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Enhancing Sugarcane Seedling Resilience to Water Stress through Exogenous Abscisic Acid: A Study on Antioxidant Enzymes and Phytohormone Dynamics

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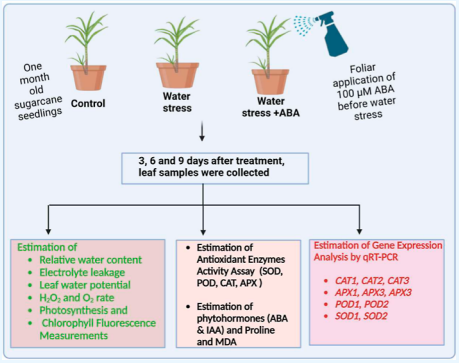
ABSTRACT: Exogenous hormones play a crucial role in regulating plant growth, development, and stress tolerance. However, the effects of exogenous abscisic acid (ABA) on sugarcane seedlings under water stress remain poorly understood. Here, in this study, a pot experiment was conducted on sugarcane seedlings 4 weeks after transplanting, employing three treatments: control (normal growth), drought (water stress), and drought + ABA (foliar application of 100 μM ABA before water stress). The main objectives of this research are to understand the effects of exogenous ABA on sugarcane seedlings under water stress conditions and to assess the changes in antioxidant enzyme activity and phytohormone levels in response to exogenous ABA. Water stress was induced in the solution culture by adding 25% (w/v) polyethylene glycol (PEG) 6000 to the Hoagland solution. Leaf samples were collected at 3, 6, and 9 days after treatment, and the photosynthetic and biochemical responses of ABA-treated plants to drought stress were investigated. The indole acetic acid (IAA) activity of the ABA-treated drought plants is compared to that of drought plants. Moreover, the endogenous ABA levels of the ABA-treated drought plants were significantly enhanced by 42.2, 39.9, and 42.3% at 3, 6, and 9 days, respectively, compared to those of drought plants. Additionally, the proline content of the ABA-treated drought plants significantly increased by 45 and 80% at 6 and 9 days, respectively, compared to that of drought plants. The expression of the catalase 1 (*CAT1*) gene was increased in the ABA-treated drought plants by 2.1-fold, 0.7-fold, and 1.37-fold at 3, 6, and 9 days, respectively, compared to that in drought plants. Similarly, the expression of superoxide dismutase, peroxidase, and ascorbate peroxidase genes of the ABA-treated drought plants also increased compared to those of the drought plants. In conclusion, foliar application of ABA mitigated the negative effects of water shortage of sugarcane plants under water stress. Applying ABA improved the antioxidant defense system of sugarcane plants under drought stress, thereby enhancing their photosynthetic activities and productivity.



1. INTRODUCTION

Global crop production faces severe challenges due to abiotic stress, resulting in a reduction of total yield by more than 50%. Sugarcane (*Saccharum* spp.), a prominent sugar crop grown in tropical and subtropical regions, is particularly vulnerable to adverse environmental conditions, particularly abiotic stresses like water scarcity and low temperatures.[1] With increasing populations and deteriorating environmental conditions, there is a shortage of arable land for crop cultivation. More than 80% of sugarcane land, especially in upland regions with limited irrigation, has been repurposed for cultivating other crops. Water scarcity is a significant obstacle to sugarcane production, highlighting the urgent need to develop sugarcane varieties that are highly water-efficient or more resistant to drought.[2] Sugarcane is cultivated as a commercial crop in many nations worldwide. However, its lengthy growth cycle imposes several environmental limitations that hinder its development.[3]

Abiotic stress has become a persistent limiting factor in sugarcane production due to abrupt climate changes. These stressors directly or indirectly impact sugarcane by altering its growth, metabolism, and development. Moreover, they can affect soil health, crop chemistry, sugar accumulation, and seed cane availability, exacerbating the effects of other abiotic or biotic stresses and resulting in increased losses.[4] Among the abiotic stressors, water stress holds the utmost significance in affecting sugarcane production. As highlighted by Zingaraetti et al.,[5] water stress occurs when plants receive inadequate water or experience an increase in transpiration rates. Drought emerges as a primary factor, globally diminishing sugarcane yield and productivity.[6]

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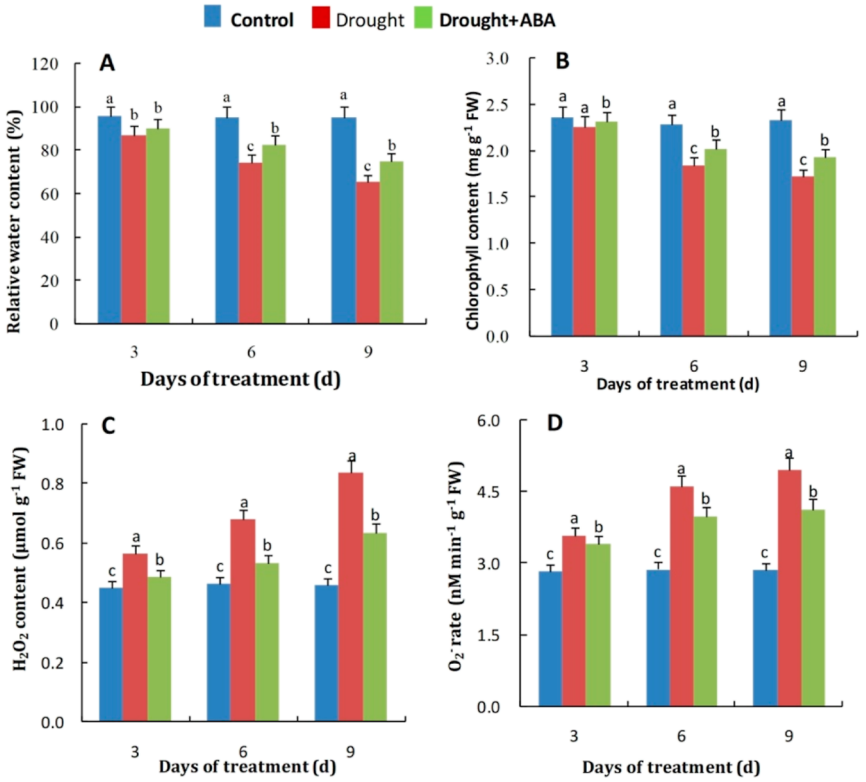

Figure 1. Effects of exogenous ABA on the relative water content (RWC) (A), total chlorophyll content (B), hydrogen peroxide (H_2O_2) content (C), and oxygen (O_2) rate (D) of sugarcane seedlings under PEG-induced drought stress at 3, 6, and 9 days after treatment (DAT). Data are presented as the means of triplicate samples. Different letters on the bars show significant differences at $P < 0.05$.

Abscisic acid (ABA) plays a critical role as a plant hormone involved in regulating genes and cell signaling. Cutler et al.[7] observed that the natural concentration of ABA increases during water shortage, serving to shield plants from rapid desiccation. Assessment of gene expression under water-deficient conditions following exogenous ABA treatment has led to the identification of several genes sharing molecular pathways associated with responses to abiotic stress.[8] The accumulation of ABA under dry conditions triggers various adaptive responses in plants. These include stomatal closure, reduced leaf and stem growth, enhanced development of deeper root systems, heightened hydraulic conductivity in roots and shoots, remobilization of assimilates, induction of senescence, maintenance of turgor pressure, upregulation of antioxidant proteins, and promotion of seed dormancy.[9−12]

Reactive oxygen species (ROS) significantly regulate many plant activities, such as growth, development, response to environmental stimuli, and programmed cell death.[13,14] An imbalance in cellular homeostasis during prolonged drought stress leads to an outburst of ROS, causing damage to cell membranes, DNA, and proteins, ultimately resulting in cell death.[15−17] Exogenous application of ABA can mitigate ROS overproduction and accumulation by modulating the expression or activity of various antioxidant enzymes. For example, significant changes in the activity of antioxidative enzymes have been observed in *Actinidia deliciosa*[18] *Capsicum annuum*,[19] maize,[20] and tomato[21] treated with exogenous ABA. Furthermore, exogenous ABA treatment has been shown to elevate the levels of phytohormones such as auxin (IAA), cytokinin (CTks), and ABA as well as enhance the transcription of genes involved in ABA production and signaling in plants under water stress conditions, including berry,[2] *Cleistocalyx operculatus*, and *Syzygium jambos*[22] sugarcane[1] under water stress. Recent studies have demonstrated the positive impact of ABA on antioxidant activity and associated enzymes under drought stress, as evidenced in *Lavandula angustifolia* cv. Munstead[23] (Safari et al., 2024). Additionally, exogenous ABA spraying has been found to significantly enhance grain filling properties, increase relevant enzyme activity to expedite starch accumulation, and regulate hormone levels in grains.[24]

However, the precise effects of exogenous ABA on the bioactive components and gene expression in sugarcane remain incompletely understood. Therefore, the present study aimed to investigate how exogenous ABA application influences antioxidant activity, related bioactive components, enzymatic activities, and gene expression in sugarcane. Furthermore, the study assessed the ability of ABA to mitigate early physiological, biochemical, and oxidative damage induced by PEG-induced drought stress in sugarcane.

2. MATERIALS AND METHODS

2.1. Plant Materials and Treatments. The greenhouse experiment was conducted at the Sugarcane Research Institute of Guangxi Academy of Agricultural Sciences (Nanning, China). Single bud sets of the sugarcane variety GT42 were initially grown in sand culture and then transplanted after 3 weeks into plastic pots (21 cm diameter and 19 cm high) containing 5 L of modified Hoagland solution [4.02 mM $Ca(NO_3)_2{\cdot}4H_2O$, 1.99 mM $Mg_2SO_4{\cdot}7H_2O$, 6.03 mM KNO_3, 1.75 mM $(NH_4){\cdot}2SO_4$, 1.03 mM KH_2PO_4, 0.15 mM EDTAFe, 10^{-3} mM H_3BO_3, 10^{-3} mM $MnCl_2{\cdot}4H_2O$, 10^{-3} mM $ZnSO_4{\cdot}7H_2O$, 10^{-4} mM $CuSO_4{\cdot}5H_2O$, and 5.0×10^{-6} mM $(NH_4)_6MoO_7O_{24}{\cdot}4H_2O$][25] and turf-humus and vermiculite

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mixture ratio is 1:4 in volume. There were three replications per treatment and five seedlings per replication, and the solution was aerated using an indigenous air pump and changed every 3 days to eliminate toxic metabolites and root debris. Treatments were administered 4 weeks after transplanting, including control (normal growth), drought (water stress), and drought + ABA (foliar application of 100 μM ABA before water stress). Water stress was induced in solution culture by incorporating 25% (w/v) polyethylene glycol (PEG) 6000 into the Hoagland solution. ABA (98% pure) was sourced from Bio Basic Inc. (Toronto, Canada). Leaf samples were collected after 3, 6, and 9 days of treatment; leaf samples were collected, instantly frozen in liquid nitrogen, and stored at −80 °C for further analysis. The overall experimental design is depicted graphically in graphics (Figure 1).

2.2. Estimation of the RWC, Electrolyte Leakage, Leaf Water Potential, Osmotic Potential, Hydrogen Peroxide, and Oxygen Level. The RWC of all the treated plants was determined using the technique developed by ref 26, where fully enlarged top leaf samples (0.1 g FW) were soaked in deionized water for 24 h to achieve full turgidity, then gently dried with filter paper to measure the turgid weight, and subsequently oven-dried for two to 3 days at 80 °C. The formula [leaf RWC (%) = (FW − DW)/(TW − DW) × 100] was applied for calculation. Electrolyte leakage (EL) was determined following the method by Murray et al.[27] with minor adjustments: ELR (%) = A/B × 100. Leaf water potential (LWP) was measured using a model 600 pressure chamber (PMS Instrument Company, Oregon). Osmotic potential (OP) was determined as described by Saneoka et al.[28] and measured using a vapor pressure osmometer (model 5500 Wescor, Logan, Utah). Hydrogen peroxide (H_2O_2) levels were measured following the method by Bates et al.[29]

2.3. Photosynthesis and Chlorophyll Fluorescence Measurements. Photosynthesis and the quantum yield of photosystem II (PSII) were assessed following the protocol outlined by Li et al.[1] The Li-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA) was utilized with a photon flux density of 1000 μmol m^{-2} s^{-1} to measure parameters including the net photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci), and fluorescence. Fluorescence measurements were conducted using the saturation pulse approach with a modulated fluorometer (FMS-2, Hansatech, Norfolk, UK). The minimum fluorescence (F0) and maximum fluorescence (Fm) were determined after subjecting dark-adapted leaves to actinic and saturated light from the fluorometer for 30 min. Light-adapted fluorescence parameters, including maximum fluorescence (Fm′), steady-state fluorescence yield (Fs), and minimum fluorescence (Fo′), were measured using a photon flux density of 1000 μmol m^{-2} s^{-1} every 20 s after far-red illumination of the previously exposed leaves. The excitation capture efficiency of open centers [Fv/Fm= (FmFo)/Fm], maximum quantum yield of PSII [Fv/Fm= (FmFo)/Fm], and effective quantum yield of PSII [PSII = F/Fm= (Fm′Fs)/Fm] were then calculated.[30]

2.4. Estimation of Antioxidant Enzyme Activities. The antioxidant activity of the crude enzyme extract was determined according to the method described by Qurashi et al.[31] Briefly, 1.0 g of leaf sample was homogenized with potassium phosphate buffer (100 mM, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, 2% polyvinylpyrrolidone, and 1 mM EDTA using a prechilled mortar and pestle. The mixture was centrifuged at 10,000g for 20 min at 4 °C to obtain the extract for measuring catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and peroxidase (POD) activities as well as malondialdehyde (MDA) levels. The modified method by Qurashi et al. was employed to measure the CAT activity. A reaction mixture containing 50 μL of crude enzyme extract, 100 mM potassium phosphate buffer (pH 7), and 20 mM hydrogen peroxide (H_2O_2) was used for the analysis. SOD activity was determined using the nitro blue tetrazolium chloride (NBT) photoreduction method. The reaction mixture contained 200 mM methionine, 75 mM riboflavin, 2 mM NBT, and 3 mM EDTA in 0.1 M potassium phosphate buffer (pH 7.8).

2.5. Estimation of Phytohormones, MDA, and Proline Concentration. Leaf samples (1 g) were crushed in 5 mL of 80% (v/v) methanol extraction buffer containing 1 mM butylated hydroxytoluene as an antioxidant. The homogenate was centrifuged at 10,000g for 20 min after incubation at 4 °C for 12 h. The supernatant was collected, and the residue was incubated at 4 °C for 1 h for re-extraction and centrifuged, and the volume of the combined supernatants was measured. The extracted solution was eluted using a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) to remove pigments. The mixture was then dissolved in 2 mL of phosphate-buffered saline containing 0.1% (v/v) Tween 20 and 0.1% (w/v) gelatin (pH 7.5) after drying in a freeze-dryer (Labconco, UK). An enzyme-linked immunosorbent assay[32] was utilized to quantify the levels of ABA and indole-3-acetic acid (IAA) in a 96-well microtiter plate precoated with coating buffer containing synthetic albumin conjugates for IAA, GA3, and ABA (China Agricultural University, Beijing, China). Each well was loaded with 50 μL of the standard or sample and an equal volume of diluted antibodies (1:2000) in the assay buffer (1000 mL of H_2O containing 8.0 g of NaCl, 0.2 g of KH_2PO_4, 2.96 g of $Na_2HPO_4{\cdot}12H_2O$, 1.0 mL of Tween-20, and 1.0 g of gelatin).

The plates were then rinsed four times with scrubbing buffer after 45 min of incubation at 37 °C (using the assay buffer without gelatin). Subsequently, 100 μL of a 1:1000 dilution of antimouse IgG linked to alkaline phosphatase was added to each well, followed by cleaning the plates after 30 min of incubation at 37 °C. Next, 100 μL of a 1.5 mg/mL *ortho*-phenylenediamine substrate solution and 0.04% (v/v) of 30% H_2O_2 (dissolved in substrate buffer; 5.10 g of $C_6H_8O_7{\cdot}H_2O$, 18.43 g of $Na_2HPO_4{\cdot}H_2O$, and 1.0 mL of Tween-20 in 1 L of water) were added to the plates. After incubating in the dark at 37 °C for 15 min, the enzyme process was halted by adding 50 μL of 2 M H_2SO_4 per well. The absorbance was measured at 490 nm using a Thermo Electron MultiskanMK3 (Pioneer Co., Shenzhen, China). The concentrations of IAA, GA3, and ABA were estimated using a logit $B/B0$ transformed standard curve, where B and $B0$ are the absorbance values in the presence and absence of the competing antigen, respectively. Lipid peroxidation was assessed by measuring the MDA concentration using Heath and Packer's[33] method, where the absorbance of the produced extracts was measured at 532 and 600 nm. Leaf samples were pulverized and extracted in 6% trichloroacetic acid, then reacted with 0.5% thiobarbituric acid, heated for 30 min at 95 °C, centrifuged, and chilled before measuring the absorbance. Proline content was determined spectrophotometrically following the method of Bates et al.[29] Briefly, leaf samples (0.5 g) were pulverized using an ice-chilled mortar and pestle in 5 mL of methanol solution (80% v/v) containing 1 mM butylated hydroxytoluene as an antioxidant.

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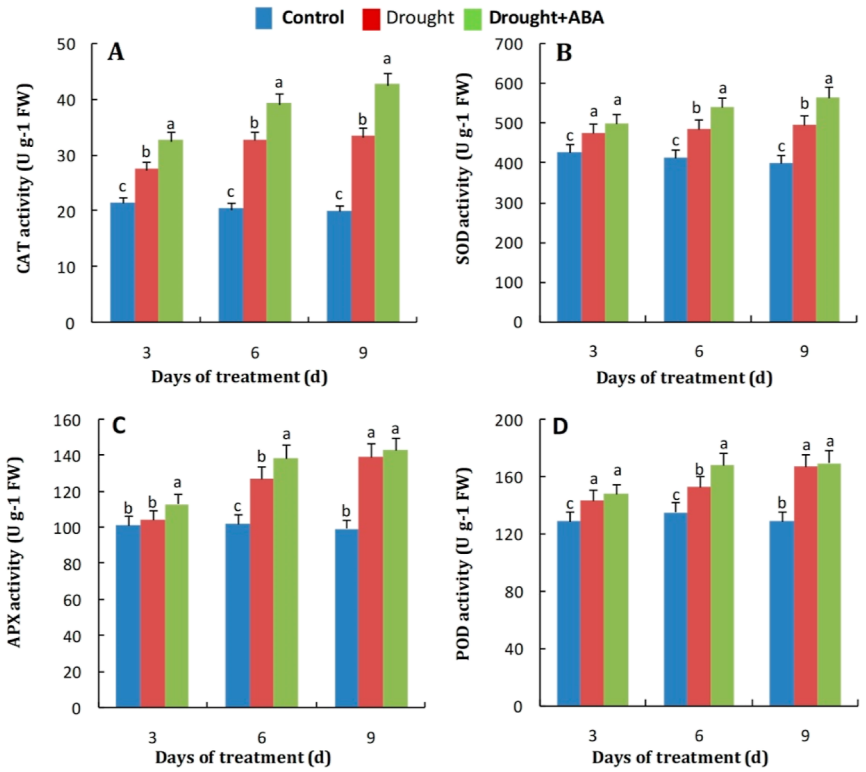

Figure 2. Effects of exogenous ABA on CAT (A), SOD (B), ascorbate peroxidase (APX) (C), and POD (D) of sugarcane seedlings under PEG-induced drought stress at 3, 6, and 9 DAT. Data are presented as the means of triplicate samples. Different letters on the bars show significant differences at $P < 0.05$.

2.6. qRT-PCR Assay of the Stress-Related Genes. The expression patterns of stress-related genes in sugarcane were analyzed using quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Total RNA extraction from the treated sugarcane leaves was performed using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). The extracted RNA was then reverse-transcribed into cDNA using the PrimeScript TMRT Reagent Kit (Takara, Shiga, Japan and China), following the manufacturer's instructions. For the qRT-PCR assay, a final reaction mixture of 20 μL was prepared, containing 2 μL of the first-strand cDNA diluted 10 times, 10 pM of sugarcane stress-specific primers, and 10 μL of SYBR Premix Ex TapTMII (Takara, Kyoto, Japan). The gene primer sequences used in the quantitative real-time PCR investigation are listed in Table S1. The assay was carried out on a real-time PCR system (Bio-Rad, Hercules, CA, USA) under the following conditions: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 15 s, 58 °C for 30 s, and 72 °C for 30 s, with a subsequent melting cycle from 60 to 95 °C. Three replicates of the reference gene, GAPDH, were included in the RT-qPCR assay. The relative expression levels of the target genes were determined using the delta−delta Ct approach.

2.7. Statistical Analysis. All the data were analyzed by using a statistical tool SPSS (Statistical Package of Social Sciences). All data are expressed as the means of three replicates with (±) standard deviation. Analysis of variance (ANOVA) was used to evaluate the significance of the experimental data, followed by Duncan's multiple range test. Differences were considered significant at the $P < 0.05$ level.

3. RESULTS

3.1. EL, LWP, and OP Contents. Table S2 presents the EL, LWP, and OP contents. Under both stress treatments, EL was significantly reduced. The LWP of ABA-treated leaves under water stress significantly increased by 31 and 33% at 6 and 9 days, respectively. Similarly, the OP of ABA-treated leaves significantly increased by 21, 24, and 25% at 3, 6, and 9 days, respectively, compared to that of drought-stressed leaves. The RWC significantly increased in ABA + drought-treated plants by 11 and 14% at 6 and 9 days, respectively, compared to that in drought-treated plants (Figure 2). However, H_2O_2 and O_2 levels were significantly increased in drought-treated plants compared to those subjected to ABA + drought (Figure 1B,C).

3.2. Antioxidant Enzyme Activities. To understand the scavenging mechanism of oxidative stress, we measured the antioxidant enzyme activities (CAT, SOD, APX, and POD) of ABA-treated sugarcane plants under water stress conditions. The results revealed that all treatments significantly affected the antioxidant enzyme activities of sugarcane plants compared to plants subjected to water stress (Figure 2). ABA + drought increased CAT by 52.1, 47.2, and 53.2% at 3, 6, and 9 days, respectively, compared to the control. CAT activities were also significantly enhanced by 18.7, 16.6, and 21.8% at 3, 6, and 9 days, respectively, in drought-stressed plants compared to those of untreated control ones. The SOD activities of ABA + drought-treated plants were significantly enhanced compared to those of the control plants. Moreover, SOD activities of ABA + drought-treated plants were significantly increased at 6 and 9 days by 11.3 and 14%, respectively, compared to those of the untreated control plants. The ABA + drought-treated plants had significantly enhanced APX activities at 3, 6, and 9

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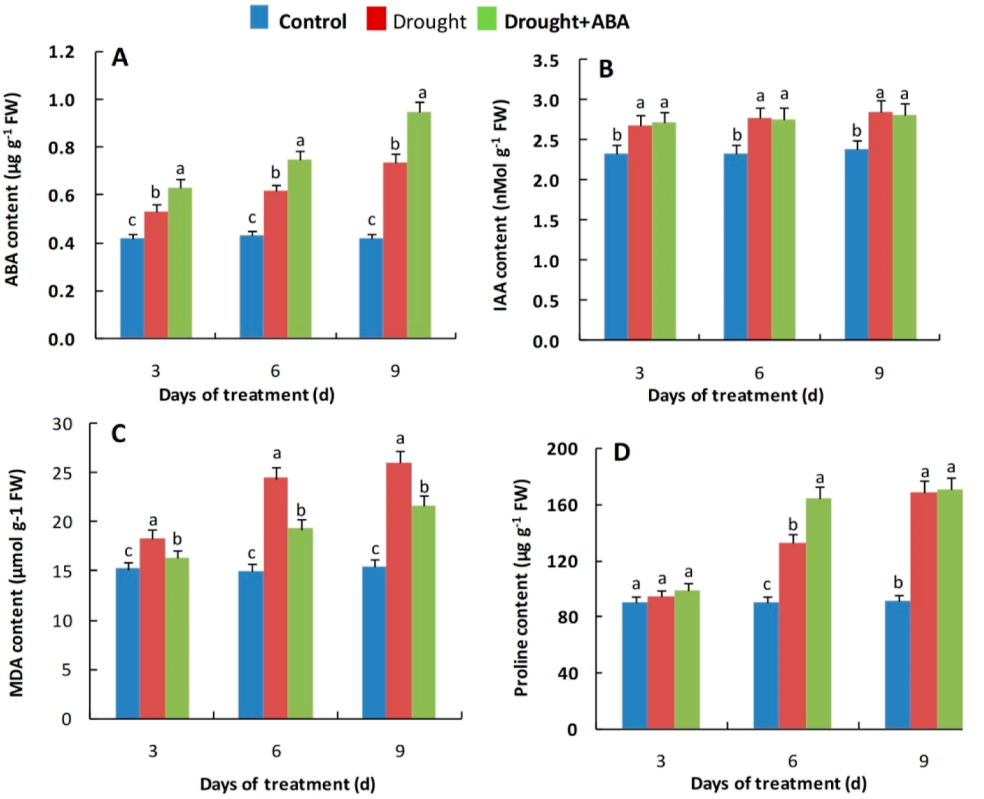

Figure 3. Effects of exogenous ABA on endogenous ABA (A), indole acetic acid (IAA) (B), MDA (C), and proline content (D) of sugarcane seedlings under PEG-induced drought stress at 3, 6, and 9 DAT. Data are presented as the means of triplicate samples. Different letters on the bars show significant differences at $P < 0.05$.

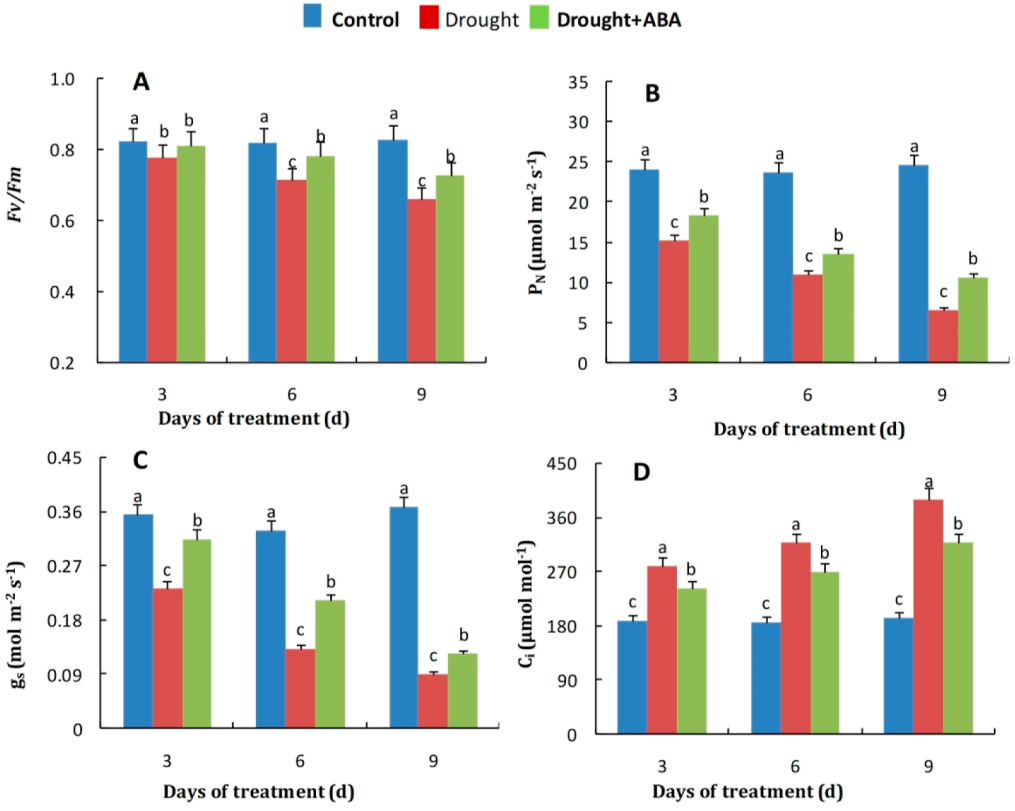

Figure 4. Effects of exogenous ABA on photochemical efficiency (Fv/Fm′) (A), net photosynthetic rate (Pn) (B), stomatal conductance (gs) (C), and CO_2 concentration (Ci) (D) of sugarcane seedlings under PEG-induced drought stress at 3, 6, and 9 DAT. Data are presented as the means of triplicate samples. Different letters on the bars show significant differences at $P < 0.05$.

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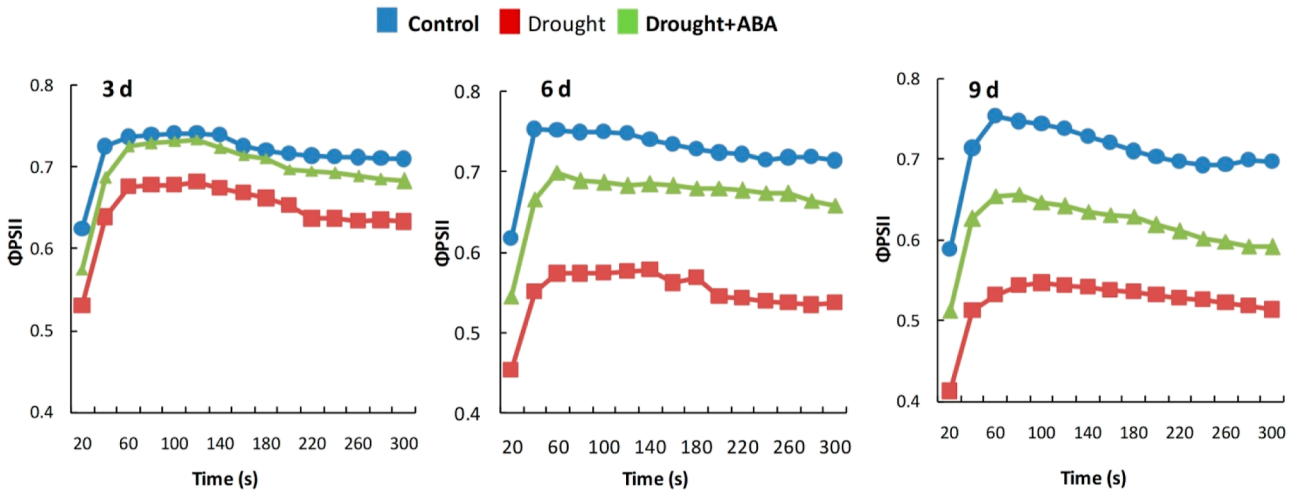

Figure 5. Effects of exogenous ABA on the quantum yield of photosystem (ΦPSII) of sugarcane seedlings under PEG-induced drought stress at 3, 6, and 9 DAT. Data are presented as the means of triplicate samples. Different letters on the bars show significant differences at $P < 0.05$.

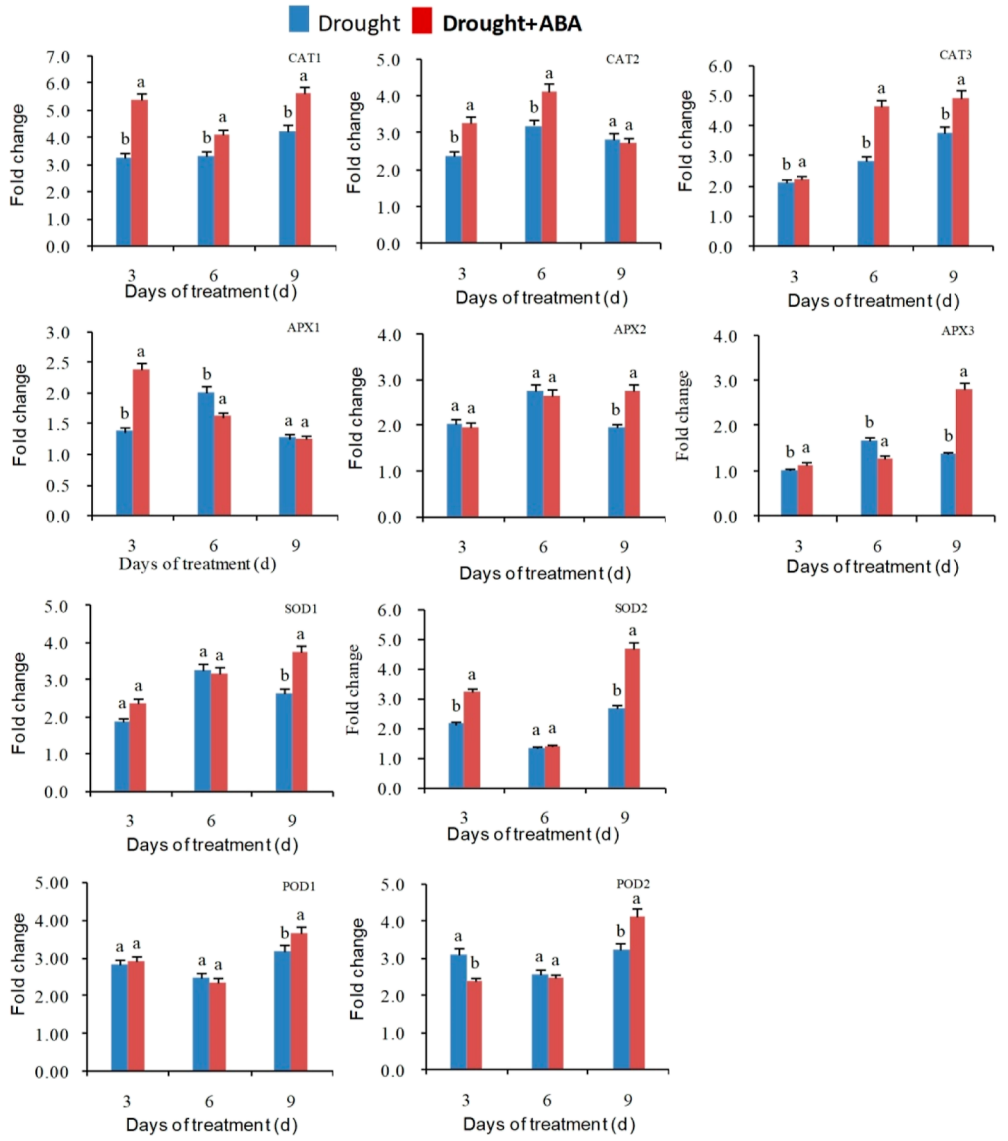

Figure 6. Effects of exogenous ABA on the expression of CAT1, CAT2, CAT 3 e, APX1, APX2, APX3, SOD1, SOD2, POD1, and POD2 genes of sugarcane seedlings under PEG-induced drought stress at 3, 6, and 9 DAT. Data are presented as the means of triplicate samples. Different letters on the bars show significant differences at $P < 0.05$.

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days compared to the control plants. The APX activities of ABA + drought-treated plants significantly increased by 8.14% at 6 days compared to those of the untreated controls. Similarly, the POD activities of ABA + drought plants were also significantly increased compared to those of the control plants, and at 6 days, the activities were increased by 21.8% compared to those of drought plants.

3.3. Phytohormones, MDA, and Proline Content. All treatments significantly affected the hormonal activities of sugarcane plants compared to water stress alone (Figure 3). ABA-treated drought plants showed a significant increase in IAA activity by 19.5, 27.3, and 33.3%, while ABA enhanced IAA activity by 42.2, 39.9, and 42.3% at 3, 6, and 9 days, respectively, compared to water-stressed plants. MDA concentrations were significantly higher in water-stressed plants compared to those in the control and ABA-treated plants. The proline content of ABA-treated drought plants significantly increased by 45 and 80% at 6 and 9 days, respectively, compared to that of the control plants. At 6 days, the proline content of ABA-treated drought plants increased by 25% compared to that of drought plants.

3.4. Photosynthesis and Chlorophyll Fluorescence Measurements. The photochemical efficiency (Fv/Fm) and net photosynthetic rate (Pn) of the plants significantly decreased with increasing water stress (Figure 4). Stomatal conductance (gs) also decreased by 34, 69, and 83% and the transpiration rate (E) by 77, 82, and 88% after 3, 7, and 9 days, respectively, compared to the control. However, the intercellular CO_2 concentration (Ci) increased by 41, 87, and 169% at 3, 7, and 9 days, respectively, with increasing water stress (mild, moderate, and severe). The efficiency of open centers (Fv/Fm) and effective quantum yield of PSII (ΦPSII) decreased with increasing water stress (Figure 5). However, the effective quantum yield significantly increased in exogenous ABA-treated plants compared to drought plants.

3.5. Gene Expression Analysis. The expression analysis of CAT, POD, SOD, and APX was performed using real-time qRT-PCR to investigate the stress tolerance mechanism of sugarcane (Figure 6). The results demonstrated that drought + ABA treatment significantly increased catalase 1 (*CAT1*) gene expression by 2.1-, 0.7-, and 1.37-fold at 3, 6, and 9 days, respectively, compared to drought-treated plants. *CAT2* expression was significantly increased at 3 and 6 days, while the *CAT3* gene was significantly increased at 6 and 9 days. Additionally, drought + ABA treatment significantly increased *APX1* expression on day 3, while *APX2* and *APX3* significantly increased on day 9 compared to drought-treated plants. *SOD1* expression was significantly increased at 9 days, while *SOD2* gene expression was significantly increased at 3 and 9 days compared to drought-treated plants. Moreover, *POD1* expression was significantly increased at 9 days, while *POD2* significantly increased at 3 and 9 days compared to drought-treated plants.

4. DISCUSSION

Abiotic stressors exert profound effects on plant morphology, biochemistry, physiology, and anatomy, impacting vital processes such as photosynthesis, respiration, growth, and development.[34] Among these stressors, drought stands out as one of the most significant, actively influencing the distribution of plant species in the wild and serving as a primary factor limiting crop yield. Drought arises from various distinct abiotic pressures and can exacerbate the impacts of other biotic or abiotic stressors on plants; hence, maintaining stable and controlled conditions is crucial for drought stress experiments.[15] This study aimed to assess the effects of drought and drought + ABA treatments on sugarcane seedlings after a month of treatment (including foliar application of 100 μM ABA before water stress). Water stress was induced by adding 25% (w/v) PEG 6000 to the Hoagland solution. Under regulated and stressful conditions, ABA plays a pivotal role in plant morphology and physiology,[35] promoting plant development by stimulating enzymes and various stress-related proteins.[36]

Our findings revealed that exogenously applied ABA increased the RWC (Figure 1). By enhancing leaf RWC and preserving the proline content of seedlings under PEG-induced drought stress, exogenous ABA countered the reduced water-use efficiency of the roots of drought-stressed soybean plants. This effect may be attributed to stomatal closure and ABA accumulation in the leaves, which reduce water loss through evaporation and dramatically decrease the soil drying rate under drought stress.[37] Additionally, in the present study, EL was significantly reduced under both stress treatments. Jiang et al.[20] reported that exogenous ABA inhibited the increase in relative EL under drought stress conditions. Compared to drought treatment alone, ABA treatment decreased relative EL in maize plants.

In the current study, we observed increased levels of MDA, oxygen (O_2), and hydrogen peroxide (H_2O_2), with H_2O_2 levels being higher in drought-stressed plants than in ABA + drought-treated plants. Faseela et al.[38] and Sohag et al.[39] reported elevated H_2O_2 and MDA levels in rice seedlings under PEG, which aligns with our findings. Excessive ROS production in response to environmental stimuli can lead to extreme oxidative damage to the cell membrane (lipid peroxidation), which can be detected by MDA. We also noted a significant increase in chlorophyll content following the application of exogenous ABA, consistent with previous reports on pearl millet.[40]

Drought stress resulted in a significant reduction in OP at full turgor, while osmotic adjustment (OA) was enhanced. Proline and soluble carbohydrates are associated with the adaptive response to drought stress, known as OA.[41] Our findings indicate that exogenous ABA significantly altered the activity of antioxidative enzymes under drought (PEG) stress (Figure 3). Exogenous ABA can scavenge ROS by increasing the activities of SOD, POD, and CAT, which are considered the first line of defense for plants against environmental stressors. SOD converts O_2 into H_2O_2, which is then broken down by CAT into H_2O and oxygen.[42] Du et al.[43] reported that exogenous ABA improved the antioxidant defense system of wheat seedlings under water stress. Jain et al.[44] observed higher expression of the SOD gene in sugarcane under drought stress. Previous studies also reported similar observations, suggesting that the improved SOD activity in drought-tolerant sugarcane genotypes may contribute to their resilience.[45] Wheat seedlings under water stress were found to produce antioxidant genes responsible for APX and glutathione reductase activity in the presence of ABA.[46] Exogenous ABA has been shown to alleviate oxidative damage caused by drought stress on cucumber seedlings by increasing antioxidant enzyme activities.[47] Qiao et al.[48] also observed that ABA application increased Asr1 gene expression, endogenous ABA levels, chlorophyll content, osmotic substances, antioxidant enzyme activity, and photosynthetic properties in corn.

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Physiological changes are the initial indicators of plant water shortage, and drought profoundly affects photosynthesis. Water stress induces photoinhibition, damaging the photosynthetic system and permanently inactivating PSII, as evidenced by significant changes in photosynthetic parameters.[49] Reduced photosynthetic activity is one of the factors influencing plant growth. To assess the impact of water deficit on the photosynthetic apparatus and gas exchange parameters in sugarcane, we measured the photosynthetic rate (Pn), stomatal conductance (gs), transpiration (Tr), and quantum yield of PSII activity. Pn, gs, and intercellular CO_2 concentration (Ci) significantly declined under water stress compared to ABA + drought-treated plants. Stomatal closure is one of the initial responses of plants to water shortage or osmotic stress, aimed at preventing excessive evaporation and maintaining regular metabolic processes in the cells.[50] Similar results were reported previously, indicating that abiotic stresses such as drought can detrimentally affect the photosynthetic organs of plants. However, ABA can mitigate these effects by increasing the number of photosynthetically active pigments under stress, enhancing photochemical reactions in leaves, protecting the photosynthetic machinery, and stabilizing the cystoid membrane, ultimately leading to an increase in the rate of photosynthesis.[51]

Proline serves as a crucial osmolyte contributing to OA. During water stress, the accumulation of compatible organic solutes, such as proline, can decrease the OP within cells, establishing an osmotic gradient.[52] The effects of endogenous phytohormones on plant responses to drought stress are commonly investigated. ABA has been reported to play a significant role in enhancing PSII efficiency under stress conditions.[53] In this study, we examined the changes in sugarcane hormones under water-stressed conditions. Exogenous ABA notably increased ABA activities at 3, 6, and 9 days. Generally, auxin, cytokinin, and ethylene tend to inhibit ABA-mediated stomatal closure, while jasmonates, brassinosteroids, and salicylic acid exhibit activities similar to those of ABA.[54]

In this study, exogenous ABA significantly upregulated the expression of CAT, APX2, APX3, POD, and SOD genes at 9 days compared to drought-treated plants (Figure 6). Catalase, essential for reducing H_2O_2 under stress, is contained within peroxisomes.[55] The maintenance of H_2O_2 levels being scavenged in root cells may be attributed to the consistent expression of CAT under both drought stress and normal conditions.[56] According to Yao et al.,[57] S-ABA treatment increased endogenous ABA levels, consequently enhancing antioxidant enzyme activity and Asr1 gene expression, thus reducing oxidative damage to maize leaves and enhancing maize seedlings' ability to withstand drought stress. In this work, we found that ABA treatment significantly altered the expression patterns of genes such as CAT, APX, and SOD. Recently, Yang et al.[58] reported that different concentrations of ABA altered the gene expression patterns of genes such as CoTOC1 and CoDMR6 in *Camellia oleifera* seedlings under drought stress. According to real-time quantitative PCR results of associated stress-related genes indicated that the regulation of related gene expression, ABA therapy increased the plant's response to stress and the ABA signaling pathway.

5. CONCLUSIONS

PEG-induced drought stress had deleterious effects on sugarcane plants. Exogenous ABA supplementation bolstered phytohormone levels and reduced H_2O_2 concentration under PEG-induced drought stress by augmenting antioxidant enzyme activity. Moreover, our examination of phytohormone dynamics unveiled intricate interactions between ABA and hormones like cytokinins and gibberellins, indicating a complex regulatory network involved in sugarcane's adaptive response to water stress. These findings offer valuable insights into potential strategies for alleviating the adverse impacts of drought on sugarcane cultivation. Overall, our results underscore the potential of exogenous ABA application as a practical approach to enhance sugarcane resilience to water stress, with promising implications for agricultural sustainability and productivity in water-limited environments. Future research endeavors could delve deeper into the molecular mechanisms underlying the interplay between ABA, antioxidant enzymes, and phytohormones in sugarcane. Additionally, exploring the efficacy of ABA application under diverse environmental conditions and across various sugarcane cultivars could yield valuable insights for optimizing agricultural practices aimed at enhancing crop resilience and productivity in water-limited environments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02341.

Gene primer sequences used for the quantitative real-time PCR analysis and effects of drought stress on electrolyte leakage, leaf water potential, and osmotic potential at full turgor (PDF)

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Notes

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