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Enhanced Seedling Growth and Physiological Performances of *Melia azedarach* L. by Foliar Application of 24-Epibrassinolide under Salt Stress

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Abstract: Salinity is a serious environmental problem following a worsening trend. This study investigates the role of 24-epibrassinolide(EBR) in regulating plant growth and physiological performances, particularly in alleviating the negative effects of salt stress. *Melia azedarach* L. seedlings from two seed sources, Sheyang (SY) and Xiashu (XS), were exposed to sea salt and treated with different concentrations of EBR within a 60-day period. The results demonstrate that appropriate EBR application improved the seedlings' stress tolerance by promoting growth and physiological systems. In terms of the relative increment, it showed that a difference of 1.45% and 1.13% in the SY and XS groups was the positive effect of the highest EBR treatment concentration. As for diameter growth, the difference observed was 2.51% and 1.80% for the SY and XS groups, respectively. In all physiological measurements, including the content of photosynthetic pigments, water relations, membrane stability, osmolytes and antioxidant enzymes, significant changes generally observed between salt stress alone and the highest EBR treatment concentration. A better performance was observed in the SY seed source, which is of a coastal nature. These findings contribute to our understanding of *Melia azedarach*'s adaptation to changing environments and provide potential for further molecular studies as well as valuable insights for forestry, agricultural and ecological research.

Keywords: *Melia azedarach*; salinity; brassinosteroid; seedling growth; physiology; stress tolerance; plant responses

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1. Introduction

Plants have to endure multiple environmental (abiotic) stresses throughout their lives because of their existence in a changing environment [1]. These abiotic stresses have a negative impact on plant growth and development by disrupting their normal cellular and molecular activities. Therefore, it is crucial to investigate plants' ability to tolerate and adapt to various constantly changing environmental conditions in the fields of agriculture, ecology and conservation research [2]. In general, mineral toxicity, heat, drought, salinity, waterlogging, cold and frost are common abiotic stresses [1]. Among them, salinity is a serious problem that follows a worsening trend and affects 20% of the world's soils [3]. In fact, according to the recent scenarios, soil salinization will intensify within the next several decades as a consequence of global climate change [4,5], remaining a challenge for the forestry and agriculture sectors.

Soil salinity first inhibits plant development through osmotic stress, followed by cellular ion unbalancing and oxidative damage caused by the generation of free radicals and other hazardous reactive oxygen species (ROS) [6–8]. After prolonged stress, plants gradually adapt with an exact mechanism as a salinity response. Salt stress responses of plants involve various physiological features, molecular networks and biochemical processes [9]. For example, when plants encounter excessive levels of ROS due to salt exposure, surpassing their defense mechanisms' capabilities, they have developed an antioxidant defense mechanism to efficiently mitigate oxidative stress damage [10]. Growth inhibition under salinity is caused by various factors, including disturbances in water and osmotic potential, as well as detrimental effects of excessive Na^+ and Cl^- ions. The excessive accumulation of these ions disrupted nutrient accumulation, enzyme activity and structure disruption, leading to damage in cell organelles and plasma membranes, as well as disruptions in photosynthesis, respiration and protein synthesis [11].

Various physiological and agronomic techniques are commonly used to lessen the impact of saline stress [12]. The presence of salinity-tolerant halophytes and the variation in salt stress responses among glycophyte genotypes suggest the existence of diverse traits for improving plants' salt tolerance [13]. However, despite these potential traits, breeding efforts focused on developing salinity-tolerant plants have limitations because of the complex nature of plants' stress tolerance mechanisms and an incomplete understanding of many underlying processes [12,13]. Consequently, the use of growth regulators has commonly been employed as an effective approach to enhance the performance of plants under stress [12]. Exogenously applying plant growth regulators is an effective way to reduce abiotic stress as hormones are essential for a plant's growth, development, and reaction to environmental stress [14]. One of them, the application of brassinosteroids (BRs), has been found to have beneficial effects in supporting growth and alleviating the negative effects of salt stress [15]. It has been investigated in various plants [16], including mustard, eggplant, pepper, maize, black locust, common bean as well as snap bean [17], soybean [18], peanut [19], strawberry [20], apple [21], *Atractylodes Macrocephala* Koidz. [22], cucumber [12] and other species. While crop research has received significant attention, studies on the salt tolerance of forest trees have lagged behind, despite trees serving a crucial role in enhancing the ecological environment [23].

Melia azedarach L. is an important tree species to conduct research on salt tolerance due to its wide distribution and ability to survive in saline soils. *Melia azedarach* is a fast-growing, moderately salt-tolerant deciduous broad-leaved tree species [23–25]. It is native to tropical Asia and has been introduced to many countries. In China, it is most abundant in the south and southwest, with a relative abundance in the east and central areas [26]. It is also widely planted in subtropical and southern temperate areas in China [25].

As a significant group of plant hormones, brassinosteroids (BRs) are well recognized for their involvement in stress mitigation and growth promotion [10]. They play an important function in regulating developmental and physiological processes and in enhancing the defense system of plants by raising the activities and levels of antioxidants in response to different abiotic stresses [15]. It is widely accepted that exogenous growth regulators, such as BRs, have the capacity to successfully ameliorate salt stress by enhancing the important parameters related to stomatal conductance, membrane permeability, osmotic control, ion homeostasis and water content [20,22]. The stress-alleviating effects of BRs depend on their concentration, which can vary based on the plant species, developmental stages and environmental conditions [27]. According to various studies, BRs and the signaling pathways of other hormones are often interconnected, facilitating the regulation of numerous physiological and developmental processes [10]. The role of BRs in modulating salt tolerance of *Melia azedarach* has remained unknown until this study. With the stress-regulating function of BRs in mind, our study aims to provide compelling evidence for the prospective management of saline lands associated with this particular tree species by formulating hypotheses related to the enhancement of salt tolerance mechanisms through the application of EBR and the potential for a greater capacity of tolerant performance in seedlings originating from coastal seed sources.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The seeds were collected in December 2021 from two different seed sources: the "Sheyang" (SY) and "Xiashu" (XS) areas of Jiangsu Province. These sources represent a coastal region and an inner region, respectively. The seeds were sown in March 2022 in a greenhouse nursery of Sheyang Tourism Investment Development Co., Ltd., Yancheng, China.

The experiment was carried out in July 2023. A total of 360 uniformly growing seedlings (6 treatments × 2 seed sources × 10 seedlings × 3 replicates) were used in a randomized block design. The six treatments the seedlings were subjected to are (i) the control (CK); (ii) 4‰ sea salt (SA); (iii) 4‰ sea salt + EBR 1 mg/L (SE1); (iv) 4‰ sea salt + EBR 0.5 mg/L (SE2); (v) 4‰ sea salt + EBR 0.1 mg/L (SE3) and (vi) 4‰ sea salt + EBR 0.05 mg/L (SE4). The treatment with 4‰ salt was prepared by dissolving natural sun-dried sea salts (NaCl content > 95%) in deionized water. The salts were obtained from Laizhou salt farm, Shandong Province. The salt application process was performed gradually over 3 days, with daily increments until a final 4‰ concentration (8 g salt/2 kg of air-dried substrate containing a mixture of organic matter, perlite and vermiculite) was reached. In the case of the control seedlings, the same amount of deionized water was applied instead of the salt solution. The EBR spraying treatment was started 15 days after salt treatment, with the respective concentration divided into 3 days, and the solution was evenly sprayed on both the front and back of the leaves. The experiment took place in a controlled greenhouse environment at a temperature of 25–30 °C. The samples for physiological determinations were collected at four different sample days, day 7, day 21, day 35 and day 60, after EBR spraying (DAS) and immediately frozen in liquid nitrogen and kept at −80 °C until their use.

2.2. Measurement of Growth Traits

The seedlings' height and ground diameter were measured at 0 DAS and 60 DAS in order to determine their growth increment. The rate of change for the samples was determined using the following formula: increment rate (%) = [(60 DAS − 0 DAS)/(60 DAS)] × 100. After the experiment, the seedlings were taken out of the pot and rinsed with water. The stems and roots of the seedlings were separated into above-ground and below-ground parts to measure their biomass. Fresh weight measurements were recorded right after harvesting.

2.3. Determination of Leaf Relative Water Content

The leaf relative water content (RWC) was measured using the method of Marriboina et al. [28]. The fresh leaves were weighed and steeped in distilled water until saturated. The leaves were then dried in an oven at 105 °C for 30 min, followed by further drying at 80 °C until a steady weight was reached, and their dry weight was recorded. Using the values obtained, the RWC of the leaves was computed as follows: RWC % = [(FW − DW)/(TW − DW)] × 100.

2.4. Determination of Relative Electrical Conductivity

The electrical conductivity was measured using a DJS-1D conductivity meter (Shanghai Lei Ci Co., Ltd., Shanghai, China). 100 mg of cleaned leaves were weighed and placed in a test tube with 20 mL of deionized water. The conductivity R1 was measured after shaking and allowing it to settle for 2 h. After 20 min of boiling in a water bath, the test tube was cooled, and the conductivity R2 was determined. The relative value was calculated using the following equation [29]: EC % = (R1/R2) × 100.

2.5. Determination of Chlorophyll and Lipid Peroxidation in Leaves

The chlorophyll content in leaves was quantified according to the technique of Xu et al. [30]. Lipid peroxidation was assessed by detecting the quantity of malondialdehyde (MDA) generated through the reaction with thiobarbituric acid [31].

2.6. Determination of Osmolytes

The proline content was determined by following the sulfosalicylic acid procedure described by Bates et al. [32]. The Coomassie brilliant blue G-250 technique was used to determine the soluble protein content [33]. The anthrone technique was used to determine the soluble sugar content [33].

2.7. Determination of Antioxidant Enzymes Activity

The nitro blue tetrazolium (NBT) technique was used to determine superoxide dismutase (SOD) [34]. The guaiacol technique was used to determine the peroxidase (POD) activity, as described by Gao [35].

2.8. Statistical Analyses

The data were examined using two-way analysis of variance (ANOVA), with the two primary factors being the various treatments and the seed sources. When performing ANOVA analysis, the Shapiro–Wilk normality test was utilized to assess the normality within the groups, which is an important assumption for ANOVA. Tukey's HSD (honestly significant difference) test was used to compare the means of variables. Correlation and principal component analyses were also performed for specific follow-up interpretations. R software version.4.2.1 [36] was used for all statistical analyses and generating the figures.

The analyzed physiological data in this study represent the mean of four sample days (day 7, 21, 35 and 60), with each day measured three times for replication, resulting in a total of 12 measurements (4 sample days $\times$ 3 replications) ($n = 12$). By incorporating data from multiple time points, the potential influence of random fluctuations that might arise on a specific day was mitigated. It also aims to facilitate the generation of comprehensive conclusions regarding the actual conditions observed throughout the entire duration of the experiment.

3. Results

3.1. Effects of Salt and EBR Treatments on Growth Traits

Melia azedarach seedlings of two different seed sources were treated with six different treatments. The seedlings' height and ground diameter were measured once at 0 DAS. The second measurement was performed at 60 DAS in order to determine their increment rates. The growth rate of the seedlings' height declined in both the SY and XS groups under salt treatment (SA) when compared to the control (CK) (Table 1).

A significant difference was observed in the SY group, while the XS group showed no significant difference. However, the results generally showed that all the different EBR treatment concentrations could increase the growth rate of the seedlings' height compared to SA seedlings. In particular, variations in the average height growth of the seedlings were observed when comparing various of EBR treatment concentrations. As the concentration of EBR decreased, the height of the seedlings correspondingly declined in both the SY and XS groups. However, no statistically significant effects were detected. At 60 DAS, the relative increment was found to be highest in CK, and lowest was found in SA seedlings in both the SY and XS groups. Specifically, in the SY group, the relative increment was 5.05% in CK and 2.90% in SA. Similarly, in the XS group, the relative increment was 4.72% in CK and 3.02% in SA, respectively. As the positive effect of the highest EBR treatment concentration (SE1), the relative increment showed a difference of 1.45% and 1.13% in the SY and XS groups when compared with SA.

Table 1. Seedling height growth under salt and EBR treatments.

Seed Source	Treatment	Height Growth (cm)	Relative Increment (%)
SY	CK	9.23 ± 2.03 a	5.05
	SA	4.97 ± 0.35 b	2.90
	SE1	7.53 ± 1.37 ab	4.35
	SE2	5.97 ± 1.21 ab	3.38
	SE3	5.13 ± 1.42 b	3.07
	SE4	4.73 ± 1.62 b	2.93
XS	CK	8.47 ± 2.41 ab	4.72
	SA	5.17 ± 0.84 b	3.02
	SE1	6.97 ± 1.33 ab	4.15
	SE2	6.03 ± 1.14 ab	3.77
	SE3	5.93 ± 0.55 ab	3.62
	SE4	5.33 ± 0.6 ab	3.18

Note: The data represent mean ± sd of 3 replications. Different letters indicate significant differences at the 0.05 level. SY, "Sheyang"; XS, "Xiashu"; CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L.

In terms of the growth of the ground diameter, significant differences were detected between CK and SA treatments in both the SY and XS groups, as shown in Table 2. At 60 DAS, the average diameter growth of control seedlings in the SY group was 1.77 mm, representing a relative growth of 9.73%. In the XS group, the average growth in CK was 1.43 mm, with a relative growth of 9.62%. On the other hand, the relative diameter growth reduced to 6.11% and 4.76% under SA treatment in the SY and XS groups, respectively. Although the diameter growth rate did not exhibit significant differences across various concentrations of EBR treatments, a general trend was observed. The average values tended to decrease from higher to lower concentrations of EBR applied, except SE3 in XS seedlings. As a positive effect of SE1 treatment, the relative increment showed a difference of 2.51% and 1.80% in the SY and XS groups when compared with SA.

Table 2. Ground diameter growth under salt and EBR treatments.

Seed Source	Treatment	Diameter Growth (mm)	Relative Increment (%)
SY	CK	1.77 ± 0.31 a	9.73
	SA	0.93 ± 0.06 bc	6.11
	SE1	1.23 ± 0.12 abc	8.62
	SE2	1.13 ± 0.15 abc	7.55
	SE3	1.03 ± 0.12 bc	7.02
	SE4	0.83 ± 0.15 bc	5.50
XS	CK	1.43 ± 0.15 ab	9.62
	SA	0.73 ± 0.15 c	4.76
	SE1	1.17 ± 0.25 abc	6.56
	SE2	0.97 ±0.31 bc	6.46
	SE3	1.07 ± 0.35 bc	7.27
	SE4	0.70 ± 0.26 c	4.96

Note: The data represent mean ± sd of 3 replications. Different letters indicate significant differences at the 0.05 level. SY, "Sheyang"; XS, "Xiashu"; CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L.

In the assessment of biomass (stem and root), no statistically significant effects were observed among the different treatments, as indicated in Table 3. However, the SA treatments exhibited lower weights compared to CK in both the SY and XS groups.

Table 3. Biomass accumulation under salt and EBR treatments.

Seed Source	Treatment	Biomass (g FW)
SY	CK	231.62 ± 39.16 a
	SA	188.36 ± 19.45 a
	SE1	214.70 ± 46.61 a
	SE2	211.66 ± 54.15 a
	SE3	215.89 ± 14.11 a
	SE4	215.00 ± 31.23 a
XS	CK	289.53 ± 68.27 a
	SA	248.38 ± 26.77 a
	SE1	271.96 ± 90.05 a
	SE2	285.06 ± 60.07 a
	SE3	253.51 ± 55.23 a
	SE4	236.58 ± 52.33 a

Note: The data represent mean ± sd of 3 replications. The lowercase letter "a" indicates no significant differences at the 0.05 level. SY, "Sheyang"; XS, "Xiashu"; CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L.

3.2. Effects of Salt and EBR Treatments on Chlorophyll Content

Chlorophyll levels can predict plant health and photosynthesis conditions. Salt stress had a profound impact on the chlorophyll content, leading to a significant reduction ($p < 0.05$), as shown in Figure 1. However, upon the application of exogenous EBR, the chlorophyll content increased significantly, approaching levels that were close to the normal condition. Notably, the measured chlorophyll content was higher in the SY group, while it was lower in the XS group, with a significant difference observed between the two seed sources across all treatments.

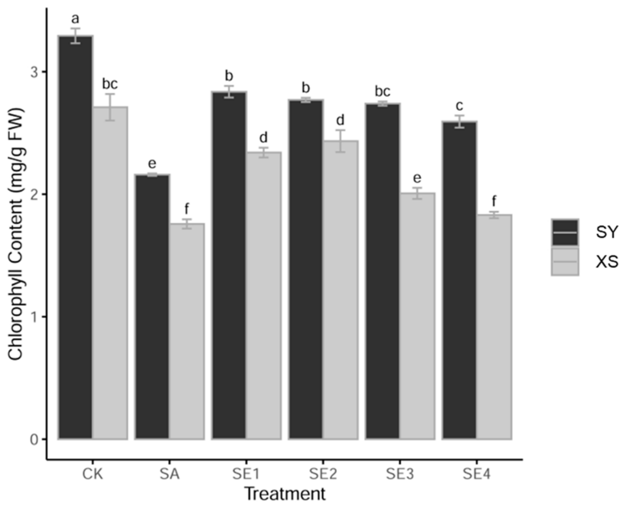


Figure 1. Effect of salt and EBR treatments on chlorophyll content of *M. azedarach* seedlings. The data represent mean ± sd (n = 12). Different letters indicate significant differences at the 0.05 level. CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L; SY, "Sheyang"; XS, "Xiashu".

3.3. Effects of Salt and EBR Treatments on Relative Water Content

In both the SY and XS groups, the relative water content of the leaves decreased significantly after salt treatment, as depicted in Figure 2. However, the average water content showed a tendency to increase in plants treated with EBR. A significant increase was observed in SE1, where the highest concentration of EBR (1 mg/L) was applied, while lower concentrations did not have a significant effect. When comparing the SY and XS groups, no statistically significant difference in relative water content was observed across all treatments.

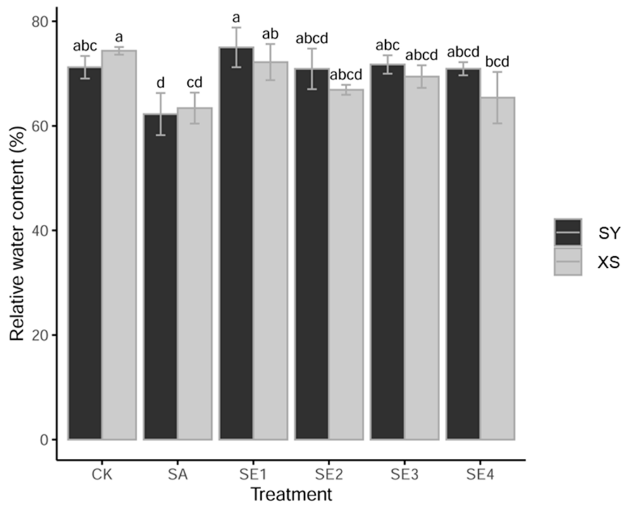


Figure 2. Effect of salt and EBR treatments on relative water content of *M. azedarach* seedlings. The data represent mean ± sd (n = 12). Different letters indicate significant differences at the 0.05 level. CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L; SY, "Sheyang"; XS, "Xiashu".

3.4. Effects of Salt and EBR Treatments on Relative Electrical Conductivity

When the salt stress treatment was applied to *Melia azedarach* seedlings in both the SY and XS seed sources, the results indicated a notable increase in relative electrical conductivity (Figure 3). A statistical analysis revealed significant differences between the control (CK) and salt stress treatment (SA) groups, as well as between CK and various EBR treatment concentrations (SE1, SE2, SE3 and SE4). Furthermore, significant differences were observed between the SA and EBR treatment groups (SE1, SE2, SE3 and SE4) within the seed source comparison. Generally, electrical conductivity decreased in EBR-treated seedlings when compared to SA seedlings.

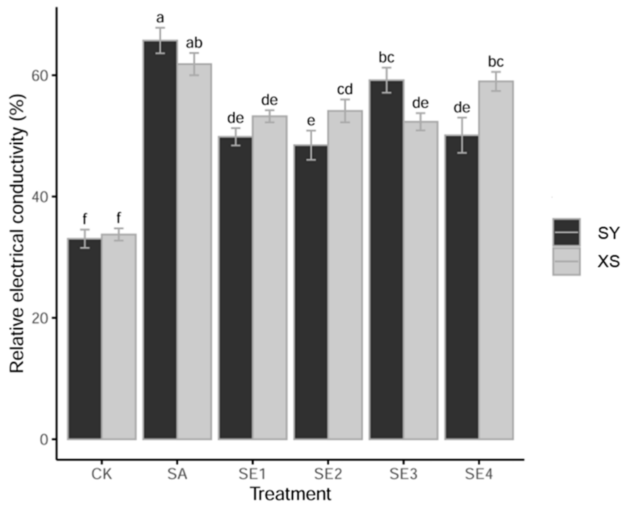


Figure 3. Effect of salt and EBR treatments on relative electrical conductivity of *M. azedarach* seedlings. The data represent mean ± sd (n = 12). Different letters indicate significant differences at the 0.05 level. CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L; SY, "Sheyang"; XS, "Xiashu".

3.5. Effects of Salt and EBR Treatments on Malondialdehyde (MDA) Content

In the presence of salt stress, the MDA concentration was considerably greater than in the control group in both SY and XS seed sources, as shown in Figure 4. In contrast, when EBR was applied under salt stress conditions, the average MDA content decreased. The salt stress treatment (SA) group and the EBR treatment groups (SE1, SE2, SE3 and SE4) of each seed source showed statistically significant differences. These findings indicate that EBR treatments have a significant effect on MDA content in both the SY and XS seedlings. When comparing the two seed sources, the XS seedlings generally exhibit a lower trend than SY seedlings, with significant differences in SE2, SE3 and SE4, while they have almost the same MDA content under control conditions.

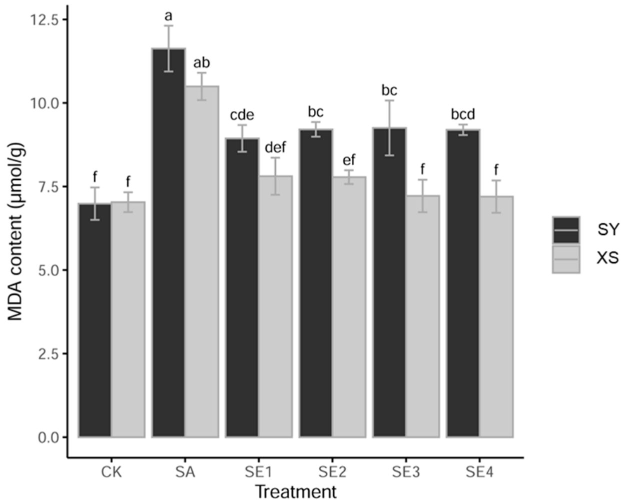


Figure 4. Effect of salt and EBR treatments on MDA content of *M. azedarach* seedlings. The data represent mean ± sd (n = 12). Different letters indicate significant differences at the 0.05 level. CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L; SY, "Sheyang"; XS, "Xiashu".

3.6. Effects of Salt and EBR Treatments on Osmolyte Content

The three important osmolytes—proline, soluble protein and soluble sugar—were measured in this study. The seedlings treated with salt had a considerably greater proline content than the control group (Figure 5a). Additionally, there was a significant difference in the proline content between the SA and SE1 treatments in the SY group, while no significant differences were observed among the remaining EBR treatments (SE2, SE3 and SE4). This suggests that only specific applications of EBR may enhance proline content under salinity; however, XS showed no significant difference between the SA and EBR treatments (SE1, SE2, SE3 and SE4).

The protein content is also a good predictor of osmotic changes in plants in response to environmental stress. The results show that the soluble protein levels in the SA seedlings of each source are much higher than those in the CK group (Figure 5b). Moreover, the application of EBR was found to enhance the levels of soluble proteins in salt-stressed *Melia azedarach* seedlings. When comparing the two seed sources, the SY group exhibited a significantly higher soluble protein content across all treatments compared to the XS group.

The soluble sugar content was notably higher in SA seedlings compared to CK (Figure 5c). This difference was statistically significant in the XS group, but not in the SY group. Interestingly, XS seedlings showed a significantly higher level of soluble sugar content across all treatments than SY seedlings. When comparing the effect of EBR on salt treatment, particularly in SE1 and SE2, there was a significant difference observed in both SY and XS seedlings.

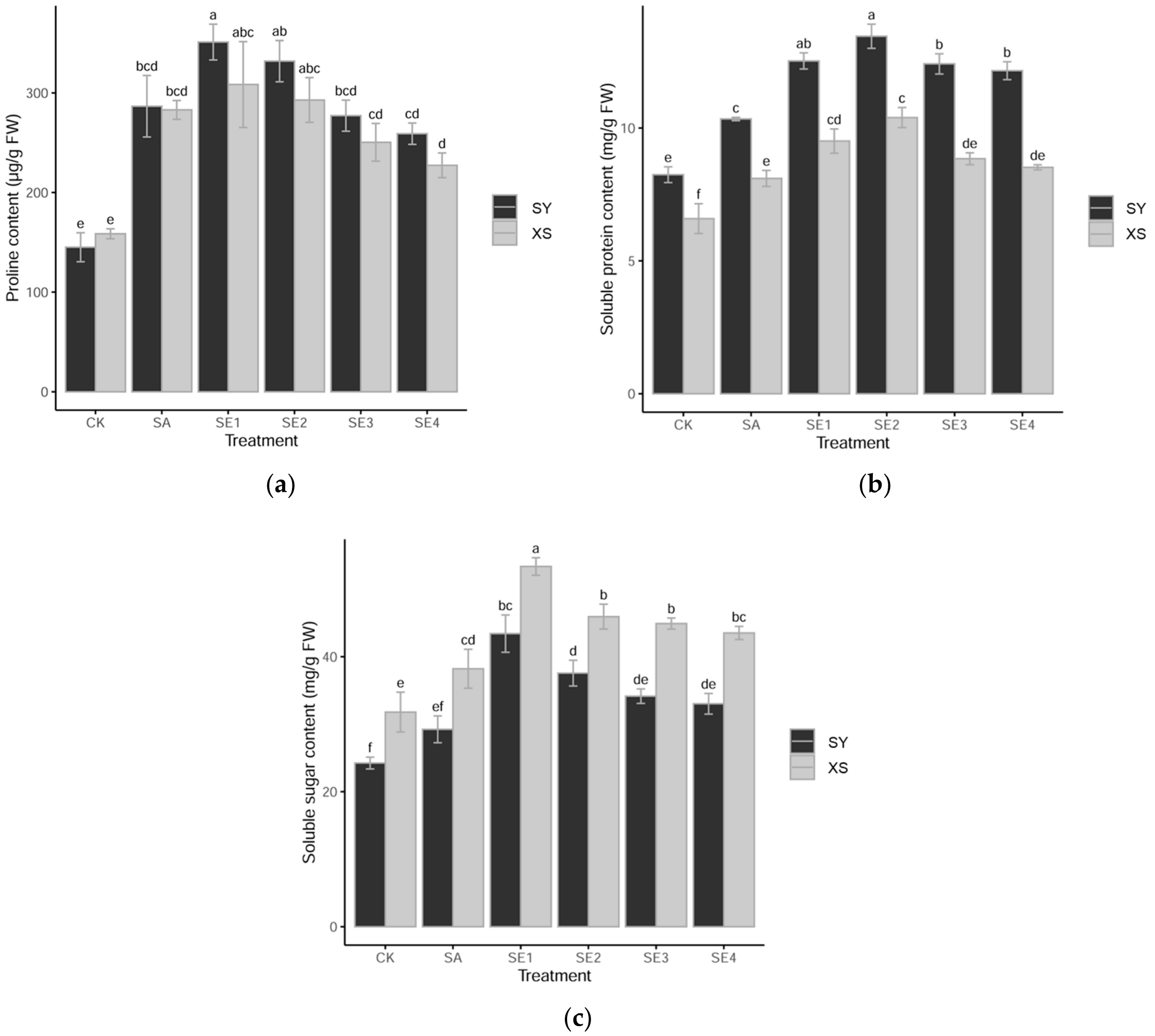
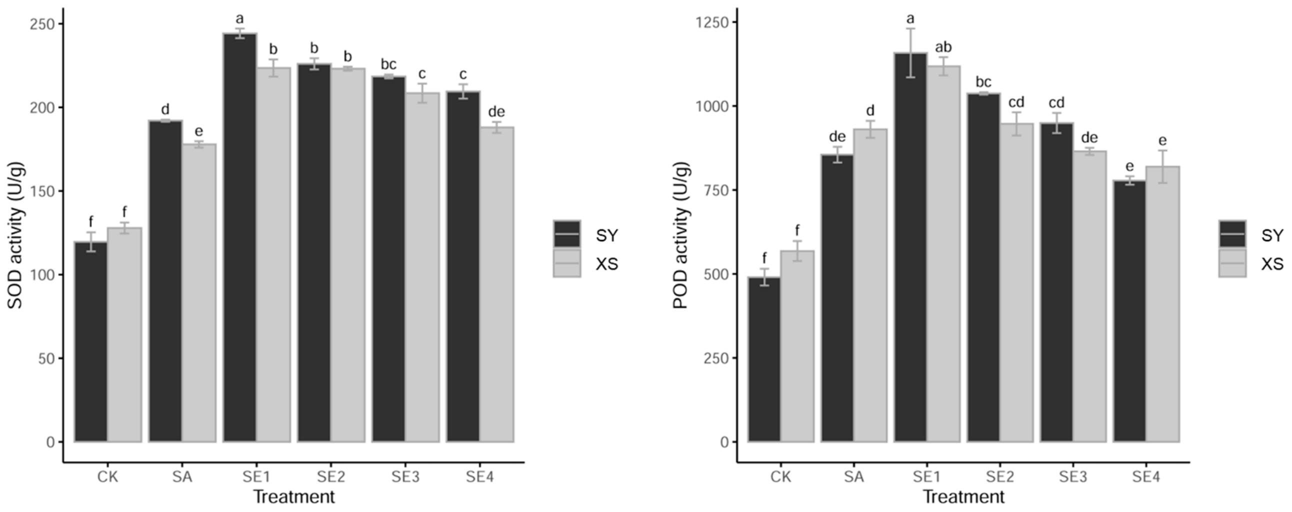


Figure 5. Effect of salt and EBR treatments on osmolyte content of *M. azedarach* seedlings. (**a**) Proline; (**b**) soluble protein; and (**c**) soluble sugar. The data represent mean ± sd (n = 12). Different letters indicate significant differences at the 0.05 level. CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L; SY, "Sheyang"; XS, "Xiashu".

3.7. Effects of Salt and EBR Treatments on Superoxide Dismutase and Peroxidase Activity

The results of the superoxide dismutase (SOD) and peroxidase (POD) activity analysis revealed a significant increase in SA seedlings in comparison to the CK group. The SE1 treatment exhibited the highest peak, which was subsequently followed by SE2, SE3 and SE4 (Figure 6). This pattern of activity was consistent in both seed sources. There were significant differences in SOD and POD activity not only between the CK and SA groups but also between CK and SE1, as well as between the SA and SE1 treatments. These findings suggest that the use of EBR at a dosage of 1 mg/L greatly affects the activity of antioxidant enzymes in *Melia azedarach* seedlings, regardless of the presence of salinity.



(**a**) (**b**)

Figure 6. Effect of salt and EBR treatments on antioxidant enzymes activity of *M. azedarach* seedlings. (**a**) SOD activity; (**b**) POD activity. The data represent mean ± sd (n = 12). Different letters indicate significant differences at the 0.05 level. CK, control; SA, 4‰ salt; SE1, 4‰ salt + EBR 1 mg/L; SE2, 4‰ salt + EBR 0.5 mg/L; SE3, 4‰ salt + EBR 0.1 mg/L; SE4, 4‰ salt + EBR 0.05 mg/L; SY, "Sheyang"; XS, "Xiashu".

3.8. Correlation Analysis

The individual results indicated that salt stress and various EBR concentrations had significant impacts on various morpho-physiological indicators of both SY and XS seedlings. Additionally, these relationships were found to change with varying treatments as well as different seed sources. Generally, these findings enhance our understanding of the interplay between different indicators and treatments.

Thereafter, to gain a more thorough knowledge of the connection between the observed characteristics in *Melia azedarach*, a Pearson's correlation analysis was performed on a set of 12 indicators. The results of the correlation analysis, as shown in Table 4, revealed that the seedlings' height and diameter growth were significantly positively correlated with the chlorophyll content and RWC of leaves. Conversely, those growth indicators were negatively correlated with electrical conductivity, MDA, proline and antioxidant enzymes (SOD and POD). Biomass accumulation was negatively correlated with MDA and soluble protein. Chlorophyll, MDA and relative water content were positively correlated. The result also indicated that a highly positive significant correlation between antioxidant enzymes (SOD and POD), osmolytes (proline, soluble protein and soluble sugar) and electrical conductivity. In turn, indicators associated with membrane stability, namely MDA and electrical conductivity, exhibited significant negative correlations with relative water content, height and diameter growth. These findings provide valuable insights into the complex nature of the important factors influencing the overall performance of *Melia azedarach* seedlings.

3.9. Principal Component Analysis

Overall, according to the interpretation of the individual statistical results, SY seedlings generally performed better under treatments compared to XS seedlings. To support this observation, we utilized the variables under the control and salt-stressed conditions to conduct a principal component analysis (PCA). The first and second components, which explained 74.0% and 15.9% of the total variance, respectively, were plotted as a biplot (Figure 7).

Table 4. Correlation of the measured experimental indicators in *Melia azedarach* seedlings.

	Hgt	Dia	BM	Chl	RWC	EC	MDA	Prol	SProt	SSuga	POD
Hgt											
Dia	0.67 ***										
BM	0.27	0.14									
Chl	0.49 **	0.67 ***	−0.07								
RWC	0.33 *	0.43 **	0.08	0.60 ***							
EC	−0.71 ***	−0.71 ***	−0.17	−0.71 ***	−0.59 ***						
MDA	−0.53 ***	−0.41 *	−0.41 *	−0.22	−0.41 *	0.62 ***					
Prol	−0.49 **	−0.42 **	−0.31	−0.19	−0.03	0.57 ***	0.52 ***				
Sprot	−0.36 *	−0.17	−0.35 *	0.31	0.18	0.27	0.40 *	0.68 ***			
SSuga	−0.19	−0.30	0.21	−0.45 **	0.04	0.32	−0.24	0.53 ***	0.06		
POD	−0.41 *	−0.35 *	−0.11	−0.25	−0.00	0.57 ***	0.35 *	0.92 ***	0.59 ***	0.69 ***	
SOD	−0.47 **	−0.43 **	−0.12	−0.18	0.03	0.56 ***	0.29	0.87 ***	0.75 ***	0.63 ***	0.90 ***

Note: Hgt, height; Dia, diameter; BM, biomass; Chl, chlorophyll; RWC, relative water content; EC, electrical conductivity; MDA, malondialdehyde; Prol, proline; Sprot, soluble protein; SSuga, soluble sugar; POD, peroxidase; SOD, superoxide dismutase. The numbers represent correlation coefficient values, where negative numbers indicate the negative correlation, 0 indicates no correlation, and positive numbers indicate the positive correlation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

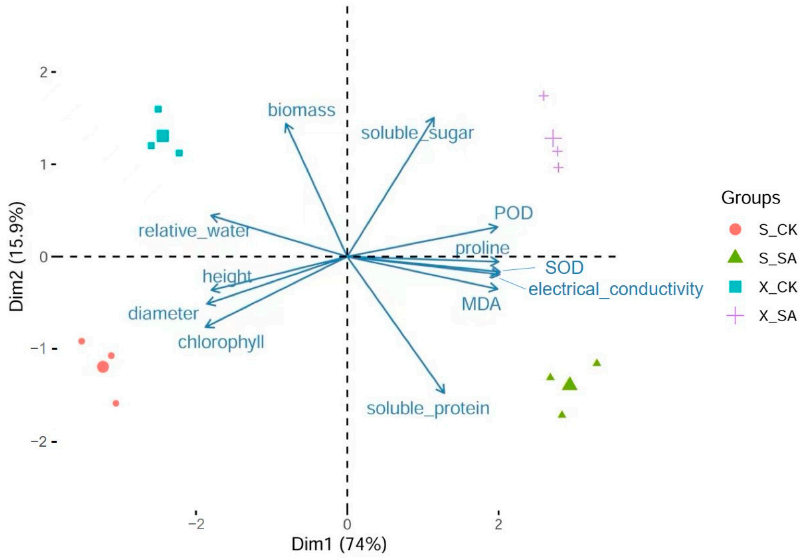


Figure 7. Principal component analysis of control and salt-stressed groups among the variables measured. S_CK, control seedlings of Sheyang; S_SA, salt-stressed seedlings of Sheyang; X_CK, control seedlings of Xiashu; X_SA, salt-stressed seedlings of Xiashu.

The biplot indicated that salt-treated seedlings were positioned on the right side of the plot and demonstrated a positive correlation with the first component. On the other hand, control seedlings were positioned on the left side of the plot and exhibited a negative correlation with the first component. This indicates that the groups displaying a stronger positive correlation with the first component were affected by salt stress. The first component demonstrated a strong positive correlation with soluble protein, electrical conductivity, MDA, SOD, POD and soluble sugar contents. Conversely, it exhibited a negative correlation with chlorophyll content, ground diameter, height, relative water content and biomass. Therefore, this biplot analysis indicated that the salt-treated seedlings that struggled under the imposed stress are positively correlated with the first component. Additionally, the degree of negative correlation with variables related to the second component, such as chlorophyll content, ground diameter, height and relative water content, indicate the level of salt tolerance performance. Accordingly, SY seedlings outperformed XS seedlings in this aspect, as seen in the biplot, which is consistent with the majority of our study's individual results.

4. Discussion

4.1. Growth Performance under Salt and EBR Treatments

Salt stress has a negative impact on plant development and overall performance [37]. Our results could highlight the negative effects of salt on seedlings' growth in terms of the reduced growth rate of height (Table 1), ground diameter (Table 2) and biomass (Table 3). These results are consistent with previous studies. For example, salt stress reduced shoot length in a wheat variety known as Kharchia Local [38]; vegetative growth in *Physalis peruviana* L. [39]; and stem diameter, shoot length and number in olive plants due to osmotic stress induced by salinity [40]. Salinity-induced osmotic stress triggers a series of reactions induced by hormones, resulting in reduced stomatal opening, CO_2 assimilation and photosynthetic rate. The diversion of energy from growth to maintain salinity balance and decrease carbon gains contributes to the decline in growth [41].

On the other hand, the application of EBR, especially at higher concentrations, had a positive impact, resulting in a relative growth increment in the indicators measured in the present study (Tables 1–3). The application a plant growth regulator is one of the most efficient techniques for alleviating abiotic stress because hormones play significant roles in plant growth and development under environmental stress [14]. In the study of Dong et al. [42], EBR application at a low concentration significantly increased the plant-growth-related parameters of wheat seedlings under 120 mM NaCl treatment, and 10 nM was found as the most effective dosage of EBR under saline conditions. Zhang et al. [22] also reported that EBR application improved germination and development in *Atractylodes macrocephala* Koidz. According to Munsif et al. [43], BRs minimized the impact of salt on plant development by significantly conserving plant pigment levels, increasing the activity of essential enzymes and up-regulating the genes which facilitates the cell elongation of kenaf plants. The salt-induced changes include nutrient uptake changes, the accumulation of excess ROS, the inhibition of cytoplasmic enzymes, the loss of turgor and hormonal imbalance. These factors collectively contribute to a reduction in cell division and elongation, resulting in reduced plant development under saline conditions [44,45]. However, BR application leads to enhanced growth parameters primarily due to the activation of cell division and cellular enlargement, facilitated by the involvement of BR-regulated genes. These genes play a role in cell wall modification, cytoskeleton development and hormone synthesis [15].

We observed that there was a reduced rate of biomass in salt-stressed plants compared to the control and EBR-treated plants, but the difference was not significant in our study. Plant biomass is not a highly sensitive parameter that reflects the long-term consequences of unfavorable environmental conditions [46]. Alternatively, Ventura et al. [47] reported that some genotypes of *Crithmum maritimum* L. showed reduced biomass production under NaCl concentrations of 50 mM and 100 mM, while its tolerant genotype increased biomass significantly in response to the 50 mM NaCl treatment. We can conclude that the impact of different treatments varies depending on species-specific, genotype-specific factors and the duration and intensity of the treatment.

4.2. Leaf Chlorophyll and Photosynthesis Status under Salt and EBR Treatments

In saline environments, plants employ various physiological mechanisms to overcome the negative effects of salt stress. This study revealed that *Melia azedarach* experienced reductions in chlorophyll content when exposed to salt. The toxic accumulation of Na^+ and Cl^- in the plants' leaves reduced chlorophyll content, ultimately limiting photo-assimilate production [48], as a result of the interaction of salt ions and pigment–protein complexes [41]. The chlorophyll decrease might be caused by increased activity of chlorophyll-degrading enzymes such as chlorophyllase [41,48]. Salt stress is believed to hinder chlorophyll synthesis or accelerate its breakdown [45].

However, the application of exogenous EBR treatment was able to effectively restore the chlorophyll content depending on the EBR concentration. The finding aligns with previous research conducted on apple [21], snap bean [17] and soybean [18]. As a result, our study supports the notion that the application of foliar BR spraying has the potential to enhance chlorophyll content and promote increased photosynthetic capacity [19]. Studies have pointed out that EBR-induced transcription and/or translation may involve the activation of certain genes responsible for synthesizing enzymes that play a role in chlorophyll synthesis [15,45]. The stability of chlorophyll content plays an important role in maintaining normal photosynthesis, improving plant resistance under stress conditions, and preventing the adverse impacts of salinity on chlorophyll biosynthesis and degradation [49].

4.3. Seedling Leaves' Water Status under Salt and EBR Treatments

Leaf relative water content (RWC) is indicative of a stable osmotic balance [50]. Under saline conditions, low cell turgidity or the limited ability to transport water from the roots to the shoots result in a relatively low RWC in leaves [18,41,51]. Generally, the relative water content decreases under salinity stress [50,52]. Our study findings align with those of the existing literature, indicating a significant decrease in RWC due to salt treatment. On the other hand, our study also indicated that a higher concentration of EBR results in a significant increase in the water status of plants under saline conditions which is similar with the results of earlier studies. According to Otie et al. [18], the application of BR spraying increased the RWC in soybean leaves, while Karlidag et al. [20] reported the similar result in strawberry, indicating its distinctive capability to counteract any water uptake deficiency by enhancing membrane stability and improving the physiological mechanisms of plants to withstand salinity-induced stress [18].

4.4. Plant Cell Membrance Stability under Salt and EBR Treatments

Plant cell membranes are the sites of sensing and the initiation of fast responses to changing abiotic factors, including saline condition [13]. Changes in relative electrical conductivity indicate the level of cell membrane damage under osmotic stress [53]. Gu et al. [53] mentioned that it has been observed that the relative electrical conductivity of various plant leaves tends to increase with higher salt concentrations. These findings are further supported by the research of Ghoname et al. [17] and by the present study on *M. azedarach*. On the other hand, our study revealed that the application of EBR through spraying reduced the average relative electrical conductivity, indicating a potential mitigation of cell membrane damage induced by salt stress. In other words, EBR potentially enhances membrane integrity by boosting the antioxidant system and protecting the membrane against free radical attacks [54].

The degree of lipid peroxidation was also assessed to evaluate salt-induced oxidative stress by quantifying malondialdehyde (MDA), which is a byproduct of lipid peroxidation [55,56]. Generally, membrane stability weakened when the MDA content increased [57]. The higher the content of MDA while under stress, the higher the degree of cell membrane damage [44]. Salinity increased the MDA content in wheat [57], *Gypsophila oblanceolate* Bark. [56], maize [10], cucumber [12] and peanut [19], which is consistent with the present study's results. Kumar et al. [44] pointed out that MDA accumulation in salt-stressed plants is due to the activation of Rubisco and PSII core proteins. This is based on the finding of a negative correlation between MDA content and electron transport, confirming a feedback mechanism between PSII and reduced MDA levels in plants [44]. However, a significant reduction in MDA content of salt-stressed *Melia azedarach* seedlings was observed after the application of exogenous EBR spraying. It has been reported that BRs have the capacity to modify the membrane structure and stability under stressful conditions [42].

4.5. Response of Osmolytes under Salt and EBR Treatments

Osmolyte accumulation under salt stress serves to maintain cell turgor pressure, protect cellular components from ionic toxicity, scavenge reactive oxygen species, preserve antioxidative enzymes and activate defense-related genes, emphasizing their critical role in plant defense mechanisms [58]. Most plants can produce and accumulate organic osmotic substances, such as proline, soluble sugar and soluble protein, in order to cope with osmotic stress caused by high salinity [22].

Proline content increments serve as a primary defense response in salt-stressed plants, ensuring the maintenance of optimal osmotic pressure within the cells [39,59]. It also plays a role as an antioxidant to scavenge ROS, safeguarding cells against damage [58,60,61]. Additionally, proline serves to stabilize membranes by maintaining the integrity of proteins and membrane structures [61]. In the present research, proline was higher in salt-treated seedlings of both seed sources compared to the control. Previous studies which were conducted on *Ailanthus altissima* Mill. [62], *Physalis Peruviana* L. [39], tomato [63], *Kochia prostrata* L. [64], *Glaux maritima* L. [53] and *Oenanthe javanica* (Blume) DC. [44] also reported that the proline content increased with increasing NaCl concentrations. Again, our study suggests that specific applications of EBR may significantly enhance proline content under saline conditions. It is also in line with the previous report that EBR can relieve the adverse effects of various stresses by enhancing photosynthesis through the upregulation of protein and proline levels [42].

The increase in protein in the cytosol and other organelles contributes to the osmotic adjustment of plants [42]. One common observation in salinity stress is the reduction in protein content. This decrease can be attributed to the reaction between amino acids in proteins and active radicals, leading to their degradation [65]. For example, in the study of pistachio seedlings [66] and cowpea [65], it is revealed that the higher concentrations of NaCl caused a higher significant decrease in protein concentration. On the other hand, El-Mashad et al. [65] observed that a low concentration of NaCl induced an opposite pattern of change in cowpea plants. This is consistent with our study which showed an average increment pattern in salt stressed seedlings. This can be regarded as *M. azedarach* seedlings possessing relatively high salinity tolerance capabilities. The present findings are also in line with the earlier research of Kumar et al. [44] conducted on water dropwort, suggesting the potential role of protein accumulation in enhancing tolerance against salt stress. Additionally, it was observed that EBR increased the content of soluble protein in *Melia azedarach* seedlings from two seed sources; therefore, it has exhibited compatibility in previous studies that demonstrated the ability of BRs to partially alleviate the inhibitory effects of salt stress on the total protein content [65,67].

Higher soluble sugar content in plants reduces the likelihood of cell water loss, enhancing plant survival and improving stress tolerance [68]. These sugars play an important role in acting as osmolytes and scavenging ROS to mitigate salt stress [58]. They can contribute up to 50% of the overall osmotic potential by accumulating compatible solutes [52] and are also essential for the preservation of chlorophyll pigments and the maintenance of optimal photosynthetic capabilities [69]. The concentration of leaf soluble sugars was increased by increasing the salinity level [64,66,70]. The increased enzymatic activities that interact with macromolecules are likely responsible for the accumulation of soluble sugars, as these enzymes play a role in regulating cellular structures and functions [44]. Zhang et al. [71] also mentioned that sugars such as sucrose, glucose, trehalose and fructose accumulated, serving important functions including osmotic protection, carbon storage and scavenging ROS under salt stress. Our study is consistent with the current literature. According to Wei et al. [72], plants counteract the detrimental effects of salt stress by accumulating soluble sugars as a means of resistance to salt damage. A strong association between sugar accumulation and tolerance to osmotic stress has been extensively documented [70]. Additionally, our study could point out that the appropriate EBR usage can increase soluble sugar content, indicating the advantage of EBR in osmotic protection and ROS scavenging abilities. In the study of Li et al. [19], the same result is reported while mentioning EBR's effect on the salt tolerance of peanut plants.

4.6. Response of Antioxidant Enzymes under Salt and EBR Treatments

Plants have the ability to remove excess ROS by producing antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) [51]. SOD is believed to be the primary defense enzyme that transforms superoxides into H_2O_2. Thereafter, POD, CAT and APX enzymes convert H_2O_2 into water and oxygen [42,44]. POD is a vital protective enzyme serving to prevent oxygen free radical damage in plants as a stress-tolerance enhancer [68]. In our study, the increase in SOD and POD activities observed in *Melia azedarach* under salt stress aligns with the findings reported by Chen et al. [73] and Hu et al. [51]. Moreover, a specific concentration of EBR has a significant effect on enhancing SOD and POD activity of both control and salt-treated seedlings. Arora et al. [67] reported that combining 28-homobrassinolide with a salt solution resulted in enhanced activities of the antioxidative enzymes compared to salt-treated seedlings, which supports our findings. Otie et al. [18] demonstrated that BR increased SOD activity regardless of salinity presence. Additionally, BR application led to the highest increase in POD activity in cucumber [12] and soybean seedlings [18]. Overall, the increase in antioxidant enzyme activities was investigated under salinity, and the application of exogenous EBR further enhanced these activities, as reported by Nejad-Alimoradi et al. [74]. As a result, BR has the potential to effectively stimulate antioxidative defensive responses, with the capability of enhancing antioxidant enzyme activity and gene expression, thereby reducing the accumulation of H_2O_2 and MDA and aiding in the removal of harmful ROS accumulation under high salinity [10,15,75].

4.7. Sheyang (SY) and Xiashu (XS) Seedling Comparison

In terms of a comparison of the two seed sources, we observed significant differences in chlorophyll, soluble protein, soluble sugar content and SOD activity among the measured variables. Out of these, the SY group showed a significantly higher chlorophyll and soluble protein content in all treatments, significantly higher SOD activity under salt stress, and the highest EBR treatment concentration compared to XS seedlings. On the other hand, the XS group exhibited a significantly higher soluble sugar content across all treatments. According to the available literature, the concentration of chlorophyll in stressed tissues serves as an indicator of salinity tolerance [41], and plants with tolerance to adverse conditions exhibit higher enzyme activities [44] and higher soluble sugars [76] than the sensitive ones. There is a postulation that if growth is more inhibited than photosynthesis, it would lead to sugar accumulation in plant tissues, and the plants that are most sensitive to this inhibition would have the highest sugar levels [77]. In this regard, insufficient evidence exists to establish a universal association between soluble sugars and salt tolerance in all plant species [76]. However, this lack of evidence does not negate the significant contribution of sugars to salt tolerance mechanisms nor preclude the potential utilization of sugar accumulation as an indicator of salt tolerance [76]. Nevertheless, through a biplot analysis, we were able to affirm our findings, providing the evidence that SY seedlings showed superior overall performance under salt stress compared to XS seedlings (Figure 7), suggesting a greater capacity for tolerance to salt stress in the coastal seed source group; however, it is important to note that these findings should be considered preliminary and require further validation through the inclusion of additional indicators or aspects.

5. Conclusions

The appropriate application of EBR spraying has demonstrated potential in alleviating salinity-induced stress in *Melia azedarach* seedlings, enhancing various growth and physiology-related parameters. In terms of height growth, the relative increment showed that a difference of 1.45% and 1.13% in the SY and XS groups was the positive effect of the addition of highest EBR treatment concentration when compared to salt-only treatment. As for diameter growth, the difference observed was 2.51% and 1.80% for the SY and XS groups, respectively. Under these two specific treatments comparison, physiological measurements such as chlorophyll content, leaf water status, plant cell membrane stability, the activities of osmolytes and antioxidant enzymes showed significant changes within each group of seed sources, except for proline determination in XS seedlings. Optimal stress mitigation with EBR was observed to be concentration dependent. While the present study found 1 mg/L to be the optimal EBR concentration, further investigation with a broader range of concentrations is required to comprehensively assess the concentration-dependent effects. Our study unveils a promising potential for future research, focusing on unraveling the underlying molecular mechanisms by which EBR enhances *Melia azedarach* seedlings' resilience to saline environments, as well as investigating the potential applications of EBR in other plant species. Understanding these mechanisms could provide valuable insights for the development of strategies to mitigate the negative impacts of salinity stress on agricultural, forestry and ecological systems. Ultimately, such advances could contribute to the sustainable management of saline environments.

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