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Exogenous calcium-induced physiological and biochemical changes in tree peony (*Paeonia* section *Moutan* DC.) under drought stress

X.Y. ZHANG*, Z.W. FANG*, H.N. LIU*, D.Q. ZHAO*,+, and J. TAO*,+

*Jiangsu Key Laboratory of Crop Genetics and Physiology, College of Horticulture and Plant Protection, Yangzhou University, Yangzhou, China**

Abstract

Tree peony (*Paeonia* section *Moutan* DC.) is an excellent ornamental plant, of which *Paeonia ostii* (*P. ostii*) has a high oil value. It is widely cultivated in China, but severe drought affects its growth. In this study, the effects of exogenous calcium on drought-induced damage of *P. ostii* were studied. The results showed that under drought stress, leaf water content showed a downward trend, while reactive oxygen species (ROS), relative electrical conductivity (REC), proline (Pro) content, and related antioxidant enzymes increased significantly. Spraying $CaCl_2$ could effectively slow leaf wilting and water loss, induced an increase in enzyme activity of the antioxidant enzyme system, and reduced the accumulation of ROS caused by drought stress. Simultaneously, REC and Pro content could be alleviated, and the degree of cell membrane damage could be reduced. In addition, $CaCl_2$ improved photosynthetic characteristics and chlorophyll fluorescence parameters. These results indicated that $CaCl_2$ reduced the harmful effects of drought stress on the growth of *P. ostii* by regulating infiltration, activating photosynthesis, and enhancing the antioxidant system. These findings suggested that $CaCl_2$ can be used to manage drought stress in *P. ostii* cultivation.

Additional key words: ascorbate peroxidase; gas exchange; physiological index; relative water content; superoxide dismutase; water stress.

Introduction

Tree peony (*Paeonia* section *Moutan* DC.) is native to China and has a high ornamental, medicinal, and economic value. Tree peony is also known as the 'king of flowers', and its seeds have the high oil value (Mao *et al*. 2017, Han *et al*. 2018). In addition, the root bark of tree peony is used in traditional Chinese medicine, and paeonol and paeoniflorin are important components for the treatment of breast cancer and neuropathic pain (Shah *et al*. 2018). Tree peony consists of nine species, with *Paeonia ostii* (*P. ostii*) receiving the most extensive promotion at the national level (Ren *et al*. 2018). At present, more than 20 provinces and autonomous regions, including Henan, Shandong, Anhui, and Hunan, are vigorously promoting the cultivation of *P. ostii* (Du 2016). The *P. ostii* root is a fleshy root that is resistant to drought and is hydrophobic. Excessive moisture can cause root rot, but insufficient water affects the growth and development of *P. ostii* and its ornamental value (Li *et al*. 2014).

Drought stress is one of the most important environmental factors affecting plants (Engelbrecht *et al*. 2007, Wahid *et al*. 2007). In recent years, as the global warming effect has intensified, drought stress has become increasingly serious in most parts of the world (Anderegg *et al*. 2012). Studies have shown that plants experiencing drought stress often show leaf wilting and sagging, and drought stress also affects the metabolism of plants, including increased reactive oxygen species (ROS) content and antioxidant enzyme activities, photosynthesis inhibition, reduced transpiration rates, and damage to various cell structures (Sperry *et al*. 1998, Lawlor 2002, Niu and Rodriguez 2009, Soares-Cordeiro *et al*. 2009, Sekmen *et al*. 2014). Researchers are currently trying to enhance the tolerance of plants under extreme water conditions using a variety of chemical agents (Hojati *et al*. 2011, Upadhyaya *et al*. 2011). In recent years, studies have found that exogenous calcium can maintain cell membrane permeability, protect enzyme activity and antioxidant content, and enhance plant antioxidant capacity (Jiang and Huang 2001, El-Beltagi and Mohamed 2013). In addition, calcium is not only a nutrient necessary for plant growth; more importantly, it acts as a messenger to regulate physiological processes, including the opening

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+Corresponding author; phone: +86-514-87997219, fax: +86-514-87347537, e-mail: dqzhao@yzu.edu.cn (D.Q. Zhao), taojun@yzu.edu.cn (J. Tao)

Abbreviations: APX – ascorbate peroxidase; C_i – intercellular CO_2 concentration; DAB – diaminobenzidine; DM – dry mass; E – transpiration 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_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; P_N – net photosynthetic rate; POD – peroxidase; Pro – free proline; q_N – nonphotochemical quenching coefficient; REC – relative electrical conductivity; ROS – reactive oxygen species; SOD – superoxide dismutase; $Y_{(II)}$ – effective quantum yield of PSII photochemistry.

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and closing of stomata (Ingram and Bartels 1996, Cousson 2009). Among them, $CaCl_2$ has long been used in agricultural production as an antitranspiration agent. Under drought stress, plants adapt to stress by regulating the concentration of calcium ions (Ma *et al*. 2005). Xu *et al*. (2013) reported that calcium induction in *Zoysia japonica* under drought stress increases the antioxidant enzyme activity and reduces the transpiration rate, thereby increasing drought tolerance. Under adverse conditions, such as drought, high temperature, and salt damage, plant internal calcium messengers improve the adaptability of plants to stress by regulating gene expression (Ma *et al*. 2005, Yin *et al*. 2018).

However, studies on the application of exogenous calcium to improve plant stress resistance have focused on crops, such as wheat (Yao *et al*. 2009) and rice (Pallavi and Rama 2005), and studies on ornamental plants, such as tree peony, have been rarely reported. Therefore, based on previous studies, this study assessed the alleviation of exogenous calcium on the growth of *P. ostii* under drought stress from the aspects of plant physiology, antioxidant enzymes, photosynthesis, and fluorescence. The purpose of this experiment was to study the effects of drought stress on the growth of *P. ostii* and the alleviation effect of exogenous $CaCl_2$ on the growth of *P. ostii* under drought stress. This study also sought to further assess the effects of exogenous calcium on plant drought resistance, especially, the impact on drought resistance of *P. ostii* and other ornamental plants.

Materials and methods

Plant materials and treatments: Three-year-old potted *Paeonia ostii* was used as the material for this study, and the experimental period was from July to August 2018. The plants were divided into two groups: one for the control (Control) and the other for $CaCl_2$ treatment. The plants in the Control group were sprayed with water at 17:00 h, and another group of plants was sprayed with $CaCl_2$. Preliminary experiments in the previous period revealed that the optimum $CaCl_2$ solution concentration was 5 mM. The corresponding reagents were sprayed on the two groups of plants until the leaves were wet and the tips of the leaves were dripping. The plants were sprayed continuously for 3 d and then subjected to natural drought treatment. Relative water content, electrical conductivity, active oxygen content, photosynthetic characteristics, and chlorophyll (Chl) fluorescence parameters were measured on days 0, 4, 8, and 12 after treatment. Finally, the leaves were collected and stored in liquid nitrogen for measurement of physiological indicators, such as proline and antioxidant enzymes.

The relative water content of the blades was measured by using a balance (*Gandg Testing Instrument Factory*, Changshou, China) and an oven (*Jinghong Laboratory Instrument Co.*, *Ltd.*, Shanghai, China). The fresh mass (FM) of the leaves was weighed, and the sample was placed in an oven at 105°C for 5 min. The temperature was then adjusted to 65°C for at least 2 h and the dry mass (DM) was weighed. Relative water content [%] = (FM – DM)/FM ×100.

Determination of relative electrical conductivity (REC) was done according to the method of Yang *et al*. (1996). Fresh leaves were washed with distilled water and a 1-cm diameter leaf tissue was removed from the leaves with a punch while avoiding the main vein as much as possible. Then, 0.1 g of the sample was weighed into a test tube, 20 ml of distilled water was added, and after standing at 25°C for 4 h, the electrical initial conductivity measurement value C1 was measured. Then, it was bathed at 100°C for 30 min, and after cooling, a conductivity value C2 was obtained. The distilled water conductivity value C0 was measured. REC [%] = (C1 – C0)/(C2 – C0) ×100.

Active oxygen content measurement: The amount of H_2O_2 accumulated in this experiment was measured by diaminobenzidine (DAB) staining (Tian *et al*. 2013). The concentration of the DAB staining solution was 0.1 mg ml^{-1}, prepared using 50 mM Tris-acetate buffer, and then the pH was adjusted to 5.0. The fresh sample was immersed in the solution and placed under dark and room temperature for 24 h. It was then removed and placed in 95% (v/v) ethanol and boiled in water for 15 min. Finally, the leaves were placed on a white paper with tweezers, the surface of the leaves was wiped dry, and photographed with a camera. The amount of superoxide anion free radical ($O_2^{\bullet-}$) accumulated was measured by a reagent kit (*Shanghai Haling Biotechnology Co.*, *Ltd.*, China). Observation and photographing were carried out under a fluorescence microscope (*Axio Imager D2*, *ZEISS*, Germany), and an excitation wavelength of 540 nm, an emission wavelength of 590 nm, and an exposure time of 17.24 ms were set. Fluorescence signal intensity was collected using *ZEN* software (*ZEISS*, Germany).

Proline content and antioxidant enzyme activities: Determination of free proline (Pro) content and antioxidant enzyme activity, including superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (POD, EC 1.11.1.7), and ascorbate peroxidase (APX, EC 1.11.1.11), by UV spectrophotometry (*BioPhotometer*, *Eppendorf*, Germany) according to the instructions in the kit (*Suzhou Corning Biotechnology Co.*, *Ltd.*, China). First, 0.1 g of fresh sample was weighed, 1 ml of the extraction solution supplied from the kit was added, and the homogenate was ground in an ice bath. Then, it was centrifuged at 8,000 rpm for 10 min at 4°C and the supernatant was taken. Finally, the corresponding reagents and supernatants were added to the cuvette according to the instructions and measured with a spectrophotometer.

For SOD activity determination the absorbance values of the control tube and the measuring tube were measured by a spectrophotometer at 560 nm to be A0 and A, respectively. Percentage of inhibition P [%] = (A0 – A)/A0 × 100, SOD activity [U g^{-1}(FM)] = 114 × P/(1 – P). For POD activity determination the absorbance value A1 at 1 min and the absorbance value A2 at 2 min at 470 nm were measured with a spectrophotometer. Then ΔA = A2 – A1 and POD activity [U g^{-1}(FM)] = 20,000 ΔA. Absorbance

values A1 and A2 at 10 and 130 s for APX determination were measured at 290 nm using a spectrophotometer. Then $\Delta A = A1 - A2$ and APX activity [μmol min^{-1} g^{-1}(FM)] = 17.9 ΔA.

Photosynthetic characteristics and Chl fluorescence parameters: Photosynthetic parameters were measured using a *LI-6400* portable photosynthetic apparatus (*Li-Cor*, Lincoln, USA). Photosynthetic parameters include net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E). Measurements were performed in fine weather conditions from 8:00–9:00 h.

Chl fluorescence parameters were measured using the slow fluorescence induction curve (saturation pulse method) measurement procedure of the *PAM-2500* portable Chl fluorescence instrument (*Walz*, Germany). Fluorescence parameters include the minimal fluorescence yield of the dark-adapted state (F_0) and nonphotochemical quenching coefficient (q_N). To obtain sufficient dark adaptation of the blade, the measurement was performed after turning off the lamp for 1 h at night. The maximal quantum yield of PSII photochemistry (F_v/F_m) and the effective quantum yield of PSII photochemistry ($Y_{(II)}$) of PSII were calculated using the instrument's data processing software *PAM Win*.

Statistical analysis: All experiments were repeated three times and randomly arranged, with all data being the average of three replicates ± standard deviation. The variance was analyzed by the *SAS/STAT* statistical analysis package (*version 6.12*, *SAS Institute*, Cary, NC, USA).

Results

Plant phenotype and leaf water content: The application of exogenous calcium can effectively alleviate the water deficit caused by drought stress on *P. ostii*. Under drought stress, *P. ostii* leaves in the Control group began to sag on the 4th day and severely withered on the 12th day (Fig. 1). *P. ostii* leaves in the $CaCl_2$ treatment group only sagged on the 12th day, and no obvious leaf wilting was observed. In this experiment, the water content in the two groups of *P. ostii* leaves exhibited different degrees of reduction. On the 12th day, the relative water content in the Control and $CaCl_2$ treatment groups decreased to a minimum of 9.8 and 41.7%, respectively (Fig. 2). Under drought conditions, the relative water content in the plants treated with exogenous calcium was significantly higher than that in the Control. Especially on the 12th day, the leaf water content in the Control was 76.4% higher than that in the $CaCl_2$ treatment group. The results showed that the spraying of $CaCl_2$ alleviated the water deficit in *P. ostii* leaves.

Proline (Pro) content and relative electrical conductivity (REC): Pro is an important osmotic adjustment substance in plants, and its content changes under drought conditions. In this experiment, the amount of Pro accumulation in both $CaCl_2$-treated and Control leaves significantly increased. However, compared with the Control, the Pro accumulation in $CaCl_2$-treated leaves was relatively low, especially on the 8th day with a 1.65-fold difference in accumulation (Fig. 2).

The REC of the Control increased significantly with the onset of drought stress, which increased 2.53 times on the 12th day. The change in the REC value in the $CaCl_2$ treatment group was less obvious than that in the Control. On the 12th day, the REC value was 35.1%, which was only 1.46 times higher than that at the beginning of the experiment (Fig. 2). The above results indicated that under drought stress, plant cells showed different degrees of damage increased, resulting in the accumulation of Pro and the increase in REC. However, spraying appropriate concentrations of exogenous calcium could effectively alleviate this stress.

Reactive oxygen species (ROS) content: Subsequently, the accumulation of $O_2^{\bullet-}$ in the leaves under drought stress was determined using a fluorescent probe. The results of fluorescent probe experiments showed that the fluorescence signals in the Control and $CaCl_2$-treated plants were weak on day 0 of drought stress. As drought treatment continued, the fluorescence signals in both groups gradually became stronger, but the fluorescence intensity in the $CaCl_2$-treated plants was significantly weaker than that in the Control (Fig. 3*A*). In addition, the accumulation of H_2O_2 in the leaves was revealed by DAB staining, wherein the deeper the color of the leaves indicated a greater damage to the leaves. The results showed that the leaf color of the two groups of plants gradually deepened as drought treatment progressed. However, the leaf color of the $CaCl_2$-treated plants was always lighter than that of the Control, and the color difference reached a maximum on the 12th day (Fig. 3*B*). The results indicate that the application of $CaCl_2$ could alleviate the accumulation of ROS caused by

Fig. 1. Phenotypic changes of control and $CaCl_2$-treated *Paeonia ostii* under drought stress.

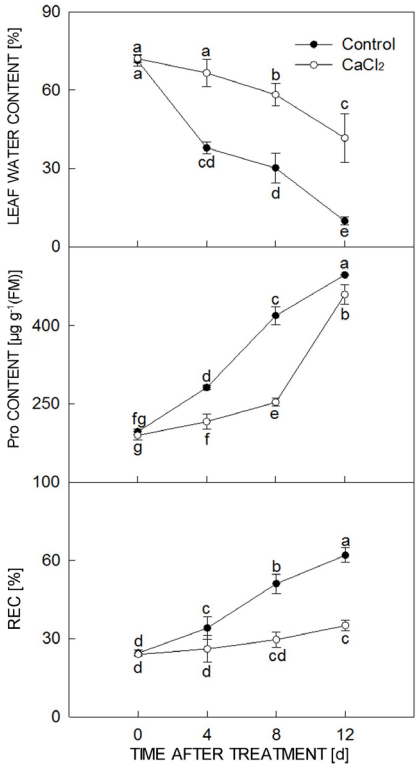

Fig. 2. Leaf water content, proline content, and relative electrical conductivity (REC) of $CaCl_2$-treated *Paeonia ostii* under drought stress. The values represent mean ± SD; *different letters* indicate significant differences according to *Duncan*'s multiple range test (P<0.05).

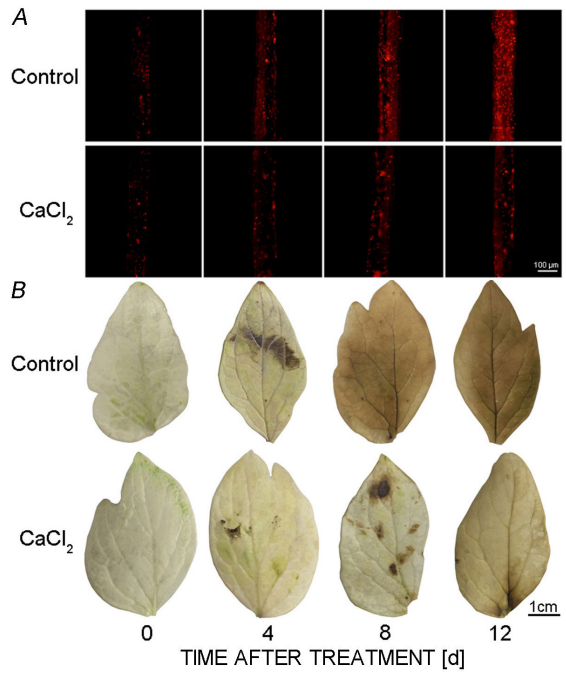

Fig. 3. Changes in reactive oxygen species accumulation in $CaCl_2$-treated *Paeonia ostii* under drought stress. $O_2^{\bullet-}$ accumulation detected by a fluorescence probe (*A*), H_2O_2 accumulation detected by diaminobenzidine staining (*B*).

drought stress and reduce the risk of ROS harming *P. ostii*.

Antioxidant enzyme activities: Corresponding to the results of ROS accumulation, exogenous calcium had a significant effect on the *P. ostii* antioxidant enzyme system under drought stress (Fig. 4). Under stress, the accumulation of ROS in plants can cause toxic effects on cells. APX, POD, SOD, and other protective enzymes can effectively scavenge reactive oxygen free radicals. In the present study, drought stress resulted in a significant increase in APX and POD activity in *P. ostii*. APX and POD activities in the plants treated with $CaCl_2$ increased compared with the Control during the 12-d period. Especially on the 12th day, APX and POD activities increased 1.26 and 1.15-fold compared with the Control, respectively. Drought stress first increased SOD activity, which began to decrease on the 4th day. The SOD activity in $CaCl_2$-treated *P. ostii* was also consistently higher than that in the Control, and its trend was consistent with the Control. Exogenous calcium could promote the antioxidant enzyme system to a certain extent and enhance the activity of antioxidant enzymes.

Photosynthetic characteristics: Drought stress affected the photosynthetic characteristics in *P. ostii* (Fig. 5). As the drought treatment continued, the P_N of $CaCl_2$-treated plants and the Control decreased significantly, but the P_N

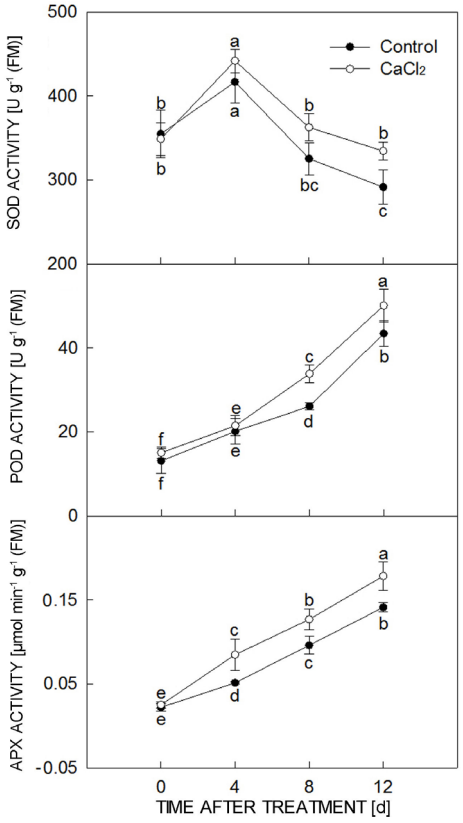

Fig. 4. Antioxidative enzyme activities in $CaCl_2$-treated *Paeonia ostii* under drought stress. The values represent mean ± SD; *different letters* indicate significant differences according to *Duncan*'s multiple range test (P<0.05). SOD – superoxide dismutase; POD – peroxidase; APX – ascorbate peroxidase.

of $CaCl_2$-treated plants was always higher than that of the Control. By the 12th day, the plants treated with $CaCl_2$ and the Control showed 0.82 and 0.20 μmol(CO_2) $m^{-2}s^{-1}$, with a decrease of 56.6 and 89.5%, respectively. The most direct effect of drought stress on plants is the selective closure of leaf stomata. With the prolongation of drought stress, the g_s of *P. ostii* leaves increased first and then decreased. On the 4th day of drought stress, the g_s reached the highest value, which was basically the same as the trend of P_N. E was consistent with g_s and also showed a trend of first increasing and then decreasing. Fig. 5 demonstrates that as the degree of drought stress increased, the C_i of *P. ostii* gradually decreased. As the degree of drought increased, the stomata closed, resulting in a decrease in C_i. Moreover, overall, the g_s, C_i, and E of the $CaCl_2$-treated plants were higher than those of the Control, and the trend was consistent with the Control.

Chl fluorescence parameters: Moreover, *P. ostii* Chl fluorescence parameters were also significantly affected by drought stress (Fig. 6). Under drought stress, the F_v/F_m and $Y_{(II)}$ of *P. ostii* showed a downward trend. F_v/F_m reflects the efficiency and potential activity of PSII conversion. The value was reduced; thus, the photochemical efficiency was lowered. $Y_{(II)}$ refers to the actual photochemical quantum efficiency of PSII and its numerical value indicates that the photoreaction center is destroyed. Simultaneously, the values for the plants treated with $CaCl_2$ were higher than those for the Control, 1.30 and 2.26 times, respectively. F_0, the initial fluorescence, indicates the level of fluorescence at the complete opening of the PSII reaction center. Drought treatment caused the increase of F_0 value for the Control plants. In contrast, the F_0 value for the $CaCl_2$-treated plants increased first and then decreased, and the change was not obvious. On the 12th day, the F_0 value for the Control was 1.20 times than that for the $CaCl_2$-treatment. In contrast regarding the nonphotochemical quenching coefficient, q_N, the value for the $CaCl_2$-treated plants increased continuously under drought stress, and the value for the Control plants first increased and then decreased on the 8th day. The difference between the two groups was the largest on the 12th day, and the value for the $CaCl_2$ treatment was 1.51 times that of the Control plants.

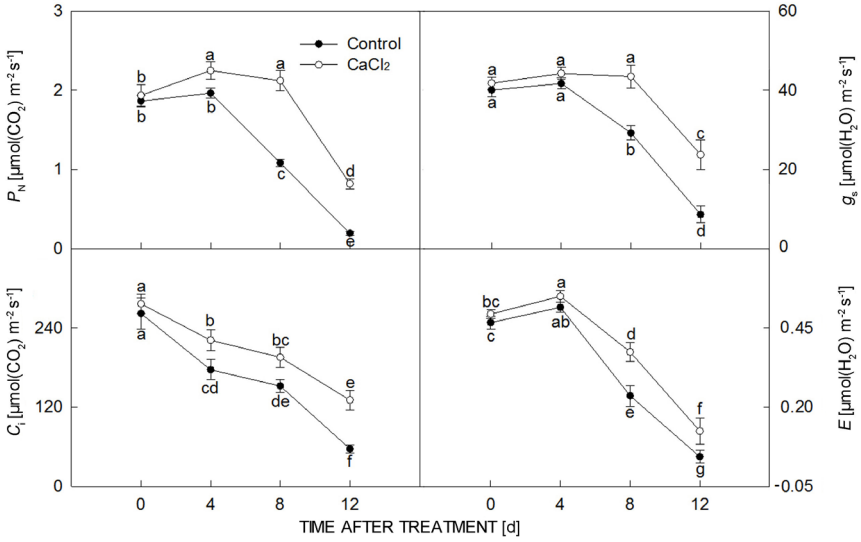

Fig. 5. Photosynthetic characteristics of $CaCl_2$-treated *Paeonia ostii* under drought stress. The values represent mean ± SD; *different letters* indicate significant differences according to *Duncan*'s multiple range test (P<0.05). P_N – net photosynthesis rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; E – transpiration rate.

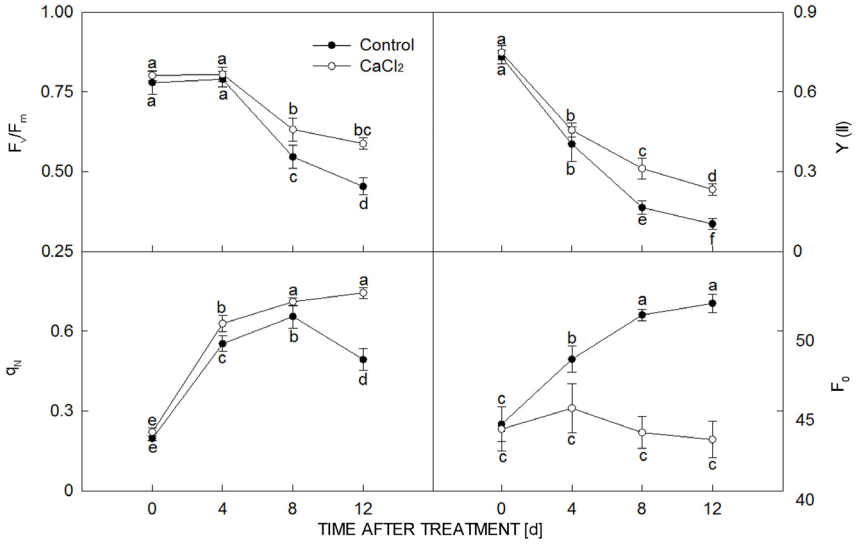

Fig. 6. Chlorophyll fluorescence parameters of $CaCl_2$-treated *Paeonia ostii* under drought stress. The values represent mean ± SD; *different letters* indicate significant differences according to *Duncan*'s multiple range test (P<0.05). F_v/F_m – maximal quantum yield of PSII photochemistry; $Y_{(II)}$ – effective quantum yield of PSII photochemistry; q_N – nonphotochemical quenching coefficient; F_0 – minimal fluorescence yield of the dark-adapted state.

Discussion

With global climate change, local temperatures increase, causing plants to encounter a variety of stresses from environmental changes in natural growth. Among them, drought is one of the main adverse factors that restricts plant growth and development. Various studies have shown that under drought stress, exogenous application of $CaCl_2$ has a protective effect on plants, which can alleviate plant damage caused by drought stress (Arshi *et al*. 2006, Shoresh *et al*. 2011). In this research, potted peony was used as material to study water content, electrical conductivity, Pro content, ROS accumulation, APX, CAT, and SOD protective enzyme activities, and photosynthetic and Chl fluorescence characteristics of peony leaves under drought stress. This study provides a theoretical reference for exploring practical measures to reduce peony drought stress.

In this study, drought stress caused severe sagging and wilting of *P. ostii* leaves: the leaves were basically withered on the 12th day. The $CaCl_2$-treated *P. ostii* plant only sagged on the 12th day, and there was no obvious wilting, indicating that $CaCl_2$ treatment could alleviate leaf damage caused by drought stress. In addition, $CaCl_2$ treatment effectively alleviated the water deficit caused by drought stress of *P. ostii* and reduced the speed of water loss. Khushboo (2018) and Xu *et al*. (2013) also found that $CaCl_2$ prevents cell dehydration damage by balancing cell penetration strength, which is consistent with the above research results.

Drought stress induces plants to produce large amounts of ROS, such as H_2O_2 and $O_2^{\bullet-}$, and excessive ROS content leads to membrane lipid peroxidation (Sarker and Oba 2018). Wang *et al*. (2009) and Issam *et al*. (2012) found that the treatment of $CaCl_2$ helps reduce ROS under stress conditions. Consistent with the above report, the accumulation of H_2O_2 and $O_2^{\bullet-}$ in *P. ostii* increased with the persistence of drought stress in this study, while the accumulation rate and concentrations of these two ROS substances were lower in $CaCl_2$-treated plants than that in the Control. In addition, lipid peroxidation caused by ROS can destroy cell membrane stability (Sarker and Oba 2018). The REC value for the $CaCl_2$-treated *P. ostii* was always lower than that of the Control. The REC value is related to cell membrane stability. The higher the value is, the more severe the cell membrane damage (Chen *et al.* 2017). Therefore, it can be considered that $CaCl_2$ can alleviate lipid peroxidation caused by ROS and enhance cell membrane stability. Pro is an important osmotic regulator in cells and is very sensitive to drought. Under drought stress, plants accumulate a large amount of Pro (Upadhyaya *et al*. 2008). In this experiment, the Pro content in $CaCl_2$-treated *P. ostii* plants was lower than that in the Control. This is different from the findings of Upadhyaya *et al*. (2008, 2011). This difference may be due to the fact that $CaCl_2$ treatment alleviates cell membrane damage, reduces REC, and reduces the osmotic regulation of Pro, resulting in a decrease in the Pro content.

To remove ROS, higher plants increase antioxidant enzyme activities, including SOD, POD, and APX, in order to enhance the role of the antioxidant defense system (Li *et al*. 2016). These antioxidant enzymes can scavenge some toxic compounds and reduce oxidative damage induced by ROS (Zhou *et al*. 2012, Kumar *et al*. 2013). In the present study, the activities of SOD, POD, and APX in $CaCl_2$-treated *P. ostii* plants increased under drought stress compared with the Control. Similarly, various studies have reported an increase in the activity of antioxidant enzymes in plants with externally supplemented $CaCl_2$ (Willekens *et al*. 1997, Wang *et al*. 2009, Upadhyaya *et al*. 2011). The results could also be mutually confirmed in the determination of ROS content, such as H_2O_2 and $O_2^{\bullet-}$. Increases in antioxidant enzymes, such as SOD, POD, and APX, inhibit the production of ROS, thereby protecting plants from oxidative damage. These results indicate that exogenous calcium can enhance the antioxidant defense system to reduce the ROS content induced by stressors, such as drought.

Studies have shown that photosynthesis may be restricted by stomatal and nonstomatal limitations under drought conditions. When g_s and C_i are simultaneously reduced, the decrease in P_N is mainly caused by stomatal limitations. In contrast, when P_N decreases as C_i increases, photosynthesis is mainly limited by nonstomatal factors (Li *et al*. 2017, Hu *et al*. 2018). In our study, P_N, g_s, and C_i decreased simultaneously on the 4th day in both groups, clearly indicating that the main cause of P_N reduction was stomata closure. Compared with the Control, the plants treated with $CaCl_2$ had higher P_N, g_s, and C_i, indicating that $CaCl_2$ treatment can improve these parameters of *P. ostii* under drought stress conditions. In addition, $CaCl_2$ has long been used in agricultural production as an antitranspiration agent. Hu *et al*. (2018) also showed that calcium controls the opening and closing of leaf stomata. In this study, $CaCl_2$ alleviated the rate of decline of E in leaves, while the decrease in E was associated with a decrease in g_s. These findings verified that exogenous calcium regulates the leaf transpiration rate through stomata to alleviate drought stress damage to *P. ostii*. Therefore, it can be considered that the effect of exogenous calcium on improving photosynthetic efficiency of *P. ostii* under drought stress is related to stomatal regulation.

Chl fluorescence parameters are often used to indicate the extent of damage to the photosynthetic apparatus (Niu *et al*. 2008). In the present study, $CaCl_2$-treated *P. ostii* F_0 was significantly reduced relative to that of the Control after 12 d. It is indicated that exogenous calcium can alleviate the damage to the PSII reaction center under drought stress to some extent. In addition, the F_v/F_m, $Y_{(II)}$, and q_N of $CaCl_2$-treated *P. ostii* increased significantly after 8 d of drought stress. This finding is consistent with the findings of Li (2018) and Wei *et al.* (2015), suggesting that exogenous calcium can partially alleviate the closure of the PSII reaction center and increase the utilization of light energy.

In conclusion, this study demonstrated that exogenous calcium conferred tolerance to drought stress in *P. ostii*. Under drought stress, $CaCl_2$ could reduce its harmful effects on the growth of *P. ostii* by regulating the infiltration, activating photosynthesis, and enhancing the antioxidant

system. These results revealed a key role of calcium chloride in the relief of drought stress and indicated that it can be used to manage drought in *P. ostii* cultivation.

References

Anderegg W.R.L., Kane J.M., Anderegg L.D.L.: Consequences of widespread tree mortality triggered by drought and temperature stress. – Nat. Clim. Change **3**: 30-36, 2012.

Arshi A., Abdin M.Z., Iqbal M.: Effect of $CaCl_2$ on growth performance, photosynthetic efficiency and nitrogen assimilation of *Cichorium intybus* L. grown under NaCl stress. – Acta Physiol. Plant. **28**: 137-147, 2006.

Chen X.M., Qiu L.L., Guo H.P. *et al*.: Spermidine induces physiological and biochemical changes in southern highbush blueberry under drought stress. – Braz. J. Bot. **40**: 841-851, 2017.

Cousson A.: Involvement of phospholipase C-independent calcium-mediated abscisic acid signaling during *Arabidopsis* response to drought. – Biol. Plantarum **53**: 53-62, 2009.

Du S.B.: [The physiological and biochemical effects on *Paeonia ostii* under waterlogging stress and restoration.] Pp. 5-6. East China Normal University, Shanghai 2016. [In Chinese]

El-Beltagi H.S., Mohamed H.I.: Alleviation of cadmium toxicity in *Pisum sativum* L. seedlings by calcium chloride. – Not. Bot. Horti. Agrobo. **41**: 157-168, 2013.

Engelbrecht B.M.J., Comita L.S., Condit R. *et al*.: Drought sensitivity shapes species distribution patterns in tropical forests. – Nature **447**: 80-82, 2007.

Han C.J., Wang Q., Zhang H.B. *et al*.: Light shading improves the yield and quality of seed in oil-seed peony (*Paeonia ostii* Feng Dan). – J. Integr. Agr. **17**: 1631-1640, 2018.

Hojati M., Modarres-Sanavy S.A.M., Ghanati F., Panahi M.: Hexaconazole induces antioxidant protection and apigenin-7-glucoside accumulation in *Matricaria chamomilla* plants subjected to drought stress. – J. Plant Physiol. **168**: 782-791, 2011.

Hu W., Tian S.B., Di Q. *et al*.: Effects of exogenous calcium on mesophyll cell ultrastructure, gas exchange, and photosystem II in tobacco (*Nicotiana tabacum* Linn.) under drought stress. – Photosynthetica **56**: 1204-1211, 2018.

Ingram J., Bartels D.: The molecular basis of dehydration tolerance in plants. – Annu. Rev. Plant Phys. **47**: 377-403, 1996.

Issam N., Kawther M., Haythem M., Moez J.: Effects of $CaCl_2$ pretreatment on antioxidant enzyme and leaf lipid content of faba bean (*Vicia faba* L.) seedlings under cadmium stress. – Plant Growth Regul. **68**: 37-47, 2012.

Jiang Y.W., Huang B.R.: Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. – J. Exp. Bot. **52**: 341-349, 2001.

Khushboo, Bhardwaj K., Singh P. *et al*.: Exogenous application of calcium chloride in wheat genotypes alleviates negative effect of drought stress by modulating antioxidant machinery and enhanced osmolyte accumulation. – In Vitro Cell. Dev.-Pl. **54**: 495-507, 2018.

Kumar D., Yusuf M.A., Singh P. *et al*.: Modulation of antioxidant machinery in α-tocopherol enriched transgenic *Brassica juncea* plants tolerant to abiotic stress condition. – Protoplasma **250**: 1079-1089, 2013.

Lawlor D.W.: Limitations to photosynthesis in water stressed leaves: Stomata *vs*. metabolism and the role of ATP. – Ann. Bot.-London **89**: 871-885, 2002.

Li J., Kong X.S., Li J.H. *et al*.: [Effect of gradual drought stress on physiological indexes of *Paeonia suffruticosa* Andr.] – Northern Horticulture: 50-53, 2014. [In Chinese]

Li P., Zhao C., Zhang Y. *et al*.: Calcium alleviates cadmium-induced inhibition on root growth by maintaining auxin homeostasis in *Arabidopsis* seedlings. – Protoplasma **253**: 185-200, 2016.

Li Z., Tan X.F., Lu K. *et al*.: The effect of $CaCl_2$ on calcium content, photosynthesis, and chlorophyll fluorescence of tung tree seedlings under drought conditions. – Photosynthetica **55**: 553-560, 2017.

Ma R., Zhang M., Li B. *et al*.: The effects of exogenous Ca^{2+} on endogenous polyamine levels and drought resistant traits of spring wheat grown under arid conditions. – J. Arid Environ. **63**: 177-190, 2005.

Mao Y., Han J., Tian F. *et al*.: Chemical composition analysis, sensory, and feasibility study of tree peony seed. – J. Food Sci. **82**: 553-561, 2017.

Niu G.H., Rodriguez D.S., Mackay W.: Growth and physiological responses to drought stress in four oleander clones. – J. Am. Soc. Hortic. Sci. **133**: 188-196, 2008.

Niu G.H., Rodriguez D.S.: Growth and physiological responses of four rose rootstocks to drought stress. – J. Am. Soc. Hortic. Sci. **134**: 202-209, 2009.

Pallavi S., Rama D.: Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. – Plant Growth Regul. **46**: 209-221, 2005.

Ren X.X., Xue J.Q., Wang S.L. *et al*.: Proteomic analysis of tree peony (*Paeonia ostii* 'Feng Dan') seed germination affected by low temperature. – J. Plant Physiol. **224**: 56-67, 2018.

Sarker U., Oba S.: Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in *Amaranthus tricolor*. – Appl. Biochem. Biotech. **186**: 999-1016, 2018.

Sekmen A.H., Ozgur R., Uzilday B., Turkan I.: Reactive oxygen species scavenging capacities of cotton (*Gossypium hirsutum*) cultivars under combined drought and heat induced oxidative stress. – Environ. Exp. Bot. **99**: 141-149, 2014.

Shah F.A., Ren Y., Yuan Y.J. *et al*.: Effect of plant age and geographical location on active paeonol and paeoniflorin accumulation in the roots of *Paeonia ostii*. – Pak. J. Bot. **50**: 1785-1790, 2018.

Shoresh M., Spivak M., Bernstein N.: Involvement of calcium-mediated effects on ROS metabolism in the regulation of growth improvement under salinity. – Free Radical Bio. Med. **51**: 1221-1234, 2011.

Soares-Cordeiro A.S., Carmo-Silva A.E., Bernardes da Silva A. *et al*.: Effects of rapidly imposed water deficit on photosynthetic parameters of three C_4 grasses. – Photosynthetica **47**: 304-308, 2009.

Sperry J.S., Adler F.R., Campbell G.S., Comstock J.P.: Limitation of plant water use by rhizosphere and xylem conductance: results from a model. – Plant Cell Environ. **21**: 347-359, 1998.

Tian F.X., Gong J.F., Zhang J. *et al*.: Enhanced stability of thylakoid membrane proteins and antioxidant competence contribute to drought stress resistance in the *tasg1* wheat stay-green mutant. – J. Exp. Bot. **64**: 1509-1520, 2013.

Upadhyaya H., Panda S.K., Dutta B.K.: Variation of physiological and antioxidative responses in tea cultivars subjected to elevated water stress followed by rehydration recovery. – Acta Physiol. Plant. **30**: 457-468, 2008.

Upadhyaya H., Panda S.K., Dutta B.K.: $CaCl_2$ improves post-drought recovery potential in *Camellia sinensis* (L) O. Kuntze. – Plant Cell. Rep. **30**: 495-503, 2011.

Wahid A., Gelani S., Ashraf M., Foolad M.R.: Heat tolerance in plants: An overview. – Environ. Exp. Bot. **61**: 119-123, 2007.

Wang Y., Yang Z.M., Zhang Q.F., Li J.L.: Enhanced chilling tolerance in *Zoysia matrella* by pre-treatment with salicylic

acid, calcium chloride, hydrogen peroxide or 6-benzylaminopurine. – Biol. Plantarum **53**: 179-182, 2009.

Wei R., Liu Y., Sui Y. *et al*.: Effects of Ca^{2+} and polyethylene glycol on the chlorophyll fluorescence parameters of transgenic *OsCaS* rice (*Oryza sativa* L.). – Photosynthetica **53**: 336-341, 2015.

Willekens H., Chamnongpol S., Davey M. *et al*.: Catalase is a sink for H_2O_2 and is indispensable for stress defense in C_3 plants. – EMBO J. **16**: 4806-4816, 1997.

Xu C.B., Li X.M., Zhang L.H.: The effect of calcium chloride on growth, photosynthesis, and antioxidant responses of *Zoysia japonica* under drought conditions. – PLoS ONE **8**: e68214, 2013.

Yang G.P., Rhodes D., Joly R.J.: Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine-containing maize lines. – Aust. J. Plant Physiol. **23**: 437-443, 1996.

Yao X.Q., Chu J.Z., Wang G.Y.: Effects of drought stress and selenium supply on growth and physiological characteristics of wheat seedlings. – Acta Physiol. Plant. **31**: 1031-1036, 2009.

Yin D.C., Qi J.Y., Deng X. *et al*.: [Effects of exogenous calcium on the metabolic system of *Pinus sylvestris* var. *mongolica* under drought stress.] – J. Shenyang Agr. Univ. **49**: 559-565, 2018. [In Chinese]

Zhou S., Hu W., Deng X. *et al*.: Overexpression of the wheat aquaporin gene, *TaAQP7*, enhances drought tolerance in transgenic tobacco. – PLoS ONE **7**: e52439, 2012.