CaCl_2$ concentration exceeded 22 mmol•L-1, the chlorophyll content was not significantly different from that of CK2, indicating that the excessive concentration of $CaCl_2$ is not conducive to the synthesis of chlorophyll in leaves of *P. polyphylla* under high temperature and strong light stress (Table 4).

Effects of $CaCl_2$ on photosynthesis parameters of *P. polyphylla*: After treatment with high temperature and high light stress, Pn, Gs and Tr of CK2 leaves were significantly decreased while Ci was significantly increased. Compared with those of CK1 at room temperature, Pn, Gs and Tr were significantly decreased by 31.79%, 61.29% and 34.70% (p<0.05), respectively, whereas Ci was increased by 16.68% (p<0.05). Under high temperature and strong light stress, the treatment with appropriate concentration of $CaCl_2$ can improve the photosynthetic efficiency of the leaves of *P. polyphylla*. Compared with high temperature and strong light stress CK2, treatments with different concentrations of $CaCl_2$ significantly increased Pn, Gs and Tr of the leaves of *P. polyphylla*, making Ci significantly lower than that of high temperature/strong light control group (CK2). When $CaCl_2$ concentration was 18mmol•L-1, the effect of this treatment on *P. polyphylla* was the highest one. Compared with those of CK2, the leaf Pn, Gs and Tr were significantly increased by 56.90%, 141.66% and 46.85%, respectively (p<0.05). Ci was significantly decreased by 21.22% (p<0.05). When the concentration of $CaCl_2$ was 26mmol•L-1, there was no significant difference in leaf Pn between treatment groups and CK2. Gs and Tr were significantly lower than those of CK2 whereas Ci was significantly higher than that of CK2, indicating that high concentration of $CaCl_2$ cannot improve the photosynthetic efficiency of *P. polyphy*lla (Table 5).

Table 3. Effects of $CaCl_2$ on activities of POD, SOD and CAT of *P. polyphylla* Sm.

$CaCl_2$ (mmol·L^{-1})	SOD (U·g^{-1}·min^{-1})	POD (U·g^{-1}·min^{-1})	CAT (mg·g^{-1}·min^{-1})
CK1	201.5 ± 4.65a	1576.25 ± 45.32a	56.31 ± 2.31a
CH2	92.35 ± 3.58e	886.32 ± 43.68cd	34.62 ± 1.35d
10	132.54 ± 5.64b	1132.56 ± 65.32c	42.32 ± 3.24b
14	156.38 ± 6.24a	1298.86 ± 65.32a	48.65 ± 1.35a
18	198.35 ± 4.74c	1356.32 ± 47.5b	54.62 ± 2.32c
22	135.35 ± 2.65d	1205.63 ± 53.21c	43.56 ± 1.32d
26	106.52 ± 3.45e	916.32 ± 34.65d	36.45 ± 0.85e

Table 4. Effects of CaCl2 on the contents of photosynthetic pigments of *P. polyphylla* Sm.

CaCl2 (mmol·L-1)	Chl a (mg·g-1)	Chl b (mg·g-1)	Chl (a+b) (mg·g^{-1})	Chla/b
CK1	1.52 ± 0.02a	0.65 ± 0.01a	2.17 ± 0.01a	2.34 ± 0.02de
CH2	0.98 ± 0.01d	0.42 ± 0.01f	1.40 ± 0.02d	2.33 ± 0.01a
10	1.12 ± 0.05c	0.50 ± 0.00cd	1.62 ± 0.01c	2.24 ± 0.01c
14	1.32 ± 0.02a	0.54 ± 0.01d	1.87 ± 0.02a	2.44 ± 0.02a
18	1.48 ± 0.04b	0.62 ± 0.02bc	2.10 ± 0.02b	2.39 ± 0.01b
22	1.12 ± 0.03d	0.51 ± 0.01b	1.63 ± 0.02d	2.22 ± 0.01cd
26	1.08 ± 0.01e	0.45 ± 0.01b	1.53 ± 0.01e	2.40 ± 0.02e

Table 5. Effects of $CaCl_2$ on photosynthetic parameters of *P. polyphylla* Sm.

$CaCl_2$ ($mmol \cdot L^{-1}$)	Pn ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	GS ($mol \cdot m^{-2} \cdot s^{-1}$)	Ci ($\mu mol \cdot mol^{-1}$)	Tr ($mmol \cdot m^{-2} \cdot s^{-1}$)
CK1	9.56 ± 0.15d	0.31 ± 0.01d	259.32 ± 5.16b	2.68 ± 0.12a
CK2	6.52 ± 0.23f	0.12 ± 0.00e	302.56 ± 6.32d	1.75 ± 0.23e
10	7.12 ± 0.25b	0.18 ± 0.01c	285.8 ± 6.21b	2.14 ± 0.24d
14	8.23 ± 0.34a	0.22 ± 0.01a	258.32 ± 7.38e	2.46 ± 0.34a
18	10.23 ± 0.34c	0.29 ± 0.01c	238.35 ± 5.45c	2.57 ± 0.16c
22	7.56 ± 0.34e	0.15 ± 0.00b	286.54 ± 6.24d	1.95 ± 0.18b
26	6.58 ± 0.16f	0.13 ± 0.01f	305.32 ± 5.24a	1.81 ±0.24f

Table 6. Effects of $CaCl_2$ on chlorophyll fluorescence parameters of *P. polyphylla* Sm.

$CaCl_2$ ($mmol \cdot L^{-1}$)	Fv/Fm	Yield	ETR	qP	NPQ
CK1	0.95 ± 0.01a	0.68 ± 0.04a	29.55 ± 1.18a	0.75 ± 0.01b	0.38 ± 0.03e
CH2	0.52 ± 0.02f	0.38 ± 0.01e	16.58 ± 2.03e	0.51 ± 0.01f	0.65 ± 0.02a
10	0.69 ± 0.01b	0.53 ± 0.02c	23.31 ± 1.46c	0.66 ± 0.02b	0.52 ± 0.01c
14	0.85 ± 0.01d	0.58 ± 0.01a	26.45 ± 1.34a	0.71 ± 0.01a	0.42 ± 0.01b
18	0.93 ± 0.02c	0.65 ± 0.01d	29.68 ± 1.35d	0.79 ± 0.02c	0.36 ± 0.01c
22	0.63 ± 0.02e	0.49 ± 0.02b	19.35 ± 1.35b	0.58 ± 0.01e	0.43 ± 0.02d
26	0.55 ± 0.01f	0.41 ± 0.02b	16.21 ± 1.89e	0.49 ± 0.02g	0.56 ± 0.02a

Table 7. Effects of CaCl2 on the growth of rootstock and the content of saponin in *Paris polyphylla* Sm.

$CaCl_2$ ($mmol \cdot L^{-1}$)	Dry weight of root and stem/shoot (mg) (g)	Dry rate of root and stem/shoot ($g \cdot g^{-1}$)	Saponin content (%)
CK1	8.65 ± 0.14c	0.36 ± 0.01c	0.54 ± 0.12a
CK2	5.68 ± 0.18b	0.32 ± 0.01e	0.34 ± 0.08b
10	6.89 ± 0.14c	0.37 ± 0.02d	0.47 ± 0.14d
14	7.68 ± 0.18a	0.42 ± 0.01a	0.52 ± 0.13a
18	9.15 ± 0.14d	0.48 ± 0.01d	0.56 ± 0.14a
22	6.87 ± 0.15c	0.35 ± 0.02c	0.51 ± 0.14dc
26	6.14 ± 0.16b	0.32 ± 0.01b	0.45 ± 0.16cb

Effect of $CaCl_2$ on chlorophyll fluorescence parameters of *P. polyphylla*: Under high temperature and strong light stress, the Fv/Fm, Yield, ETR and qP of the leaves of *P. polyphylla* were significantly decreased by 45.26%, 44.11%, 43.89% and 32.00% ($p<0.05$), respectively, whereas non-photochemical quenching (NPQ) was significantly increased by 71.05% as compared with those of CK1 at room temperature, indicating that photosynthetic system II (PSII) was damaged by high temperature and strong light stress. Application of exogenous $CaCl_2$ could significantly affect leaf PSII of *P. polyphylla* under high temperature and strong light stress. Compared with those of CK2, Fv/Fm, Yield, ETR and qP in leaves of *P. polyphylla* were significantly higher than those in CK2 at different concentrations of $CaCl_2$, and NPQ was significantly lower than those of CK2. Among them, Fv/Fm, Yield, ETR and qP in leaves of *P. polyphylla* treated with 18 mmol•L-1 $CaCl_2$ were significantly higher than those of the groups treated with $CaCl_2$ at other concentrations. NPQ was significantly lower than those of groups treated with $CaCl_2$ at other concentrations. As compared with those of CK2, leaf Fv /Fm, Yield, ETR, and qP were significantly increased by 78.84%, 71.05%, 78.25%, and 54.91%, respectively whereas NPQ was significantly reduced by 44.62%. When $CaCl_2$ concentration reached 26 mmol•L-1, there were no significant differences in leaf Fv/Fm, ETR, qP and NPQ of P. polyphylla between the CaCl2-treated groups and CK2 (Table 6).

Effects of application of $CaCl_2$ on the dry weight, drying rate and content of active ingredient of roots and stem/shoot of *P. polyphylla*: High temperature and strong light stress could inhibit the yield of root and stem/shoot and the accumulation of saponin in the leaves of *P. polyphylla*. Compared with those of CK1 at room temperature, the dry weight, the dry rate of roots and stem/shoot and the content of saponin in CK2 group were significantly decreased by 34.33%, 44.44% and 29.63%, respectively. The application of different concentrations of $CaCl_2$ contributed to the growth of roots and accumulation of saponins in the leaves of *P. polyphylla*, *which* were increased first but then decreased with increasing $CaCl_2$ concentration. Among them, root and stem/shoots and saponins content in 18 $mmol \cdot L^{-1}$ $CaCl_2$-treated group were significantly higher than those of the other treatment groups. As compared with those of CK2, they were increased significantly by 61.10%, 51.86% and 47.37%, respectively. When the concentration of $CaCl_2$ reached 26 $mmol \cdot L^{-1}$, the dry weight of roots and stem, the dry rate of root/stem and the content of saponin were not significantly different from those of CK2, indicating that while application of $CaCl_2$ can promote the growth of roots and accumulation of saponins in the leaves of *P. polyphylla*, when its concentration was too high, its effect will drop (Table 7).

Discussion

Under high temperature and strong light environment, the light energy that can be absorbed and utilized by plant leaves increases. If the plant leaves cannot convert and dissipate all the captured light energy in time, it will cause excess light energy, and more and more electrons will pass through photosynthetic electron transfer chain to O_2 and generates ROS, such as H_2O_2 and $O_2^{-\bullet}$ through the Mehler reaction (Asada, 2006). When the level of ROS in plant leaves exceeds a certain threshold, the structures of intracellular macromolecules are destroyed, which affects plant's normal physiological functions. This is reflected by the changes in plant morphology, which shows that the leaves gradually lose water and become wilting, and leaf spots appear. The damage caused by long-term high temperature and strong light stress will also eventually cause plant death (Luo *et al*., 2011). In the present study, we found that after 10-day treatment of high temperature and strong light stress, the MDA content and relative conductivity of CK2 leaves were significantly higher than those of normal temperature shading (CK1) (Sun *et al*., 2015). This may be due to the reason that under high temperature and strong light stress, the contents of H_2O_2 and $O_2^{-\bullet}$ in the leaves of *P. polyphylla* in CK2 group were significantly increased as compared with those of CK1, and mem brane lipid peroxidation occurred in cell membrane, leading to an increase in MDA content; the structure and function of cell membrane were destroyed, causing the electrolyte to flow out of the cells and an increased conductivity. This study showed that treatment with Ca^{2+} at appropriate concentration can effectively alleviate the membrane lipid peroxidation damage of the leaves of *P. polyphylla* under high temperature and strong light, reduce MDA content and relative conductivity, and enhance the adaptability of *P. polyphylla* to high temperature and strong light environment. On the one hand, Ca^{2+} enhances plant's stress resistance by participating in the formation of biological membrane and accelerating the self-repair of biological membranes, thereby stabilizing the structure of biological membranes and enhancing the ability of biological membranes to resist H_2O_2 and $O_2^{-\bullet}$oxidation (Liu *et al*., 2105). This aspect may be achieved by activating the downstream genes and physiological and biochemical reactions through the Ca^{2+} signal transduction pathway (Tang *et al*., 2015). However, when the concentration of Ca^{2+} was too high, the damage caused by high temperature and strong light stress to *P. polyphylla* would be aggravated, and the MDA content and relative conductivity of the cells will be significantly increased. Wang *et al*., (2010) believed that this was due to the reason that the excessive Ca^{2+} will cause the alterations in structure and function of cell membrane system, resulting in metabolic disorders in the cells.

The generation of a large amount of ROS under high temperature and strong light stress can also cause damage to the plant photosynthetic system, resulting in a decrease in plant Pn and Fv/Fm (Ding *et al*., 2012). It was found that leaf Pn and Fv/Fm of CK2 were significantly lower than that of CK1. On the one hand, it may be due to the reason that ROS can destroy the structure of chlorophyll pyrrole ring and chloroplast capsule membrane, and can induce stomatal closure of plant leaves, hindering photosynthesis (Mcainsh *et al*., 1996). The smooth progress of Pn causes the decrease of Pn. On the other hand, it may be also due to the reason that ROS can damage the structure of the D1 protein of PSII active center, resulting in a decrease in the utilization and conversion efficiency of light energy (Yamamoto *et al*., 2008). Recent studies have shown that ROS cause damage to D1 protein in the reaction center of PSII, which is not a direct injury but affects the activity of the reaction center of PSII by inhibiting the repair process of D1 protein (Krishna *et al*., 2013). The decrease of leaf Pn resulted in insufficient accumulation of photosynthetic products, which led to a significant decrease in the dry weight and saponin content of the roots of structure and function. This study showed that the application of appropriate concentration of exogenous Ca^{2+} could effectively alleviate the damage to PSII of *P. polyphylla*, significantly increase the Pn of *P. polyphylla*, and significantly increase the accumulation of photosynthesis products and active components of *P. polyphylla*. The protective effect of Ca^{2+} on the photosynthetic system of *P. polyphylla* may be related to the effective enhancement of ROS-scavenging capacity by Ca^{2+} and the inhibition of ROS on the reaction center of PSII (Yang *et al*., 2013). The recovery of the reaction center of PSII accelerates photosynthetic electron transfer and alleviates the damage caused by high temperature and strong light stress. The actual photochemical efficiency, ETR and qP of PSII, and the reduction of NPQ, make the absorbed light energy flow more to the dark reaction. At the same time, the enhanced ROS-scavenging capacity of *P. polyphylla* is beneficial to the synthesis of chlorophyll, and can make the stomatal opening and closing tend to be normal, which is conducive to photosynthesis. However, when the Ca^{2+} concentration exceeds 20 mol•L^{-1}, it has no obvious protective effect on the photosynthetic system of *P. polyphylla*, and may even aggravate damage caused by high temperature and strong light stress. This may be due to the reason that when the Ca^{2+} concentration is too high to a certain extent, it may cause damage to the structure and function of the photosynthetic system membrane, resulting in the photosynthetic electron transport chain being blocked and the light energy utilization efficiency being reduced (Wang *et al*., 2010).

Antioxidant enzymes, including SOD, POD and CAT, are the first lines of defense against ROS in plants to protect against stress. The higher the enzymatic activities of SOD, POD and CAT are, the stronger the plant's capacity to adapt to the environment (Wang *et al*., 2009). In this study, we observed that the activities of SOD, POD and CAT of *P. polyphylla* growing under high temperature and strong light stress were significantly lower than those of CK1. This may be due to the reason that under high temperature and strong light stress, a large amount of ROS will destroy the active center of the enzyme, change the structure of the enzymes or inhibit the expression of the enzyme, resulting in the decreased activities of antioxidant enzymes. Studies have shown that Ca^{2+} treatment at

appropriate concentration can effectively improve the activities of antioxidant enzymes of *P. polyphylla*, and enhance the adaptability of *P. polyphylla* to high temperature and strong light environment. Ca^{2+} enhances activities of antioxidant enzymes mainly through the Ca^{2+} signal transduction pathway (Zhu *et al*., 2004). Yang *et al*., (2002) found that Ca2+ can directly increase enzymatic activity of CAT by binding to calmodulin (CaM). Ca^{2+} can also increase the activities of protein kinases, promote the accumulation of antioxidants and compatible substances to reduce ROS damage under environmental stress and improve plant stress resistance (Jiang & Zhang., 2003). However, when the concentration of Ca^{2+} is too high, it will antagonize other metal ions absorbed by plants (Li *et al*., 2013), which will hinder the synthesis of antioxidant enzymes, resulting in a decrease in enzymatic activities. Cell osmotic adjustment is another important protective mechanism for plants to resist external environmental stress. Under high temperature and strong light stress, plants can reduce intracellular osmotic potential by increasing the content of intracellular osmotic adjustment substances, and increase plant water-holding capacity (Zhao & Tan, 2005). Proline and soluble protein are common osmotic regulators in plants. They can participate in the osmotic regulation of plant cells and in the clearance of intracellular ROS (Wang *et al*., 2019; Lü *et al*., 2019). In this study, we found that the appropriate concentration of $CaCl_2$ treatment significantly increased the contents of proline and soluble protein in the leaves of *P. polyphylla*, compared with CK2 treated with high temperature and intense light stress, indicating that Ca^{2+} can induce proline and soluble protein within cells. Synthesis and accumulation, which indirectly increases the free water content in the cells, maintains the cell swell pressure, protects the bio-membrane to a certain extent, reduces the peroxidation level of the bio-membrane, and enhances the resistance of the to the high temperature. The ability of strong light stress is consistent with the results of Zhao & Tan (2005). On the effects of Ca^{2+} on wheat growth, active oxygen accumulation and photo inhibition.

High-temperature and intense light is an important environmental factor that restricts the growth of *P. polyphylla*. In this study, we found that application of $CaCl_2$ at appropriate concentration could further increase the contents of proline and soluble protein and significantly increased the activities of antioxidant enzymes, reduced the content of H_2O_2 and the production rate of $O_2^{-\bullet}$, and reduced the damage caused by ROS to photosynthetic system. Therefore, it is conducive to the growth and photosynthesis of *P. polyphylla* under high temperature and strong light stress. For the purposes of this study, 18 $mmol \bullet L^{-1}$ $CaCl_2$ treatment significantly reduced the damage caused by high temperature and strong light stress to *P. polyphylla* and increased its root/shoot yield and content of active ingredients.

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