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The effect of $CaCl_2$ on calcium content, photosynthesis, and chlorophyll fluorescence of tung tree seedlings under drought conditions

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Abstract

The study investigated the effects of different $CaCl_2$ concentrations (2, 5, and 10 mM) on photosynthetic enzymatic activities, photosynthesis, and chlorophyll fluorescence of tung tree seedlings under drought conditions. Plants were sprayed with either $CaCl_2$ or distilled water until run-off. Irrigation was then withheld to induce drought stress. The strength of drought stress was evaluated by relative leaf water content and soil water content, which was 27.3 and 9.5% on day 0 and day 12, respectively. Drought stress decreased activities of ribulose-1,5-bisphosphate carboxylase/oxygenase and phospho*enol*pyruvate carboxylase, chlorophyll (*a*+*b*) content, net photosynthetic rate, stomatal conductance, transpiration rate, electron transport rate, the maximal quantum yield of PSII photochemistry, and effective quantum yield of PSII in tung tree seedlings. The $CaCl_2$ pretreatments alleviated the negative effect of drought stress to some degree on all the parameters mentioned above.

Additional key words: calcium chloride; gas exchange; *Vernicia fordii*, water stress.

Introduction

Drought is a major environmental factor that limits productivity, distribution, and survivability of plants (Engelbrecht *et al*. 2007). In recent years, along with global climate change, the frequency, duration and severity of drought has increased in many regions of the world (*e.g.*, Anderegg *et al*. 2012, Flato *et al.* 2013). Plants experiencing drought stress often exhibit serious physiological and biochemical dysfunctions including reduced photosynthesis, decreased transpiration rate (*E*), generation of reactive oxygen species, and damage to various cellular structures (Sperry *et al*. 1998, Lawlor 2002, Ramachandra *et al.* 2004, Xu *et al*. 2008, Niu and Rodriguez 2009, Soares-Cordeiro *et al*. 2009). However, plants adopt different strategies in order to adapt to drought conditions. For example, stomata closure is considered a primary mechanism for regulating water content when plants are under drought conditions (de Souza *et al*. 2005, Kowitcharoen *et al*. 2015, Zhao *et al*. 2015). Similarly, endogenous abscisic acid and ascorbic acid concentrations in leaves and shoots increase in response to drought stress (Synková and Valcke 2001, Kowitcharoen *et al*. 2015). Many authors reported that drought stress causes a decrease in the chlorophyll (Chl) content, maximal quantum yield of PSII photochemistry (F_v/F_m), and electron transport rate (ETR), but increases dark respiration in wheat (Ahmed *et al*. 2002, Nikolaeva *et al*. 2010, Akhkha *et al*. 2011, Santos and Silva 2015).

Plant biologists have used various chemicals, such as calcium chloride (Upadhyaya *et al*. 2011, Xu *et al*. 2013), plant growth regulators (Shan *et al*. 2010, Hojati *et al*. 2011), and other substances (Zhao *et al*. 2007, Ishibashi *et al*. 2011) to alleviate the harmful effects of drought stress.

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Abbreviations: C_i – intercellular CO_2 concentration; Chl – chlorophyll; DM – dry mass; DAT – day of treatment; *E* – transpiration rate; ETR – electron transport rate; FM – fresh mass; F_0 – minimal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; PEPC – phospho*enol*pyruvate carboxylase; P_N – net photosynthetic rate; RWC – relative leaf water content; SPAD values – corresponding to content of chlorophyll (*a*+*b*); SWC – soil water content; Φ_{PSII} – effective quantum yield of PSII.
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Several studies have shown that calcium is involved in the regulation of plant responses under adverse environmental conditions related to drought (Ma *et al*. 2005, Shao *et al*. 2008, Xu *et al*. 2013), heat (Bamberg *et al*. 1998, Wang and Li 2006, Bhattacharjee 2008), salt (Arshi *et al*. 2006, Amor *et al*. 2010), and heavy metals (Antosiewicz and Hennig 2004, Siddiqui *et al*. 2011). Previous studies indicated calcium appears to play an important role in plant defense mechanisms against drought stress. Cousson (2009) revealed Ca^{2+}-signaling was required for the acquisition of drought resistance in *Arabidopsis.* Xu *et al*. (2013) reported calcium-induced drought tolerance in *Zoysia japonica* and increased biomass, Chl content, net photosynthetic rate (P_N), and antioxidant enzyme activities.

Tung tree (*Vernicia fordii* Hemsley) is a member of the Euphorbiaceae family, a native tree species which has been cultivated for more than 1,000 years in China. Along with oil-tea tree (*Camellia oleifera*), walnut (*Juglans regia*), and Chinese tallow tree (*Sapium sebiferu*m), it is one of the four major woody oil trees in China (Tan *et al*. 2011). Tung oil, which is extracted from tung seeds, exhibits traits that are highly valued by the industry. Tung oil has fast drying properties, is lightweight, and has excellent adhesion and glossiness. Additionally, tung oil is resistant to heat, acids, alkalines, and cracking as a result of frost. It is resistant to many chemical treatments, and – unlike other drying oils – it does not darken with age. These properties make tung oil a valuable drying ingredient of paints, varnishes, and other coatings and finishes (Cao and Shockey 2012). Tung tree is a promising biomass species for resolving energy shortage problems (Tan *et al*. 2011). However, various studies indicate water-use efficiency, Chl content and production efficiency decrease in tung tree when subjected to abiotic stresses, especially drought stress (Zhou *et al*. 2012, Li and Zhu 2014). Thus, the growth and yield of tung tree are affected by drought stress. However, only a few studies have investigated the effects of $CaCl_2$ on the physiology of tung tree under drought.

In this experiment, we studied the effects of exogenous calcium on physiology and growth of one-year-old tung tree seedlings under drought conditions. Our primary objective was to explore the mechanism behind Ca^{2+}-induced alleviation of drought stress on the photosynthetic process in tung tree. For this purpose, we measured photosynthesis as well as the Chl fluorescence parameters and Chl content by SPAD value of tung tree.

Materials and methods

Plant materials and treatments: Tung tree seedlings (*Vernicia fordii* Hemsley) were cultivated in plastic containers (15 cm × 15 cm) containing wet sand under natural conditions at Central South University of Forestry and Technology, Changsha, China. When the seedlings formed a true leaf, they were transplanted to (15 cm × 15 cm) plastic pots filled with a mixture of commercial soil and sand (1:1 ratio) in a nonopaque rain-shelter greenhouse for 1 month before starting the experiment. During the acclimation period, all seedlings were watered 3–4 times per week and fertilized once a week with Hoagland's solution. Once the experiment began, fertilization was stopped.

A total of 72 tung tree seedlings were used in the experiment, which were randomly divided into four groups; each treatment group consisted of three replicates of 18 plants. Aqueous 2, 5, 10 mM $CaCl_2$ solutions (*AR*, *SCR*, Shanghai, China) were sprayed on the leaves of three groups until run-off twice a day for 3 d. The control plants were similarly sprayed with distilled water to run-off. Drought was induced by withholding water for 12 d after $CaCl_2$ application (DAT). Thus, the treatments were as follows:

Treatment	Specification
Control	No $CaCl_2$ and drought
2 mM	2 mM $CaCl_2$ pretreatment and drought
5 mM	5 mM $CaCl_2$ pretreatment and drought
10 mM	10 mM $CaCl_2$ pretreatment and drought.

On the 0, 4, 8, 12 DAT, photosynthesis and Chl fluorescence parameters were measured. Immediately, after photosynthesis measurements, the leaves were cut, weighed, wrapped up in tin foil, frozen in liquid nitrogen, and stored at –80°C until analysis. The collected leaf sections were used for measurements of enzyme activities.

Relative leaf water content and soil water content: The relative leaf water content (RWC) was determined at each leaf developmental stage according to the following equation: RWC = [(FM – DM)/(SM – DM)] × 100%. An analytical balance (0.1 mg precision) (*CP522C*, *OHAUS*, Shanghai, China) was used to record the fresh mass (FM), saturated mass (SM), and dry mass (DM) of leaves. The saturated mass was obtained after 24 h of leaf immersion in distilled water in the dark. The DM was obtained after drying leaves in an oven (*DHG-9202*, *SANFA*, Shanghai, China) for 48 h at 60°C. Soil water content (SWC) was determined after the treatment period according to the following equation: SWC = [(soil wet mass) – (soil dry mass)]/(soil wet mass) × 100%. After the treatment period was complete, the soil wet mass was weighed immediately. The soil DM was obtained after drying soil in an oven (*DHG-9202*, *SANFA*, Shanghai, China) for 48 h at 80°C.

Determination of Ca^{2+} content and relative Chl content: The leaves were dried in an oven at 105°C for 30 min and ground after drying at 65°C to a constant mass. The content of Ca^{2+} in leaves of tung tree was measured according to the method described in Chinese National

Standard LY/T 1270–1999. Ca^{2+} was measured by atomic absorption spectrometry (*TAS-990*, *PGENERAL*, Beijing, China). Relative Chl content was measured using a SPAD meter (*SPAD-502*, *Minolta*, Tokyo, Japan).

Enzyme extraction and assays: Fresh leaves (0.5 g) were ground with 3 mL of ice-cold 100 mM Tris-HCl buffer (pH 8.2) (*T8230*, *Solarbio*, Beijing, China) containing 5% glycerin, 1 mM EDTA (*Amresco0105*, *Solarbio*, Beijing, China), 7 mM mercaptoethanol, and 1% PVP (*K-30*, *Solarbio*, Beijing, China). The homogenates were centrifuged at 4°C for 20 min at 15,000 × g and the supernatant was stored at 4°C for enzyme activity assay. The carbon assimilatory enzymes Rubisco (EC 4.1.1.39) and phospho*enol*pyruvate carboxylase (PEPC, EC 4.1.1.31) were assayed according to Siegel (1975) and Shi *et al*. (1979), respectively. The absorbance was measured at 340 nm by spectrophotometer (*UV 9100 D*, *LabTech*, Beijing, China) and activity was expressed as μmol(CO_2) min^{-1} g^{-1}(FM).

Leaf gas exchange was measured on the uppermost fully expanded leaves using a *LI-6400XT* portable photosynthesis system (*LI-COR*, *Lincoln*, NE, USA) during midday (9:00–11:00 h) at each measurement period (one leaf per plant; six plants per replicate). Light was provided by LEDs emitting in the blue-red light spectrum with a constant saturating light intensity of 1,500 μmol(photon) m^{-2} s^{-1} and an ambient CO_2 concentration of 400 μmol mol^{-1}. The temperature of the leaf cuvette was 30°C and the relative humidity was 70%.

Measurement of Chl fluorescence: Fluorescence parameters were measured with *LI-6400-40LCF* (*LI-COR*, *Lincoln*, NE, USA). Three leaves of each treatment were dark-adapted for 30 min prior to the fluorescence measurements. The minimal Chl fluorescence (F_0) level was measured after applying a far-red pulse for 6 s and the maximal fluorescence (F_m) was registered after applying a 0.8 s saturating flash. Maximal photochemical efficiency of PSII (F_v/F_m) was expressed as: $F_v/F_m = (F_m - F_0)/F_m$ (Genty *et al*. 1989).

Statistical analysis: All data were obtained from three replicates and the results presented were the mean values. All data were subjected to one way analysis of variance (*ANOVA*) with *SPSS 17.0* software. When the main effect was a significant, *Duncan*'s multiple range test was performed at the 0.05 level of significance.

Results

Leaf relative water content and soil water content: The RWC values showed significant reduction in control groups at 8 and 12 DAT (Fig. 1*A*). Compared with the control, at 12 DAT, leaf RWC of plants pretreated with 5 mM $CaCl_2$ significantly increased by 11.4%; however, no significant differences were found between concentrations of 2, 5, and 10 mM $CaCl_2$ during the whole experiment (Fig. 1*A*). The SWC decreased significantly, as expected, as drought was prolonged, and there was no difference observed between the $CaCl_2$ pretreatment group and the control (Fig. 1*B*).

Ca^{2+} content and relative Chl content: Application of $CaCl_2$ had no significant advantage for Ca^{2+} accumulation compared with the control at the beginning of drought stress, while the $CaCl_2$ pretreatment resulted in an increase in Ca^{2+} content at 4 DAT (Fig. 2*A*). At 8 and 12 DAT, the plants pretreated with $CaCl_2$ demonstrated similar Ca^{2+}

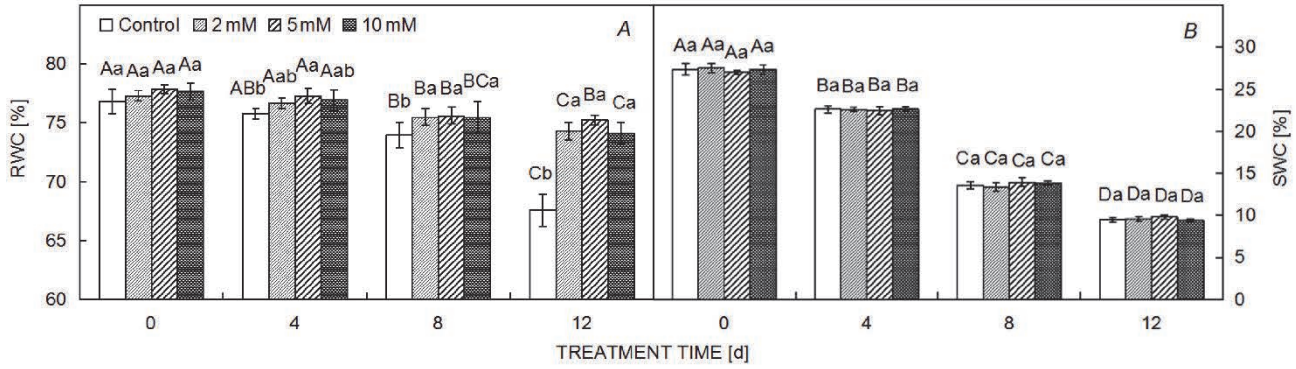

Fig. 1. Effects of $CaCl_2$ on RWC (*A*) in tung tree seedlings and SWC (*B*) under drought stress. (*1*) No $CaCl_2$ and drought; control, (*2*) 2 mM $CaCl_2$ pretreatment and drought; 2 mM, (*3*) 5 mM $CaCl_2$ pretreatment and drought; 5 mM, and (*4*) 10 mM $CaCl_2$ pretreatment and drought; 10 mM. The data in the figure represent mean ± SE ($n = 3$). Different *capital letters* indicate statistical differences between drought treatment stages, and *lowercase letters* indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to *Duncan*'s multiple range test (DMRT).

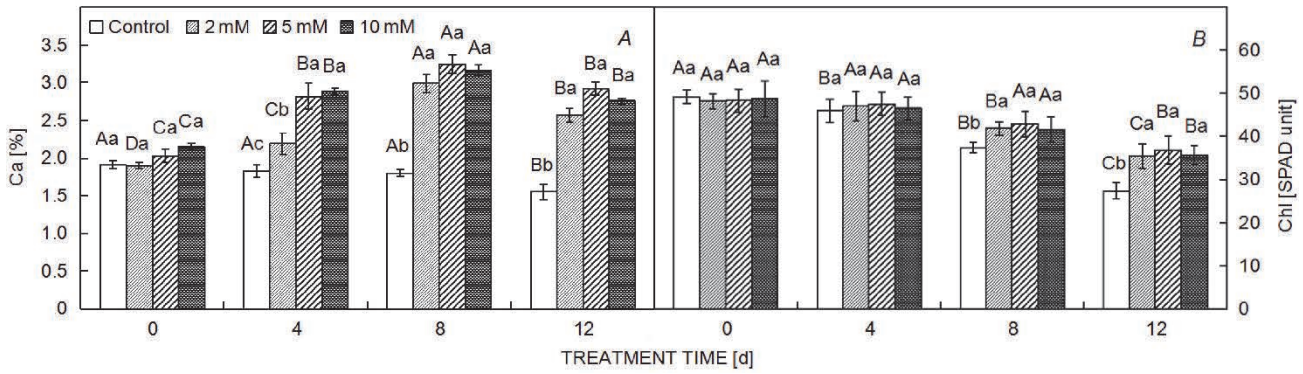

Fig. 2. Effects of $CaCl_2$ on Ca content, Chl SPAD value in leaves of tung tree seedlings under drought stress. (*1*) No $CaCl_2$ and drought; control, (*2*) 2 mM $CaCl_2$ pretreatment and drought; 2 mM, (*3*) 5 mM $CaCl_2$ pretreatment and drought; 5 mM, and (*4*) 10 mM $CaCl_2$ pretreatment and drought; 10 mM. The data in the figure represent mean ± SE (n = 3). Different *capital letters* indicate statistical differences between drought treatment stages, and *lowercase* letters indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to *Duncan*'s multiple range test (DMRT).

contents, which were 80 to 88% higher, respectively, than that of the control. The SPAD value in all pretreatments decreased as the drought stress persisted (Fig. 2*B*). However, the $CaCl_2$ pretreatment alleviated the loss of Chl induced by water stress. Compared with the stressed control, SPAD values significantly increased in the $CaCl_2$-pretreated plants at 8 and 12 DAT. Meanwhile, no significant differences in SPAD values were observed in $CaCl_2$ treatments and stressed control at 4 DAT.

Rubisco and PEPC: The drought stress decreased the activity of both Rubisco and PEPC enzymes and this effect became more pronounced with time (Fig. 3). The plants in all groups pretreated with $CaCl_2$ overcame the drought-induced reduction in activities of the CO_2-assimilatory enzymes, which was especially evident at 8 and 12 DAT. Compared with the control, Rubisco and PEPC activities of the tung tree leaves increased in the $CaCl_2$-pretreated plants at 8 and 12 DAT.

Leaf gas exchange: The P_N, g_s, and E of all treatments and the control decreased as the drought stress continued. However, the C_i of the control first decreased and then increased, while the $CaCl_2$-pretreated plants exhibited no obvious changes except for 8 DAT (Fig. 4). At 0 DAT, there was no significant difference between the $CaCl_2$ pretreatment and control in P_N and E, while the plants pretreated with 5 mM $CaCl_2$ showed a dramatic increase in g_s compared with the control and other treatments. Meanwhile, g_s of the $CaCl_2$-pretreated plants was significantly higher than that of the control at 4 DAT and g_s of plants pretreated with 5 mM $CaCl_2$ was higher than the control at 8 and 12 DAT. Plants pretreated with 2 mM $CaCl_2$ did not show significant differences in g_s compared with the control.

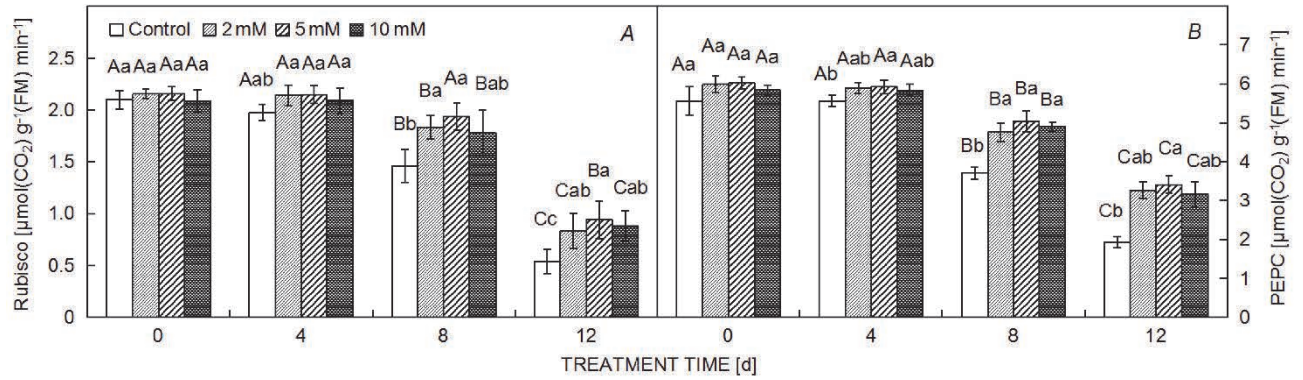

Fig. 3. Effects of $CaCl_2$ on enzymatic activity of Rubisco and phospho*enol*pyruvate carboxylase (PEPC) in tung tree seedlings under drought stress. (*1*) No $CaCl_2$ and drought; control, (*2*) 2 mM $CaCl_2$ pretreatment and drought; 2 mM, (*3*) 5 mM $CaCl_2$ pretreatment and drought; 5 mM, and (*4*) 10 mM $CaCl_2$ pretreatment and drought; 10 mM. The data in the figure represent mean ± SE (n = 3). Different *capital letters* indicate statistical differences between drought treatment stages, and *lowercase letters* indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to *Duncan*'s multiple range test (DMRT).

Chl fluorescence: Drought stress increased the F_0 in tung tree leaves 8 and 12 DAT after withholding irrigation (Fig. 5*A*). However, F_v/F_m, Φ_{PSII}, and ETR of plants without $CaCl_2$ pretreatment decreased gradually with time under drought stress (Fig. 5*B–D*). No difference was detected between $CaCl_2$ pretreatment and control for F_v/F_m and Φ_{PSII} at 0 and 4 DAT (Fig. 5*B*,*C*). In addition, 5 mM $CaCl_2$ pretreatment increased ETR compared with the control group. Drought stress markedly reduced both F_v/F_m and Φ_{PSII} at 12 DAT, and the values of F_v/F_m and Φ_{PSII} in plants with $CaCl_2$ pretreatment, except for those at 2 mM $CaCl_2$, were higher than that of the control.

Discussion

RWC is an appropriate estimate of plant water status in terms of cellular hydration under drought conditions. RWC started to decrease at 4 DAT, but there was no statistical difference until 8 DAT, while SWC decreased by 49.9 and 64.9% on 4 and 8 DAT, respectively, compared to that on 0 DAT. On 8 DAT, leaf RWC in the plants with $CaCl_2$ pretreatment was 2.2% higher than that of the control, indicating ameliorative effect of the $CaCl_2$ pretreatment. With the proceeding stress, SWC decreased by 9.6%, RWC in the plants pretreated with $CaCl_2$ was 12.0% higher than the control at 12 DAT, but was 2.9 % lower than that on 0 DAT. The results indicated that RWC of tung tree seedlings improved by the $CaCl_2$ pretreatment under drought stress.

Calcium is involved in the regulatory mechanisms that help plants adjust to adverse environmental conditions and play an important role in maintaining the stability of cell membrane phospholipids and proteins (Ma *et al*. 2005, Kim *et al*. 2009, Upadhyaya *et al*. 2011). In this study, Chl SPAD values at 8 and 12 DAT were reduced by drought because of the destruction of pigments and the instability of the pigment-protein complexes (Levitt 1980, Xu *et al*. 2013); however, exogenous $CaCl_2$ application alleviated the drought effect (Fig. *2B*). This result is similar to observations found in zoysiagrass (*Zoysia japonica*), where Chl content was enhanced by $CaCl_2$ pretreatment under drought stress (Xu *et al*. 2013). At 4 DAT, the P_N and g_s with $CaCl_2$ pretreatment were higher than the control, while the carboxylation of both Rubisco and PEPC did not increase compared with the control until 8 and 12 DAT. The main reason was that drought stress led to a slight stomatal closure at 4 DAT, which decreased the P_N. Along with the increase of drought stress, both Rubisco and PEPC increased in the plants pretreated with $CaCl_2$ at 8 and 12 DAT compared with the control. The results indicated that Ca^{2+} could alleviate stress-induced damages and increase photosynthetic performance in tung tree. One reason might be that the Ca^{2+} treatment prevented the dehydration damage of cellular structure by maintaining the osmotic strength of the cytoplasm in plants (Gzik 1996, Arshi *et al*. 2006, Yang *et al*. 2016).

Many studies have indicated that $CaCl_2$ could act as a physiological treatment to increase plant drought tolerance (Tan *et al*. 2011, Xu *et al*. 2013, Yang *et al*. 2016). Barbosa *et al*. (2015) reported that the carboxylation of Rubisco and PEPC were the main limiting factors for photosynthesis under drought stress. Synková *et al*. (2006) reported that reduction of P_N was caused by the decrease of Rubisco activity and stomata closure. The results of our study showed that the carboxylation activity of both Rubisco and PEPC was significantly reduced at 8 to 12 DAT (Fig. 3). Drought stress significantly reduced P_N and E in the control group, whereas exogenous $CaCl_2$ application diminished this decrease (Fig. 4*A*,*D*). The change of g_s in the plants pretreated with $CaCl_2$ exhibited a similar trend, but g_s significantly decreased after 4 DAT. However, exogenous $CaCl_2$ application reduced the decrease (Fig. 4*B*).

Decreasing C_i and the inhibition of Rubisco enzyme activity and ATP synthesis lead to a decrease of P_N (Dulai *et al*. 2006). The present investigation indicated that the $CaCl_2$ pretreatment could improve the P_N of tung tree under drought stress conditions. Many studies have indicated that $CaCl_2$ could increase plant tolerance, especially to drought stress (Ma *et al*. 2005, Xu *et al*. 2013). Ca^{2+} may regulate stomata movement, decrease respiration intensity, and activate the NAD kinase, prompting NADP production processes (Jones *et al*. 1967). Under a water deficit, there are stomatal and nonstomatal limitations of photosynthesis; when g_s and C_i decrease at the same time, the P_N decline is mainly caused by stomatal limitation. If g_s decreases while C_i increases, the photosynthesis is limited mainly by nonstomatal factors (Farquhar and Sharkey 1982). The simultaneous decline of P_N, g_s, and C_i in the control group at 4 DAT clearly indicated that the stomata closure was the main factor responsible for reduction in the P_N. Later on, after 12 DAT under drought, the rise in C_i let us hypothesize that the drop in P_N was mainly due to nonstomatal factors, such as a reduction of CO_2-assimilatory enzyme activity. We found that at the beginning of drought stress, 5 mM $CaCl_2$-treated leaves had higher g_s than those of other treatments, but P_N and E did not increase significantly. The increased g_s in this study by the $CaCl_2$ pretreatment may be due to opening of stomatal aperture as reported by Allen *et al*. (2001).

Chl fluorescence is an indicator of PSII functioning and electron transfer from PSII to PSI and has been used frequently to estimate damage to the photosynthetic system (Niu *et al*. 2008). An increase in F_0 was observed on 8 DAT only for the control, and the F_0 with $CaCl_2$ pretreatment were lower than the control after 12 DAT. Our results showed that 5 mM $CaCl_2$ treatment significantly increased F_v/F_m and Φ_{PSII} in tung tree seedlings after 8 DAT of drought stress (Fig. 5*B*,*C*). In this study, we found that $CaCl_2$ pretreatment significantly increased ETR from 8 to 12 DAT. This finding was consistent with previous studies, which demonstrated that Ca^{2+} could

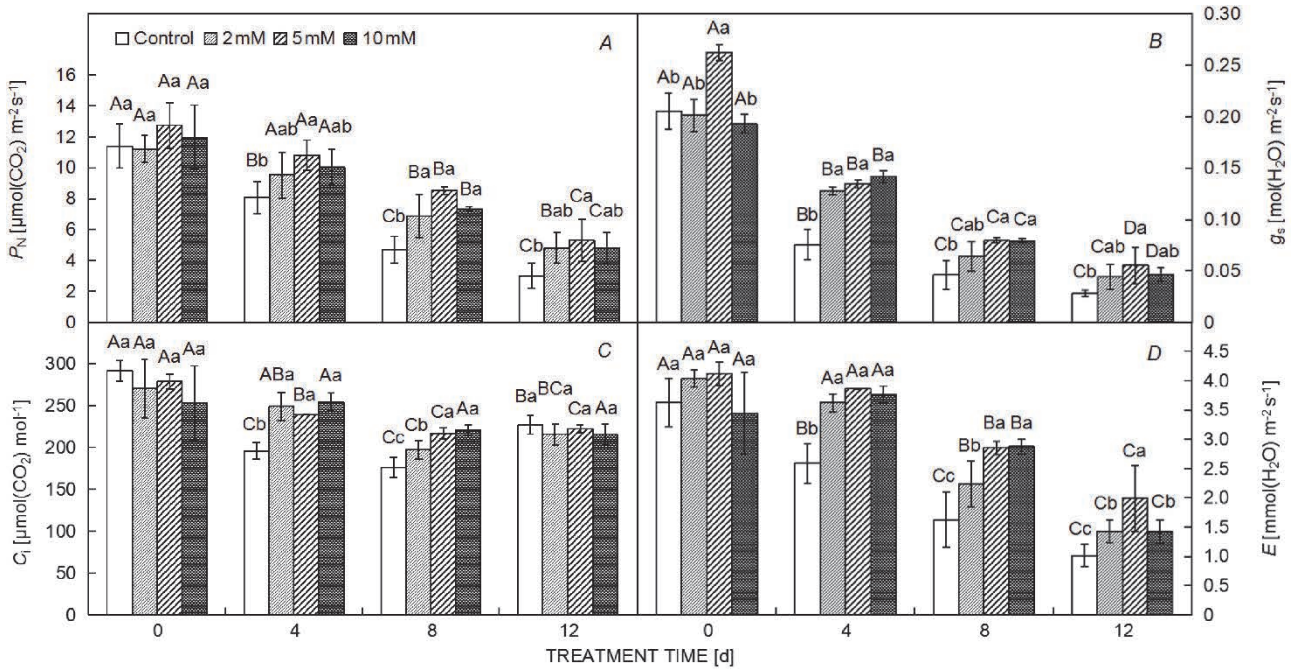

Fig. 4. Effects of $CaCl_2$ on net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) in tung tree seedlings under drought stress. (*1*) No $CaCl_2$ and drought; control, (*2*) 2 mM $CaCl_2$ pretreatment and drought; 2 mM, (*3*) 5 mM $CaCl_2$ pretreatment and drought; 5 mM, and (*4*) 10 mM $CaCl_2$ pretreatment and drought; 10 mM. The data in the figure represent mean ± SE (n = 3). Different *capital letters* indicate statistical differences between drought treatment stages, and *lowercase letters* indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to *Duncan*'s multiple range test (DMRT).

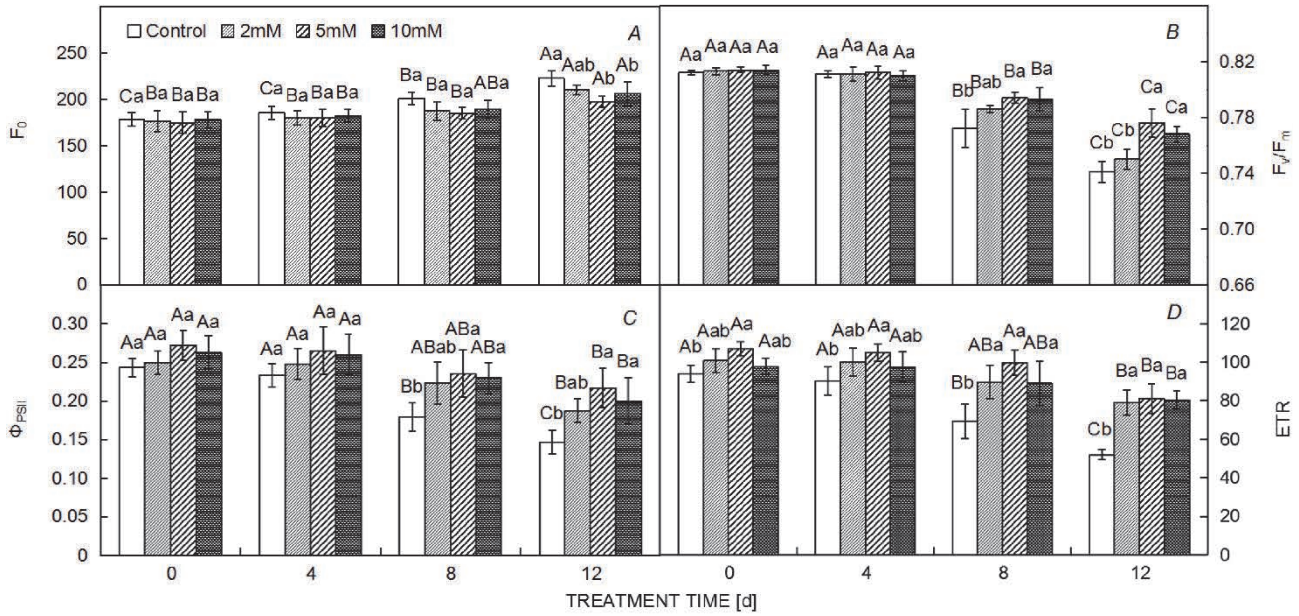

Fig. 5. Effects of $CaCl_2$ on minimal fluorescence yield of the dark-adapted state (F_0), maximal quantum yield of PSII photochemistry (F_v/F_m), effective quantum yield of PSII (Φ_{PSII}) and electron transport rate (ETR) in tung tree seedlings under drought stress. (*1*) No $CaCl_2$ and drought; control, (*2*) 2 mM $CaCl_2$ pretreatment and drought; 2 mM, (*3*) 5mM $CaCl_2$ pretreatment and drought; 5 mM, and (*4*) 10 mM $CaCl_2$ pretreatment and drought; 10 mM. The data in the figure represent mean ± SE (n = 3). Different *capital letters* indicate statistical differences between drought treatment stages, and *lowercase letters* indicate significant difference between Ca treatments at the same drought treatment stage, at $P \leq 0.05$ according to *Duncan*'s multiple range test (DMRT).

partially alleviate PSII reaction center closure and improve utilization of light energy (Wei *et al*. 2015). Previous studies showed that different $CaCl_2$ concentrations significantly influenced plant tolerance of drought stress, which indicated F_v/F_m was higher in *Zoysia japonica* pretreated with 10 mM $CaCl_2$ than in other treatments (Xu *et al*. 2013). Amor *et al*. (2010) reported that 3.5 mM Ca^{2+} was more effective than 20 mM in lowering NaCl stress for *Cakile maritime*. These studies indicate that different $CaCl_2$ concentrations result in different levels of plant tolerance towards abiotic stresses. Compared to 2 and 10 mM, we found that 5 mM Ca^{2+} relieved the most effectively the injury of drought stress.

Conclusion: In conclusion, drought stress had negative effects on tung tree seedlings causing reduced relative Chl content, activities of Rubisco and PEPC, photosynthesis, and photochemical efficiency of PSII. Exogenous $CaCl_2$ application improved drought tolerance in tung tree seedlings and 5 mM $CaCl_2$ was more effective than 2 and 10 mM. The findings of the present study are of great importance as new techniques are needed to improve the survivability of tung tree under drought conditions. Further studies are necessary to investigate the drought tolerance of tung tree under field conditions.

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