

Article

Physiological Responses of *Handeliodendron bodinieri* (Levl.) Rehd. to Exogenous Calcium Supply under Drought Stress

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Abstract: The soils in karst regions, with carbonate rocks as the background material, are characterized by two main factors, drought and high calcium levels. *Handeliodendron bodinieri* (Levl.) Rehd is an endemic plant species in this area of China. However, few studies have been carried out on the adaptation mechanism of *H. bodinieri* to drought and high calcium soil. To reveal the physiological responses of *H. bodinieri* to exogenous calcium under drought stress, 10% PEG-6000 was used to simulate drought stress, and the effects of exogenous calcium at different concentrations on the physiology of *H. bodinieri* seedlings under drought stress were studied. The results showed that drought stress significantly reduced the relative water content and water potential of *H. bodinieri* seedlings. Malondialdehyde (MDA) content, O₂⁻ and H₂O₂ production rates significantly increased under drought stress. The addition of exogenous calcium significantly reduced MDA content and O₂⁻ and H₂O₂ production rates. Moreover, peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APX) and glutathione peroxidase (GPX) activities increased significantly under drought stress. At the same time, the accumulation of osmotic regulators such as soluble sugar, betaine and free proline also increased significantly. The addition of exogenous calcium further increased the activity of antioxidant enzymes and the accumulation of osmotic regulatory substances. Consequently, the oxidative stress and osmotic stress induced by drought decreased. Finally, exogenous calcium enhanced the photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) of *H. bodinieri* under drought stress and significantly improved water use efficiency (WUE). This study confirmed that the application of exogenous calcium can enhance the water condition, photosynthetic capacity, osmotic regulation and antioxidant capacity of *H. bodinieri* under drought stress. Of the treatments, the 10 mmol·L⁻¹ CaCl₂ treatment is more likely to improve survival of *H. bodinieri* under drought tolerance. This study provides an important reference for describing the adaptation mechanism and appropriate conservation of *H. bodinieri* under drought and high calcium conditions.

Keywords: exogenous calcium; drought stress; physiological response; calcicole; *Handeliodendron bodinieri* (Levl.) Rehd

1. Introduction

The karst region in China is approximately 3.44 million km², which is the most concentrated area of karst globally [1]. In this area, the soil layer is shallow and discontinuous, and the rock exposure rate is high. The soil is rich in calcium and is alkaline, with a high infiltration rate, and the ecological environment is extremely fragile [2]. In the karst region in southwestern China, an area of 12×10^4 km² has experienced rock desertification, which is a key area for ecological management and a difficult area for vegetation restoration [3]. The rainwater quickly infiltrates into the ground. After a few days of high temperature and sunny days, the soil water content is greatly reduced, and the surface often has intermittent droughts [4]. The available soil moisture in karst areas can only meet transpiration requirements for one to two weeks after the plant is saturated at field moisture capacity [5]. Therefore, drought stress has become one of the main factors restricting the growth of karst habitats.

Drought may cause Gs rates to decrease, which would result in a lower photosynthetic rate, transpiration rate and photosystem reaction centre activity of plants [6,7], which would result in increased reactive oxygen species (ROS) [8], which would induce plants to establish antioxidant defense systems that scavenge ROS and alleviate oxidative damage by increasing the content of antioxidants [9–11]. In addition, plants can resist drought stress by accumulating certain osmotic adjustment substances, such as proline, soluble sugar, and betaine [12]. Under moderate drought conditions, the water use efficiency (WUE), antioxidant enzyme activity and osmotic adjustment substance content of endemic karst plant seedlings at four different life forms increased, indicating that these defense systems play an important role in plant drought resistance [13]. Calcium is one of the essential nutrients for plants and it is referred to as “the total regulator of plant cell metabolism” [14]. It can bind to calmodulin and participate in mediating many physiological metabolic pathways in plants under stress [15]. Calcium and calmodulin are involved in plant sensing, transmitting, responding and expressing stress signals, and in improving plant resistance [16,17]. Calcium can improve the adverse effects of water deficits on plants by increasing antioxidant enzyme activity and reducing membrane lipid peroxidation [18]. Calcium can also induce the synthesis of more osmotic adjustment substances to improve plant water status [19], improve stomatal movement of plant leaves, increase photosynthetic electron transfer and increase WUE [20]. At the same time, calcium acts as the second messenger and signalling molecule in the physiological and biochemical reactions of cells. Under drought stress, high temperatures and salt damage, calcium can improve the adaptability of plants to stress by regulating the expression of genes [21–23]. The limestone soil in the karst area of China has a high calcium content. Therefore, the plants in this area are generally drought and calcium tolerant, forming endemic karst vegetation [24]. However, whether high calcium in karst soil can enhance drought and calcium tolerance in plants remains to be confirmed, and the physiological mechanism of calcium involvement in drought resistance of calcicole has not yet been elucidated.

Handeliendron bodinieri (Levl.) Rehd. is an endemic calcicole tree in the southwestern karst region of China. It is listed as a national grade I protected plant with high ecological function and economic value [25]. At present, there are few studies on improving drought resistance of calcicole plants by exogenous calcium. Research on the drought resistance of *H. bodinieri* has not been reported. The purpose of this study was to study the physiological responses of *H. bodinieri* to exogenous calcium supply under drought stress. We hypothesized that calcium could alter the water status tolerance, antioxidant enzyme activity, osmotic adjustment and photosynthetic capacity of *H. bodinieri* to enhance its survival ability under drought stress.

2. Materials and Methods

2.1. Plant Materials and Stress Treatments

The seedlings were collected from the karst habitat (106°33′7.81″ E, 24°47′26.6″ N) in Leye County, Baise city, Guangxi Province, China. The altitude is 900 m–1300 m, the climate is subtropical humid. The average annual precipitation is 1100 mm–1500mm. The average number of precipitation days is

210 d, 78.3% of which occur from May to September. The average annual temperature is approximately 16.3 °C (23.4 °C in July and 7.4 °C in January). The frost-free period is long, an average of 279–345 d. The soil type is black calcareous soil with high calcium content. In October 2015, the mature seeds of *H. bodinieri* were collected and dried in the drying chamber. In March 2016, the seedlings were stored in sand storage and transferred to the pot containing black calcareous soil (4875mg/Kg calcium content in the soil) with a specification of 18*18*20. Cutting were grown in a greenhouse with a temperature of 28/23 °C (day/night), a daily light cycle of 16/8 h, and a relative humidity of about 65%. In August 2018, we used 10% PEG-6000 to simulate drought stress, and three exogenous calcium gradients were set up for a total of five treatments (Table 1). According to different treatments, 10% PEG-6000, CaCl₂ of different concentrations and 1/2 strength Hoagland nutrient solution were prepared into 6L solution with a PH of 7.0 (to simulate neutral black calcareous soil). 1/2 strength Hoagland nutrient solution of the following composition: 2.5 mM KNO₃; 2.5 mM Ca(NO₃)₂; 1 mM MgSO₄; 0.5 mM KH₂PO₄; 0.025 mM H₃BO₃; 0.005 mM MnCl₂·4H₂O; 0.4 μM ZnSO₄·7H₂O; 0.2 mM Na₂MoO₄·2H₂O; 0.15 μM CuSO₄·5H₂O and 20 μM Fe(III)–citrate-hydrate. Set three replicates for each treatment. Six three-year-old seedlings per repeat, 18 seedlings per treatment, 90 seedlings in total. At the 24 h, 48 h, 72 h and 96 h of drought stress under drought stress at 10:00–12:00 a.m., a leaf with similar size and the same illumination was selected from each of the three repeats of each treatment for photosynthesis and water status determination. At 96 h of drought stress, the roots of each repeated seedling were mixed for the determination of H₂O₂, O₂⁻, soluble sugar, betaine, proline, SOD, POD, CAT, APX and GPX.

Table 1. Treatments of the experiment.

Treatment	PEG-6000	CaCl ₂ Concentration (mmol·L ⁻¹)
1	0	0
2	10%	0
3	10%	2 mmol·L ⁻¹ CaCl ₂
4	10%	5 mmol·L ⁻¹ CaCl ₂
5	10%	10 mmol·L ⁻¹ CaCl ₂

2.2. Leaf Relative Water Content and Water Potential

In each treatment, 3 pieces of fresh leaves with the same growth potential were selected (FW). Then, the leaves were placed in water and soaked in the dark for 24 h. After the leaves were removed, the water on the surface of the leaves was quickly filtered with filter paper and weighed (TW). The leaf was placed in an oven at 85 °C for 48 h to a constant weight, and the weight (DW) was recorded. The leaf RWC was calculated using the following formula: $RWC(\%) = ((FW - DW) / (TW - DW)) \times 100$. An instantaneous leaf WP was measured by a WP4C Dewpoint Potential Metre (Decagon, Pullman, WA, USA), choosing three leaves of similar size and illumination in each repetition of each treatment. Then, leaves were cooled to 25 °C and laid flat on the bottom of the sample cup, so that the whole sample was covered with the leaves. The redundant parts of the leaves were folded to the bottom in a select precise mode for measurement. After 15 min for the balance, wait for the yellow light to flash for reading.

2.3. Reactive Oxygen Species (ROS) and Malondialdehyde (MDA) Content Determination

The content of hydrogen peroxide (H₂O₂) was determined according to the method of Sergiev [26], and the content of superoxide anion (O₂⁻) was determined by thiobarbituric acid colorimetry [27].

2.4. Measurement Osmoregulation Substances

Total soluble sugars were measured using the sucrose standard and the indolinone sulfuric acid method [28], free proline content was measured using the ninhydrin method [28], and glycine betaine was determined by the periodic colorimetric method [29].

2.5. Measurement of Antioxidant Enzyme Activities

Superoxide dismutase (SOD) activity was determined by the nitrogen blue tetrazolium (NBT) method [30], catalase (CAT) activity was determined by sodium thiosulfate titration [30], and peroxidase (POD) activity was determined by the guaiacol chromogenic method [30]. Ascorbate peroxidase (APX) was determined by the method of Nakano [31], and glutathione peroxidase (GPX) activity was measured according to the method of Lawrence and Burk [32].

2.6. Photosynthetic Parameters

The photosynthetic rate (P_n), transpiration rate (T_r) and stomatal conductance (G_s) of plants were measured by LI-COR 6400 (LI-COR Inc., Lincoln, NE, USA) at 24 h, 48 h, 72 h and 96 h after drought stress, and the WUE was estimated using the ratio of net photosynthesis to the amount of water transpiration (P_n/T_r).

2.7. Statistical Analyses

SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. One-way analysis of variance (ANOVA) was used, and Duncan's multi-range test was performed to test the significant differences between the measurements with different treatments. The values are expressed as the mean \pm standard error (SE) of the three repeated samples. Significant differences were determined at $p < 0.05$.

3. Results

3.1. Relative Water Content and Water Potential

Compared with the control treatment (Figure 1a), drought stress significantly reduced the relative water content of leaves ($p < 0.05$). However, the application of exogenous calcium could delay the reduction in the relative water content of leaves. With increasing drought stress duration, the relative water content of the leaves decreased significantly ($p < 0.05$), but the relative water contents of the *H. bodinieri* leaves treated with different concentrations of exogenous calcium were always higher than that those treated without exogenous calcium. At 72 h of drought stress, the relative water content of *H. bodinieri* leaves treated with $10 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ increased the most, reaching 26.12%. With the increase in drought stress duration, the $10 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ treatment at 96 h most improved the relative water content of leaves. Compared with the control treatment (Figure 1b), in the drought stress treatments, the water potential in the *H. bodinieri* leaves increased significantly from 24 h to 96 h after drought stress ($p < 0.05$), indicating that drought stress resulted in increased water potential in *H. bodinieri*. Compared to that without the exogenous calcium treatment, the water potential of leaves significantly decreased from 24 h after the $10 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ treatment, from 48 h after the $2 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ treatment, and from 72 h after the $5 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ treatment ($p < 0.05$). Among the treatments, the $10 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ treatment resulted in the water potential of the leaves of the *H. bodinieri* seedlings decreasing the most notably after 96 h of drought stress, reaching 40.93%. Exogenous calcium can effectively improve the water status of *H. bodinieri* under drought stress and reduce the damage caused by drought stress.

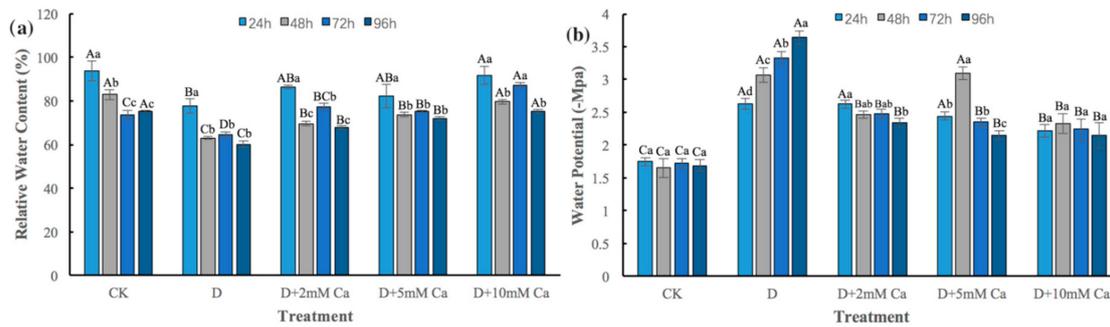


Figure 1. Effects of exogenous calcium on the relative water content (a) and water potential (b) of *H. bodinieri* under drought stress. Note: Ck: treatment 1 (control); Drought: treatment 2; Drought + 2 mM Ca: treatment 3; Drought + 5 mM Ca: treatment 4; Drought + 10 mM Ca: treatment 5. Different capital letters indicate a significant differences between. Treatments within each sampling time, and lower case letters indicate a significant difference between sampling time within each treatment ($p < 0.05$). The figure data are the average standard error of the three replicates, and the same is below.

3.2. Reactive Oxygen Species (ROS) and Malondialdehyde (MDA)

Compared with those of the control (Figure 2a,b), the O_2^- and H_2O_2 production rates of the *H. bodinieri* seedlings in the drought stress treatments increased significantly ($p < 0.05$), the O_2^- content increased by 33.32%, and the H_2O_2 content increased by 51.78%, indicating that drought constitutes oxidative stress on *H. bodinieri*. The production rates of O_2^- and H_2O_2 under different concentrations of exogenous calcium significantly increased compared with those in the control ($p < 0.05$) but significantly decreased ($p < 0.05$) compared with the non-exposed exogenous calcium treatment. When 5 $mmol \cdot L^{-1}$ $CaCl_2$ was added, the decrease in O_2^- content was the largest, which decreased by 16.00%. When 2 $mmol \cdot L^{-1}$ $CaCl_2$ was added, the H_2O_2 content decreased the most, at 21.62%. However, the difference in the content of O_2^- and H_2O_2 in *H. bodinieri* in the different concentrations of exogenous calcium treatments was not significant. The results indicated that exogenous calcium could alleviate the accumulation of reactive oxygen species in the seedlings of *H. bodinieri* under drought stress and reduce the damage caused by drought stress. The MDA content of *H. bodinieri* in the drought stress treatments was significantly higher than that in the control (Figure 2c), indicating that drought stress caused membrane lipid peroxidation in *H. bodinieri*. The MDA content in the treatment with different concentrations of exogenous calcium significantly decreased ($p < 0.05$), and the MDA content was similar to that of the control group treated with 5 and 10 $mmol \cdot L^{-1}$ $CaCl_2$. The *H. bodinieri* treated with 2 $mmol \cdot L^{-1}$ $CaCl_2$ was applied. Of the ROS, the MDA content decreased the most, reaching 25.52%. Exogenous calcium can reduce the degree of lipid peroxidation on the cell membrane of *H. bodinieri* under drought stress.

3.3. Osmotic Regulation of Substance Content

Compared with those in the control group (Figure 3), the soluble sugar, betaine and free proline content in *H. bodinieri* in the drought stress treatments increased significantly ($p < 0.05$).

Different concentrations of $CaCl_2$ significantly improved the soluble sugar, betaine and free proline content ($p < 0.05$). In comparison with those treated without $CaCl_2$, the soluble sugar, betaine and free proline treated with 10 $mmol \cdot L^{-1}$ $CaCl_2$ were the highest and increased by 12.29%, 12.55% and 18.49%, respectively. The content of soluble protein did not significantly change between the different concentrations of exogenous calcium treatments, while the contents of betaine and free proline significantly increased when treated with 10 $mmol \cdot L^{-1}$ $CaCl_2$ than with 2 and 5 $mmol \cdot L^{-1}$ $CaCl_2$ ($p < 0.05$). Exogenous calcium can effectively alleviate the osmotic stress caused by drought and reduce the damage degree of *H. bodinieri*. Of the treatments, the 10 $mmol \cdot L^{-1}$ $CaCl_2$ treatment provided the highest osmolyte content.

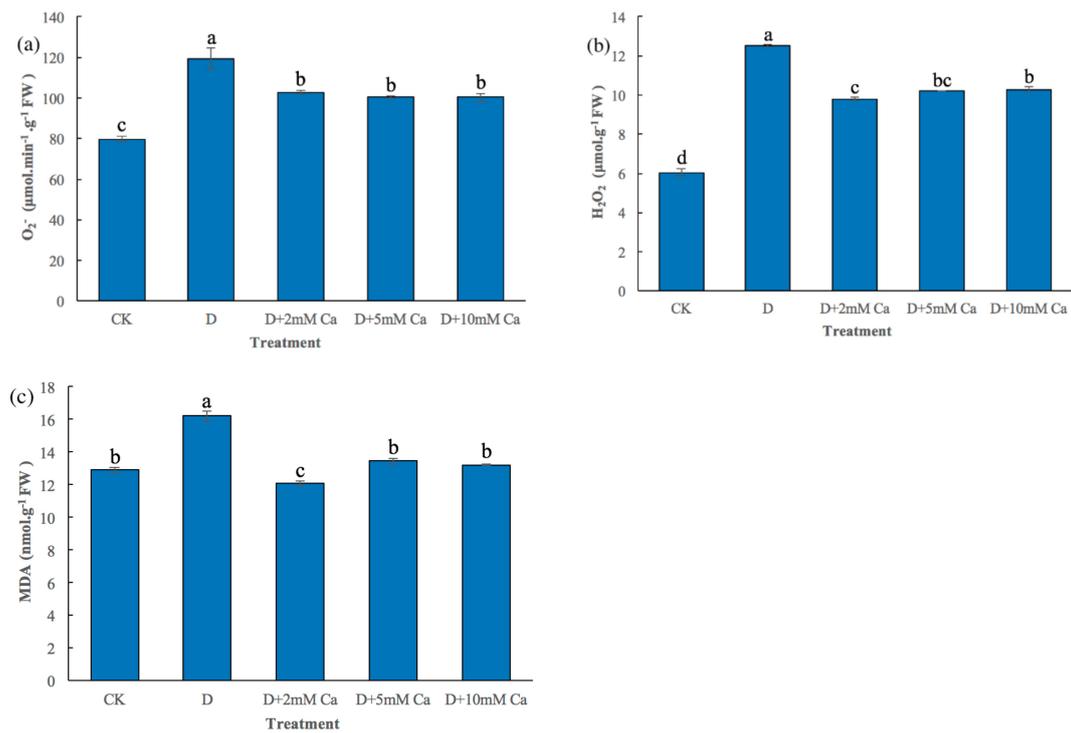


Figure 2. Effects of exogenous calcium on the production rates of O_2^- (a) and H_2O_2 (b) and MDA content (c) in *H. bodinieri* under drought stress. Note: Different lower case letters indicate a significant differences between treatment within each sampling time ($p < 0.05$). The figure data are the average standard error of the three replicates, and the same is below.

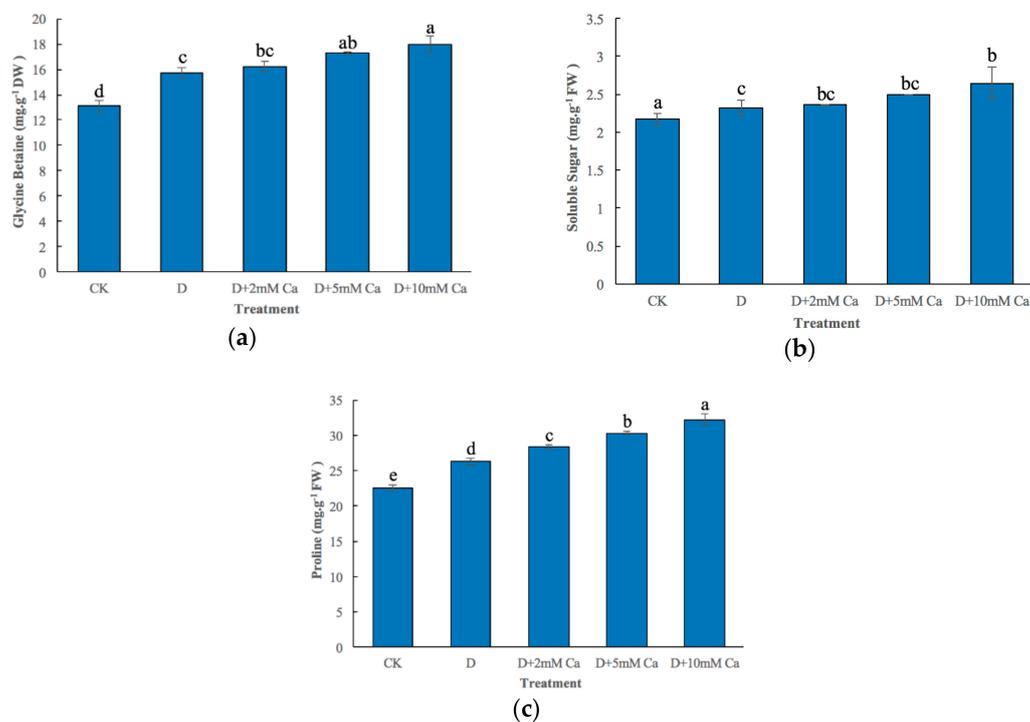


Figure 3. Effects of exogenous calcium on the content of proline (a), soluble sugar (b) and glycine. Betaine (c) in *H. bodinieri* under drought stress.

3.4. Antioxidant Enzyme Activity

Compared with the control treatment (Figure 4), the drought stress treatment resulted in increases in the activities of SOD, POD, CAT, APX and GPX in the *H. bodinieri* leaves, and the changes in various indexes were different under different exogenous calcium treatments. With the increase in exogenous calcium concentration, POD activity increased (Figure 4a), reaching a maximum in the treatment with 10 mmol·L⁻¹ CaCl₂, but the difference was not significant compared with that without exogenous calcium. SOD and CAT activity reached their maximums under the 5 mmol·L⁻¹ CaCl₂ and 10 mmol·L⁻¹ CaCl₂ treatments, with increases of 22.46% and 12.04%, respectively (Figure 4b,c), which was a significant difference compared with that without exogenous calcium treatment ($p < 0.05$). The application of exogenous calcium under drought stress significantly increased the activity of APX and GPX (Figure 4d,e), but with the increase in exogenous calcium concentration, the activity of APX and GPX decreased. Among the enzymes, APX activity reached a maximum in the 2 mmol·L⁻¹ CaCl₂ treatment, with an increase of 12.17%, which was significantly different from that in the other exogenous calcium treatments ($p < 0.05$).

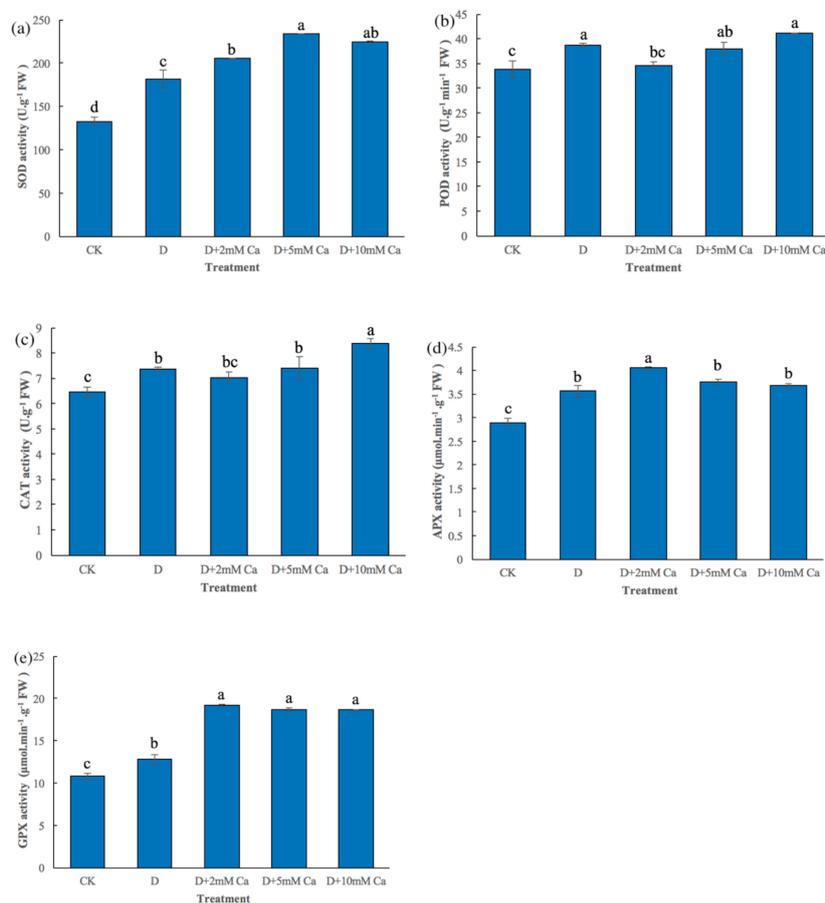


Figure 4. Effects of exogenous calcium on peroxidase (POD) (a), superoxide dismutase (SOD) (b), catalase (CAT) (c), ascorbate peroxidase (APX) (d) and glutathione peroxidase (GPX) (e) activities in *H. bodinieri* under drought stress.

Similarly, GPX activity reached its maximum in the 2 mmol L⁻¹ CaCl₂ treatment, with an increase of 33.19%, but there was no significant difference with that in the other exogenous calcium treatments. Exogenous calcium at an appropriate concentration can induce increased activity of SOD, POD, CAT, APX and GPX in *H. bodinieri* seedlings with potential to further alleviate the damage caused by drought.

3.5. Photosynthetic Parameters

Compared with the control treatment (Figure 5), the drought stress treatments caused the photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs) in the *H. bodinieri* leaves to decrease significantly after 24 h ($p < 0.05$). The addition of exogenous calcium slowed drought stress. Pn, Tr and Gs decreased with the increase in exogenous calcium concentration at 24 h in the early stage of drought stress. The difference in exogenous calcium treatment rates was not significant. With the increase in drought stress duration, Pn, Tr and Gs significantly ($p < 0.05$) increased in exogenous calcium concentration at 96 h. The Pn, Tr, and Gs indexes significantly increased ($p < 0.05$) in the treatments with exogenous calcium application in comparison with those in the treatment without exogenous calcium application, with maximum increases of 61.17%, 57.90% and 58.44%, respectively. Compared with the control group (Figure 5), in the drought stress treatments, the WUE of the seedlings in the early stage of drought stress was not significant. With the increase in stress duration to 96 h, the WUE of seedlings was significantly higher than that of the control group ($p < 0.05$). Of the plants treated with exogenous calcium, the plants treated with $10 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ had the highest WUE, which was 7.64% higher than that without the application of exogenous calcium.

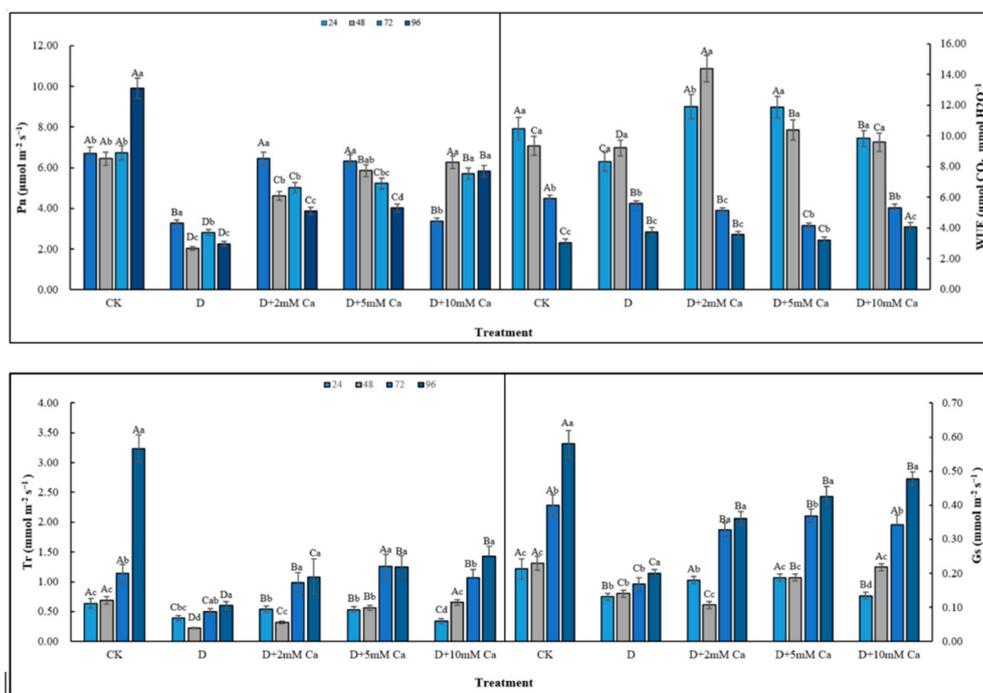


Figure 5. Effects of exogenous calcium on the photosynthetic parameters of *H. bodinieri* under drought stress. Note: Different capital letters indicate a significant differences between treatments ($p < 0.05$) within each sampling time, and lower case letters indicate a significant difference between sampling time ($p < 0.05$) within each treatment. The figure data are the average standard error of the three replicates, and the same is below.

At the initial stage of drought stress, a low concentration of exogenous calcium can alleviate the damage caused by drought stress. With the increase in stress duration time, the appropriate concentration of exogenous calcium can increase the Pn, Tr and Gs of *H. bodinieri* leaves and improve the light availability and photosynthetic ability of plants. The improvement in plant WUE was reflected in the late stage of drought stress, with the $10 \text{ mmol}\cdot\text{L}^{-1} \text{ CaCl}_2$ treatment performing best, but the improvement was less than that of the other indicators.

4. Discussion

Drought is the main stress plants face in the karst environment, and drought stress can affect the water status of plants. The relative water content and water potential of plant leaves are two important indexes to evaluate plant water status. In this study, drought stress significantly decreased the relative water content and water potential of *H. bodinieri*. However, the relative water content and water potential of *H. bodinieri* leaves treated with different concentrations of exogenous calcium increased relative to the droughted control, indicating that exogenous calcium alleviated the damage of drought to *H. bodinieri*. In a study on *Zoysia japonica* [18], *Phaseolus vulgaris* L [33], *Zea mays* L. [34] and *Vernicia fordii* (Hemsl.) Airy Shaw [35], exogenous calcium effectively improved the water status of plants under drought stress. Martínez-Ballesta et al. [36] showed that the addition of exogenous calcium in NaCl solution can lessen the decrease in aquaporin function and abundance of *Capsicum frutescens* L., suggesting that exogenous calcium may play an important role in regulating the transport of water in plants under abiotic stress. For the purpose of this paper, it seems appropriate to putatively suggest that increases in water potential and RWC are caused by increased expression of aquaporin, as demonstrated in pepper plants subjected to osmotic stress induced by NaCl.

Osmotic regulation is one of the main adaptation strategies plants use to resist stress. When plants are subjected to water stress, soluble sugar, free proline and betaine, which are the main osmotic regulators in vivo, accumulate in large quantities, increase the osmotic potential of cells, and improve their osmotic regulation ability and water retention capacity [37]. Muhammad et al. sprayed exogenous calcium on corn leaves. The results showed that exogenous calcium increased the content of free proline in corn and improved the drought resistance of corn [38]. Some studies have shown that CaCl_2 decreases proline concentration by increasing proline degradation enzymes and decreasing the proline biosynthesis level in plants under drought stress [17]. The accumulation of betaine can alleviate the osmotic stress effect on plants.

Exogenous calcium can further increase the content of free proline and betaine in wheat under drought stress, reduce cell membrane damage and reduce the destructive effect of osmotic stress on plants [39]. Under drought stress, the content of soluble sugar increases significantly, which may be a response to tissue damage or a method to reduce plant oxidative stress [40]. Upadhyaya et al. found that adding exogenous calcium could increase the content of soluble sugar in *Camellia sinensis* under drought stress [41]. In this study, different concentrations of exogenous calcium increased the contents of soluble sugar, free proline and betaine in *H. bodinieri* seedlings. The results showed that exogenous calcium promoted the accumulation of many osmotic regulators and played an important role in improving the water status of plants under drought stress. However, studies by Hayat show that the accumulation of proline can cause toxic effects on plants [42]. Therefore, it is still necessary to study the effects of exogenous calcium on soluble sugar, free proline and betaine under long-term drought stress.

Calcium has a variety of protective effects on plants under drought stress; and calcium not only is involved in osmotic regulation but also reduces membrane lipid peroxide, enhances enzyme activity and regulates active oxygen metabolism [43].

Some studies have shown that calcium can maintain the integrity of the cell membrane, thus enhancing plant tolerance to drought [44]. In this study, the addition of exogenous calcium significantly reduced the content of H_2O_2 and O_2^- in the *H. bodinieri* leaves under drought stress and also significantly reduced the content of MDA, minimizing the lipid peroxidation of the *H. bodinieri* cell membranes and effectively protecting the plants. Under drought stress, a variety of antioxidant enzymes are synthesized in a plant to maintain the balance of active oxygen production and clearance. The activity of superoxide dismutase (SOD) is related to plant self-protection when the plants are subjected to oxidative stress [45]; POD and CAT systems may operate at their maximum rates to consume, to decompose and remove excess peroxide and H_2O_2 [46] in the plant. APX is involved in the ascorbic ASA-GSH cycle and is an efficient detoxification and antioxidant system for H_2O_2 . GPX catalyses the reduction of H_2O_2 and membrane lipid peroxide products with glutathione as an electron donor. In this study, the activities of SOD, POD, CAT, APX and GPX in *H. bodinieri* under drought stress increased,

and the indexes were significantly improved by applying different concentrations of exogenous calcium, except that of POD, which indicated that calcium could reduce and delay the oxidative damage caused by drought. Chen et al. [47] showed that exogenous calcium could reduce the oxidative stress of cucumber seedlings under drought stress and increase the antioxidant enzyme activity of cucumber seedlings, which was consistent with the results of this study. In a study on *Quercus glauca* Thunb, a calcicole plant, the activities of antioxidant enzymes such as SOD, GR and GPX decreased with a prolonged stress duration [48]. There was no significant increase in POD content, which may have been due to the duration of the stress.

Photosynthesis is sensitive to water stress, and abiotic stress often leads to a decrease in the plant photosynthetic rate and WUE [49]. Calcium plays an important role in the regulation of plant photosynthesis, including photosynthetic electron transport, chlorophyll fluorescence, stomatal movement and other pathways [50]. In this study, with the increase in time, the Pn, Gs and Tr of *H. bodinieri* seedlings under drought stress decreased significantly. The addition of exogenous calcium minimized the downward trend of these three indexes, which indicated that exogenous calcium could significantly reduce the rate and amplitude of photosynthetic related indexes and reduce the physiological damage caused by drought; these results are consistent with the results related to seedlings of *Koelreuteria bipinnata* Franch [51]. WUE is a comprehensive index used to evaluate plant growth under drought stress. A high WUE means that less water is needed to fix the unit mass of carbon dioxide and that there is a high water productivity. In this study, there was no significant difference in the WUE content between the two groups at the early stage of drought stress. However, there was a significant increase at 96 h, where the 10 mmol·L⁻¹ CaCl₂ treatment was significantly higher than that in the non-calcium treatment, which indicated that an appropriate concentration of exogenous calcium could improve the WUE of plants after drought stress for a certain period of time; these results were consistent with the results of a study on *Sophora davidii* (Franch.) in the karst mountain area [52]. Huang et al. studied the regulation of exogenous calcium on the photosynthetic system of *Lonicera japonica*, and the results indicated that calcium plays a key role in maintaining photochemical efficiency and chlorophyll content [53]. Hu et al. studied the proteome pathway of *Pinus massoniana* Lamb and *Liquidambar formosana* Hance under acid rain stress, and the results indicated that calcium plays an important role in increasing the genes involved in photosynthetic electron transport and carbon sequestration [54]. The expression of the D1 protein, which is one of the core subunits of the light system ii (ii), is increased by exogenous calcium under herbicide stress [55]. Therefore, exogenous calcium may increase the photosynthetic capacity of *H. bodinieri* by increasing the expression of the related genes involved in photosynthetic electron transfer, carbon assimilation and the photochemical pathway, and the specific mechanism for tolerance acquisition should be further studied.

The beneficial effects of different concentrations of exogenous calcium on the water status, osmotic regulating substances, active oxygen metabolism, antioxidant enzyme activity and photosynthesis of *H. bodinieri* under simulated drought stress increased. However, different concentrations of exogenous calcium have different effects on these factors. Of the treatments, the 2 mmol·L⁻¹ CaCl₂ treatment had the largest effect on MDA, H₂O₂, APX and GPX in *H. bodinieri* seedlings. The 5 mmol·L⁻¹ CaCl₂ treatment had the largest effect on O₂⁻ and SOD in the *H. bodinieri* seedlings. The 10 mmol·L⁻¹ CaCl₂ treatment had the largest effect on the photosynthesis, soluble sugar, betaine, NS free proline indexes, and on relative water content, water potential and CAT of *H. bodinieri* seedlings. Therefore, it can be speculated that under the 10% PEG-6000 simulated short-term drought stress, adding the 10 mmol·L⁻¹ CaCl₂ treatment had the most obvious increase in drought resistance of *H. bodinieri* seedlings.

An appropriate concentration of exogenous calcium can improve the drought resistance of *H. bodinieri* seedlings under short-term drought stress, which is consistent with our hypothesis. The high calcium soil environment in the karst area increased the survival probability of calcicole under intermittent drought by improving plant water status, antioxidant capacity, osmotic regulation and photosynthesis. In addition, the length and intensity of plant species and drought stress duration

can affect drought resistance, so the mechanism of exogenous calcium in different karst-related species under different drought durations and intensities needs to be further studied.

5. Conclusions

Drought stress significantly decreased the production rate and MDA content of reactive oxygen species such as H_2O_2 and O_2^- ; increased the activities of antioxidant enzymes such as POD, CAT, SOD, APX and GPX; and increased the contents of osmotic regulators such as proline, soluble sugar and betaine. The results showed that water deficit led to a decrease in the water status and photosynthetic capacity of the leaves, and the degree of oxidative stress increased. *H. bodinieri* coped with drought by increasing the activity of its antioxidant enzymes and the content of osmotic regulators. After applying different concentrations of exogenous calcium, the relative water content of the leaves was significantly higher than that under drought stress; the contents of MDA, H_2O_2 and O_2^- were significantly lower than those under drought stress; and the activities of the antioxidant enzymes and the content of osmotic regulators in the leaves further increased. The results showed that exogenous calcium was involved in osmotic regulation to increase leaf water potential, promote leaf water maintenance, increase plant photosynthetic capacity and antioxidant capacity of leaves, and enhance the overall drought resistance of *H. bodinieri* seedlings. Therefore, the soil with high calcium content in karst areas may enhance the drought resistance of *H. bodinieri* seedlings in arid environments by maintaining plant water status, inducing the production of osmotic regulators, reducing oxidative stress and increasing photosynthesis. Under the short-term drought stress simulated by 10% PEG-6000, of the treatments, the $10 \text{ mmol}\cdot\text{L}^{-1}$ CaCl_2 treatment had the most obvious effect on the drought resistance of *H. bodinieri* seedlings. This study provides an important reference for the screening of drought-resistant calcicole plants and the mechanisms protecting *H. bodinieri* in rocky desertified areas.

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