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Foliar Application of Trehalose or 5-Aminolevulinic Acid Improves Photosynthesis and Biomass Production in Drought Stressed *Alpinia zerumbet*

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Abstract: *Alpinia zerumbet* is an important medicinal and ornamental plant species. Drought stress is a major concern for sustainable horticulture crop production under changing climate scenarios. Trehalose (Tre) and 5-aminolevulinic acid (ALA) are osmoprotectants that play important roles in mitigating plant stresses. In this study, the effects of foliar application of 25 mM Tre or 10 mg L⁻¹ ALA on biochemical and physiological parameters of *A. zerumbet* seedlings and their growth were evaluated under well-watered and drought-stressed (65% of field capacity) conditions. Drought caused reductions in physiological parameters and plant growth. These decreases were accompanied by increases in leaf free proline and glycine betaine concentrations and peroxidase activities. Foliar application of Tre or ALA remediated physiological and biochemical parameters and plant growth. Overall, foliar application of ALA or Tre proved to be beneficial for mitigating drought stress in *A. zerumbet*.

Keywords: *Alpinia zerumbet*; drought stress; trehalose; 5-aminolevulinic acid

1. Introduction

Drought stress is a major constraint in crop production worldwide. Drought stress negatively affects growth and development of ornamental and medicinal plants, which ultimately threatens crop productivity and quality. Under current climate change models, more frequent droughts and a persistent reduction in irrigation water are becoming major challenges to crop production, especially in arid and semi-arid regions [1]. Human population growth exacerbates water scarcity through increases in the expansion of urban,

industrial, and recreational activities. These increased activities result in less available fresh water per capita and for agricultural purposes [2]. Thus, irrigated crop production faces increasing threats from water shortages [3]. A strategy for sustaining crop productivity in the face of reduced water availability is to improve plant drought-stress tolerance [4].

Plants grown under drought stress experience a variety of morpho-physiological and biochemical changes that have serious consequences for plant productivity, including stomatal conductance and CO₂ assimilation. The consequences arise due to disruption of biochemical reactions, such as the synthesis of photosynthetic pigments [5]. Drought stress also triggers the production of reactive oxygen species (ROS), such as superoxide anion, singlet oxygen, and hydrogen peroxide. Drought-stress-induced ROS are highly reactive and lead to oxidative damage of cell structures and affect biosynthesis of sugars, proteins, nucleic acids, lipids, and other molecules [6–8].

Severe or long-term exposure of plants to abiotic stresses can overwhelm endogenous mechanisms that plants have to cope with these stresses [9]. In the case of drought stress, plants have a variety of complex responses and tolerance mechanisms. These mechanisms include drought escape by an abrupt transition towards the reproductive phase before drought-induced death, or drought avoidance of water loss through reduced transpiration, enhanced water uptake, and/or osmotic adjustments. The natural antioxidant defense systems in plants that reduce drought-induced ROS toxicity work directly or in combination with other antioxidants to detoxify ROS [6–8]. Enzymatic antioxidants, such as superoxide dismutases (SOD) and peroxidases (POD) are of primary importance in detoxifying ROS in plants [6–8]. Activating these enzymes is therefore an important defense against drought stress.

The search continues for effective and economic strategies to minimize the deleterious effects of drought stress and increase drought tolerance in crops to help ensure sustainable productivity [4,10,11]. Exogenous applications of a variety of osmoprotectants, nutrients, plant-growth regulators, and oxidant scavengers before or at the onset of drought are considered to be beneficial in mitigating the negative effects of drought on plant performance, thereby enhancing plant productivity [8,10,11]. Trehalose (Tre) is a non-reducing glucose disaccharide (α -D-glucopyranosyl-1,1- α -D-glucopyranoside) that is produced by a variety of organisms including plants and is present in a wide range of concentrations [12,13]. Trehalose has been reported to play a critical role in coordinating plant metabolism with growth and development [14,15]. Its accumulation in plants alters sugar metabolism. Trehalose is also an osmoprotectant under stress conditions, including drought [16,17]. Recent studies have demonstrated that exogenous application of Tre induces significant drought tolerance in many plant species, including radish (*Raphanus sativus*), wheat (*Triticum aestivum*), rice (*Oryza sativa*), sunflower (*Helianthus annuus*), mung bean (*Vigna radiata*), and cucumber (*Cucumis sativus*) [16–20].

Like Tre, the natural and environmentally-friendly compound 5-aminolevulinic acid (ALA) plays an important role in plants as an osmoprotectant during stress [21,22]. It is a biosynthetic precursor of heterocyclic tetrapyrrole molecules, including vitamin B12, bilins, and heme, and has been considered to be a potential growth regulator in plants [23–25]. ALA is synthesized from glutamate and is a precursor of chlorophyll, so it is indispensable to photosynthesis [21]. In addition to harvesting light for photosynthesis, tetrapyrroles in plants are signaling factors, electron carriers, and catalysts of redox reactions [21].

Alpinia zerumbet (Pers.) B.L.Burtt & R.M.Sm., commonly known as shell ginger, is an aromatic evergreen monocotyledonous herb in the family Zingiberaceae [26]. It is a perennial rhizomatous plant that can be easily cultivated vegetatively from its rhizomes in tropical and subtropical regions and is widely used as a landscape or ornamental plant [26]. Its attractive leaves and showy fragrant flowers make it a perfect choice as a potted foliage plant [27]. It is also widely used in traditional medicine for its antimicrobial, anti-cancer, anti-inflammatory, and bacteriostatic properties [26]. *A. zerumbet* grows in rich, moist loamy soils enriched with organic matter. It grows in partial shade to full sun and needs abundant moisture. In Pakistan, *A. zerumbet* seedlings tend to suffer from drought stress, resulting in

major economic losses in both ornamental and medicinal/industrial production. There are no studies available on Tre or ALA-mediated drought protection of *A. zerumbet*.

In the present study, we hypothesized that exogenous application of Tre or ALA would enhance drought tolerance in *A. zerumbet* via physiological and/or biochemical modulations to allow the plant to maintain growth under water-limiting conditions. Therefore, vegetative, physiological, and biochemical responses of *A. zerumbet* seedlings to foliar application of Tre or ALA during drought stress (65% of field capacity) were investigated to determine if these treatments could improve drought tolerance in *A. zerumbet*.

2. Materials and Methods

2.1. Plant Material, Experimental Conditions, and Treatments

Healthy *A. zerumbet* seedlings grown in plastic bags were purchased from a commercial nursery (Best Garden Nursery, Faisalabad, Pakistan). The seedlings were transplanted singly into 7 L earthen pots (25 cm top diameter, 19 cm base diameter, 20 cm height) filled with sandy loam soil composed of 65% sand, 24.5% silt, and 10.5% clay by volume with an electrical conductivity (EC) of $1.88 \text{ dS} \cdot \text{m}^{-1}$ and pH of 7.7. The soil contained 74 mg g^{-1} nitrogen (N); 8.2 mg g^{-1} phosphorus (P) and 156 mg g^{-1} potassium (K). Its saturation percentage was 30% on 11 March 2018. Following transplanting, the pots were kept at constant relative humidity (61.42%) and temperature (16.7°C) under a shade structure at Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan ($31^\circ 30' \text{ N}$, $73^\circ 10' \text{ E}$, altitude 213 m). The daily photoperiod ranged from 12 to 13 h. The mean light intensity received by the *A. zerumbet* plants was about $150 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, with a 95% quantile of $1230 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Plants were irrigated based on soil water holding capacity. Pots were weighed to establish field capacity (FC) and then weighed again after 2 days to replace the water lost to maintain the desired field capacity (W0: 100% FC; W1: 65% FC). Transplants were watered regularly to 100% FC for 4 weeks, then assigned to well-watered (100% FC) or drought-stressed (65% FC) groups. After 6 weeks under these watering regimes, exogenous chemicals were applied to 100% FC and 65% FC plants as a foliar spray once a week for 4 weeks. The treatments were: distilled water (DW) having 0.05% Tween-20 (control); 25 mM trehalose + 0.05% (*v/v*) Tween-20 (Tre); and 10 mg L^{-1} 5-aminolevulinic acid + 0.05% (*v/v*) Tween-20 (ALA). The choice of the Tre and ALA concentrations was based on a preliminary experiment where Tre at 5, 10, 15, 20, 25, 30, and 35 mM and ALA at 2, 4, 6, 8, 10, 12, and 14 mg L^{-1} were evaluated, and Tre at 25 mM and ALA at 10 mg L^{-1} provided vigorous growth of leaves and shoots. Both young and old leaves of drought-stressed plants were homogeneously sprayed with treatment solutions between 7:00 and 8:00 at a constant pressure of 40 psi using an agricultural pressure sprayer. The application volume varying from 25 to 30 mL plant^{-1} . Plant measurements, and leaf and rhizome samples were taken two weeks after the final chemical application. Experiments were arranged in a completely randomized design, with two levels of irrigation (W0 and W1) and one level of each Tre or ALA treatment. There were six replicates (pots) per treatment.

2.2. Leaf Gas Exchange Parameters and Chlorophyll Concentration

Net photosynthetic rate (P_n), stomatal conductance (g_s), internal CO_2 concentration (C_i), and transpiration rate (E) were measured on a sunny day between 10:00 and 12:30 on four fully-expanded mature leaves of each plant, with constant light intensity ($1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$), CO_2 concentration ($400 \mu\text{mol}$), and airflow ($500 \mu\text{mol s}^{-1}$), using a portable CO_2 infrared gas analyzer (LCi-SD, ADC Bio-scientific Ltd., Hoddesdon, UK).

Chlorophyll concentration was determined following Arnon [28]. Approx. 0.5 g of fresh leaf material was minced and extracted overnight in the dark with 80% (*v/v*) acetone at -4°C . The extract was centrifuged at $10,000 \times g$ for 5 min. The absorbance of the

supernatant was read at 645 nm and 633 nm in a spectrophotometer. Chlorophyll *a* and *b* were calculated as described by Arnon (1949) using the formula:

$$\text{Chlorophyll } a \text{ (mg g}^{-1} \text{ fresh weight)} = \{12.7(\text{OD}_{663}) - 2.69(\text{OD}_{645}) \times (V/1000 \times W)\}$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1} \text{ fresh weight)} = \{22.9(\text{OD}_{645}) - 4.68(\text{OD}_{663}) \times (V/1000 \times W)\}$$

where OD was optical density at wavelength 645 or 663 nm, V was final volume, and W was the weight of the fresh leaf sample.

2.3. Biochemical Parameters

Glycine betaine concentration was measured following the method of Grieve and Grattan [29]. Leaf material (0.5 g) was grounded for each replicate and shaken occasionally in 10 mL toluene (0.5%) and kept at 4 °C overnight. After filtration and centrifugation, 1 mL of filtrate was added to 1 mL of 2 N sulfuric acid and 0.1 mL of 200 µL potassium triiodide (KI₃) in a test tube. Samples were cooled at 4 °C for 1 h before adding 2.8 mL ice-cold deionized H₂O and 5 mL 1,2-dichloroethane. The absorbance of the lower organic layer was recorded spectrophotometrically at 365 nm (UV-1900 spectrophotometer; BMC, Quebec, QC, Canada).

Leaf free proline was determined in fresh leaf samples using the methodology of Bates et al. [30]. A mature leaf sample (0.5 g) was isolated from the middle portion of each replicate plant and grounded. Sample was mixed with 10 mL 3% (*w/v*) sulfosalicylic acid (MP Biomedicals Inc., Solon, OH, USA). After filtering, 2.0 mL of filtrate was mixed in a test tube with 2.0 mL acid ninhydrin solution and 2.0 mL glacial acetic acid (MP Biomedicals, Inc., Singapore). Acid ninhydrin was prepared by mixing 1.25 g ninhydrin (C₆H₄COCOCOH₂O, BDH AnalaR, BDH Chemical Ltd., Poole, UK) with 30 mL glacial acetic acid and 20 mL of 6 M H₃PO₄. The optical density of the filtrate was measured at 520 nm using a UV-1900 spectrophotometer (BMC, Montreal, QC, Canada).

Antioxidant enzymes were extracted from 0.5 g of fresh leaf sample by homogenizing at 4 °C in 4 mL 0.05 M sodium phosphate buffer, pH 7.8, containing 2% (*w/v*) polyvinylpyrrolidone and 1.0 mM EDTA. Homogenates were centrifuged at 10,000× *g* for 20 min at 4 °C and fresh aliquots were immediately used in assays. The POD activity was measured as described by Chance and Maehly [31]. The reaction mixture contained 0.1 mL protein extract, 0.4 mL 20 mM guaiacol, 0.5 mL 40 mM H₂O₂, and 2 mL 50 mM sodium phosphate buffer. Absorbance was read at 470 nm in 20-s intervals with a UV-1900 spectrophotometer (BMC, Montreal, QC, Canada). The absorbance slope was used to calculate POD activity. The SOD activity was determined according to Van Rossum [32]. The reaction mixture contained 0.4 mL distilled water, 0.1 mL methionine, 0.1 mL Triton-X, 0.25 mL sodium phosphate buffer (pH 7.8), 0.5 mL nitro blue tetrazolium, 0.5 mL riboflavin, and 0.5 mL protein extract. The mixture was kept in the light for 20 min before recording the absorbance at 560 nm.

2.4. Growth Parameters

Shoot fresh weight (SFW), shoot dry weight (SDW), shoot diameter, shoot length (SL), leaf fresh weight (LFW), leaf dry weight (LDW), leaf area, rhizome fresh weight and rhizome dry weight were measured at the end of the experiment. Shoot length was determined with a ruler, and leaf area was measured using a portable area meter (LI-3000C, LICOR, Lincoln, NE, USA). Dry weight was measured after drying material at 80 °C to a constant weight.

2.5. Data Analysis

Data were checked for normality and homogeneity of variances using SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). All data were then subjected to two-way analysis of variance (ANOVA) by SPSS. Mean differences in treatments were separated by LSD at *p* < 0.05. All data are presented as means ± standard error (S.E.) with *n* = 6.

3. Results

3.1. Plant Growth Responses to Drought and Treatment with Trehalose or 5-Aminolevulinic Acid

Under well-watered conditions (100% FC), SFW of plants treated with either Tre or ALA was greater than that of the untreated control plants (Figure 1A). The SFW of *A. zerumbet* grown under drought stress (65% FC) was significantly reduced compared to plants grown at 100% FC. The foliar application of either Tre or ALA relieved the loss in SFW at 65% FC, since SFW of treated drought-stressed plants was similar to the level seen in the untreated plants at 100% FC. Thus, the treatments did not stimulate growth above that of the untreated control plants at 100% FC. Drought stress also significantly reduced shoot dry weight (SDW) compared to plants grown at 100% FC (Figure 1B). Foliar application of Tre to drought-stressed plants recouped this loss in SDW. Interestingly, ALA treatment of drought-stressed plants enhanced SDW more than Tre treatment to levels similar to those in the Tre-treated and ALA-treated plants at 100% FC.

Figure 1. Shoot fresh weight (A), shoot dry weight (B), shoot diameter (C), and shoot length (D) of well-watered (100% FC, Control) and drought-stressed (65% FC, Drought) *Alpinia zerumbet* treated with foliar applications of water alone, 25 mM trehalose (Tre) or 10 mg L⁻¹ 5-aminolevulinic acid (ALA). Values are means $\pm$ S.E. ($n = 6$). Different letters above the bars indicate significant differences based on LSD analysis at $p < 0.05$.

Both Tre and ALA treatment resulted in an increase in shoot diameter at 100% FC, while drought stress caused a significant reduction in this trait (Figure 1C). Foliar treatment with Tre or ALA had a compensatory effect on shoot diameter in drought-stressed plants by maintaining the shoot diameter seen in the well-watered control plants. Shoot length was also significantly less in the drought-stressed plants than in the well-watered control

group (Figure 1D). Foliar treatment with Tre or ALA increased shoot length significantly in both well-watered and drought-stressed plants compared to their untreated counterparts. However, the increase in stem length in the drought-stressed plants was only to the same level as observed for the untreated well-watered plants.

Application of Tre or ALA caused an increase in both leaf fresh weight and leaf dry weight in plants at 100% FC, with ALA treatment causing leaf fresh weight to increase more than Tre treatment (Figure 2A,B). Both leaf fresh and dry weight were significantly lower when plants were grown at 65% FC compared to plants grown at 100% FC (Figure 2A,B). Treatment with Tre or ALA prevented this loss but did not cause an increase over the well-watered control.

Both Tre and ALA treatments caused an increase in leaf area in well-watered *A. zerumbet* plants (Figure 2C). Drought stress did not significantly impact leaf area. However, Tre or ALA treatment did not significantly increase leaf area at 65% FC, as they did at 100% FC.

Rhizome fresh and dry weights increased in response to Tre and ALA treatments in well-watered plants. Drought decreased both rhizome dry weight and fresh weight. Treatment with Tre or ALA prevented the decrease (Figure 3A,B).

3.2. Plant Physiological Responses

Application of Tre or ALA increased P_n under well-watered conditions (Table 1). Other photosynthetic parameters at 100% FC, such as g_s , E and C_i , also increased in response to Tre or ALA treatment. Drought stress reduced P_n , g_s , E and C_i . All of these decreases, with the exception of E , were relieved by the application of Tre or ALA to a level similar to the untreated well-watered plants. Treatment of drought-stressed plants with Tre and ALA also caused an increase in E , but only to 70% or 80% of the untreated plants at 100% FC.

Table 1. Effects of foliar application of 25 mM trehalose (Tre) or 10 mg L^{−1} 5-aminolevulinic acid (ALA) on leaf gas exchange parameters in *Alpinia zerumbet* seedlings grown under well-watered (Control, CK) and drought-stress (Drought) conditions. Values are means ± S.E. ($n = 6$). Different letters within a column indicate significant differences among means according to LSD test at $p < 0.05$.

Treatments ^a	CO ₂ Assimilation Rate (P_n) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	Stomatal Conductance (g_s) ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Transpiration (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	Intercellular CO ₂ Concentration (C_i) ($\mu\text{mol mol}^{-1}$)
Control (CK)	5.94 ± 0.38b	0.53 ± 0.04b	1.41 ± 0.05c	237.5 ± 9.63b
CK + Tre	8.21 ± 0.39c	0.70 ± 0.12c	1.73 ± 0.11cd	272.6 ± 14.75c
CK + ALA	9.00 ± 0.38c	0.73 ± 0.49c	1.94 ± 0.07d	289.1 ± 6.12c
Drought	4.04 ± 0.18a	0.12 ± 0.05a	0.62 ± 0.02a	183.1 ± 7.1a
Drought + Tre	5.34 ± 0.35b	0.54 ± 0.14b	0.99 ± 0.04b	226.1 ± 7.12b
Drought + ALA	5.98 ± 0.33b	0.68 ± 0.12b	1.14 ± 0.03b	240.1 ± 11.2b

Figure 2. Leaf fresh weight (A), leaf dry weight (B), and leaf area (C) of well-watered (100% FC, Control) and drought-stressed (65% FC, Drought) *Alpinia zerumbet* treated with foliar applications of water alone, 25 mM trehalose (Tre) or 10 mg L⁻¹ 5-aminolevulinic acid (ALA). Values are means $\pm$ S.E. ($n = 6$). Different letters above the bars indicate significant differences based on LSD at $p < 0.05$.

Figure 3. Rhizome fresh weight (A) and dry weight (B) for well-watered (100% FC, Control) and drought-stressed (65% FC, Drought) *Alpinia zerumbet* treated with foliar applications of water alone, 25 mM trehalose (Tre) or 10 mg L⁻¹ 5-aminolevulinic acid (ALA). Values are means $\pm$ S.E. ($n = 6$). Different letters above the bars indicate significant differences based on LSD analysis at $p < 0.05$.

3.3. Plant Biochemical Responses

Chlorophyll a and b concentrations increased in response to Tre or ALA treatment in leaves of well-watered *A. zerumbet*. Water deficit stress had no significant impact of these photosynthetic pigments. In contrast to the increases seen in well-watered plants, Tre or ALA treatment did not cause a change in chlorophyll a or b concentration under drought stress (Figure 4).

Figure 4. Chlorophyll a (A) and chlorophyll b (B) concentrations in leaves of well-watered (100% FC, Control) and drought-stressed (65% FC, Drought) *Alpinia zerumbet* treated with foliar applications of water alone, 25 mM trehalose (Tre) or 10 mg L⁻¹ 5-aminolevulinic acid (ALA). Values are means $\pm$ S.E. ($n = 6$). Different letters above the bars indicate significant differences based on LSD analysis at $p < 0.05$.

Leaf concentrations of glycine betaine and proline increased in well-watered plants in response to Tre treatment, but not in response to ALA treatment (Figure 5A,B). Leaf concentrations of both glycine betaine and proline increased significantly under drought stress compared to untreated well-watered plants to levels similar to those in well-watered plants treated with Tre, but higher than those plants treated with ALA. Both Tre and ALA treatments of drought-stressed plants caused a further, but similar, increase in the leaf concentrations of both glycine betaine and proline.

Figure 5. Leaf glycine betaine (A) and proline (B) concentrations in well-watered (100% FC, Control) and drought-stressed (65% FC, Drought) *Alpinia zerumbet* treated with foliar applications of water alone, 25 mM trehalose (Tre) or 10 mg L⁻¹ 5-aminolevulinic acid (ALA). Values are means $\pm$ S.E. ($n = 6$). Different letters above the bars indicate significant differences based on LSD analysis at $p < 0.05$.

Superoxide dismutase (SOD) and peroxidase (POD) activities increased in *A. zerumbet* leaves in response to both Tre and ALA treatments under well-watered conditions (Figure 6A,B). The imposition of water deficit had no effect on SOD activity but caused an increase in POD activity. The application of Tre or ALA to drought-stressed plants caused an increase in both SOD and POD activities. The activity of POD increased similarly in response to both Tre and ALA treatments to the levels greater than those in the treated well-watered plants. The activity of SOD in the Tre-treated drought-stressed plants was also stimulated greater than that of the Tre-treated well-watered plants. On the other hand, ALA treatment of drought-stressed plants only caused SOD activity to increase to reach a level similar to that in the well-watered plants treated with ALA.

Figure 6. Superoxide dismutase (SOD) (A) and peroxidase (POD) (B) activity in leaves of well-watered (100% FC, Control) and drought-stressed (65% FC, Drought) *Alpinia zerumbet* treated with foliar applications of water alone, 25 mM trehalose (Tre) or 10 mg L⁻¹ 5-aminolevulinic acid (ALA). Values are means $\pm$ S.E. ($n = 6$). Different letters above the bars indicate significant differences based on LSD analysis at $p < 0.05$.

4. Discussion

Drought is a major constraint affecting plant growth and development [33]. Its impact depends upon the severity of the stress and species of plant. The level of stress, particularly at the seedling stage, is critical to determining the severity of the impact on plant growth and productivity [34]. Thus, by adopting strategies that ameliorate the impacts of drought at the seedling stage, one can achieve a crop harvest even when plants experience stressful conditions. In the present study, drought stress significantly reduced the growth of *A. zerumbet* seedlings by decreasing shoot fresh mass, shoot dry mass, shoot length, leaf fresh mass, leaf dry mass and leaf area. This impairment in morphological growth due to drought may be due to a number of factors, including a shortage of moisture in tissues, inhibition of the activity of apical meristems, reduction in cell enlargement, or inequality in the distribution of phytohormones [35]. Foliar application of Tre or ALA helped relieve the growth deficits of *A. zerumbet* plants experiencing moisture deficit. Our results showed that application of either Tre or ALA enhanced plant growth by increasing shoot fresh mass, shoot dry mass, leaf fresh mass, leaf dry mass and leaf area in *A. zerumbet* plants exposed to water limitation. Foliar application of ALA also improved shoot and root

growth in wheat [36], oilseed rape [37], *Raphanus sativus* [38], and *Leymus chinensis* [39]. The external application of ALA improved growth parameters in treated plants compared to non-treated plants [40]. Our findings that Tre application alleviates the adverse effects of drought stress on *A. zerumbet* were similar to those reported for various abiotic stresses in rice [41], sunflower [17], and wheat [42]. The mechanism through which Tre treatment enhanced plant biomass production may be through the induction of enzymes involved in carbohydrate storage in photosynthetic tissues [43]. The metabolic processes induced by Tre have been found to safeguard turgor pressure and mineral balance by scavenging ROS [44].

The rhizome fresh and dry masses of *A. zerumbet* plants significantly decreased when grown under drought stress. Application of Tre and ALA alleviated these effects. As there are no reports in the literature regarding Tre and ALA effects on rhizome growth, the mechanism underlying this amelioration is unclear. It is possible that the onset of soil desiccation induces stomatal closure as a first line of defense. Consequently, impairment in CO₂ absorption, carbon assimilation and transpiration would be expected. This situation would affect photosynthesis [45]. In the present analysis, drought stress caused negative impacts on *Pn*, *Ci*, *E*, and *gs* of *A. zerumbet* plants. Both Tre and ALA treatments were able to reverse these impacts. Similar to our results, a significant reduction in *Pn*, *gs* and *E* was reported by Nishihara et al. [46] in spinach under saline conditions, while there was an increase in the rate of photosynthesis upon foliar application of ALA in both stressed and non-stressed plants. The role of ALA as a precursor in chlorophyll biosynthesis has been suggested to be a mechanism underlying its ability to rehabilitate photosynthesis under unfavorable conditions [47]. Indeed, an increase in chlorophyll a and b concentration was seen in ALA-treated control *A. zerumbet* plants. However, this increase was not observed in ALA-treated plants subjected to drought. The presence of ALA maintained the osmotic potential of leaf tissues and regulated stomatal limitation in date palm subjected to salt stress [48]. Abiotic stresses often impaired light harvesting capacity and perturbed carbon assimilation [49]. In *Brassica napus*, application of ALA maintained thylakoid membrane intactness and increased photosynthesis [50]. The application of Tre to sunflower mitigated the negative impacts of drought stress on gas exchange parameters, but WUE was decreased [17]. In contrast, pre-soaking of radish with Tre resulted in increased WUE [18]. Similar findings were reported by Ali and Ashraf [51] in maize leaves under water deficit stress. Thus, the application of ALA or Tre reduced the impact of abiotic stress on gas exchange parameters.

The onset of stress conditions often causes a decrease in photosynthetic and accessory pigments and/or disrupts stomatal activity [4]. As a result, the photosynthetic rate declines. In *A. zerumbet*, there was a significant increase in both chlorophyll a and b in well-watered plants treated with Tre and ALA; as expected, water deficit caused a significant decline in leaf concentrations of both chlorophyll a and b. However, this decrease due to drought was not reversed by ALA or Tre treatments. This is in contrast to many plants under salinity stress, where application of ALA caused an increase in chlorophyll a and b concentrations [48,50,52]. The adverse circumstances of stress induced the production of reactive oxygen species which impaired the chlorophyll compounds in stressed plants [53]. The foliar application of Tre under water deficit stress markedly improved the photosynthetic pigments in sunflower plants [17]. In drought stressed radish plants, Tre pre-soaking application increased chlorophyll a [18]. So, it is inferred that treatment of both ALA and Tre remained beneficial for photosynthetic pigments under drought stress.

Drought conditions typically induce biosynthesis of various cytosolic osmolytes that not only protect cellular organelles from damage, but also assist in the retention of cellular osmotic potential [4]. Leaf concentrations of both glycine betaine and proline increased in *A. zerumbet* subjected to moisture deficit. These concentrations increased further in drought-stressed plants treated with foliar Tre or ALA. Both treatments cause similar increases for both osmolytes. In water stressed wheat, leaf concentrations of proline and glycine betaine significantly increased upon ALA treatment [36]. These osmoprotectants safeguard

biological membranes and proteins and carry out essential cellular processes even under extreme stress [9]. Application of ALA also increases mineral nutrient uptake and the accumulation of proline even in brackish environments. Like ALA, Tre treatment also caused increased proline and glycine betaine under stress conditions in sunflower [17] and *Brassica campestris* [44] plants, and in priming of radish seeds [18]. These analyses support the conclusion that both ALA and Tre alleviate drought stress by stimulating biosynthesis of osmoprotectants like glycine betaine and proline.

Drought stress causes plant cells to produce toxic free radicals that damage cellular structures, including biological membranes. To cope with these strong reactive oxygen species, plants tend to accumulate antioxidants [6–8]. Antioxidant enzymes, such as SOD, POD, and catalase are often synthesized by plants to combat reactive oxygen species [7,8]. In *A. zerumbet*, water deficit induced POD activity, but did not have a strong effect on SOD activity. Foliar application of Tre and ALA caused an increase in both SOD and POD activities. However, in drought-stressed plants, Tre treatment caused a greater increase in SOD activity than ALA treatment. Typically, ALA and Tre has a key role in up-regulating plant defense strategies against abiotic stress, for example in drought-stressed cucumber [54], and in cold stressed melon seedlings [55]. Treatment with ALA also caused increases in antioxidant enzyme activities in salt-stressed spinach [46]. In contrast, though, ALA had no effect on these antioxidant enzymes in water stressed wheat [36]. Similarly, to ALA, foliar application of Tre also stimulated SOD and POD activities in water stressed radish root [16].

5. Conclusions

The growth of *A. zerumbet* was adversely affected by water stress at 65% of field capacity. Foliar application of Tre and ALA relieved these adverse growth effects and stimulated a number of physiological and biochemical parameters. Overall, foliar application of either 10 mg L^{−1} ALA or 25 mM Tre to the drought-stressed plants largely prevented the reduction in shoot dry mass, shoot diameter and length, leaf fresh and dry matter, rhizome fresh and dry weights, and photosynthetic parameters, increased glycine betaine and proline contents, and enhanced the activity of SOD and POD. Further studies should determine if co-application of ALA and Tre at appropriate concentrations could provide synergetic effects on the alleviation of drought stress in *A. zerumbet* plants. Regardless, foliar application of ALA or Tre could be an alternative strategy for mitigating drought stress in *A. zerumbet*.

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References

1. Razzaq, A.; Wani, S.H.; Saleem, F.; Yu, M.; Zhou, M.; Shabala, S. Rewilding crops for climate resilience: Economic analysis and de novo domestication strategies. *J. Exp. Bot.* **2021**, erab276. [\[CrossRef\]](#)
2. Galindo, A.; Collado-González, J.; Griñán, I.; Corell, M.; Centeno, A.; Martín-Palomo, M.J.; Carbonell-Barrachina, A.A. Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. *Agric. Water Manag.* **2018**, *202*, 311–324. [\[CrossRef\]](#)
3. Sposito, G. Green water and global food security. *Vadose Zone J.* **2013**, *12*. [\[CrossRef\]](#)
4. Zulfiqar, F.; Chen, J.; Finnegan, P.M.; Younis, A.; Nafees, M.; Zorrig, W.; Hamed, K.B. Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth. *Plants* **2021**, *10*, 1078. [\[CrossRef\]](#) [\[PubMed\]](#)

5. Wang, Y.; Wei, S.; Wang, J.; Su, X.; Suo, B.; Qin, F.; Zhao, H. Exogenous application of 5-aminolevulinic acid on wheat seedlings under drought stress enhances the transcription of *psbA* and *psbD* genes and improves photosynthesis. *Braz. J. Bot.* **2018**, *41*, 275–285. [\[CrossRef\]](#)
6. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Zulfiqar, F.; Raza, A.; Mohsin, S.M.; Mahmud, J.A.; Fujita, M.; Fotopoulos, V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants* **2020**, *9*, 681. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Parvin, K.; Bhuiyan, T.F.; Anee, T.I.; Nahar, K.; Hossen, M.S.; Zulfiqar, F.; Alam, M.M.; Fujita, M. Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. *Int. J. Mol. Sci.* **2020**, *21*, 8695. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Zulfiqar, F.; Ashraf, M. Bioregulators: Unlocking their potential role in regulation of the plant oxidative defense system. *Plant Mol. Biol.* **2021**, *105*, 11–41. [\[CrossRef\]](#)
9. Zulfiqar, F.; Akram, N.A.; Ashraf, M. Osmoprotection in plants under abiotic stresses: New insights into a classical phenomenon. *Planta* **2020**, *251*, 3. [\[CrossRef\]](#)
10. Zulfiqar, F.; Casadesús, A.; Brockman, H.; Munné-Bosch, S. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. *Plant Sci.* **2020**, *295*, 110194. [\[CrossRef\]](#)
11. Zulfiqar, F.; Russell, G.; Hancock, J.T. Molecular hydrogen in agriculture. *Planta* **2021**, *254*, 56. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Baena-González, E.; Lunn, J.E. SnRK1 and trehalose 6-phosphate—Two ancient pathways converge to regulate plant metabolism and growth. *Curr. Opin. Plant Biol.* **2020**, *55*, 52–59. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Fichtner, F.; Lunn, J.E. The role of trehalose 6-phosphate (Tre6P) in plant metabolism and development. *Annu. Rev. Plant Biol.* **2021**, *72*, 737–760. [\[CrossRef\]](#)
14. Sharma, M.P.; Grover, M.; Chourasiya, D.; Bharti, A.; Agnihotri, R.; Maheshwari, H.S.; Pareek, A.; Buyer, J.S.; Sharma, S.K.; Schütz, L.; et al. Deciphering the role of trehalose in tripartite symbiosis among rhizobia, arbuscular mycorrhizal fungi, and legumes for enhancing abiotic stress tolerance in crop plants. *Front. Microbiol.* **2020**, *11*, 509919. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Wang, W.; Chen, Q.; Xu, S.; Liu, W.C.; Zhu, X.; Song, C.P. Trehalose-6-phosphate phosphatase E modulates ABA-controlled root growth and stomatal movement in *Arabidopsis*. *J. Integr. Plant Biol.* **2020**, *62*, 1518–1534. [\[CrossRef\]](#)
16. Shafiq, S.; Akram, N.A.; Ashraf, M. Does exogenously-applied trehalose alter oxidative defense system in the edible part of radish (*Raphanus sativus* L.) under water-deficit conditions? *Sci Hort.* **2015**, *185*, 68–75. [\[CrossRef\]](#)
17. Kosar, F.; Akram, N.A.; Ashraf, M.; Sadiq, M.; Al-Qurainy, F. Trehalose-induced improvement in growth, photosynthetic characteristics and levels of some key osmoprotectants in sunflower (*Helianthus annuus* L.) under drought stress. *Pak. J. Bot.* **2018**, *50*, 955–961.
18. Akram, N.A.; Waseem, M.; Ameen, R.; Ashraf, M. Trehalose pretreatment induces drought tolerance in radish (*Raphanus sativus* L.) plants: Some key physio-biochemical traits. *Acta Physiol. Plant.* **2016**, *38*, 3. [\[CrossRef\]](#)
19. Abdallah, M.S.; Abdelgawad, Z.A.; El-Bassiouny, H.M.S. Alleviation of the adverse effects of salinity stress using trehalose in two rice varieties. *S. Afr. J. Bot.* **2016**, *103*, 275–282. [\[CrossRef\]](#)
20. Akram, N.A.; Noreen, S.; Noreen, T.; Ashraf, M. Exogenous application of trehalose alters growth, physiology and nutrient composition in radish (*Raphanus sativus* L.) plants under water-deficit conditions. *Braz. J. Bot.* **2015**, *38*, 431–439. [\[CrossRef\]](#)
21. Niu, K.; Ma, H. The positive effects of exogenous 5-aminolevulinic acid on the chlorophyll biosynthesis, photosystem and calvin cycle of Kentucky bluegrass seedlings in response to osmotic stress. *Environ. Exp. Bot.* **2018**, *155*, 260–271. [\[CrossRef\]](#)
22. Wang, H.; Liu, Z.; Luo, S.; Li, J.; Zhang, J.; Li, L.; Xie, J. 5-Aminolevulinic acid and hydrogen sulphide alleviate chilling stress in pepper (*Capsicum annuum* L.) seedlings by enhancing chlorophyll synthesis pathway. *Plant Physiol. Biochem.* **2021**, *167*, 567–576. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Rasheed, R.; Yasmeen, H.; Hussain, I.; Iqbal, M.; Ashraf, M.A.; Parveen, A. Exogenously applied 5-aminolevulinic acid modulates growth, secondary metabolism and oxidative defense in sunflower under water deficit stress. *Physiol. Mol. Biol. Plant.* **2020**, *26*, 489–499. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Sher, A.; Tahira, A.S.; Sattar, A.; Nawaz, A.; Qayyum, A.; Hussain, S.; Manaf, A. Foliage application of 5-aminolevulinic acid alleviates drought stress in sunflower (*Helianthus annuus* L.) through improving stay green and antioxidant enzymes activities. *Acta Physiol. Plant.* **2021**, *43*, 22. [\[CrossRef\]](#)
25. Cai, C.; He, S.; An, Y.; Wang, L. Exogenous 5-aminolevulinic acid improves strawberry tolerance to osmotic stress and its possible mechanisms. *Physiol. Plant* **2020**, *168*, 948–962. [\[CrossRef\]](#)
26. Zulfiqar, F.; Chen, J.; Younis, A.; Abideen, Z.; Naveed, M.; Koyro, H.W.; Siddique, K.H. Biochar, compost, and biochar-compost blend applications modulate growth, photosynthesis, osmolytes, and antioxidant system of medicinal plant *Alpinia zerumbet*. *Front. Plant Sci.* **2021**, *12*, 707061. [\[CrossRef\]](#)
27. Chen, J.; McConnell, D.B.; Henny, R.J.; Norman, D.J. The foliage plant industry. *Hort. Rev.* **2005**, *31*, 45–110.
28. Arnon, D.T. Copper enzyme in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1–15. [\[CrossRef\]](#)
29. Grieve, C.M.; Grattan, S.R. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil* **1983**, *70*, 303–307. [\[CrossRef\]](#)
30. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil.* **1973**, *39*, 205–207. [\[CrossRef\]](#)
31. Chance, B.; Maehly, A.C. Assay of catalases and peroxidases. *Methods Enzymol.* **1955**, *11*, 764–775.

32. Van Rossum, M.W.P.C.; Alberda, M.; Van der Plas, L.H.W. Role of oxidative damage in tulip bulb scale micropropagation. *Plant Sci.* **1997**, *130*, 207–216. [[CrossRef](#)]
33. De Kauwe, M.G.; Medlyn, B.E.; Tissue, D.T. To what extent can rising (CO₂) ameliorate plant drought stress? *New Phytol.* **2021**, *231*, 2118–2124. [[CrossRef](#)] [[PubMed](#)]
34. Ayyaz, A.; Miao, Y.; Hannan, F.; Islam, F.; Zhang, K.; Xu, J.; Farooq, M.A.; Zhou, W. Drought tolerance in *Brassica napus* is accompanied with enhanced antioxidative protection, photosynthetic and hormonal regulation at seedling stage. *Physiol. Plant* **2021**, *172*, 1133–1148. [[CrossRef](#)]
35. Zhu, Y.; Luo, X.; Nawaz, G.; Yin, J.; Yang, J. Physiological and biochemical responses of four cassava cultivars to drought stress. *Sci. Rep.* **2020**, *10*, 6968. [[CrossRef](#)]
36. Kosar, F.; Akram, N.A.; Ashraf, M. Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. *S. Afr. J. Bot.* **2015**, *96*, 71–77. [[CrossRef](#)]
37. Naeem, M.S.; Jin, Z.L.; Wan, Z.L.; Liu, D.; Liu, H.B.; Yoneyama, K.; Zhou, W.J. 5-aminolevulinic acid improves photosynthetic gas exchange capacity and ion uptake under salinity stress in oilseed rape (*Brassica napus* L.). *Plant Soil.* **2010**, *332*, 405–415. [[CrossRef](#)]
38. Hotta, Y.; Tanaka, T.; Takaoka, H.; Takeuchi, Y.; Konnai, M. New physiological effects of 5-aminolevulinic acid in plants: The increase of photosynthesis, chlorophyll content, and plant growth. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 2025–2028. [[CrossRef](#)]
39. Anjum, S.A.; Li, J.H.; Lv, J.; Zong, X.F.; Wang, L.; Yang, A.J.; Yan, R.; Ali, Z.; Song, J.X.; Wang, S.G. Regulation mechanism of exogenous ALA on growth and physiology of *Leymus chinensis* (Trin.) under salt stress. *Chil. J. Agr. Res.* **2016**, *76*, 314–320. [[CrossRef](#)]
40. Ostrowska, A.; Grzesiak, M.T.; Hura, T. Exogenous application of growth stimulators improves the condition of maize exposed to soil drought. *Acta Physiol. Plant* **2021**, *43*, 63. [[CrossRef](#)]
41. Shahbaz, M.; Abid, A.; Masood, A.; Waraich, E.A. Foliar-applied trehalose modulates growth, mineral nutrition, photosynthetic ability, and oxidative defense system of rice (*Oryza sativa* L.) under saline stress. *J. Plant Nutr.* **2017**, *40*, 584–599. [[CrossRef](#)]
42. Ibrahim, H.A.; Abdellatif, Y.M. Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress. *Ann. Agric. Sci.* **2016**, *61*, 267–274. [[CrossRef](#)]
43. Winger, A.; Delatte, T.L.; O'Hara, L.E.; Primavesi, L.F.; Jhurrea, D.; Paul, M.J.; Schlupepman, H. Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. *Plant Physiol.* **2012**, *158*, 1241–1251. [[CrossRef](#)]
44. Alam, M.M.; Nahar, K.; Hasanuzzaman, M.; Fujita, M. Trehalose-induced drought stress tolerance: A comparative study among different *Brassica* species. *Plant Omics.* **2014**, *7*, 271–283.
45. Neumann, P.M. Coping mechanisms for crop plants in drought prone environments. *Ann. Bot.* **2008**, *101*, 901–907. [[CrossRef](#)]
46. Nishihara, E.; Kondo, K.; Parvez, M.M.; Takahashi, K.; Watanabe, K.; Tanaka, K. Role of 5-aminolevulinic acid (ALA) on active oxygen-scavenging system in NaCl-treated spinach (*Spinacia oleracea*). *J. Plant Physiol.* **2003**, *160*, 1085–1091. [[CrossRef](#)] [[PubMed](#)]
47. Korkmaz, A.; Korkmaz, Y.; Demirkiran, A.R. Enhancing chilling stress tolerance of pepper seedlings by exogenous application of 5-aminolevulinic acid. *Environ. Exp. Bot.* **2010**, *67*, 495–501. [[CrossRef](#)]
48. Youssef, T.; Awad, M.A. Mechanisms of enhancing photosynthetic gas exchange in date palm seedlings (*Phoenix dactylifera* L.) under salinity stress by a 5-aminolevulinic acid-based fertilizer. *J. Plant Growth Regul.* **2008**, *27*, 1. [[CrossRef](#)]
49. Nilsen, E.T.; Orcutt, D.M. *The Physiology of Plants under Stress: Abiotic Factors*; Wiley: New York, NY, USA, 1996.
50. Naeem, M.S.; Warusawitharana, H.; Liu, H.; Liu, D.; Ahmad, R.; Xu, L.; Zhou, W. 5-Aminolevulinic acid alleviates the salinity-induced changes in *Brassica napus* as revealed by the ultrastructural study of chloroplast. *Plant Physiol. Biochem.* **2012**, *57*, 84–92. [[CrossRef](#)]
51. Ali, Q.; Ashraf, M. Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: Growth, photosynthesis, water relations and oxidative defense mechanism. *J. Agron. Crop. Sci.* **2011**, *197*, 258–271. [[CrossRef](#)]
52. Pavlovic, A.; Demko, V.; Durcan, M.; Hudák, J. Feeding with aminolevulinic acid increased chlorophyll content in Norway spruce (*Picea abies*) in the dark. *Photosynthetica* **2009**, *47*, 631–634. [[CrossRef](#)]
53. Baker, N.R.; Harbinson, J.; Kramer, D.M. Determining the limitations and regulation of photosynthetic energy transduction in leaves. *Plant Cell Environ.* **2007**, *30*, 1107–1125. [[CrossRef](#)] [[PubMed](#)]
54. Li, D.M.; Zhang, J.; Sun, W.J.; Li, Q.; Dai, A.H.; Bai, J.G. 5-Aminolevulinic acid pretreatment mitigates drought stress of cucumber leaves through altering antioxidant enzyme activity. *Sci. Hort.* **2011**, *130*, 820–828. [[CrossRef](#)]
55. Liu, T.; Shi, J.; Li, M.; Ye, X.; Qi, H. Trehalose triggers hydrogen peroxide and nitric oxide to participate in melon seedlings oxidative stress tolerance under cold stress. *Environ. Exp. Bot.* **2021**, *184*, 104379. [[CrossRef](#)]