The Induction of Salt Stress Tolerance by Gibberellic Acid Treatment in *Stevia rebaudiana* Bertoni Plants

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**Abstract:** Salinity poses a perpetual threat to agricultural lands, presenting an ongoing challenge to global food security. The present study aimed to explore the potential benefits of gibberellic acid (GA3) in enhancing stevia’s tolerance to salt stress. The experimental treatments comprised a control group (C) with 0 mM NaCl, salt stress (S) with 80 mM NaCl, 50 ppm of GA3 (G1), 100 ppm of GA3 (G2), as well as combinations of GA3 with salt stress (G1+S and G2+S). Exposure to saline water (80 mM NaCl) significantly decreased plant growth, water status, and photosynthetic attributes. However, it also led to notable increases in proline, glycine betaine, malondialdehyde (MDA), and antioxidant enzyme activities compared to the control treatment. Application of 100 ppm of GA3 effectively alleviated salt stress by enhancing plant performance under saline conditions, as evidenced by increased aerial (54%) and root (31%) dry weights compared to the control. Additionally, GA3 treatment resulted in elevated activities of polyphenol oxidase (24%), peroxidase (12%), superoxide dismutase (31%), and catalase (11%) while reducing MDA content by 41%, electrolyte leakage by 37%, and hydrogen peroxide by 34%. The use of phytohormones such as GA3 emerges as a promising strategy for mitigating salt stress-induced damage. It not only enhances plant performance but also reduces oxidative stress, offering protection against the detrimental effects of soil salinization.

**Keywords:** gibberellic acid; *Stevia rebaudiana*; water status; photosynthetic attributes; antioxidant activity

1. Introduction

Water and soil salinization pose constant challenges to crop production worldwide. Salt stress adversely affects plants through various mechanisms, including ion toxicity, osmotic shock, and oxidative stress [1,2]. These combined stresses significantly hinder plant growth, presenting considerable challenges to their ability to thrive. Plant morphology, physiology, and molecular and biochemical aspects undergo considerable changes under salt stress conditions [3,4]. This disruption occurs directly by reducing plant growth rates and yield or indirectly by causing osmotic, ionic, and nutritional constraints [1]. Reactive oxygen species (ROS) production under salt stress can lead to nucleic acid destruction
and lipid peroxidation [2]. However, plants have an ROS scavenging system that includes antioxidant enzymes such as polyphenol oxidase, peroxidase, superoxide dismutase, and catalase, which help mitigate the adverse effects of salt stress. Hence, there is a pressing need to explore innovative tools aimed at enhancing the resilience of plants to salinity. Plant growth regulators have been widely employed over the last few decades to mitigate the challenges associated with salt stress in crops. Gibberellic acid (GA3), a plant growth-stimulating hormone, regulates various physiological and biochemical processes in plants [5,6], including seed germination, plant biomass, leaf expansion, plant height, cell division, net absorption rate, flowering, and photosynthesis systems [7–10]. Additionally, GA3 plays a crucial role in protecting plants against salt stress. For instance, Shahzad et al. [11] reported that GA3 application improved maize growth, chlorophyll content, total soluble protein levels, and K+ ion concentration while mitigating oxidative stress and reducing Na+ ions accumulation under salt stress. In another study, exogenous GA3 significantly alleviated salt stress in rice by reducing oxidative stress damage and increasing plant growth and biomass production [12]. Stevia (Stevia rebaudiana Bertoni) is a medicinal plant and natural sweetener belonging to the Asteraceae (Compositae) family. Indigenous to Paraguay in South America, stevia is cultivated in numerous countries worldwide [13]. The plant garners significant attention due to its high sweetening power and non-caloric nature [13], making it one of the most economically important plants globally. Stevia leaves contain diterpene glycosides (steviosides and rebaudiosides), extensively used as a natural, non-caloric sweetener, particularly beneficial for diabetics [13]. Numerous investigations have explored the detrimental effects of salt stress on stevia [14,15]. The exposure of Stevia rebaudiana Bertoni to salt stress influences all the morphological, physiological, and biochemical parameters through the reduction of the biomass, the disturbance of the photosynthetic apparatus, and the deterioration of the plant’s cell membrane [14]. To the best of our knowledge, there is currently no research investigating the impact of foliar application of GA3 on stevia plants subjected to salt stress. Therefore, this study aimed to evaluate the potential influence of GA3 foliar spray on the growth, physiological parameters, ionic homeostasis, and antioxidant system of stevia plants under salinity stress.

2. Materials and Methods

2.1. Plant Growth Conditions and Treatments

The seedlings were cultivated in a greenhouse at the Faculty of Science Semlalia, Cadi Ayyad University, Marrakesh, Morocco, under a 16/8 h day/night cycle. The environmental conditions included a relative humidity of 68%, a temperature maintained at 24 °C, and a light intensity of 500 µm−2·s−1. Stevia (Stevia rebaudiana Bertoni) seeds were disinfected in 5% (v/v) NaClO solution for 10 min, followed by rinsing with sterile distilled water. Germination was carried out at 25 °C in peat-filled honeycombed plates. After 2 months, the germinated seeds were transferred into plastic pots filled with sterilized sand/peat mixture at a ratio of 2:1.

The experimental treatments, each with 10 replicates, included:

- Control (C): 0 mM NaCl
- Salt stress (S): 80 mM NaCl
- 50 ppm GA3 (G1)
- 100 ppm GA3 (G2)
- Combination of GA3 with salt stress (G1+S and G2+S)

To prevent osmotic shock, the NaCl concentration was gradually increased (by 20 mM per irrigation) until reaching 80 mM NaCl. The plants received a foliar spray of GA3 solution (8 mL/pot) mixed with Tween-20. In contrast, the control plants were sprayed with an equal volume of distilled water (8 mL/pot) mixed with Tween-20.

2.2. Parameters Measured

At the end of the experiment (8 weeks), the plants were harvested (Figure 1) to measure the fresh and dry weights, shoot height, and leaf area of stevia plants (5 plants/measurement).
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Figure 1. Stevia plants at harvest.

2.3. Mineral Ions Estimation (K, Ca, and Na)

The mineral ions (K, Ca, and Na) in plant leaves were determined as described by Yin et al. [16]. The K, Ca, and Na contents were quantified after digestion with H$_2$SO$_4$ and H$_2$O$_2$ using the flame photometric method (AAS3, Carl Zeiss Jena).

2.4. Chlorophyll Fluorescence and Stomatal Conductance Measurements

Chlorophyll fluorescence (Fv/Fm) was evaluated using a portable fluorometer (chlorophyll fluorometer, OPTI-SCIENCES, OS30p, Hudson, NH, USA). Five repetitions of each treatment were performed. Stomatal conductance (mmol/m$^2$·s) was assessed between 10:00 and 12:00 a.m. using five distinct mature leaves per treatment. Measurements were conducted using a portable porometer (leaf porometer, Decagon Devices, Pullman, Washington, DC, USA).

2.5. Chlorophyll Content Determination

Chlorophyll a (Cha) and chlorophyll b (Chb) were analyzed following the protocol outlined by Arnon [17]. The absorbance of the acetone extracts was measured, respectively, at 663 nm and 645 nm. The results were quantified and expressed as mg/g fresh weight (FW).

2.6. Leaf Relative Water Content

Leaf relative water content (RWC) was determined according to the Turner and Begg [18] method. The mature leaves were weighed to record the fresh weight (FW). Turgid weight (TW) was obtained after immersing the leaves for 24 h in distilled water at...
4 °C. Dry weight (DW) was assessed after drying in an oven at 80 °C for 72 h. The RWC was calculated by the following formula: RWC (%) = [(FW − DW) / (TW − DW)] × 100.

2.7. Evaluation of Proline, Glycine Betaine, and Total Soluble Sugar Contents

Proline content was analyzed by homogenized plant leaves (1 g) in sulphosalicylic acid (3%). The mixture was heated in a boiling water bath (100 °C for 1 h). The samples were cooled on ice to stop the reaction then extracted with toluene, and spectrophotometer absorbance at 520 nm was recorded [19]. For glycine betaine content determination [20], leaf samples (500 mg) were extracted in 10 mL of distilled water and centrifuged. Following dilution with 2N H₂SO₄ (1:1), the extracts were incubated for 1 h in an ice bath. Subsequently, cold potassium iodide reagent (prepared by mixing 7.85 g of I₂ with 10 g of KI in 50 mL of water) was added to the mixture and incubated at 4 °C for 16 h. After centrifugation, the resulting precipitate was dissolved in 7 mL of 1,2-dichloroethane. The total glycine betaine content was then measured at 365 nm. The total soluble sugars were evaluated according to the method given by Dubois et al. [21]. A total of 0.1 g of leaf powder was extracted by ethanol (80%) and centrifuged at 5000 rpm for 10 min. Then, 0.2 mL of supernatant was combined with 0.2 mL of phenol and 1 mL of concentrated sulfuric acid. The optical density was determined at 465 nm.

2.8. Lipid Peroxidation, Electrolyte Leakage, and Hydrogen Peroxide

Lipid peroxidation was assessed by quantifying the malondialdehyde (MDA) content following the method outlined by Rao and Sresty [22]. The MDA concentration was determined by measuring the absorbance at 532 and 600 nm and applying a molar absorption coefficient of 155 mM⁻¹ cm⁻¹ for calculation. For electrolyte leakage [23], 100 mg of fresh material was rinsed with distilled water and placed in closed tubes containing 10 mL of deionized water. After incubation (25 °C for 24 h), the first electrical conductivity (EC1) was measured. The second electrical conductivity (EC2) was recorded after incubation in a water bath (120 °C, 20 min). The electrolyte leakage was calculated using the following equation: EL (%) = (EC1/EC2) × 100. Hydrogen peroxide was quantified following the method described by Velikova et al. [24], which involved measuring the absorbance at 390 nm.

2.9. Measurement of Antioxidant Enzyme Activities

Antioxidant enzymes activity including peroxidase (POX), polyphenoloxidase (PPO), superoxide dismutase (SOD), and catalase (CAT), were assessed in the leaves of stevia plants. POX activity was evaluated using guaiacol and hydrogen peroxide (H₂O₂) as substrates. The reaction mixture included 100 µL of enzyme extract, 300 µL of guaiacol (20 mM), and 2 mL of phosphate buffer (0.1 M, pH 6). The reaction was initiated by adding 200 µL of H₂O₂ (0.3%), and the formation kinetics of tetra-guaiacol were monitored at 470 nm [25]. PPO activity was determined following the procedure outlined by Oktay et al. [26]. The reaction mixture consisted of 600 µL of catechol (0.1 M), 100 µL of enzyme extract, and 3 mL of phosphate buffer (0.1 M, pH 7). For the assessment of SOD activity, the method described by Beauchamp and Fridovich [27] was utilized. This involved measuring the enzyme’s capacity to inhibit the photochemical reduction of nitroblue tetrabromide, with the absorbance of the reaction product recorded at 560 nm. The CAT assay adhered to the protocol outlined by Montavon et al. [28].

2.10. Statistical Analysis

The experimental data underwent statistical analysis using analysis of variance with SPSS version 23.0 for Windows. The significance of differences between means was determined using the least significant difference (LSD) test.
3. Results

3.1. Effect of Gibberellic Acid on Growth Traits

Under salt stress, the shoot height, leaf area, and biomass of stevia plants were significantly reduced. However, foliar application of GA3 treatments resulted in a notable enhancement of plant growth under saline conditions (Table 1). Particularly, plants treated with 100 ppm of GA3 exhibited the highest values, leading to a significant improvement in stevia’s shoot height (36%), shoot dry weight (54%), root dry weight (31%), and leaf area (40%) compared to the control under salt stress.

Table 1. Effects of salt stress and gibberellic acid treatments on shoot height, shoot dry weight, root dry weight, and leaf area of stevia. G0: 0 ppm GA3, G1: 50 ppm GA3, and G2: 100 ppm GA3.

<table>
<thead>
<tr>
<th></th>
<th>Shoot Height (cm)</th>
<th>Shoot Dry Weight (g)</th>
<th>Root Dry Weight (g)</th>
<th>Leaf Area (cm²/Plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 mM NaCl</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G0</td>
<td>65.66 ± 2.31 b</td>
<td>4.17 ± 0.10 c</td>
<td>4.80 ± 0.07 b</td>
<td>629.11 ± 12.8 c</td>
</tr>
<tr>
<td>G1</td>
<td>81.65 ± 3.28 a</td>
<td>5.01 ± 0.09 b</td>
<td>5.66 ± 0.04 a</td>
<td>671.03 ± 16.20 b</td>
</tr>
<tr>
<td>G2</td>
<td>82.50 ± 2.16 a</td>
<td>5.48 ± 0.11 a</td>
<td>5.68 ± 0.10 a</td>
<td>680.54 ± 12.16 a</td>
</tr>
<tr>
<td><strong>80 mM NaCl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0</td>
<td>38.82 ± 1.78 e</td>
<td>1.51 ± 0.08 e</td>
<td>2.30 ± 0.05 d</td>
<td>316.16 ± 14.11 f</td>
</tr>
<tr>
<td>G1</td>
<td>42.00 ± 1.12 d</td>
<td>2.73 ± 0.07 d</td>
<td>3.31 ± 0.06 c</td>
<td>498.23 ± 10.43 e</td>
</tr>
<tr>
<td>G2</td>
<td>60.43 ± 1.47 c</td>
<td>3.28 ± 0.11 d</td>
<td>3.34 ± 0.02 c</td>
<td>520.07 ± 11.52 d</td>
</tr>
</tbody>
</table>

Means ± SE sharing similar letters within the same parameter are statistically non-significant at p < 0.05 (LSD).

3.2. Effect of Gibberellic Acid on Nutrients

Under NaCl stress, stevia exhibited a significant reduction in K and Ca contents, as well as the K/Na ratio, compared to the non-saline conditions. On the other hand, Na content showed a significant increase in plants subjected to salt stress (Table 2). Nevertheless, GA3 treatment significantly inhibited the absorption of Na while enhancing the content of K and Ca, resulting in an increased K/Na ratio. A higher increase in K (31%) and Ca (41%) contents as well as K/Na ratio (50%) was noted in plants treated with 100 ppm of GA3, as compared to their corresponding controls.

Table 2. Effects of salt stress and gibberellic acid treatments on sodium, potassium, and calcium contents, and K/Na ratio of stevia. G0: 0 ppm GA3, G1: 50 ppm GA3, and G2: 100 ppm GA3.

<table>
<thead>
<tr>
<th></th>
<th>Sodium Content (mg/g DW)</th>
<th>Potassium Content (mg/g DW)</th>
<th>Calcium Content (mg/g DW)</th>
<th>K/Na Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0 mM NaCl</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G0</td>
<td>9.99 ± 0.18 d</td>
<td>26.99 ± 0.32 b</td>
<td>5.97 ± 0.09 c</td>
<td>2.71 ± 0.02 b</td>
</tr>
<tr>
<td>G1</td>
<td>10.04 ± 0.12 d</td>
<td>28.30 ± 0.12 a</td>
<td>7.44 ± 0.03 b</td>
<td>2.81 ± 0.03 a</td>
</tr>
<tr>
<td>G2</td>
<td>10.07 ± 0.15 d</td>
<td>28.46 ± 0.53 a</td>
<td>7.91 ± 0.13 a</td>
<td>2.79 ± 0.01 a</td>
</tr>
<tr>
<td><strong>80 mM NaCl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G0</td>
<td>22.17 ± 1.09 a</td>
<td>14.16 ± 0.11 e</td>
<td>3.01 ± 0.11 f</td>
<td>0.63 ± 0.04 e</td>
</tr>
<tr>
<td>G1</td>
<td>17.54 ± 0.22 b</td>
<td>18.15 ± 0.82 d</td>
<td>4.76 ± 0.10 e</td>
<td>1.03 ± 0.02 d</td>
</tr>
<tr>
<td>G2</td>
<td>16.15 ± 0.51 c</td>
<td>20.47 ± 0.10 c</td>
<td>5.10 ± 0.07 d</td>
<td>1.26 ± 0.03 c</td>
</tr>
</tbody>
</table>

Means ± SE sharing similar letters within the same parameter are statistically non-significant at p < 0.05 (LSD).

3.3. Effect of Gibberellic Acid on Photosynthesis-Related Performance

Salt stress and GA3 treatments significantly influenced the chlorophyll content (Cha and Chb), stomatal conductance (gs), and maximum quantum yield of photosystem II (Fv/Fm) (Figure 2). In unstressed plants, treatment with 100 ppm of GA3 increased gs and Fv/Fm by 50 and 10%, respectively, compared to their control. However, there was a rise in gs by approximately 58 and 69% for G1+S and G2+S treatments, respectively, compared to the stressed group (S). The combination of NaCl and GA3 significantly increased the Fv/Fm ratio by 17 and 25% for G1+S and G2+S treatments, respectively, compared to the
stressed group (S). Under salt stress conditions, the highest values of Cha and Chb were observed in G1+S and G2+S treatments, with enhancement of 9 and 20%, respectively, as compared to the control plants.

Figure 2. Effect of foliar spray with GA3 on gs (a), Fv/Fm (b), Cha (c), and Chb (d) of stevia plants under salt stress. Six treatments were applied with 0 mM NaCl (C), 80 mM NaCl (S), 50 ppm GA3 (G1), 100 ppm GA3 (G2), G1+S, and G2+S. Results were expressed as the mean ± SD of 5 independent replicates. Values followed by different lowercase letters are significantly different according to an LSD test (p ≤ 0.05).

3.4. Effect of Gibberellic Acid on Osmotic Molecules and Leaf Relative Water Content

To elucidate how GA3 might positively affect stevia plants, leaf relative water content (RWC) and osmotic regulators (total soluble sugars, proline, and glycine betaine) were measured (Figure 3). Salt stress induced significant changes in RWC, glycine betaine, proline, and total soluble sugars content. Specifically, untreated plants subjected to salt stress exhibited a significant decline in RWC by 41% and an increase in glycine betaine, proline, and total soluble sugars content by 34%, 65%, and 40%, respectively, compared to unstressed plants (0 mM NaCl). The RWC reached approximately 66% and 70% for G1+S and G2+S, respectively, compared to S (50%).

3.5. Effect of Gibberellic Acid on Oxidative Damage

To analyze oxidative damage in stevia leaves, malondialdehyde (MDA), electrolyte leakage (EL), and hydrogen peroxide content (H$_2$O$_2$) were examined (Figure 4). GA3-treated plants exhibited lower MDA, EL, and H$_2$O$_2$ content under 80 mM of NaCl. The maximum decrease in MDA, EL, and H$_2$O$_2$ content (41%, 37%, and 34%, respectively) was observed at 100 ppm of GA3.
Figure 3. Effect of foliar spray with GA3 on RWC % (a), glycinebetaine (b), proline (c), and total soluble sugars (d) content of stevia plants under salt stress. Six treatments were applied with 0 mM NaCl (C), 80 mM NaCl (S), 50 ppm GA3 (G1), 100 ppm GA3 (G2), G1+S, and G2+S. Results were expressed as the mean ± SD of 5 independent replicates. Values followed by different lowercase letters are significantly different according to an LSD test ($p \leq 0.05$).

Figure 4. Effect of foliar spray with GA3 on malondialdehyde (MDA) (a), electrolyte leakage (b), and hydrogen peroxide content (c) of stevia plants under salt stress. Six treatments were applied with 0 mM NaCl (C), 80 mM NaCl (S), 50 ppm GA3 (G1), 100 ppm GA3 (G2), G1+S, and G2+S. Results were expressed as the mean ± SD of 5 independent replicates. Values followed by different lowercase letters are significantly different according to an LSD test ($p \leq 0.05$).
3.6. Effect of Gibberellic Acid on Antioxidant Enzyme Activities

Antioxidant enzymes activities (i.e., PPO, POX, CAT, and SOD) were significantly influenced by both salt stress and GA3 treatments (Figure 5). The foliar application of 50 ppm or 100 ppm of GA3 led to a significant increase in PPO, POX, CAT, and SOD activities. The most significant enhancements were noted in plants treated with 100 ppm GA3 under saline conditions. These increases amounted to 24%, 12%, 11%, and 31% over the untreated plants in PPO, POX, CAT, and SOD activities, respectively.

Figure 5. Effect of foliar spray with GA3 on polyphenoloxidase (a), peroxidase (b), catalase (c), and superoxide dismutase (d) of stevia plants under salt stress. Six treatments were applied with 0 mM NaCl (C), 80 mM NaCl (S), 50 ppm GA3 (G1), 100 ppm GA3 (G2), G1+S, and G2+S. Results were expressed as the mean ± SD of 5 independent replicates. Values followed by different lowercase letters are significantly different according to an LSD test (p ≤ 0.05).

4. Discussion

Salinity is a serious obstacle impeding optimal plant growth and physiological functioning. Its presence in the soil disrupts fundamental processes essential for plant development, i.e., water uptake, photosynthesis, and mineral nutrition. The current study highlights the significant detrimental effects of salt stress on growth and performance of stevia plants. Our findings demonstrate that exposure to 80 mM of NaCl significantly inhibited plant growth and biomass accumulation compared to unstressed controls. This aligns with numerous previous investigations that have underscored the negative impacts of salt stress on growth in various plant species [15,29,30]. Specifically, salt stress led to reductions in shoot length, dry biomass, and leaf area, indicating compromised plant health under saline conditions. The decline in plant biomass can be attributed to the elevated concentration of Na ions outside the cellular membrane, hindering water and nutrient absorption by plant roots. Furthermore, increased Na ion accumulation in roots induces osmotic stress and oxidative damage and disrupts ionic homeostasis, further compromising plant health [15,31]. Plant growth regulators and signaling molecules like GA3 have the potential to overcome the harmful impacts of abiotic stresses on plants. Spraying with
100 ppm of GA3 resulted in significant improvement in stevia’s shoot height, shoot dry weight, root dry weight, and leaf area under salt stress conditions [32]. Previous studies have also documented the positive influence of GA3 on various crop species [11,33–35], indicating its potential as a stress mitigation tool. GA3 is recognized for its ability to stimulate cell division and/or elongation, resulting in enlarged leaf area, enhanced photosynthetic rate, and upregulated invertase activity [6,36,37], which may explain the observed improvement in growth attributes in GA3-treated stevia plants. Research conducted by Gao et al. [38] indicates that DELLA (aspartic acid–glutamic acid–leucine–leucine–alanine) family proteins, which act as primary negative regulators of GA3, play crucial roles in mediating environmental signals and other plant hormone signaling pathways. The significant influence of GA3 in regulating plant growth under salt stress conditions is largely attributed to the growth-restraining effects mediated by DELLA proteins upon exposure to such stressors. When plants are exposed to salt stress, there is a decrease in the levels of GA3, accompanied by an increase in the accumulation of DELLA proteins [38,39]. Consequently, the growth and developmental processes of wild-type plants are inhibited by salt stress. Through their interaction, exogenous GA3 and DELLA proteins modulate plant responses to salt stress, thereby enhancing stress tolerance and promoting plant growth in saline environments [40]. Furthermore, GA3 has the potential to stimulate the accumulation of endogenous hormones or interact with other plant hormones [41], thus promoting improved plant growth. These interactions have the capacity to impact gene expression, signaling pathways, and metabolic processes, thereby enhancing the plant’s capacity to adapt to adverse environmental conditions.

Our findings also revealed that GA3 application led to an increase in Ca and K ions in stevia plants under salt stress. This enhancement of nutrient content may contribute to improved nutrient homeostasis and cell membrane integrity by GA3 application. Similar results reported that GA3 supply enhances plant growth and biomass production by maintaining ionic homeostasis under salt stress [42–44].

Salt stress induced significant damage to stomatal conductance and photosynthesis, resulting in reduced chlorophyll content and photosystem II efficiency. Salt can exert its impact by disrupting the chlorophyll–protein complex either through chlorophyll oxidation or by damaging the enzymes responsible for chlorophyll synthesis [44,45]. However, these parameters showed improvement with GA3 application, indicating its role in mitigating stomatal conductance and photosynthetic damage under salt stress. Likewise, in sorghum plants, Ali et al. [46] found that the application of GA3 in combination with a salt stress treatment resulted in higher values of chlorophyll content than those observed in the case of salt stress alone. Additionally, GA3 application led to an increase in stomatal conductance, further supporting its positive impact on photosynthesis consistent with the findings of Keawmanee et al. [47], who found that gibberellin could improve the ultrastructural morphogenesis of plastids and enhance chlorophyll content. Furthermore, our study highlights the importance of osmolytes in preserving membrane integrity and neutralizing free radicals during salt stress. GA3 supplementation enhanced osmolyte accumulation (proline, glycinebetaine, and total soluble sugars) in stevia plants, contributing to improved water uptake and osmotic balance [48,49]. Moreover, GA3 application effectively mitigated salt-induced oxidative damage, as evidenced by reduced levels of reactive oxygen species and membrane lipid peroxidation. This enhancement can be attributed to the upregulation of antioxidant enzymes, including PPO, POX, SOD, and CAT activities, further highlighting the role of GA3 in enhancing plant tolerance to salt stress. In support of this, Dinler et al. [50] conducted a study demonstrating the synergistic effects of GA3 and salinity on antioxidant enzyme activities. They observed that the combined treatment of GA3 (100, 300, and 500 ppm) and salinity (350 mM NaCl) induced the activities of key antioxidant enzymes, including SOD, APX, CAT, and GST, while maintaining relative water content and biomass yield. This suggests that the simultaneous application of GA3 and salinity acts as a signaling agent, promoting antioxidant enzyme activities and ultimately enhancing plant resilience to salt stress (Figure 6).
5. Conclusions

The present study provides valuable insights into the potential of GA3 as a strategy for mitigating stress in stevia plants under salt stress conditions. Specifically, treatment with 100 ppm GA3 significantly improved plant growth and physiological performance under saline conditions. This treatment led to substantial increases in the dry weights of aerial and root parts and elevated the activities of key antioxidant enzymes. Moreover, GA3 application reduced oxidative damage markers such as malondialdehyde, electrolyte leakage, and hydrogen peroxide levels. These findings suggest that GA3 not only alleviates the adverse effects of salt stress but also boosts the plant’s antioxidative defense mechanisms. Therefore, the utilization of phytohormones like GA3 represents a promising strategy for protecting crops against salinity-induced damage, thereby promoting more resilient agricultural practices and improved crop yields in saline environments.

Author Contributions: A.M. conceived the original research plan and designed and supervised the experiments; I.J. performed the experiments and collected and analyzed the data; R.B.-L., M.A., and A.E. reviewed and edited the manuscript; I.J. drafted the first version of the manuscript with editorial input from R.B.-L., M.A., and A.E.; and A.M. finalized the paper. All authors have read and agreed to the published version of the manuscript.

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