Acta Botanica Brasilica, 2025, 39: e20230217
doi: https://doi.org/10.1590/1677-941X-ABB-2023-0217

Original Article

Influence of exogenous abscisic acid on physiological responses of *Dendrobium candidum* under drought stress

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ABSTRACT

Abscisic acid (ABA) is a naturally occurring plant hormone, and it is also a known stress hormone that acts in plant responses to abiotic stresses, especially drought. *Dendrobium candidum* production losses due to drought have prominent economic effects. The current study investigates the role of ABA in the drought stress tolerance of *D. candidum*. The influence of drought stress and foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) on physiological and biochemical parameters was analyzed. The stems and leaves of *D. candidum* were collected on Day 0, Day 6, and Day 12. The osmotic regulation ability, photosynthetic maintenance ability, and antioxidant ability of *D. candidum* under drought stress were measured. Additionally, the changes in growth and physiological characteristics of *D. candidum* under drought stress were studied after applying three concentrations of ABA. Exogenous ABA application effectively ameliorates the adverse effects of drought stress to improve drought resistance. In conclusion, 10 mg/l ABA resulted in leaf water status maintenance, photosynthetic performance, and antioxidative ability under drought stress.

Keywords: ABA, antioxidant ability, *Dendrobium candidum*, drought stress, drought resistance.

Introduction

Dendrobium candidum Kimura et Migo, has been used as a natural, nutritional food ingredient and traditional Chinese herb for thousands of years (Cao *et al.*, 2019). The application of *D. candidum* was recorded in Shennong's Classic of Materia Medica at the end of the Eastern Han Dynasty (25 C.E.–220 C.E.). It has been used to nourish the stomach, regulate intestinal microbiota, enhance immunity, and reduce blood lipids (Cui *et al.*, 2014; Mittraphab *et al.*, 2016). Modern pharmacological studies have shown that *D. candidum* has many effects (*e.g.*, hypoglycaemic and antiaging effects; Li *et al.*, 2018). In recent years, the reserves of *D. candidum* have decreased yearly. The reasons for the decline include damage to the natural habitat of wild *D. candidum* such as uncontrolled excavation and mining activities, and lack of protective measures for its living environment. These factors have led to environmental deterioration in *D. candidum* production areas and a reduction in its wild populations. Second, *D. candidum* has strict requirements for environmental conditions, low reproductive capacity, and slow growth under natural conditions. Drought can inhibit

Received September 05, 2023; Accepted January 07, 2025
Editor-in-Chief: Thaís Elias Almeida; Associate Editor: Marina Scalon

How to cite:
Tian FX, Xu GL, Wang CX, Wei YQ, Zhou S. 2025. Influence of exogenous abscisic acid on physiological responses of *Dendrobium candidum* under drought stress. Acta Botanica Brasilica 39: e20230217. doi: 10.1590/1677-941X-ABB-2023-0217

the survival and growth of *D. candidum* seedlings, which is the main factor affecting their survival and distribution (Xu *et al.*, 2019a). Therefore, enhancing the drought resistance ability of *D. candidum* seedlings and finding an effective method to alleviate drought damage are key to improving the survival rate and yield of its seedlings, an urgent problem to be solved in *D. candidum* planting and production.

Phytohormones are considered the main signals during stress conditions (Hasanagic *et al.*, 2020). Exogenous application of hormones has been shown to mitigate the negative effects of drought stress and improve crop drought resistance by mediating adjustments to leaf physiology and metabolism (Izabela *et al.*, 2014; Muller & Munne-Bosch, 2021). Moreover, abscisic acid (ABA) is a naturally occurring plant hormone. ABA has also been reported to play an essential role in maintaining plant growth under different stress conditions (Vishal & Kumar, 2018), especially drought (Wang *et al.*, 2013). ABA plays a vital role in plants under drought conditions by signaling stomatal closure and reducing shoot expansion, and ABA is involved in vigorous root growth and other modifications (Fang & Xiong, 2015). Exogenous ABA application has been used as a drought mitigation practice in many experiments since it has been shown to reduce drought-induced deficit stress in plants through changes in their antioxidant system (Li *et al.*, 2011). Additionally, ABA application has been shown to significantly increase the chlorophyll content and net photosynthetic rates (A) of drought-stressed leaves (Chen *et al.*, 2018), regulate leaf carbon metabolism, and enhance plant drought resistance (Dekkers *et al.*, 2008). Furthermore, some studies have reported that ABA spraying altered the carbohydrate components in drought-stressed leaves (Li *et al.*, 2016; Pattanagul, 2011) and assimilated partitioning among plant tissues under drought (Travaglia *et al.*, 2007). ABA levels rapidly increase at the onset of drought and trigger a complex and multilayered signaling cascade, leading to the transcription of drought-responsive genes that enable the plant to maintain and adapt physiological processes under drought (Ali *et al.*, 2020).

Drought stress is one of the most severe abiotic stresses across the world and seriously affects plant growth (Xu *et al.*, 2019b; Hassan *et al.*, 2020). *Dendrobium candidum* is an epiphytic orchid and grows on trunks and cliffs. Owing to this water-scarce environment, *D. candidum* has evolved sophisticated defense mechanisms against severe drought stress, such as abundant metabolites and facultative crassulacean acid metabolism (CAM), an evolutionary adaptation of photosynthesis to reduce water loss under drought (Xiao *et al.*, 2007). Huang *et al.* (2021) reported that plant hormone biosynthesis and signal transduction, particularly ABA, may play a vital role in the regulation of facultative CAM in *D. candidum*.

Based on previous reports on the positive effects of ABA on enhancing drought resistance of plants (Ramya *et al.*, 2022; Hu *et al.*, 2022), it was hypothesized that exogenous ABA application would also improve leaf osmotic regulation ability, photosynthetic maintenance ability, and antioxidant ability in *D. candidum* seedlings under drought stress. This experiment simulates the drought situation of *D. candidum* and applies different concentrations of ABA to explore its physiological characteristics under drought stress conditions, to determine the optimal ABA concentration. It also provides technical support for the large-scale promotion and application of *D. candidum*.

Materials and methods

Plant materials and stress treatment

The materials used in this experiment were all 3-year-old robust seedlings of *D. candidum* in the growth stage. The plants were growing at Nanyang National Agricultural Science and Technology Park (112.430498 N, 32.948114 E) in Henan province (China) in the autumn of 2022. Osmotic stress was induced using 20% polyethylene glycol (PEG)-6000 solution (-1.45 MPa) with water as control (Pattanagul, 2011).

The *D. candidum* seedlings were divided into four groups, with five pots in each group. Each pot was transplanted with 4-5 plants per pot. The culture medium in the pot was a mixture of pine needles, sawdust, and coconut bricks soaked in water for two days. Four groups of *D. candidum* were grown in a chamber at 25 °C with a 16/8 h light/dark cycle (300-400 μmol photons m^{-2} s^{-1}) and a relative humidity of 75-80%. In the early stage, they were watered every two days to restore their growth. Day 0 was defined as the day when new leaves of *D. candidum* seedlings began to grow. The four groups of *D. candidum* roots were irrigated with 20% PEG-6000 every two days under drought stress (DS). At the same time, leaves of group C0 were sprayed with water every two days under control treatment (CK), leaves of group C5 were sprayed with 5 mg/l ABA, leaves of group C10 were sprayed with 10 mg/l ABA, and leaves of group C15 were sprayed with 15 mg/l ABA. Subsequently, *D. candidum* was sampled and stored in chronological order at 0, 6, and 12 days. After determining water potential, relative water content, chlorophyll a fluorescence, and photosynthetic gas exchange parameters on Days 0, 6, and 12, four sets of *D. candidum* leaves were collected and weighed for the measurements of the physiological and biochemical parameters in the laboratory. The selected material was wrapped in tin foil and identified with a marker. The samples were placed in liquid nitrogen for 30 min before being placed in a -20 °C refrigerator for posterior use.

Determination of water potential

Leaf water potential (Ψw) was measured with an HR-33 T dewpoint microvoltmeter (Wescor, Logan, UT, USA) after equilibration in the chamber for 2.5 h. The water potential was determined in the psychrometer mode following the manufacturer´s method.

Determination of relative water content

The relative water content (RWC) of leaves was determined according to Ma *et al.* (2006). Relative water content was calculated using the following equation (FW–DW) / (TW–DW) × 100%. Meanwhile, FW is the fresh weight, TW is the saturated weight after soaking the samples in water for at least 12 hours and DW is the dry weight after the shaping at 105°C for 15 minutes and then drying samples at 65°C for 24h.

Measurements of soluble sugar and proline contents

Soluble sugar content was analyzed with anthrone reagent with a Bausch and Lomb Spectrophotometer (Yemm & Willis, 1954). To measure the soluble sugar content, frozen leaf material (0.3 g) was extracted with 10 ml H_2O at 100 °C for 10 min. The extracts were filtered and analyzed for soluble sugar content using the anthrone-sulfuric acid method. Briefly, 1 ml of the extract was mixed with 1 ml of H_2O, 0.5 ml of anthrone reagent (1 g anthrone and 50 ml ethyl acetate), and 5 ml of oil of vitriol and then heated at 100 °C for 1 min. After cooling, the mix was analyzed by UV spectrophotometry at 630 nm. Proline was measured according to Shan *et al.* (2007).

Determination of photosynthetic pigments

Photosynthetic pigments were extracted from 0.05 g of fresh leaves in 10 ml of 80% aqueous acetone. The homogenate was filtered, and 1 ml of the suspension was diluted by the addition of 2 ml of acetone. The chlorophyll a (chl a), chlorophyll b (chl b), and carotenoid contents were determined using a Hitachi U-2001 spectrophotometer (Hitachi Ltd., Japan) at three wavelengths (663.2, 646.8, and 470.0 nm). The concentrations of pigments (mg/g FW) were calculated using a previously reported equation (Lichtenthaler, 1987).

Chlorophyll a fluorescence analysis

The actual PSII efficiency under irradiance (ΦPSII) and the maximal photochemical efficiency of PSII (Fv/Fm) were measured with a portable pulse-modulated fluorometer (FMS-2, Hansatech Instruments Ltd., King's Lynn, UK). For quenching analyses, the leaves were illuminated with actinic light intensities of 800 μmol photons/m^2 s for 16 min (which was found to be sufficient for the induction of steady-state light conditions) and subsequently kept for an additional 10 min in the dark (Jiang *et al.*, 2003).

Determination of photosynthetic gas exchange parameters

Photosynthetic gas exchange parameters were measured according to Guo *et al.* (2008). Leaves were analyzed for photosynthetic gas exchange parameters, including net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs), using a portable photosynthetic system (LI-6800, UK) at 9:00-11:00 am, with a photon flux density (PFD) of ~1200 μmol photons/m^2 s. Environmental conditions in the leaf chamber comprised CO_2 concentration of 400 μl l^{-1}, block temperature 25 ◦C, and chamber flow rate of 300-500 μmol s^{-1} to maintain relative humidity between 40% and 60%. The orientation of the leaves was adjusted to achieve this PFD. The measurements lasted approximately 10 min, during which no significant environmental change was observed. These measurements were made for 6 leaves from *D. candidum*.

Determination of malondialdehyde content

The malondialdehyde (MDA) level was assayed according to Quan *et al.* (2004). Leaves (50-100 mg) were homogenized in 5 ml of 10% trichloroacetic acid (TCA), and centrifuged at 12 000 × g for 10 min. Four milliliters of 0.6% thiobarbituric acid (TBA) in 10% TCA was added to 2 ml of the supernatant. The mixture was heated in boiling water for 15 min, and then quickly cooled in an ice bath. After centrifugation at 12 000 × g for 10 min, the absorbance of the supernatant at 450, 532, and 600 nm was determined with a spectrometer. The concentration of MDA was calculated by the following formula: C (μmol l^{-1}) = 6.45 (OD_{532}-OD_{600})-0.56OD_{450}

Determination of relative electrical conductivity

Electrolyte leakage was measured according to Cao *et al.* (2007). Six leaf discs (0.8 cm in diameter) were placed into 10 ml of distilled water, vacuumed for 30 min, and then surged for 3 h to measure the initial electrical conductivity (S1) (25 °C). A test tube was filled with leaf discs and distilled water, and the mixture was cooked (100 °C) for 30 min and then reduced to room temperature (25 °C) to determine the final electrical conductivity (S2). The relative electrical conductivity (REC) was evaluated as REC = S1 × 100/S2.

Staining detection of superoxide radical ($O_2^{\cdot-}$) accumulation

$O_2^{\cdot-}$ accumulation was visualized with nitroblue tetrazolium (NBT) staining methods (Zong *et al.*, 2009). Detached leaves were infiltrated with 6 mM NBT. Chlorophyll was removed from the leaves prior to imaging by infiltrating them with lactoglycerol-ethanol (1:1:4, v/v/v) and boiling in water for 5 min. The location of formazan deposits was visualized by subtracting background (nonformazan) pixels from the leaf image.

Hydrogen peroxide (H_2O_2) staining with diaminobenzidine

Detached leaves were infiltrated with 6–8 ml diaminobenzidine (DAB) solution (1 mg/ml DAB, Sigma pH 3.8) with NaOH according to the methods of Giacomelli *et al.* (2007) with slight modification. The leaves were then left in the solution overnight in the dark. The following day, the solution containing the leaves was boiled for a few minutes and then incubated at room temperature for 3 h under mild agitation. The leaves were then cleared with chlorhydrate (10 ml of water added to 25 g chlorhydrate).

Determination of $O_2^{\cdot-}$ and H_2O_2

The assay for $O_2^{\cdot-}$ was performed using the method described by Sui *et al.* (2007). The H_2O_2 content was determined according to the method proposed by Sairam & Srivastava (2002). The H_2O_2 concentration was estimated by measuring the spectrum absorbance of the titanium-hydroperoxide complex and using a standard curve plotted using a known concentration of H_2O_2.

Determination of antioxidant enzyme activity

The activities of the following enzymes were determined according to previously described methods: superoxide dismutase (SOD; EC 1.15.1.1; He *et al.* (2005), ascorbate peroxidase (APX; EC 1.11.1.11; Cakmak & Marschner (1992), catalase (CAT; EC 1.11.1.6; Durner & Klessig (1996), and guaiacol peroxidase (POD; EC 1.11.1.7; Scebba *et al.* (2001). Enzyme activity assays were carried out using a UV-visible spectrophotometer (UV-2550, Shimadzu, Japan) at 25 °C. The specific activity for all enzymes is expressed as U/mg (protein). The protein content was assayed according to Bradford (1976) using BSA as a standard.

Statistical analysis

Data analyses were performed using SPSS 16.0 (SPSS Inc., USA) software. Data was presented as the mean ± SE of 3 replicates and statistical significance was determined by the Tukey test ($P < 0.05$).

Results

Comparison of osmoregulation ability parameters

We measured the water potential and relative water content in the leaves of *D. candidum* seedlings under control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) (Fig. 1a-c). Under drought stress for 0 days, there was no significant difference in the four groups of *D. candidum*. After 6 days of drought stress, the water potential of the four groups of *D. candidum* decreased. The C10 group of *D. candidum* had a lower water potential (-1.5 MPa) after 12 days of drought stress (Fig. 1a). After 12 days of drought stress, the relative water content decreased. The C10 group of *D. candidum* had higher relative water content (97%) after 12 days of drought stress (Fig. 1b). The C10 group of *D. candidum* was more likely to absorb water from the environment under stress. The lower Ψs in the C10 group may be related to the accumulation of soluble protein and sugars.

The osmoregulation substances

We further measured the contents of soluble protein, soluble sugar, and free proline. Under drought stress for 0 days, the soluble protein, soluble sugar, and free proline contents among the four groups of *D. candidum* were relatively small (Fig. 2a-c). After 6 days of drought stress, the chlorophyll, soluble protein, soluble sugar, and free proline contents were low, and they decreased gradually over time under drought conditions. After 12 days of drought stress, the soluble protein, soluble sugar, and free proline contents of Group C10 *D. candidum* were relatively higher.

Changes in chlorophyll content and photosynthetic fluorescence parameters

Under drought stress for 0 days, the chlorophyll content, ΦPSII, and Fv/Fm among the four groups of *D. candidum* were relatively low (Fig. 3a-c). After 12 days of drought stress, the C10 group had the highest chlorophyll content (2.1 mg/g FW) (Fig. 3a). After 6 days of drought stress, ΦPSII were low, and they decreased gradually over time under drought conditions (Fig. 3b). Comparing the data, it can be concluded that the ΦPSII of Group C0 *D. candidum* was relatively higher, while after ABA processing, Group C10 *D. candidum* had the highest ΦPSII (Fig. 3b). No significant difference in Fv/Fm of PSII photochemistry was observed between the four groups of *D. candidum* at 6 d and 12 d under drought stress (Fig. 3c).

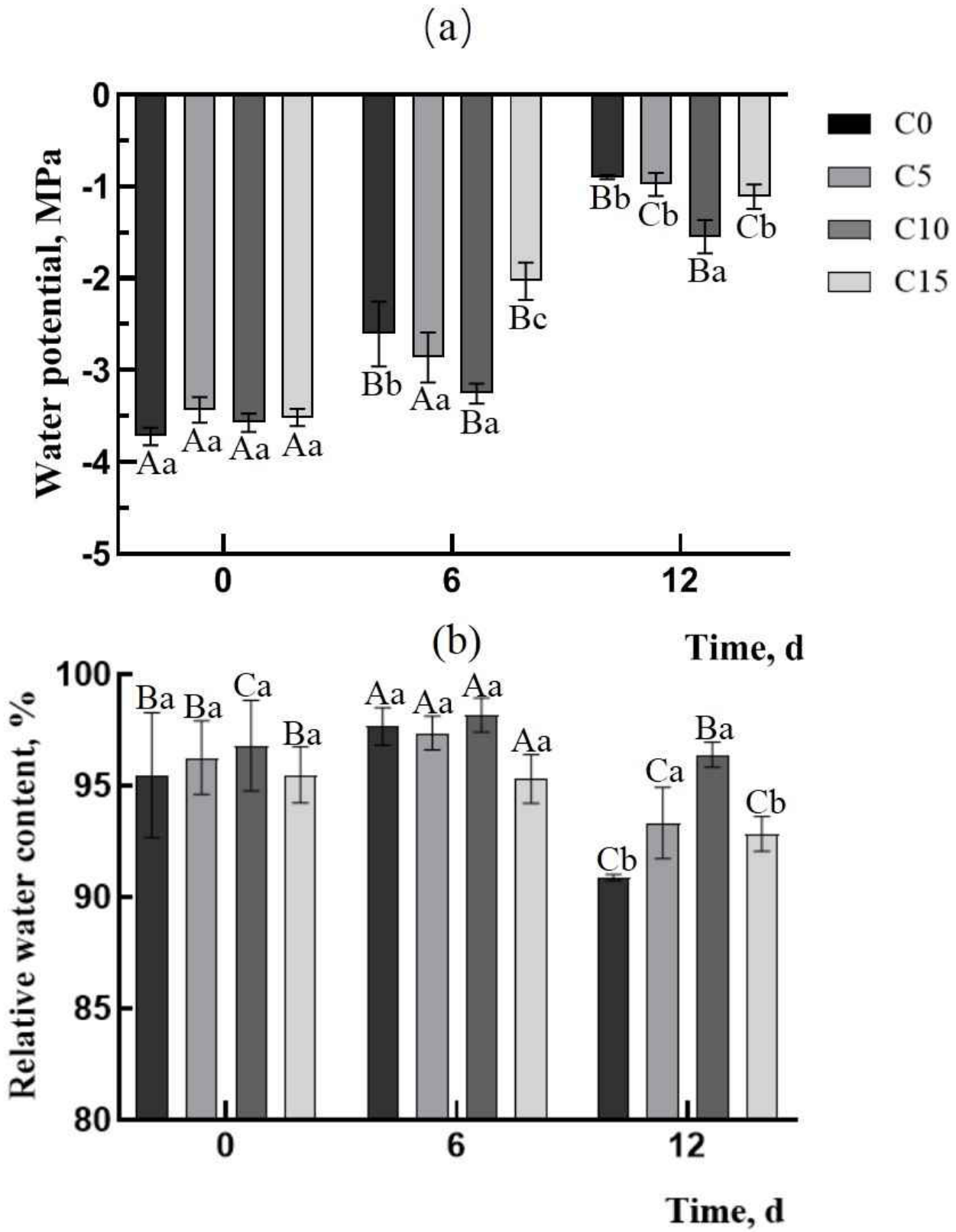

Figure 1. Changes in (a) water potential, (b) relative water content in leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

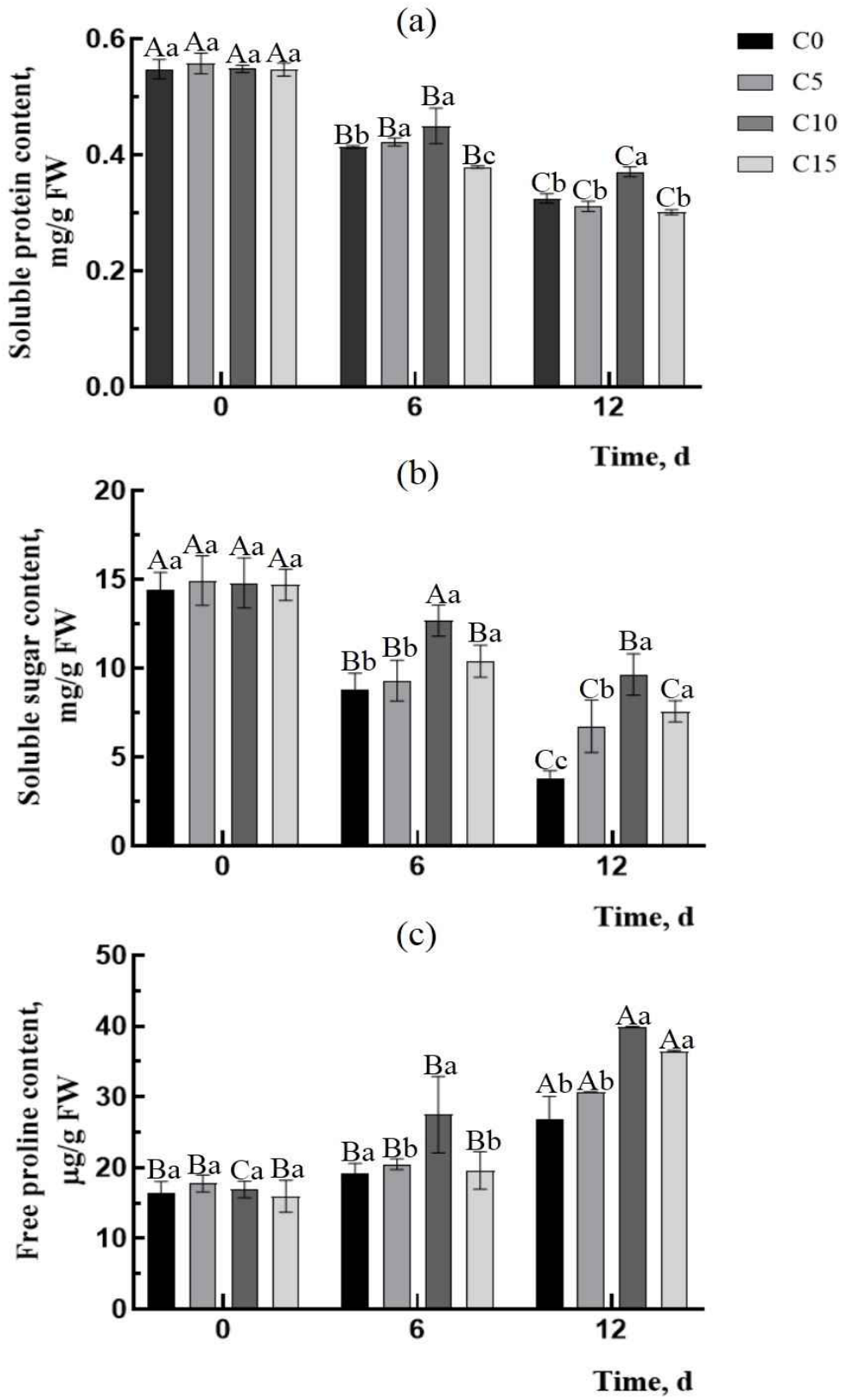

Figure 2. Changes in (a) soluble protein, (b) soluble sugar, and (c) free proline contents in the leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

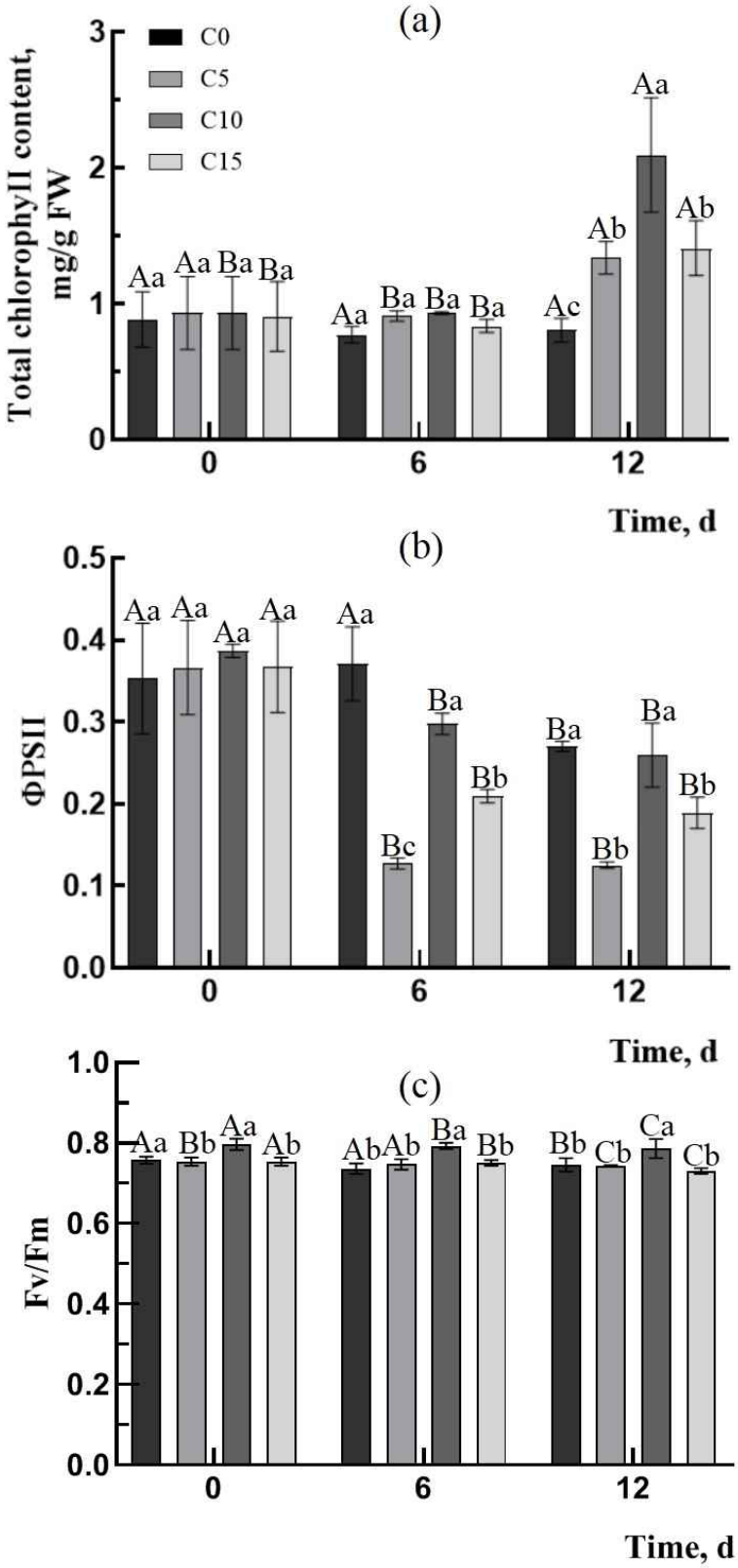

Figure 3. The (a) chlorophyll content (a), (b) ΦPSII, and (c) Fv/Fm in the leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

Changes in the net photosynthetic rate and its related gas parameters

With the increase of days under drought stress, the A, E, and gs of the four groups decreased, but the C10 group of *D. candidum* remained at a high level (Fig. 4a, c, d). The Ci values of the four groups increased after ABA processing, and the Ci of Group C0 *D. candidum* was relatively higher (Fig. 4b).

MDA content and relative electrical conductivity

After 0 days of drought stress, there was a small difference in the MDA content and the relative electrical conductivity of the four groups of *D. candidum* (Fig. 5a and b). The MDA content and the relative electrical conductivity gradually increased over time under drought conditions. After 12 days of drought stress, the MDA content and the relative electrical conductivity of the C10 group were the lowest with increasing drought time, indicating that its antioxidant capacity was stronger.

$O_2^{\cdot-}$ accumulation and H_2O_2

There was no significant difference in staining among the four groups of *D. candidum* leaves (Fig. 6a). At 6 days, the C10 group of *D. candidum* leaves showed the shallowest staining compared to the other three groups, so the $O_2^{\cdot-}$ accumulation and H_2O_2 in the C10 group of *D. candidum* leaves was the lowest. At 12 days, the staining of leaves in the C10 group of *D. candidum* was also the shallowest compared to the other three groups of leaves.

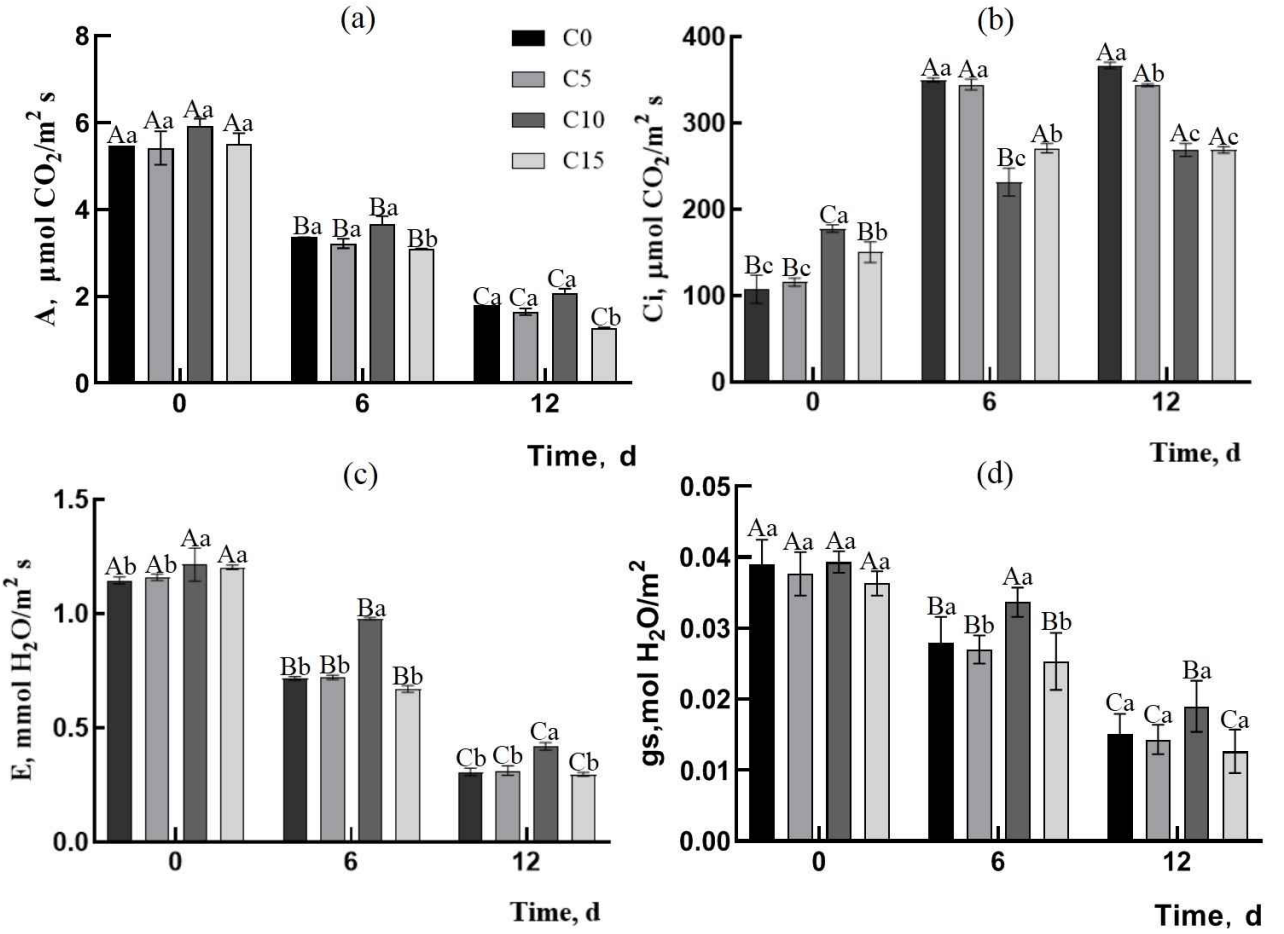

Figure 4. (a) Net photosynthetic rate (A), (b) intercellular CO_2 concentration (Ci), (c) transpiration rate (E, mmol H_2O/m^2 s), and (d) stomatal conductance (*gs*) in the leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance. Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

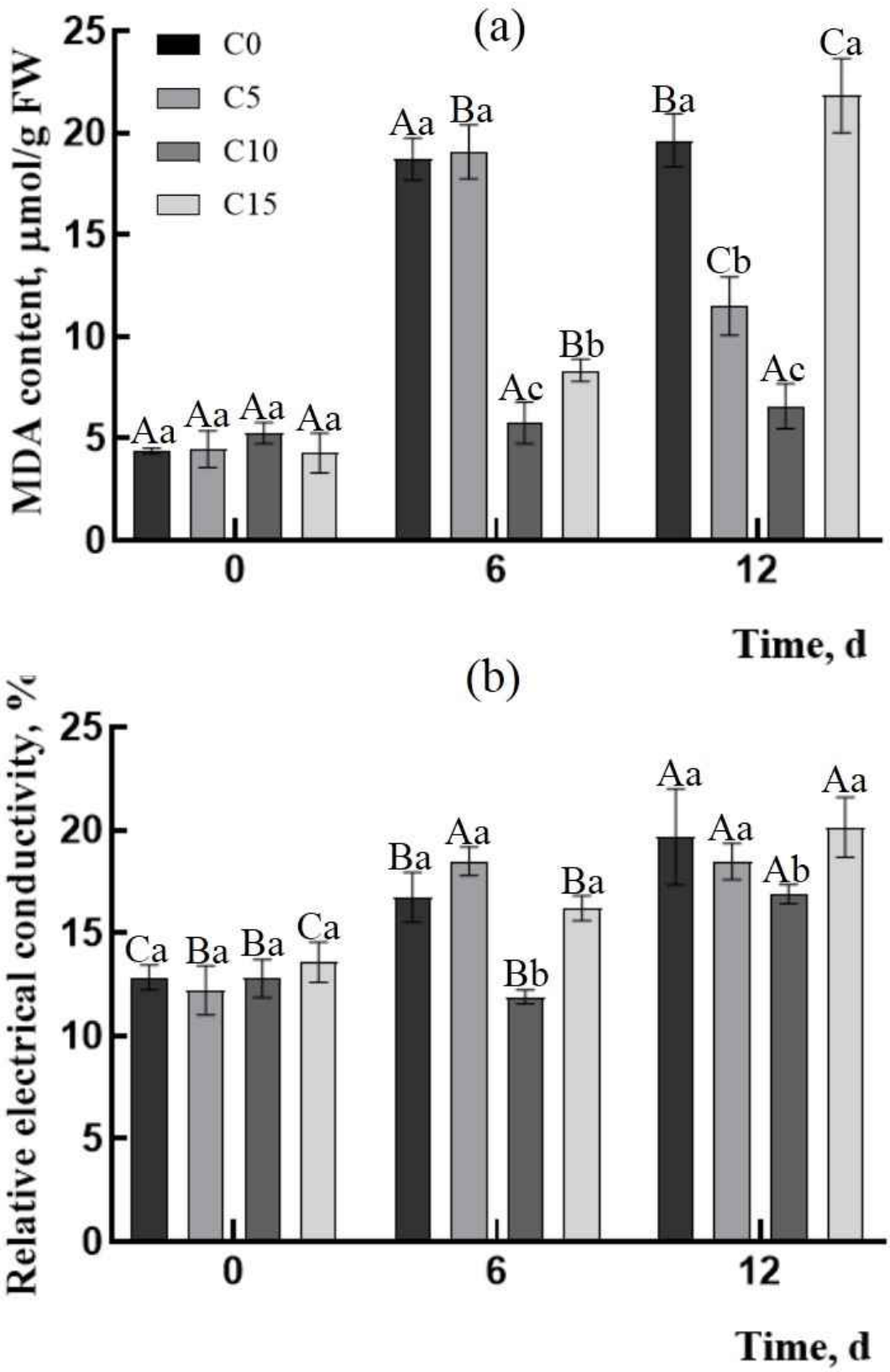

Figure 5. Changes in (a) malondialdehyde content and (b) relative electrical conductivity in the leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

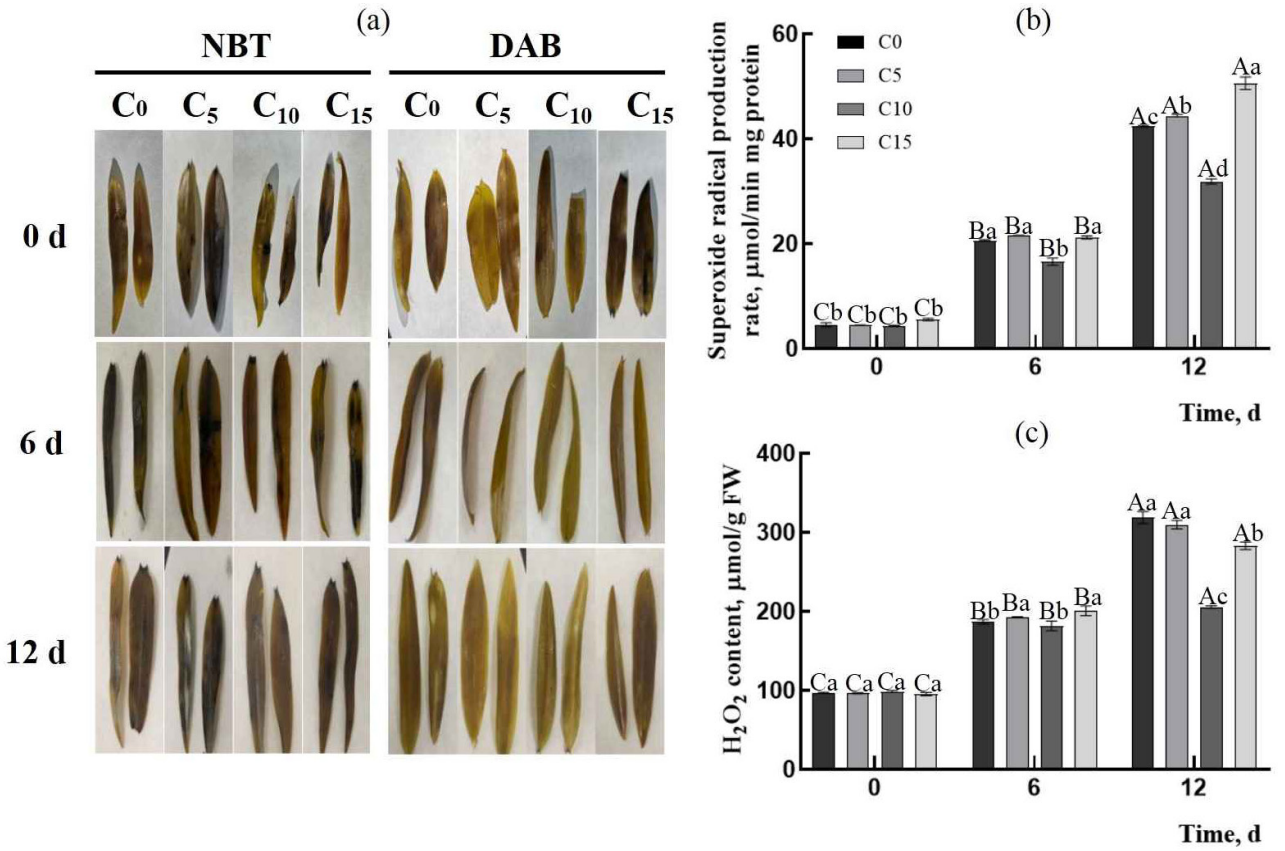

Figure 6. Changes in (a) $O_2^{\cdot-}$ accumulation and H_2O_2 accumulation, (b) $O_2^{\cdot-}$ production rate, and (c) H_2O_2 contents in the leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates, and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

To confirm further differences in antioxidant activities between the four groups of *D. candidum* leaves, quantification of ROS accumulation was performed (Fig. 6b). After 0 days of drought stress, there was a small difference in the H_2O_2 content and $O_2^{\cdot-}$ production rate among the four groups. After 6 days of drought stress, the $O_2^{\cdot-}$ production rate and H_2O_2 content increased. After 12 days of drought stress, the $O_2^{\cdot-}$ production rate and H_2O_2 content in the four groups increased significantly. By comparing the data, the $O_2^{\cdot-}$ production rate and H_2O_2 content of the C10 group of *D. candidum* increased with increasing drought time. Still, they were smaller compared to the other three groups, indicating that the antioxidant capacity of the C10 group was the strongest.

SOD, POD, CAT and APX activities

The contents of SOD, POD, CAT, and APX at 0 d of drought stress in *D. candidum* were not significantly different (Fig. 7a-d). After 6 days of drought stress, the content of the four antioxidant enzymes in the four groups increased. The C2 group had the highest content of antioxidant enzymes and the strongest antioxidant ability. After 12 days of drought stress, the content of the four antioxidant enzymes in the four groups of *D. candidum* increased again. The content of antioxidant enzymes in the C10 group was still the highest. Therefore, the antioxidant capacity of the C10 group is the best and can best alleviate the impact of drought stress on *D. candidum*.

Discussion

In this study, we found that exogenous foliar ABA may partly alleviate the adverse effects of drought-induced oxidative and osmotic stress in *D. candidum* plants by increasing the levels of antioxidant enzymes. ABA mitigates the detrimental effects of drought on *D. candidum* plants via antioxidant activity and ROS production levels. Additionally, ROS accumulation in plants impairs organelle integrity, oxidation of cellular components, and even cell death. Thus, foliar application of ABA at 10 mg/l reduced *D. candidum*'s adverse impacts and enhanced osmoregulation ability, photosynthetic pigments, and biochemicals.

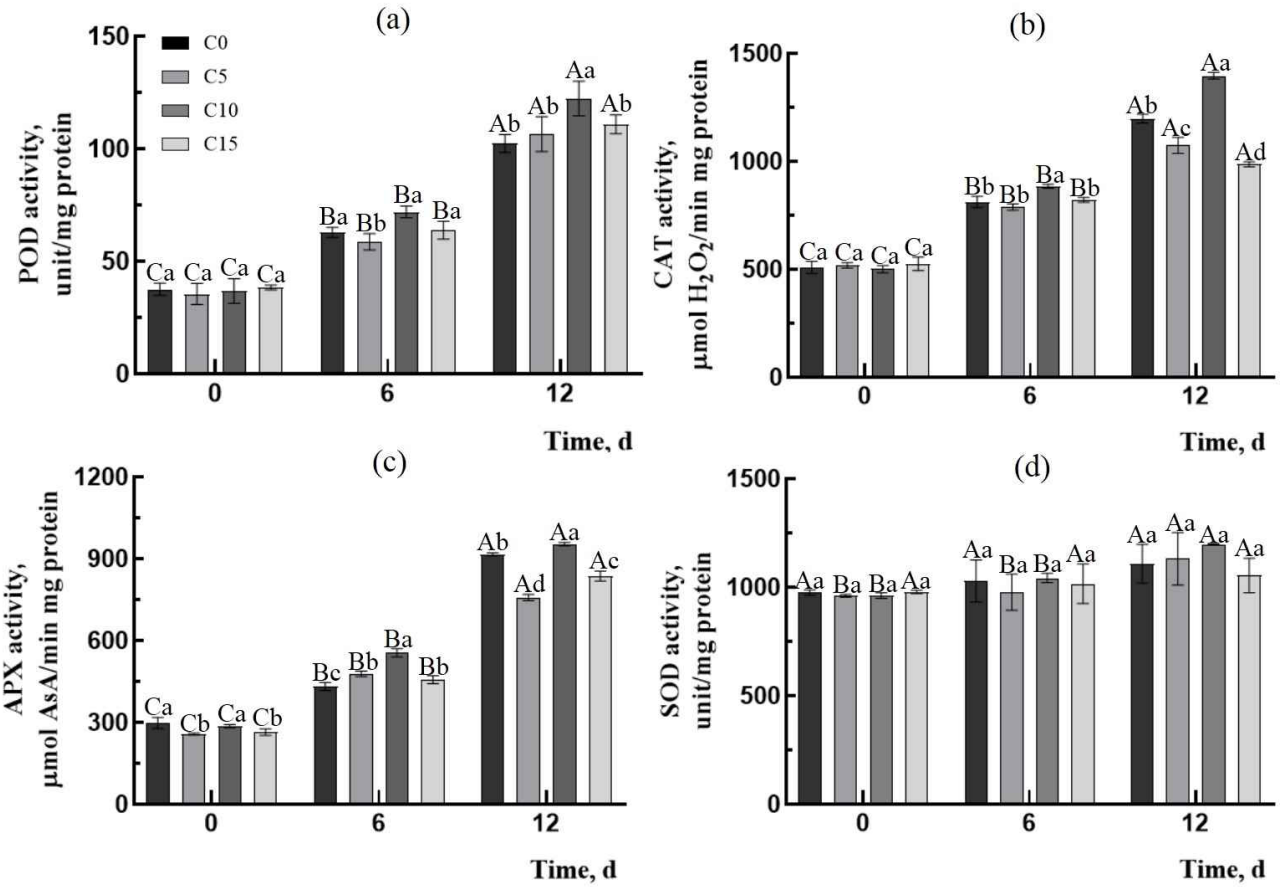

Figure 7. Activities of (a) SOD, (b) POD, (c) CAT, and (d) APX in the leaves of *Dendrobium candidum* seedlings under the control treatment (CK) and drought stress (DS) after foliar spray with ABA at different concentrations (5 mg/l, 10 mg/l, 15 mg/l) for 0, 6 and 12 days. Data is presented as the mean ± SE of three replicates and statistical significance was determined by the Tukey test ($P < 0.05$). Note: Capital letters indicate inter-group significance and lowercase letters indicate intra-group significance.

The osmoregulation ability of *D. candidum* was measured by measuring the water potential, osmotic potential, relative water content, soluble protein, soluble sugar, and free proline. After comparing several sets of data, it was found that the osmoregulation ability of *D. candidum* is greatly affected by drought stress. Finally, through analysis, it was found that the C10 group of *D. candidum* has better osmoregulation ability. The lower Ψs in the C10 group may be related to the accumulation of soluble protein and sugars (Fig. 1a-b). After comparing several sets of data, it was found that the osmoregulation ability of *D. candidum* is greatly affected by drought stress. Finally, through analysis, it was found that the C10 group of *D. candidum* has better osmoregulation ability. After 6 days of drought stress, the chlorophyll content, soluble protein, soluble sugar, and free proline were low, and they decreased gradually over time under drought conditions. After 12 days of drought stress, the soluble protein, soluble sugar, and free proline contents of Group C10 *D. candidum* were relatively higher (Fig. 2a-c). Water stress increased the reduction of sugar, amino acid, and carbohydrate contents in ABA-treated *Oryza sativa* plants. Accumulation of these osmolytes in stressed plants may serve as a storage form of nitrogen that is reutilized when the stress is over and plays a part in osmotic adjustments (Pattanagul, 2011; Ramachandran *et al.*, 2021).

The photosynthetic maintenance ability of *D. candidum* was measured. Comparing the data, it can be concluded that the ΦPSII of Group C0 *D. candidum* were relatively higher, while after ABA processing, Group C10 had the highest ΦPSII (Fig. 3b). With the increase of days under drought stress, the A, E and gs of the four groups of *D. candidum* decreased, but the C10 group of *D. candidum* remained at a high level (Fig. 4a, c, d). The Ci values of the four groups of *D. candidum* increased after ABA processing, and the Ci of Group C0 *D. candidum* was relatively higher (Fig. 4b). The C10 group of *D. candidum* was able to better maintain photosynthetic capacity under drought stress and survived better than the other three groups. The previously reported induction of water stress also resulted in a gradual increase in chl-a and chl-b concentrations in ABA-treated plants. The application of ABA regulates a higher level of photosynthetic pigment content in pearl millet plants (Awan

et al., 2021). However, carotenoid content was increased in withheld water-induced drought-stressed rice, and foliar application of ABA enhanced the carotenoid contents (Ramachandran & Arulbalachandran, 2018). In the present study, gradually increased photosynthetic pigments were recorded in response to the foliar application of increasing ABA concentrations. Moreover, abscisic acid stimulated the biosynthesis of chlorophyll pigments, significantly reducing the inhibitory effect of drought stress by improving photosynthetic pigments (Sirhindi *et al.*, 2016).

Abiotic stresses such as drought, cold, and salinity lead to the production and accumulation of ROS. Plants have evolved complex protective antioxidant systems to maintain the ROS balance under adverse conditions for survival (Han *et al.*, 2022; Zhang *et al.*, 2022; Xu *et al.*, 2022). The efficient removal of ROS is a key factor for plant stress tolerance. MDA, a membrane lipid peroxide, usually accompanies plant responses to environmental stress, reflecting the extent of membrane peroxidation (Bu *et al.*, 2017). In this work, after 12 days of drought stress, the MDA content and the relative electrical conductivity of the C10 group remained at a steady level and increased rapidly with increasing drought stress in the other groups (Fig. 5a and b). After 12 days of drought stress, the increase in the $O_2^{\cdot}$ production rate and H_2O_2 content in the four groups of *D. candidum* increased significantly. By comparing the data, the increase in the $O_2^{\cdot}$ production rate and H_2O_2 content of the C10 group increased with increasing drought time but were smaller compared to the other three groups, indicating that the antioxidant capacity of the C10 group of *D. candidum* was the strongest (Fig. 6a and b).

The increased SOD and POD activities have a positive correlation with drought tolerance in many species (Han *et al.*, 2022; Yuan *et al.*, 2022). CAT and APX are involved in decomposing the H_2O_2 generated by SOD into water and molecular oxygen (Reddy *et al.*, 2004). After 12 days of drought stress, the content of the four antioxidant enzymes in the four groups of *D. candidum* increased again. The content of antioxidant enzymes in the C10 group of *D. candidum* was still the highest. Therefore, the antioxidant capacity of the C10 group of *D. candidum* is the best and can better alleviate the impact of drought stress on *D. candidum* (Fig. 7a-d). Drought decreased the SOD activity under drought stress was also observed in previous studies, such as in pea (*Pisum sativum* L.) plants at different growth stages (Osman, 2015), pea varieties (Farooq *et al.*, 2021), and *Cerasus humilis* Moris (Ren *et al.*, 2016). A notable high CAT and APX activity was also found in drought-tolerant maize (*Zea mays* L.) seeds (Huang & Song, 2013) and tobacco (*Nicotiana tabacum* L.) plants treated with spermidine compared with control plants under drought stress (Xu *et al.*, 2022).

Huang *et al.* (2021) showed a significant positive correlation between plant hormone biosynthesis and signal transduction with facultative CAM, especially ABA. ABA accumulates after drought stress and is an essential factor that positively regulates plant drought-stress responses (Sato *et al.*, 2018). In our study, after comparing the osmotic regulation ability, photosynthetic maintenance ability, and antioxidant ability of the four groups of *D. candidum*, it was found that the foliar application of 10 mg/l ABA prevents and mitigates drought damage in *D. candidum* by limiting lipid peroxidation in the cellular membrane. ABA has the attributed ability to induce the antioxidant system and reduce ROS species. Overall, the results indicated that ABA treatment could reduce the effects of drought stress on *D. candidum*.

Exogenous ABA application effectively ameliorates the adverse effects of drought stress to improve drought resistance. In conclusion, 10 mg/l ABA resulted in leaf water status maintenance, photosynthetic performance, and antioxidative ability under drought stress. Therefore, this study provides a basic understanding of the role of ABA and its potential use in the production of *D. candidum*. Further studies are needed to determine the expression of stress-responsive genes under drought conditions and exogenous applications of ABA.

Acknowledgements

This research was funded by National Natural Science Foundation of China (No. 32301615), the Training Program for Young Backbone Teachers in Higher Education Institutions in Henan Province (2024GGJS105) and the Nanyang Soft Science Project (RKX012).

Authors' Contributions

Feng-xia Tian: conceptualization, visualization, supervision, writing original draft. Guang-ling Xu and Suo Zhou: conceptualization and visualization. Cai-xia Wang and Yu-qing Wei: writing review & editing.

Conflict of Interest

The author(s) declare that they have no competing interests.

References

Ali S, Hayat K, Iqbal A, Xie L. 2020. Implications of abscisic acid in the drought stress tolerance of plants. Agronomy 10: 1323. doi: 10.3390/agronomy10091323

Awan SA, Khan I, Rizwan M, Zhang X, Brestic M, Khan A. 2021. Exogenous abscisic acid and jasmonic acid restrain polyethylene glycol-induced drought by improving the growth and antioxidative enzyme activities in pearl millet. Physiologia Plantarum 172: 809-819. doi: 10.1111/ppl.13247

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254. doi: 10.1016/0003-2697(76)90527-3

Bu CF, Wang C, Yang YS, Zhang L, Bowker MA. 2017. Physiological responses of artificial moss biocrusts to dehydration-rehydration process and heat stress on the Loess Plateau, China. Journal of Arid Land 9: 419-431. doi: 10.1007/s40333-017-0057-8

Cakmak I, Marschner H. 1992. Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascrobate peroxidase and glutathione reductase in bean leaves. Plant Physiology 98: 1222-1227. doi: 10.1104/pp.98.4.1222

Cao H, Ji Y, Li S *et al.* 2019. Extensive metabolic profiles of leaves and stems from the medicinal plant *Dendrobium officinale* Kimura et Migo. Metabolites 9: 215. doi: 10.3390/metabo9100215

Cao WH, Liu J, He XJ *et al.* 2007. Modulation of ethylene responses affects plant salt-stress responses. Plant Physiology 143: 707-719. doi: 10.1104/pp.106.094292

Chen Z, Wang Z, Yang Y, Li M, Xu, B. 2018. Abscisic acid and brassinolide combined application synergistically enhances drought tolerance and photosynthesis of tall fescue under water stress. Scientia Horticulturae 228: 1-9. doi: 10.1016/j.scienta.2017.10.004

Cui HY, Murthy HN, Moh SH, Cui YY, Lee EJ, Paek KY. 2014. Production of biomass and bioactive compounds in protocorm cultures of *Dendrobium candidum* Wall ex Lindl using balloon type bubble bioreactors. Industrial Crops and Products 53: 28-33. doi: 10.1016/j.indcrop.2013.11.049

Dekkers BJ, Schuurmans JA, Smeekens SC. 2008. Interaction between sugar and abscisic acid signalling during early seedling development in *Arabidopsis*. Plant Molecular Biology 67: 151. doi: 10.1007/s11103-008-9308-6

Durner J, Klessig DF. 1996. Salicylic acid is a modulator of tobacco and mammalian catalases. Journal of Biological Chemistry 271: 28492-28501. doi: 10.1074/jbc.271.45.28492

Fang Y, Xiong L. 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cellular and Molecular Life Sciences 72: 673. doi: 10.1007/ s00018-014-1767-0

Farooq M, Ahmad R, Shahzad M *et al.* 2021. Differential variations in total flavonoid content and antioxidant enzymes activities in pea under different salt and drought stresses. Scientia Horticulturae 287: 110258. doi: 10.1016/j.scienta.2021.110258

Giacomelli L, Masi A, Ripoll DR, Lee MJ. 2007. *Arabidopsis thaliana* deficient in two chloroplast ascorbate peroxidases shows accelerated light-induced necrosis when levels of cellular ascorbate are low. Plant Molecular Biology 65: 627-644. doi: 10.1007/s11103-007-9227-y

Guo QF, Zhang J, Gao Q, Xing SC, Li F, Wang W. 2008. Drought tolerance through over expression of monoubiquitinin transgenic tobacco. Journal of Plant Physiology 165: 1745-1755. doi: 10.1016/j.jplph.2007.10.002

Han Y, Hou Z, Zhang X, Yan K, Liang Z, He Q. 2022. Important changes in germination, seedling tolerance, and active components content due to drought stress on three licorice (*Glycyrrhiza*) species. Industrial Crops and Products 175: 114240. doi: 10.1016/j.indcr op.2021.114240

Hasanagic D, Koleska I, Kojic D, Vlaisavljevic S, Janjic N, Kukavica B. 2020. Long term drought effects on tomato leaves: Anatomical, gas exchange and antioxidant modifications. Acta Physiologiae Plantarum 42: 1. doi: 10.1007/s11738-020-03114-z

Hassan N, Ebeed H, Aljaarany A. 2020. Exogenous application of spermine and putrescine mitigate adversities of drought stress in wheat by protecting membranes and chloroplast ultra-structure. Physiology Molecular Biology of Plants 26: 233-245. doi: 10.1007/s12298-019-00744-7

He P, Osaki M, Takebe M. 2005. Endogenous hormones and expression of senescence related genes in different senescent types of maize. Journal of Experimental Botany 56: 1117-1128. doi: 10.1093/jxb/eri103

Hu W, Zhang J, Wu Z *et al.* 2022. Effects of single and combined exogenous application of abscisic acid and melatonin on cotton carbohydrate metabolism and yield under drought stress. Industrial Crops and Products 176: 114302. doi:10.1016/j.indcrop.2021.114302

Huang H, Song S. 2013. Change in desiccation tolerance of maize embryo during development and germination at different water potential PEG-6000 in relation to oxidative process. Plant Physiology and Biochemistry 68: 61-70. doi: 10.1016/j.plaphy.2013.02.029

Huang H, Wang H, Tong Y, Wang Y. 2021. Identification and characterization of HD-Zip genes reveals their roles in stresses responses and facultative crassulacean acid metabolism in *Dendrobium catenatum*. Scientia Horticulturae 9: 1452. doi: 10.1016/j.scienta.2021.110058

Izabela M, Ilona CM, Edyta S *et al.* 2014. Alleviation of osmotic stress effects by exogenous application of salicylic or abscisic acid on wheat seedlings. International Journal of Molecular Sciences 14: 13171. doi: 10.3390/ijms140713171

Jiang CD, Gao HY, Zou Q. 2003. Changes of donor and acceptor side in photosystem 2 complex induced by iron deficiency in attached soybean and maize leaves. Photosynthetica 41: 267-271. doi: 10.1023/B:PHOT.0000011960.95482.91

Li XP, Xu QQ, Liao WB *et al.* 2016. Hydrogen peroxide is involved in abscisic acid-induced adventitious rooting in cucumber (*Cucumis sativus* L.) under drought stress. Journal of Plant Biology 59: 536. doi: 10.1007/s12374-016-0036-1

Li XW, Chen HP, He YY *et al.* 2018. Effects of rich-polyphenols extract of *Dendrobium loddigesii* on anti-diabetic, anti-inflammatory, anti-oxidant, and gut microbiota modulation in db/db mice. Molecules 23: 3245. doi: 10.3390/molecules23123245

Li Y, Zhao H, Duan B, Korpelainen H, Li C. 2011. Effect of drought and ABA on growth, photosynthesis and antioxidant system of *Cotinus coggygria* seedlings under two different light conditions. Environmental and Experimental Botany 71: 107. doi: 10.1016/j.envexpbot.2010.11.005

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148: 350. doi: 10.1016/0076-6879(87)48036-1

Ma QQ, Wang W, Li YH, Li DQ, Zou Q. 2006. Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum* L.) by foliar-applied glycinebetaine. Journal of Plant Physiology 163: 165-175. doi: 10.1016/j.jplph.2005.04.023

Mittraphab A, Muangnoi C, Likhitwitayawuid K, Rojsitthisak P, Sritularak B. 2016. A new bibenzyl-phenanthrene derivative from *Dendrobium signatum* and its cytotoxic activity. Natural Product Communications 11: 657. doi: 10.1177/1934578x1601100526

Muller M, Munne-Bosch S. 2021. Hormonal impact on photosynthesis and photo protection in plants. Plant Physiology 185: 1500. doi: 10.1093/plphys/kiaa119

Osman HS. 2015. Enhancing antioxidant-yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. Annals of Agricultural Sciences 60: 389-402. doi: 10.1016/j.aoas.2015.10.004

Pattanagul W. 2011. Exogenous abscisic acid enhances sugar accumulation in rice (*Oryza sativa* L.) under drought stress. Asian Journal of Plant Sciences 10: 212. doi: 10.3923/ajps.2011.212.219

Quan RD, Shang M, Zhang H, Zhao YX, Zhang JR. 2004. Improved chilling tolerance by transformation with *betA* gene for the enhancement of glycinebetaine synthesis in maize. Plant Science 166: 141-149. doi: 10.1016/j.plantsci.2003.08.018

Ramachandran M, Arulbalachandran D, Dilipan E, Ramya S. 2021. Comparative analysis of abscisic acid recovery on two varieties of rice (*Oryza sativa* L.) under drought condition. Biocatalysis and Agricultural Biotechnology 33: 102006. doi: 10.1016/j.bcab.2021.102006

Ramachandran M, Arulbalachandran D. 2018. Exogenous abscisic acid mediated morphological characteristics, photosynthetic pigments and antioxidant metabolism under drought stress in rice (*Oryza sativa* L.). Madras Agricultural Journal 105(7-9): 385-389. doi: 10.29321/MAJ.2018.000167

Ramya S, Arulbalachandran D, Ramachandran M. 2022. Influence of exogenous abscisic acid on morpho-physiological and yield of maize (*Zea mays* L.) under drought stress. Plant Science Today: 9: 288-300. doi: 10.14719/pst.1413.

Reddy AR, Chaitanya KV, Vivekanandan M. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology 161: 1189-1202. doi: 10.1016/j.jplph.2004.01.013

Ren J, Sun LN, Zhang QY, Song XS. 2016. Drought tolerance is correlated with the activity of antioxidant enzymes in *Cerasus humilis* seedlings. Biomed Research International 7: 2038-2047. doi: 10.1155/2016/9851095

Sairam PK, Srivastava GC. 2002. Changes in antioxidant activity in subcellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Science 162: 897-904. doi: 10.1016/S0168-9452(02)00037-7

Sato H, Takasaki H, Takahashi F *et al.* 2018. *Arabidopsis thaliana* NGATHA1 transcription factor induces ABA biosynthesis by activating *NCED3* gene during dehydration stress. Proceedings of the National Academy of Sciences of the United States of America 115: 11178-11187. doi: 10.1073/pnas.1811491115

Scebba F, Sebastiani L, Vitagliano C. 2001. Activities of antioxidant enzymes during senescence of *Prunus armeniaca* leaves. Biologia Plantarum 44: 41-46. doi: 10.1023/A:1017962102950

Shan DP, Huang JG, Yang YT *et al.* 2007. Cotton GhDREB1 increases plant tolerance to low temperature and is negatively regulated by gibberellic acid. New Phytologist 176: 70-81. doi: 10.1111/j.1469-8137.2007.02160.x

Sirhindi G, Mir MA, Abd-Allah EF, Ahmad P, Gucel S. 2016. Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity and gene expression in *Glycine max* under nickel toxicity. Frontiers in Plant Science 7: 591. doi: 10.3389/ fpls.2016.00591

Sui N, Li M, Liu XY, Wang N, Fang W, Meng QW. 2007. Response of xanthophyll cycle and chloroplastic antioxidant enzymes to chilling stress in tomato over-expressing glycerol-3-phosphate acyltransferase gene. Photosynthetica 45: 447-454. doi: 10.1007/s11099-007-0074-5

Travaglia C, Cohen AC, Reinoso H, Castillo C, Bottini R. 2007. Exogenous abscisic acid increases carbohydrate accumulation and redistribution to the grains in wheat grown under field conditions of soil water restriction. Journal of Plant Growth Regulation 26: 28. doi: 10.1007/s00344-007-9018-3

Vishal B, Kumar PP. 2018. Regulation of seed germination and abiotic stresses by gibberellins and abscisic acid. Frontiers in Plant Science 9: 838. doi: 10.3389/fpls.2018.00838

Wang GJ, Miao W, Wang JY, Ma, DR, Li JQ, Chen WF. 2013. Effects of exogenous abscisic acid on antioxidant system in weedy and cultivated rice with different chilling sensitivity under chilling stress. Journal of Agronomy and Crop Science 199: 200. doi: 10.1111/jac.12004

Xiao Y, Zhang Y, Dang K, Wang D. 2007. Growth and photosynthesis of *Dendrobium candidum* Wall. Ex Lindl. plantlets cultured photoautotrophically. Propagation of Ornamental Plants 7: 89-96.

Xu XR, He XH, Yi Yin. 2019a Effects of calcium ion on the protective enzyme system of *Dendrobium officinale* kirnura callus under drought stress. Molecular Plant Breeding, 17: 3709-3716. doi: 10.13271/j.mpb.017.003709

Xu C, McDowell NG, Fisher RA *et al.* 2019b. Increasing impacts of extreme droughts on vegetation productivity under climate change. Nature Climate Change 9: 948-953. doi: 10.1038/s41558-019-0630-6.

Xu J, Cai M, Li J, Chen B *et al.* 2022. Physiological, biochemical and metabolomic mechanisms of mitigation of drought stress-induced tobacco growth inhibition by spermidine. Industrial Corps and Products 181: 114844. doi: 10.1016/j.indcrop.2022.114844

Yemm EW, Willis AJ. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochemical Journal 57: 508-514. doi: 10.1042/bj0570508

Yuan Y, Liu L, Gao Y *et al.* 2022. Comparative analysis of drought-responsive physiological and transcriptome in broomcorn millet (*Panicum miliaceum* L.) genotypes with contrasting drought tolerance. Industrial Crops and Products 177: 114498. doi: 10.1016/j.indcrop.2021.114498

Zhang Q, Li B, Chen Q *et al.* 2022. Non-targeted metabolomic analysis of the variations in the metabolites of two genotypes of *Glycyrrhiza uralensis* Fisch. under drought stress. Industrial Corps and Products 176: 114402. doi: 10.1016/j.indcro p.2021.114402

Zong XJ, Li DP, Gu LK, Li DQ, Liu LX, Hu XL. 2009. Abscisic acid and hydrogen peroxide induce a novel maize group C MAP kinase gene, *ZmMPK7*, which is responsible for the removal of reactive oxygen species. Planta 229: 485-495. doi: 10.1007/s00425-008-0848-4