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Ecophysiological Responses of Calcicole *Cyclobalanopsis glauca* (Thunb.) Oerst. to Drought Stress and Calcium Supply

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Abstract: Water deficit and high calcium (Ca^{2+}) content and are two typical soil characteristics in the Karst region. However, the problem of whether high Ca^{2+} in Karst calcareous soil could increase drought tolerance in calcicole plants has not been solved. We investigated the ecophysiological responses of *Cyclobalanopsis glauca* (Thunb.) Oerst. cuttings to short-term drought stress and Ca^{2+} application. Drought stress (10% PEG-6000) markedly reduced relative water content (RWC) and water potential (WP), and enhanced the levels of reactive oxygen species (ROS) production (H_2O_2 and $O_2^{\bullet-}$) and malondialdehyde (MDA) content in *C. glauca* leaves. Under drought treatment, exogenous Ca^{2+} application (20 mM $CaCl_2$) markedly increased the RWC and WP, and reduced the H_2O_2, $O_2^{\bullet-}$, and MDA content. Furthermore, water deficit induced a significant increase in the activities of antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione peroxidase (GPX), and increased the accumulation of osmoregulation substances. External Ca^{2+} alleviated drought-induced oxidative stress and osmotic stress with further increased activities of antioxidant enzymes, and enhanced the accumulation of osmoregulation substances. In addition, exogenous Ca^{2+} treatment alleviated the reduction of the photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), and chlorophyll content (SPAD), and further increased water use efficiency (WUE) under drought stress. This study confirms that exogenouos Ca^{2+} application induces improvements in the water status, osmotic adjustment, antioxidant defense, and photosynthesis efficiency of *C. glauca* under drought stress.

Keywords: ecophysiological responses; calcicole; *Cyclobalanopsis glauca* (Thunb.) Oerst.; drought stress; exogenous calcium

1. Introduction

The Karst region in southwest China is one of three distribution centers around the world [1]. Karst habitats are characterized by a double-layer hydrogeological structure and high porosity of the underlying limestone rock with serious surface water leakage, and very shallow and patchy soil with a low water retention capacity [2,3]. Rainfall rapidly disappears into subterranean streams through shafts, sinkholes, and dolines [4]. Previous studies showed that available soil water is only sufficient for plant transpiration needs for one or two weeks following saturated field capacity [5,6]. Drought stress has become one of the most constraining factors for plants in Karst habitats [4].

Plant ecophysiological characteristics might be changed by soil water deficit. Drought could induce a decrease in the relative water content (RWC) and water potential (WP) of plants [7]. Additionally, it can trigger the overproduction of reactive oxygen species (ROS), resulting in oxidative stress [8,9]. Water deficit can also reduce chlorophyll content, gas exchange parameters, stomatal conductance, and photosystem reaction center activity, resulting in the suppression of photosynthesis [10]. To cope with oxidative stress, plants have established antioxidant defense systems to eliminate ROS, including numerous antioxidant enzymes and non-enzymatic antioxidants [11]. Moreover, plants accumulate certain compatible solutes such as proline, soluble sugar, and glycine betaine to deal with drought stress [12]. Under moderate drought conditions, four Karst adapted plants with different growth forms enhanced water use efficiency (WUE), antioxidant enzymes activities, and osmoregulation substances, indicating the essential roles of these defense systems involved in drought resistance [13].

Calcium has been approved to ameliorate the adverse effects of water deficit on plants, which might be related to increased antioxidant enzyme activities and reduced membrane lipid peroxidation [14–19]. Moreover, Ca^{2+} application also improves plant water status by inducing more osmotic adjustment substance synthesis [15,18,20]. Additionally, it can improve stomatal movement and increase photosynthetic electron transport, thereby increasing WUE [21]. As a vital second messenger, Ca^{2+} also plays key roles in plant responses to drought stress [22–24].

Karst habitats are also characterized by the high soil calcium content of their limestone background. The calcium-rich environment reserves a lot of calcicole plants, which can live on limestone soil. However, whether the high exogenous calcium in the Karst environment could enhance the drought tolerance of calcicole plants remains to be proved. Furthermore, the possible ecophysiological mechanisms of Ca^{2+} involved in the drought tolerance of calcicole plants has not been illuminated. *Cyclobalanopsis glauca* (Thunb.) Oerst. (*C. glauca*) is an endemic calcicole tree species in the Karst regions of southwestern China. Because of its good ecological function and economic value, *C. glauca* has been used as a pioneer tree species for vegetation restoration in the Karst region. It has been reported that drought stress would damage *C. glauca* by aggravating oxidative stress, deteriorating water status, and inhibiting photosynthesis [25,26]. The aim of this study is to investigate the effects of exogenous Ca^{2+} on the tolerance of *C. glauca* to water deficit. We hypothesized that exogenous Ca^{2+} will improve water status, antioxidant enzyme activities, osmotic adjustment substances, and photosynthetic efficiency in *C. glauca*, enhancing its tolerance under drought stress.

2. Materials and Methods

2.1. Plant Materials and Stress Treatments

One-year-old cuttings of *C. glauca* were sampled from a twenty-eight-year-old seed bearer in a Karst habitat (24°47′26.6″ N, 106°33′7.81″ E) in Leye county, Guangxi Province, China. The long-term annual mean temperature of the sampling site was 16.5 °C (10 °C in January and 34 °C in July). The long-term annual mean precipitation was 1200 mm, 78.3% of which occurred from May to September. The soil type was black calcareous soil with a high calcium concentration. Cuttings were grown in a greenhouse where the temperature was 28/23 °C (day/night), the daily photoperiod was 16/8 h, and the relative humidity was about 65%. Six-month-old cuttings were subjected to different treatments in solutions containing 1/2 strength Hoagland solution at pH 6.0 as follows: drought (10% PEG-6000), drought with exogenous Ca^{2+} (10% PEG-6000 + 20 mM $CaCl_2$), and the control. Each treatment and control consisted of three replicates and lasted for 0, 12, 24, 48, and 72 h, respectively. One fully expanded leaf per individual with a similar size was used for photosynthesis and water status determination per replicate. Moreover, two leaves per individual were mixed for other physiological analyses.

2.2. Leaf Relative Water Content and Water Potential

Leaf samples were collected immediately to measure their fresh weight (FW) and then submerged in distilled water for 24 h to acquire the turgid weight (TW). Subsequently, the samples were placed at a temperature of 70 °C for 72 h at constant weight to obtain the dry weight (DW). All the leaf samples were weighted using an electronic scale (0.001 g precision). The leaf RWC was calculated using the following formula: RWC (%) = ((FW − DW/TW − DW) × 100). An instantaneous leaf WP was measured at 10:00 a.m. using a WP4C Dewpoint Potential Meter (Decagon, Pullman, WA, USA).

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

Hydrogen peroxide (H_2O_2) content was determined according to Sergiev et al. [27]. 0.3 g of the fresh leaf samples were homogenized in an ice bath with 4 mL of 0.1% (*w*/*v*) trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C and 0.5 mL supernatant was added to 0.5 mL 50 mM potassium phosphate buffer (pH 6.8) and 1 mL 1 M potassium iodide. The absorbance of the supernatant was read at 560 nm. The H_2O_2 content was obtained using a standard curve.

Superoxide anion ($O_2^{\bullet -}$) was measured through a method described by Meng et al. [28]. Frozen leaf samples of 0.5 g were homogenized in an ice bath with 5 mL 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C. The mixture made up of 0.5 mL supernatant and 0.5 mL phosphate buffer (pH 7.8), and 1 mL 1 mM hydroxyl ammonium chloride was incubated at 25 °C for 1 h. Then, 1 mL 17 mM p-amino benzene sulphonic acid and 1 mL 7 mM α-naphthylamine were added to the mixture and incubation was carried out at 25 °C for 20 min. Finally, the equivalent volume of ethyl ether was added to the mixture and then it was centrifuged at 1500 rpm for 5 min. The absorbance of the water phase was measured at 530 nm.

The MDA content was determined using the method of Cao et al. [29]. The extract was dissolved in 5 mL 10% TCA and centrifuged at 12,000 rpm for 10 min, and the supernatant was then transferred to a 5 mL centrifuge tube and diluted to 4 mL with 10% TCA. The 1 mL supernatant was mixed with 1 mL 20% TCA containing 0.5% (*w*/*v*) thiobarbituric acid (TBA). The mixture was heated in boiling water for 15 min and cooled immediately; the mixture was then centrifuged at 12,000 rpm for 10 min. The absorbance of the final supernatant was measured at 532 nm, 600 nm, and 450 nm. The MDA value was estimated by means of an extinction coefficent (155 mM^{-1} cm^{-1}).

2.4. Measurement Osmoregulation Substances

The total soluble sugar was measured following the anthrone sulphuric acid method [30] using sucroseas standard. Briefly, 0.5 g smashed samples were placed in 15 mL conical tubes containing 10 mL distilled water. The anthrone reagent was prepared using 0.1 g anthrone dissolved in 50 mL 95% sulphuric acid. The absorbance of the reaction solution was measured at 620 nm using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) after a water bath set at 100 °C for 10 min. The free proline content was measured following the ninhydrin method [30]. Briefly, 0.2 g samples were smashed in 5 mL 3% (*w*/*v*) sulfosalicylic acid. The reaction mixture contained equal volumes of proline, ninhydrin reagent, and glacial acetic acid. The absorbance of the reaction solution was measured at 520 nm using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) after a water bath set at 100 °C for 1 h. Glycine betaine was determined using the periodide colorimetric method according to Grieve and Grattan [31].

2.5. Measurement of Antioxidant Enzyme Activities

For enzyme extracts and assays, 0.5 g frozen leaf samples were homogenized in 8 mL 50 mM cold potassium phosphate buffer (KPB) (pH 7.8) containing 0.1 mM EDTA and 1% (*w*/*v*) polyvinypolypyrrolidone phosphate buffer (pH 6) in a chilled mortar. The homogenate was then centrifuged at 12,000 prm for 20 min at 4 °C, and the resulting supernatant was used to assay

the following enzyme activities and measured using a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan).

Superoxide dismutase (SOD) activity was determined using the nitroblue tetrazolium (NBT) method as described by Beauchamp and Fridovich [32]. The assay medium contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 µM NBT, and 2 µM riboflavin. The reaction mixture was placed under a 15 W fluorescent lamp with 120 µmol m^{-2} s^{-1} for 15 min. The absorbance was read at 560 nm.

Catalase (CAT) activity was assayed following the method described by Chance and Maehly [33], with some modification. The reaction mixture contained 50 mM KPB (pH 7.0), 200 mM H_2O_2, and enzyme extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm for 1 min.

Peroxidase (POD) activity was determined using the method described by Upadhyaya et al. [34], with some modification. The reaction mixture contained 100 mM KPB (pH 7.0), 10 mM H_2O_2, 20 mM guaiacol, and 1 mL of the enzyme extract. Changes in the absorbance of the reaction solution at 470 nm were measured.

The activity of ascorbate peroxidase (APX) was determined as a decrease in absorbance at 290 nm for 1 min, with minor modification [35]. The reaction was started by adding 50 µL extraction enzyme, 1.25 mL 50 mM KPB (pH 7.8), 500 µL 2mM H_2O_2, and 200 µL ascorbic acid (ASA).

The activity of the glutathione reductase (GR) was determined using the method of Cakmak et al. [36]. The absorbance of the reaction solution was measured at 340 nm. The reaction mixture contained 1 mM ethylene diamine tetraacetic acid (EDTA), 0.5 mM glutathione disulfide (GSSG), 0.15 mM nicotinamide adenine dinucleotide phosphate (NADPH), 100 mM sodium phosphate buffer (pH 7.8), and 0.15 mL enzyme extract. The protein content was determined using Bradford's [37] method.

Glutathione peroxidase (GPX) activity was assayed according to the method of Lawrence and Burk [38]. The reaction mixture contained 0.2 mM NADPH, 1 mM sodium azide (pH 7.0), 1 mM glutathione (GSH), 1 unit glutathione reductase and 2 mM H_2O_2 and enzyme extract. The reaction was started by adding H_2O_2 and the absorbance was measured at 340 nm.

2.6. Photosynthetic Parameters

Chlorophyll content was determined using a SPAD-502 chlorophyll meter (Minolta Co., Tokyo, Japan). The photosynthesis rate (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were measured at 10:00 a.m. using an infrared gas analyzer LI-COR 6400 (LI-COR Inc., Lincoln, NE, USA). The measurements were implemented under certain conditions of CO_2 concentration (380 ppm), photosynthetic photon flux density (PPFD) of 800 µmol photons m^{-2} s^{-1} at 25 °C. WUE was estimated using the ratio between net photosynthesis and the amount of water transpiration (Pn/Tr).

2.7. Statistical Analyses

Statistical analyses were performed with SPSS version19.0 (SPSS Inc., Chicago, IL, USA). Statistical differences between measurements for different treatments were tested using an analysis of one-way variance (ANOVA) followed by Duncan's multiple range test. Values are presented as the means ± standard deviation (SD) of three replicate samples. Significant differences were determined at $p < 0.05$ levels.

3. Results

Plant leaves with PEG-6000 and Ca^{2+} treatment after 72 h are shown in Figure 1. Compared with the control, the drought-treated leaf margin appeared to be yellowing and showed milder symptoms when supplemented with Ca^{2+}.

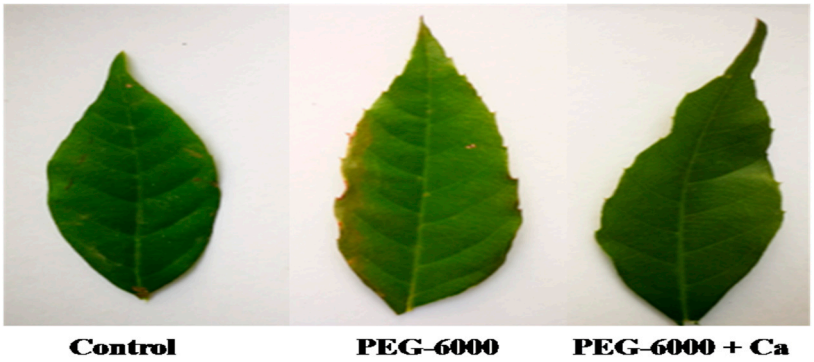

Figure 1. Typical pictures of plant leaf with PEG-6000 and Ca^{2+} treatment after 72 h.

3.1. Relative Water Content (RWC) and Leaf Water Potential (WP)

Compared with the control, the leaf RWC was significantly decreased at 24 h for drought stress and 48 h for Ca^{2+} addition ($p < 0.05$), respectively (Figure 2a). Meanwhile, significant decreases in the leaf instantaneous WP were also observed for drought stress and Ca^{2+} addition, with the lowest value of −3.4 MPa and −2.67 MPa at 72 h, respectively (Figure 2b). Under drought condition, the Ca^{2+}-treated plants had a higher RWC than the untreated one, with up to a 4.67% increase at 72 h (Figure 2a). Compared with the drought, significant higher WPs were also observed in the Ca^{2+}-treated plants at all treatment times.

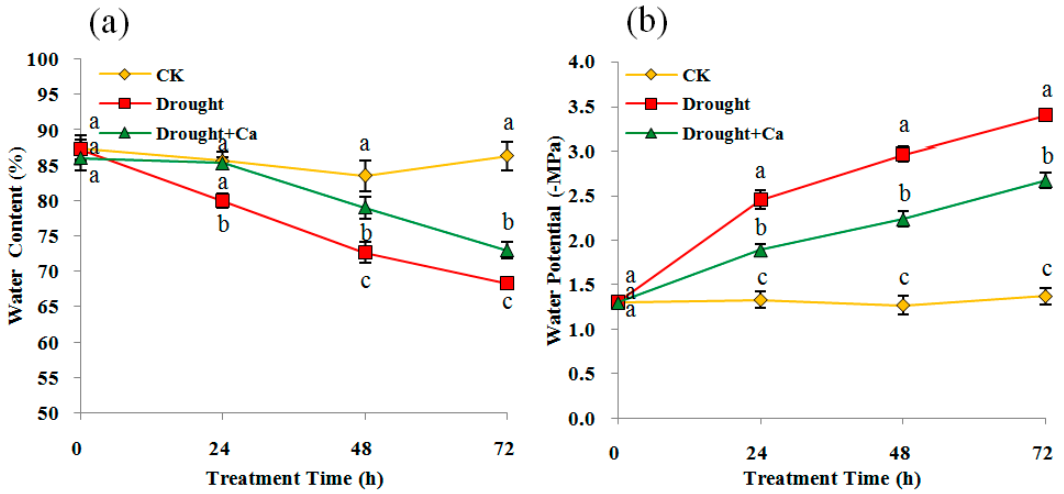

Figure 2. (**a**) Relative water content (RWC), (**b**) Water potential (WP) in *C. glauca* leaves under drought stress and amended with Ca^{2+}. Different small letters indicate a significant difference between treatments ($p < 0.05$) within each sampling time. Control group with no drought or Ca^{2+} treatment, CK; Drought-treated plants with no Ca^{2+} amendment, Drought; and Ca^{2+} amended drought-treated plants, Drought + Ca. All data show the means ± SD of three replicates.

3.2. $O_2^{\bullet-}$ and H_2O_2 Generation and Lipid Peroxidation

H_2O_2 and $O_2^{\bullet-}$ are the two main forms of ROS produced under adverse conditions. Compared with the control, $O_2^{\bullet-}$ and H_2O_2 contents were significantly increased ($p < 0.05$) at 12 h in drought stress and 24 h in Ca^{2+}-treated plants, respectively (Figure 3a,b). A sharp accumulation of $O_2^{\bullet-}$ (141.56%) and H_2O_2 (96.39%) over the control plants was found in drought conditions at 72 h (Figure 3a,b). Drought-treated plants supplemented with Ca^{2+} significantly ($p < 0.05$) reduced the $O_2^{\bullet-}$ accumulation starting at 12 h (Figure 3b), and significantly ($p < 0.05$) reduced the H_2O_2 accumulation starting at 12 h with an exception for 24 h (Figure 3a). The maximum reduction of $O_2^{\bullet-}$ and H_2O_2 contents with Ca^{2+} treatment were 31.82% and 17.25% at 12 h, respectively (Figure 3a,b).

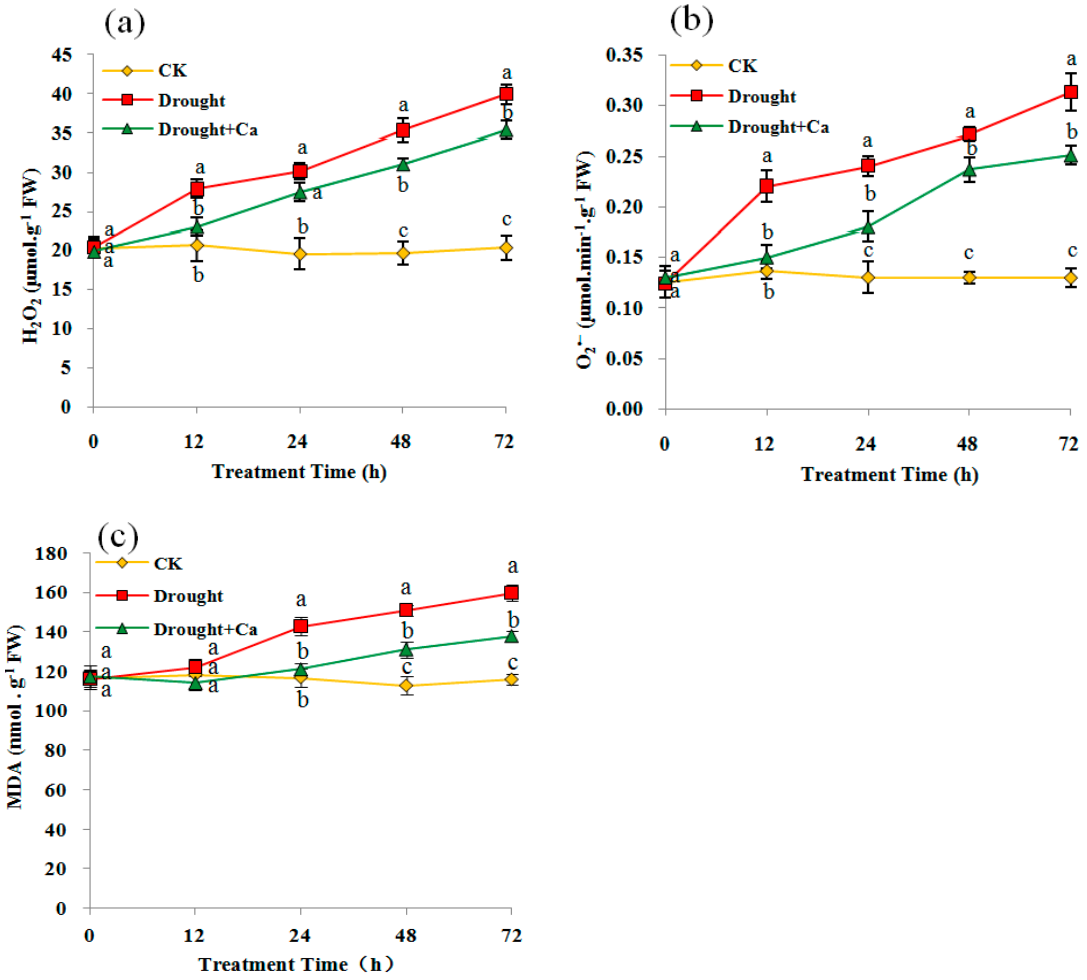

Figure 3. (**a**) H_2O_2 content, (**b**) $O_2^{\bullet-}$ content, (**c**) Lipid Peroxidation (MDA content) in *C. glauca* leaves under drought stress and amended with Ca^{2+}. The details of the treatments are given in the legend of Figure 2.

The MDA content in drought-stressed plants was significantly higher ($p < 0.05$) than the control plants starting at 24 h, with the sharpest accumulation of 38.37% over the control plants at 72 h (Figure 3c). Compared to the drought stress, the Ca^{2+}-treated plants showed a significantly lower MDA content starting at 24 h, which reduced the MDA accumulation by 13.94% at 72 h (Figure 3c).

3.3. Osmoregulation Substances

Compared to the control, the total proline and soluble sugar contents were significantly higher ($p < 0.05$) under drought stress starting at 12 h, and gradually increased as drought stress intensified, with the maximum increases of 64.21% and 59.09% at 72 h, respectively (Figure 4a,b). Drought-treated plants supplemented with Ca^{2+} significantly ($p < 0.05$) further increased the accumulation of proline and soluble sugar contents (Figure 3a,b). Glycine betaine only increased significantly ($p < 0.05$) under moderate drought stress (12, 24 h) (Figure 4c). Compared with the drought treatment, Ca^{2+}-treated plants significantly increased the glycine betaine contents starting at 24 h, with the maximum increase up to 26.87% at 48 h (Figure 4c).

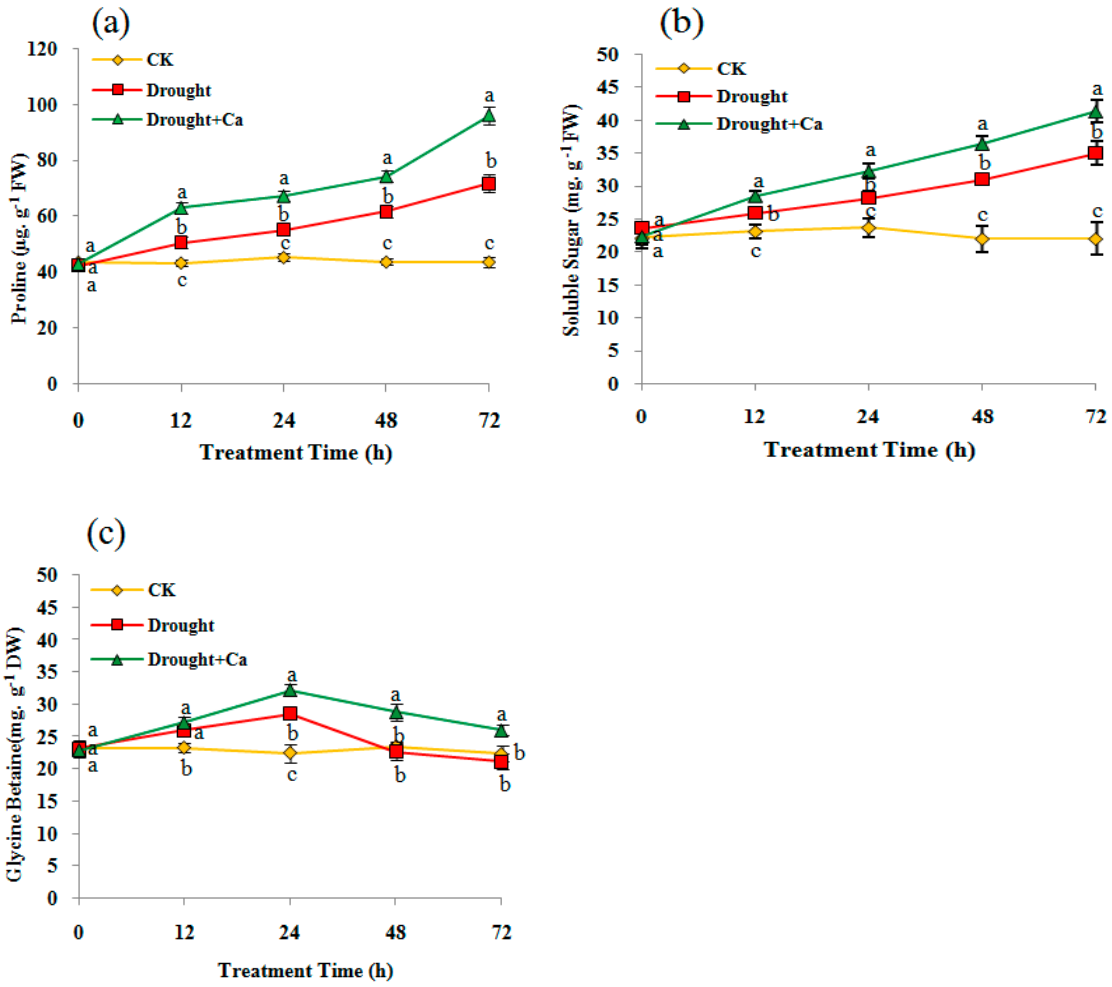

Figure 4. (**a**) Proline content, (**b**) Soluble sugar content, (**c**) Glycine betaine content in *C. glauca* leaves under drought stress and amended with Ca^{2+}. The details of the treatments are given in the legend of Figure 2.

3.4. Antioxidant Enzymes

Drought stress and exogenous Ca^{2+} positively regulated the antioxidant enzyme activities in the *C. glauca* cuttings. Under drought stress, the activities of POD, SOD and GPX were significantly induced ($p < 0.05$) starting at 12 h, while the activities of APX and GR significantly increased at 24 h (Figure 5a,b,d–f). The activities of CAT increased from 12 h to 48 h, but showed no differences compared to the control at 72 h (Figure 5c). Compared to the drought stress, Ca^{2+} treatment significantly increased ($p < 0.05$) POD, CAT, APX, and GPX activities starting at 12 h (except for POD at 72 h) and POD and GR activities starting at 24 h (Figure 5a–f). For instance, Ca^{2+}-treated plants remarkably increased POD and CAT activities as the drought condition intensified, with the maximum increases of 80.76% and 143.17% at 72 h, respectively (Figure 5a,c). The data showed that exogenous Ca^{2+} mainly increased the activities of the major antioxidant enzymes activities in *C. glauca* cuttings under water deficit.

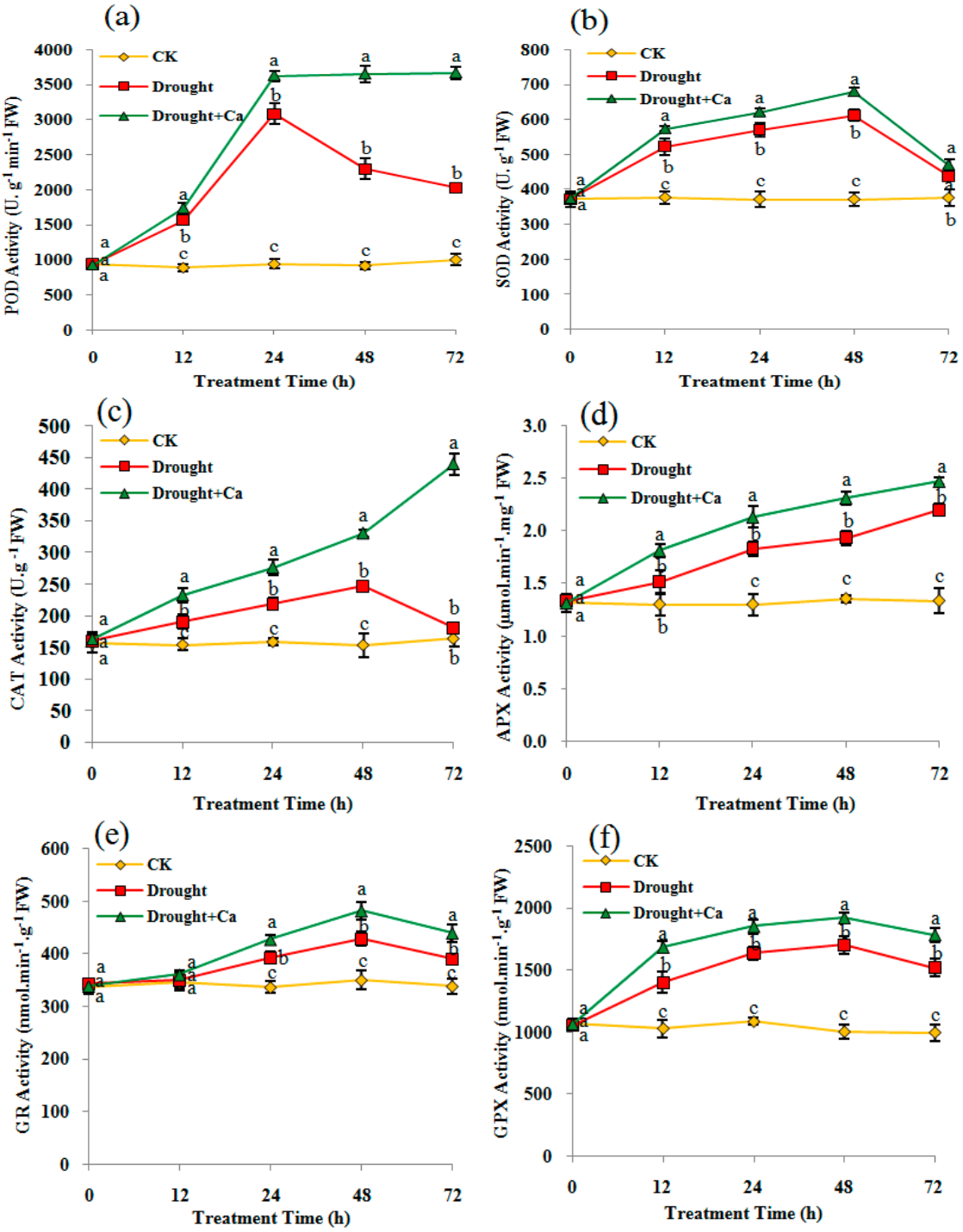

Figure 5. (**a**) Peroxidase (POD) activity, (**b**) Superoxide dismutase (SOD) activity, (**c**) Catalase (CAT) activity, (**d**) Ascorbate peroxidase (APX) activity, (**e**) Glutathione reductase GR activity, (**f**) Glutathione peroxidase (GPX) activity in *C. glauca* leaves under drought stress and amended with Ca^{2+}. The details of the treatments are given in the legend of Figure 2.

3.5. Photosynthetic Parameters

The SPAD value decreased as the drought stress persisted. However, a significant increase ($p < 0.05$) in SPAD was observed in Ca^{2+}-treated plants compared with the drought treatment across all stress time points (Table 1). Moreover, PEG-6000 induced drought stress significantly ($p < 0.05$) inhibited the Pn, Tr, and Gs of *C. glauca* compared to the control treatment, with the exception of a significant

($p < 0.05$) increase in WUE (Table 1). The largest decreases in Pn, Tr, and Gs under water deficit were 55.07%, 63.92%, and 55.08% at 72 h, respectively (Table 1). Ca^{2+} application significantly ($p < 0.05$) improved the above photosynthetic parameters at all treatment times (Table 1). Compared with the drought stress, the Pn and WUE in Ca^{2+}-treated plants increased by 57.55% and 13.55% at 72 h, respectively (Table 1).

Table 1. Photosynthetic parameters in *C. glauca* leaves under drought stress and amended with Ca^{2+}.

Treatment Time (h)	Treatment Type	SPAD SPAD Value	Pn ($\mu mol\ m^{-2}\ s^{-1}$)	Tr ($mmol\ m^{-2}\ s^{-1}$)	Gs ($mmol\ m^{-2}\ s^{-1}$)	WUE ($\mu mol\ CO_2\ mmol\ H_2O^{-1}$)
0	CK	50.50 ± 1.80 a	5.63 ± 0.47 a	3.57 ± 0.15 a	0.23 ± 0.03 a	1.58 ± 0.07 a
	Drought	51.00 ± 3.61 a	5.77 ± 0.23 a	3.67 ± 0.15 a	0.23 ± 0.01 a	1.57 ± 0.02 a
	Drought + Ca^{2+}	50.33 ± 1.53 a	5.83 ± 0.15 a	3.70 ± 0.10 a	0.23 ± 0.02 a	1.58 ± 0.02 a
24	CK	50.40 ± 2.62 a	6.00 ± 0.44 a	3.50 ± 0.20 a	0.22 ± 0.01 a	1.71 ± 0.05 a
	Drought	45.00 ± 1.50 b	4.40 ± 0.26 b	2.42 ± 0.10 b	0.16 ± 0.01 b	1.82 ± 0.02 b
	Drought + Ca^{2+}	47.63 ± 0.81 c	5.01 ± 0.25 c	2.77 ± 0.15 c	0.20 ± 0.01 c	1.81 ± 0.05 c
48	CK	49.01 ± 1.73 a	5.60 ± 0.43 a	3.53 ± 0.23 a	0.23 ± 0.01 a	1.58 ± 0.02 a
	Drought	42.33 ± 0.76 b	3.70 ± 0.36 b	2.04 ± 0.10 b	0.12 ± 0.01 b	1.81 ± 0.01 b
	Drought + Ca^{2+}	46.13 ± 1.46 c	4.38 ± 0.38 c	2.21 ± 0.12 c	0.15 ± 0.01 c	1.98 ± 0.00 c
72	CK	49.03 ± 3.61 a	6.07 ± 0.32 a	3.51 ± 0.10 a	0.22 ± 0.02 a	1.73 ± 0.05 a
	Drought	40.57 ± 1.36 b	3.13 ± 0.29 b	1.75 ± 0.13 b	0.10 ± 0.02 b	1.85 ± 0.08 b
	Drought + Ca^{2+}	43.00 ± 1.73 c	3.73 ± 0.25 c	1.99 ± 0.12 c	0.13 ± 0.01 c	1.95 ± 0.20 c

Note: Control group with no drought or Ca^{2+} treatment, CK; Drought-treated plants with no Ca^{2+} amendment, Drought; and Ca^{2+}-amended drought-treated plants, Drought + Ca^{2+}. Photosynthesis rate (Pn), stomatal conductance (Gs), transpiration rate (Tr), chlorophyll content (SPAD), and further increased water use efficiency (WUE). Different small letters indicate a significant difference between treatments ($p < 0.05$) within each sampling time.

4. Discussion

The present study effectively confirmed that exogenous Ca^{2+} could increase the drought tolerance of *Cyclobalanopsis glauca* (Thunb.) Oerst. (*C. glauca*). Compared to plants with simple drought treatment, leaves with Ca^{2+} application showed less yellowing, indicating a positive role of Ca^{2+} in increasing comprehensive drought resistance in plants. Relative water content (RWC) and water potential (WP) are two appropriate indicators in estimating the water status of plants. Drought induced significant declines in RWC and WP in *C. glauca*. External Ca^{2+} application alleviated drought damage, as manifested by the increases in these two factors compared to simply drought-treated plants. These findings partially support the results reported in *Zoysia japonica* Steud. [15], *Nitraria tangutorum* Bobr. [39], *Zea mays* L. [20,40], *Triticum aestivum* L. [40] and *Trifolium repens* L. [16], indicating a crucial role of Ca^{2+} in improving water conditions under drought stress. External Ca^{2+} application can mitigate the inhibition of aquaporin under osmotic stress in *Zea mays* [41], which plays an essential role in facilitating the transport of water [42]. Thus, whether the relative high water status maintained by Ca^{2+} in this study partly contributed to the increased aquaporin expression should be further studied.

Osmotic adjustment is an important mechanism for plants to maintain water status under drought conditions [43]. Accumulations of proline, soluble sugars, and glycine betaine are known to be associated with drought tolerance in plants [15,18,44]. The calcium-induced accumulation of proline, soluble sugar, and glycine betaine indicates the important role of calcium in alleviating the impact of drought [15,18]. For instance, Ca^{2+} application induced proline accumulation in *Zoysia japonica* under drought conditions [15]. Foliar calcium spray accelerated the accumulation of proline content in maize, which enhanced its tolerance to drought stress [45]. Indeed, Knight et al. [46] reported that a gene involved in proline biosynthesis could be upregulated by exogenous Ca^{2+} application under drought stress, implying a molecular function of Ca^{2+} involved in regulating the drought-resistant gene. Among the compatible solutes, glycine betaine is recognized as a particularly effective protectant against abiotic stress [44]. Calcium chloride application further increased the glycine betaine content in *Catharanthus roseus* L., thereby conferring greater osmoprotection under drought stress [14]. Chen et al. [47]

found that *Populus euphratica* Oliv. accumulated an amount of soluble sugar for osmoregulation to counter drought stress in a desert riparian forest. Xi et al. [48] reported that exogenous Ca^{2+} addition could increase soluble sugar content of *Vitis vinifera* L. during water stress. In the present study, we found that Ca^{2+} application resulted in a further increase in the concentrations of all three osmoregulation substances. This result indicated that calcium plays a vital role in improving water status by accelerating the accumulation of numerous osmoregulation substances during drought stress. However, massive accumulation of proline has been found to have toxic effects on plants [49]. Thus, research on accumulation and the effect of osmoregulation regulated by Ca^{2+} under prolonged drought stress is still needed.

The production and scavenging of ROS are unbalanced under water deficit conditions, causing oxidative damage to plants [8,9]. Therefore, drought stress induced peroxidation of membrane lipids as the excessive accumulation of ROS. MDA is considered as an important criterion of the peroxidation of membrane lipids [50]. Calcium is known to maintain membrane integrity, consequently enhancing drought tolerance in plants [51]. The absence of Ca^{2+} significantly increased the H_2O_2 and $O_2^{\bullet -}$ across all treatment times, accompanied by a significant enhancement of MDA in *C. glauca*. Plants synthesize numerous antioxidant enzymes to maintain the balance of the formation and elimination of ROS [52]. SOD is the first line of ROS defense that catalyzes $O_2^{\bullet -}$ dismutation into H_2O_2 and O_2 [53]. CAT and POD are the primary antioxidant enzymes for H_2O_2 scavenging in plant cells [54]. APX is involved in the ascorbate-glutathione (ASA-GSH) cycle, which is an efficient antioxidant system for the detoxification of H_2O_2. GR also plays a major role in maintaining the pool of reduced glutathione through H_2O_2 removal and by activating the ascorbate-glutathione cycle. GPX catalyze the reduction of H_2O_2 and lipid peroxides using GSH as an electron donor [55]. In the present study, drought stress induced enhancements of the antioxidant enzyme activities analyzed, and Ca^{2+} treatments further improved their activities. These results indicate that calcium plays a crucial role in detoxifying drought-induced oxidative stress by improving numerous antioxidant enzyme activities in *C. glauca*. It is note worthy that some antioxidant enzymes (such as SOD, GR, and GPX) showed decreased activities with the extension of the time of stress even in Ca^{2+} application treatments. The results may imply that the effects of Ca^{2+} in upregulating these antioxidant enzyme activities would be limited under prolonged drought stress.

Photosynthesis is the basis of plant growth and development, and water is recognized as one of the most important factors affecting photosynthesis [56]. Drought stress depressed stamota aperture, Rubisco activity, and chlorophyll biosynthesis, resulting in a reduction of the photosynthesis rate [10]. Ca^{2+} plays a vital role in the regulation of plant photosynthesis, including photosynthetic electron transport, the Calvin cycle, and chlorophyll stabilization [57,58]. In the present study, the application of Ca^{2+} significantly improved photosynthesis-related parameters such as Pn, Tr, Gs, WUE, and SPAD in *C. glauca*. These findings are consistent with previous studies on *Sophora viciifolia* Hance. [59], *Arachis hypogaea* L. [60], and *Cucumis sativus* L. [61]. Ramalho et al. [62] investigated the effects of Ca deficiency on *Coffea arabica* L. photosynthesis, indicating a pivotal role of Ca^{2+} in maintaining photochemical efficiency and chlorophyll content. Hu et al. [58,63] studied the proteomic responses of *Pinus massoniana* L. and *Liquidambar formosana* Hance. under acid rain stress with different calcium treatments, which confirmed that Ca^{2+} plays a vital role in the upregulation of genes involved in photosynthetic electron transport and carbon fixation. Under atrazine stress, Ca^{2+} application upregulated the expression of D1 protein in *Pennisetum giganteum* A. Rich., which has been recognized as one of the core subunits in photosystem II (PSII) [64]. Thus, external Ca^{2+} treatment might upregulate genes involved in electron transport, carbon assimilation, and PSII photochemical efficiency, which jointly improve the photosynthetic capacity of *C. glauca* under drought stress.

Supporting our hypothesis, high exogenous calcium could enhance the short-term drought tolerance of calcicole plant *C. glauca*. By improving the water status and capacities of ROS scavenging and photosynthesis, the Karst calcium-rich habitat might increase the survival chances of native plants under intermittent drought conditions. Even so, water status, ROS contents, and Pn in drought-treated

plants with Ca^{2+} application hardly reached the control level, indicating a limited role of Ca^{2+} in drought resistance. Moreover, as plant species, and the duration and intensity of drought stress could make such defense systems variable [65], far more studies still need to be undertaken under multiple drought conditions across various species.

5. Conclusions

In this study, drought stress induced a significant decrease in RWC, WP, Pn, Gs, Tr, and SPAD, and enhanced the levels of ROS production (H_2O_2 and $O_2^{\bullet -}$) and MDA content in calcicole *Cyclobalanopsis glauca* (Thunb.) Oerst. (*C. glauca*) leaves, implying that a reduction in water status and photosynthesis capacity combined with an increased oxidative stress were induced after exposure to water deficit conditions. Enhanced activities of antioxidant enzymes (POD, CAT, SOD, APX, GR, and GPX) and increased accumulation of osmoregulation substances (proline, soluble sugar, and glycine betaine) were also induced under drought treatment, suggesting that an antioxidant defense with osmotic adjustment was induced to enhance drought tolerance in *C. glauca*. Furthermore, short-term exogenous Ca^{2+} application alleviated the harmful ecophysiological effects produced by drought. Ca^{2+} amendment lowered the drought-induced oxidative stress with an increased activity of all the antioxidant enzymes mentioned. External Ca^{2+} further stimulated the accumulation of osmoregulation substances, which improved water status under short-term drought conditions. The Pn, Gs, Tr, SPAD, and WUE levels were also increased with external Ca^{2+} treatment, indicating that an improvement in photosynthesis capacity could be induced through Ca^{2+} application. In conclusion, high soil calcium content might enhance the tolerance of the calcicole *C. glauca* in a drought environment by maintaining water status, mitigating oxidative stress, and improving photosynthesis capacity.

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References

1. Wang, S.; Liu, Q.; Zhang, D. Karst Rocky Desertification in southwestern China: Geomorphology, landuse, impact and rehibilitation. *Land Degrad. Dev.* **2004**, *15*, 115–121. [CrossRef]
2. Li, S.; Birk, S.; Xue, L.; Ren, H.; Chang, J.; Yao, X. Seasonal changes in the soil moisture distribution around bare rock outcrops within a karst rocky desertification area (Fuyuan County, Yunnan Province, China). *Environ. Earth Sci.* **2016**, *75*, 1482. [CrossRef]
3. Li, S.; Ren, H.D.; Xue, L.; Chang, J.; Yao, X.H. Influence of bare rocks on surrounding soil moisture in the karst rocky desertification regions under drought conditions. *Catena* **2014**, *116*, 157–162. [CrossRef]
4. Cao, J.; Yuan, D.; Tong, L.; Azim, M.; Yang, H.; Huang, F. An overview of Karst ecosystem in Southwest China: Current state and future management. *J. Resour. Ecol.* **2015**, *6*, 247–256.
5. Zhou, Y.C.; Pan, G.X. Adaptation and adjustment of Maolan forest ecosystem to karst environment. *Carsol. Sin.* **2001**, *20*, 47–52.
6. Li, A.D.; Lu, Y.F.; Wei, X.L.; Yu, L.F. Studies on the regime of soil moisture under different microhabitats in Huajiang karst valley. *Carsol. Sin.* **2008**, *27*, 56–62.
7. Chen, D.; Wang, S.; Cao, B.; Cao, D.; Leng, G.; Li, H.; Yin, L.; Shan, L.; Deng, X. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in Maize seedlings. *Front. Plant Sci.* **2015**, *6*, 1241. [CrossRef] [PubMed]

8. Reddy, A.R.; Chaitanya, K.V.; Vivekanandan, M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol.* **2004**, *161*, 1189–1202. [CrossRef]
9. Cruz, M.H. Drought stress and reactive oxygen species: Production, scavenging and signaling. *Plant Signal Behav.* **2008**, *3*, 156–165. [CrossRef]
10. Parry, M.A.; Andralojc, P.J.; Khan, S.; Lea, P.J.; Keys, A.J. Rubisco activity: Effects of drought stress. *Ann. Bot.* **2002**, *89*, 833–839. [CrossRef] [PubMed]
11. Gopi, R.; Lakshmanan, G.A.; Panneerselvam, R. Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci.* **2011**, *171*, 271–276.
12. Deng, X.; Liu, Y.; Xu, X.; Liu, D.; Zhu, G.; Yan, X.; Wang, Z.; Yan, Y. Comparative proteome analysis of wheat flag leaves and developing grains under water deficit. *Front. Plant Sci.* **2018**, *9*, 425. [CrossRef] [PubMed]
13. Liu, C.; Liu, Y.; Guo, K. Ecophysiological adaptations to drought stress of seedlings of four plant species with different growth forms in karst habitats. *Chin. J. Plant Ecol.* **2011**, *35*, 1070–1082.
14. Jaleel, C.A.; Manivannan, P.; Sankar, B.; Kishorekumar, A.; Gopi, R.; Somasundaram, R.; Panneerselvam, R. Water deficit stress mitigation by calcium chloride in *Catharanthus roseus*: Effects on oxidative stress, proline metabolism and indole alkaloid accumulation. *Colloids Surf. B Biointerfaces* **2007**, *60*, 110–116. [CrossRef] [PubMed]
15. Xu, C.; Li, X.; Zhang, L. The Effect of Calcium Chloride on Growth, Photosynthesis, and Antioxidant Responses of *Zoysia japonica* under Drought Conditions. *PLoS ONE* **2013**, *8*, e68214. [CrossRef] [PubMed]
16. Wang, C.Q. Exogenous calcium alters activities of antioxidant enzymes in *Trifolium repens* L. leaves under peg-induced water deficit. *J. Plant Nutr.* **2010**, *33*, 1874–1885. [CrossRef]
17. Moustakas, M.; Sperdouli, I.; Kouna, T.; Antonopoulou, C.I.; Therios, I. Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. *Plant Growth Regul.* **2011**, *65*, 315. [CrossRef]
18. Kang, J.; Zhao, W.; Zheng, Y.; Zhang, D.M.; Zhou, H.; Sun, P. Calcium chloride improves photosynthesis and water status in the C4 succulent xerophyte *Haloxylon ammodendron* under water deficit. *Plant Growth Regul.* **2017**, *82*, 1–12. [CrossRef]
19. Li, Z.; Tan, X.F.; Lu, K.; Liu, Z.M.; Wu, L.L. The effect of $CaCl_2$ on calcium content, photosynthesis, and chlorophyll fluorescence of tung tree seedlings under drought conditions. *Photosynthetica* **2017**, *55*, 553–560. [CrossRef]
20. Marques, D.J.; Ferreira, M.M.; da Silva Lobato, A.K.; Guedes de Carvalho, J.; de Assuncao Carvalho, J.; Alves de Freitas, W.; Ribeiro Bastos, A.R.; Pereira, F.J.; Mauro de Castro, E. $CaSiO_3$ improves water potential and gas exchange but not contribute to the production parameters of maize plants exposed to different irrigation depths. *Aust. J. Crop Sci.* **2015**, *8*, 1257–1265.
21. Wang, W.H.; Chen, J.; Liu, T.W.; Chen, J.; Han, A.D.; Simon, M.; Dong, X.J.; He, J.X.; Zheng, H.L. Regulation of the calcium-sensing receptor in both stomatal movement and photosynthetic electron transport is crucial for water use efficiency and drought tolerance in Arabidopsis. *J. Exp. Bot.* **2014**, *65*, 223–234. [CrossRef] [PubMed]
22. David, L.; Raoul, R.; Alain, P. Calcium in plant defence–signalling pathways. *New Phytol.* **2006**, *171*, 249–269.
23. Shao, H.B.; Song, W.Y.; Chu, L.Y. Advances of calcium signals involved in plant anti-drought. *C. R. Biol.* **2008**, *331*, 587–596. [CrossRef] [PubMed]
24. Zou, J.J.; Wei, F.J.; Wang, C.; Wu, J.J.; Ratnasekera, D.; Liu, W.X.; Wu, W.H. Arabidopsis calcium-dependent protein kinase CPK_{10} functions in ABA and Ca^{2+}-mediated stomatal regulation in response to drought stress. *Plant Physiol.* **2010**, *154*, 1232–1243. [CrossRef] [PubMed]
25. Zhang, Z.F.; You, Y.M.; Huang, Y.Q.; Li, X.K.; Zhang, J.C.; Zhang, D.N.; He, C.X. Effects of drought stress on *Cyclobalanopsis glauca* seedlings under simulating karst environment condition. *Acta Ecol. Sin.* **2012**, *32*, 6318–6325. [CrossRef]
26. Zhang, Z.F.; You, Y.M.; Huang, Y.Q.; Li, X.K.; Zhang, J.C.; Zhang, D.N.; He, C.X. Changes of *Cyclobalanopsis glauca* water parameters under drought stress in karst environment. *Chin. J. Ecol.* **2011**, *30*, 664–669.
27. Sergiev, I.; Alexieva, V.; Karanov, E. Effect of spermine, atrazine and combination between them on some endogenous protective systems and stress markers in plants. *C. R. Acad. Bulg. Sci.* **1997**, *51*, 121–124.
28. Meng, X.; Wang, J.R.; Wang, G.D.; Liang, X.Q.; Li, X.D.; Meng, Q.W. An R2R3-MYB gene, *LeAN2*, positively regulated the thermo-tolerance in transgenic tomato. *J. Plant Physiol.* **2015**, *175*, 1–8. [CrossRef] [PubMed]

29. Cao, Y.; Luo, Q.; Tian, Y.; Meng, F. Physiological and proteomic analyses of the drought stress response in *Amygdalus mira* (Koehne) Yü et Lu roots. *BMC Plant Biol.* **2017**, *17*, 53. [CrossRef] [PubMed]
30. Gao, J. *Experimental Techniques of Plant Physiology*; World Publishing Corporation: Xi'an, China, 2000.
31. Grieve, C.M.; Grattan, S.R. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil* **1983**, *70*, 303–307. [CrossRef]
32. Beauchamp, C.; Fridovich, I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* **1971**, *44*, 276–287. [CrossRef]
33. Chance, B.; Maehly, A.C. Assay of catalase and peroxidases. *Methods Enzymol.* **1955**, *1*, 764–775.
34. Upadhyaya, A.; Sankhla, D.; Davis, T.D.; Sankhla, N.; Smith, B.N. Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. *J. Plant Physiol.* **1985**, *121*, 453–461. [CrossRef]
35. Nakano, Y.; Asada, K. Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.* **1981**, *22*, 867–880.
36. Cakmak, I.; Strbac, D.; Marschner, H. Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. *J. Exp. Bot.* **1993**, *44*, 127–132. [CrossRef]
37. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
38. Lawrence, R.A.; Burk, R.F. Glutathione peroxidase activity in selenium deficient rat liver. *Biochem. Biophys. Res. Commun.* **1976**, *71*, 952–958. [CrossRef]
39. Yuan, X.T.; Liu, W.; Xuan, Y.N.; Zhang, Y.Y.; Yan, Y.Q. Physiological effects of exogenous Ca^{2+} on *Nitraria tangutorum* under salt stress. *Plant Physiol. J.* **2014**, *50*, 88–94.
40. Harsh, N. Accumulation of osmolytes and osmotic adjustment in waterstressed wheat (*Triticum aestivum*) and maize (*Zea mays*) as affected by calcium and its antagonists. *Environ. Exp. Bot.* **2003**, *50*, 253–264.
41. Wu, Y.; Liu, X.; Wang, W.; Zhang, S.; Xu, B. Calcium regulates the cell-to-cell water flow pathway in maize roots during variable water conditions. *Plant Physiol. Biochem.* **2012**, *58*, 212–219. [CrossRef] [PubMed]
42. Maurel, C.; Verdoucq, L.; Luu, D.T.; Santoni, V. Plant aquaporins: Membrane channels with multiple integrated functions. *Annu. Rev. Plant Biol.* **2008**, *59*, 595–624. [CrossRef] [PubMed]
43. Serraj, R.; Sinclair, T.R. Osmolyte accumulation: Can it really help increase crop yield under drought conditions? *Plant Cell Environ.* **2002**, *25*, 333–341. [CrossRef] [PubMed]
44. Chen, T.H.; Murata, N. Glycinebetaine: An effective protectant against abiotic stress in plants. *Trends Plant Sci.* **2008**, *13*, 499–505. [CrossRef] [PubMed]
45. Muhammad, N.; Muhammad, S.N.; Rashid, A.; Muhammad, Z.I.; Muhammad, Y.A.; Yasir, H.; Shah, F. Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. *Arch. Agron. Soil Sci.* **2018**, *64*, 116–131.
46. Knight, H.; Trewavas, A.J.; Knight, M.R. Calcium signaling in *Arabidopsis thaliana* responding to drought and salinity. *Plant J.* **1997**, *12*, 1067–1078. [CrossRef] [PubMed]
47. Chen, Y.; Zhou, H.; Chen, Y. Adaptation strategies of desert riparian forest vegetation in response to drought stress. *Ecohydrology* **2013**, *6*, 956–973. [CrossRef]
48. Xi, Z.M.; Sun, W.J.; Zhang, Z.W. Effect of exogenous Ca^{2+} on drought resistance physiological indexes of wine grape cultivar Pinot Noir under water stress. *J. Northwest A&F Univ.* **2007**, *35*, 137–140.
49. Shamsul, H.; Qaiser, H.; Mohammed, N.A.; Arif, S.W.; John, P.; Aqil, A. Role of proline under changing environments. *Plant Signal. Behav.* **2012**, *7*, 1456–1466.
50. Jiang, Y.; Huang, B. Effect of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. *J. Exp. Bot.* **2001**, *52*, 341–349. [CrossRef] [PubMed]
51. Agarwal, S.; Sairam, R.K.; Srivastava, G.C.; Tyagi, A.; Meena, R.C. Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings. *Plant Sci.* **2005**, *169*, 559–570. [CrossRef]
52. Foyer, C.H.; Shigeoka, S. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol.* **2011**, *155*, 93–100. [CrossRef] [PubMed]
53. Alscher, R.G.; Erturk, N.; Heath, L.S. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **2002**, *53*, 1331–1341. [CrossRef] [PubMed]
54. Li, L.; Yi, H. Effect of sulfur dioxide on ROS production, gene expression and antioxidant enzyme activity in *Arabidopsis* plants. *Plant Physiol. Biochem.* **2012**, *58*, 46–53. [CrossRef] [PubMed]

55. Yoshimura, K.; Miyao, K.; Gaber, A.; Takeda, T.; Kanaboshi, H.; Miyasaka, H.; Shigeoka, S. Enhancement of stress tolerance in transgenic tobacco plants overexpressing *Chlamydomonas glutathione* peroxidase in chloroplasts or cytosol. *Plant J.* **2004**, *37*, 21–33. [CrossRef] [PubMed]
56. Chaves, M.M.; Flexa, J.; Pinheiro, C. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Ann. Bot.* **2009**, *103*, 551–560. [CrossRef] [PubMed]
57. Hochmal, A.K.; Schulze, S.; Trompelt, K.; Hippler, M. Calcium-dependent regulation of photosynthesis. *Biochim Biophys Acta* **2015**, *1847*, 933–1003. [CrossRef] [PubMed]
58. Hu, W.J.; Wu, Q.; Liu, X.; Shen, Z.J.; Chen, J.; Liu, T.W.; Chen, J.; Zhu, C.Q.; Wu, F.H.; Chen, L.; et al. Comparative proteomic analysis reveals the effects of exogenous calcium against acid rain stress in *Liquidambar formosana* Hance leaves. *J. Proteome Res.* **2015**, *15*, 216–228. [CrossRef] [PubMed]
59. Zhao, L.; Wang, P.; Chen, C.; Song, G.; Tang, H. Effect of Exogenous Ca^{2+} on the growth, physiological characters of *Sophora viciifolia* seedlings in Karst mountain area under the drought stress. *J. Nucl. Agric. Sci.* **2017**, *31*, 2039–2046.
60. Gu, X.; Sun, L.; Gao, B.; Sun, Q.; Liu, C.; Zhang, J.; Li, X. Effects of calcium fertilizer application on peanut growth, physiological characteristics, yield and quality under drought stress. *Chin. J. Appl. Ecol.* **2015**, *26*, 1433–1439.
61. Chen, L.L.; Wang, X.F.; Liu, M.; Yang, F.J.; Shi, Q.H.; Wei, M.; Li, Q.M. Effect of calcium and ABA on photosynthesis and related enzymes activities in cucumber seedlings under drought stress. *Chin. J. Appl. Ecol.* **2016**, *27*, 3996–4002.
62. Ramalho, J.C.; Rebelo, M.C.; Santos, M.E.; Antunes, M.L.; Nunes, M. Effects of calcium deficiency on *Coffea arabica*. Nutrient changes and correlation of calcium levels with some photosynthetic parameters. *Plant Soil* **1994**, *172*, 87–96. [CrossRef]
63. Hu, W.J.; Chen, J.; Liu, T.W.; Wu, Q.; Wang, W.H.; Liu, X.; Shen, Z.J.; Simon, M.; Chen, J.; Wu, F.H.; et al. Proteome and calcium-related gene expression in *Pinus massoniana* needles in response to acid rain under different calcium levels. *Plant Soil* **2014**, *2380*, 285–303. [CrossRef]
64. Erinle, K.O.; Jiang, Z.; Ma, B.; Li, J.; Chen, Y.; Ur-Rehman, K.; Shahla, A.; Zhang, Y. Exogenous calcium induces tolerance to atrazine stress in Pennisetum seedlings and promotes photosynthetic activity, antioxidant enzymes and *psbA* gene transcripts. *Ecotoxicol. Environ. Saf.* **2016**, *132*, 403–412. [CrossRef] [PubMed]
65. Guo, J.; Yang, Y.; Wang, G.; Yang, L.; Sun, X. Ecophysiological responses of Abies fabri seedlings to drought stress and nitrogen supply. *Physiol. Plantarum* **2010**, *139*, 335–347.