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# Enhancing Wheat Growth and Yield through Salicylic Acid-Mediated Regulation of Gas Exchange, Antioxidant Defense, and Osmoprotection under Salt Stress

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**Abstract:** Salinity is a major challenge for agricultural productivity, adversely affecting crop growth and yield. In recent years, various techniques have been developed to increase crop tolerance to salinity, including seed priming. This study was carried out to assess the effects of salicylic acid (SA) priming (0-, 10- and 20-mM) in comparison with hydropriming on growth, physio-biochemical activities, and yield of two wheat varieties (AARI-11 and Ujala-15) under 0- and 170-mM sodium chloride (NaCl) toxicity. The exposure of wheat plants to NaCl led to a significant reduction in various growth factors, including fresh weight (40%), total chlorophyll (39%), stomatal conductance (42%), shoot $Ca^{2+}$ (39%), and 1000-grain weight (34%). In contrast, salt stress triggered the activities of POD, SOD, CAT, glycine-betaine, phenolics, and proline. The application of 20 mM SA through seed priming was found to greatly improve the fresh root weight, chlorophyll *b*, POD activities, shoot $Ca^{2+}$, and overall yield (up to 71, 66, 35, 57, and 44%, respectively) under salt stress. While hydropriming also enhanced wheat tolerance to salinity.

**Keywords:** salt stress; salicylic acid; antioxidant enzymes; phenolics; yield



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## 1. Introduction

Soil salinity is a widespread challenge that negatively impacts crop growth and productivity globally [1]. The presence of excessive amounts of soluble salts, including sodium ($Na^+$), calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), chloride ($Cl^-$), and trace amounts of carbonates and nitrates, in the top layer of soil surpassing a threshold level (0.15 g salt/100 g soil) is considered salinity [2].

This spread of soil salinity has become a major global challenge, impacting crop growth and productivity. Over 900 million hectares of land are currently affected [3,4], with the trend expected to increase at a rate of 2 Mha per year [5]. This situation is projected to worsen over the next 30 years, potentially leading to a 50% increase in affected land and a decline in agricultural yield [6]. Arid and semi-arid agricultural regions are comparatively more affected by salinity. The adverse impact of soil salinity is particularly pronounced in arid and semi-arid agricultural regions. The use of low-quality irrigation water leads to the accumulation of salts in the soil [7], which in turn affects the plant's ability to absorb water and nutrients. This decreased availability of resources leads to enzyme dysfunction, hormonal imbalances, disruption of photosynthetic apparatus, and ion toxicity, among other negative effects on plant growth and development [8].

Globally, saline soils result in a loss of crop yield of $25 billion per annum [9]. Among these crops, wheat (*Triticum aestivum* L.) is the second most cultivated cereal crop and is also a staple food for one-third of the world [10]. Therefore, increasing wheat yield is crucial for ensuring food security and addressing global food challenges. One effective strategy is to cultivate salt-tolerant wheat varieties, but the complexity of the wheat genome [11] may make it a hindrance to developing more salt-resistant varieties. Another strategy is remediation, which involves using certain plants or microorganisms to clean up and boost crop yield. However, this approach can be economically challenging [12].

Another option is to alter the plant responses to stress with specific treatments. One advanced mechanism that plants use to respond to different environmental stress conditions is crosstalk through hormones, i.e., jasmonic acid, abscisic acid, auxin, and salicylic acid [13]. Research on the application of SA on plants revealed its positive role in thermoregulation, abiotic and biotic stress tolerance, DNA repair, high yield, and seed germination [14–17]. The disclosure of the positive role of SA in enhancing the defense mechanisms of tobacco (*Nicotiana tabacum*) plants infected with tobacco mosaic virus [17] has opened up new gateways for researching this phytohormone for plant defense mechanisms.

SA is a member of the phenolic compound family and acts as a non-enzymatic antioxidant. This endogenous phytohormone contributes to plants' resistance to drought, salinity, and cold stress [18]. Under stressful conditions, SA regulates plant metabolism and other functions, such as the production of osmoprotectants, stomatal closure, increased antioxidant activities, ion uptake, reduced ethylene production, and transpiration [19,20]. Sedaghat et al. [21] suggested that the application of SA improved wheat growth under drought stress. Min and Lee [22] found that SA-treated plants produced more secondary metabolites under freezing stress compared with non-stressed plants. El Dakak and Hassan [23] investigated and proposed that SA mitigated the Cd-induced toxicity in maize (*Zea mays*). However, the impact of larger-scale and higher-dose applications of SA is still uncertain. Previous research has limitedly investigated the impact of varying salicylic acid levels as a pre-treatment for salinity tolerance in diverse wheat varieties. Based on this, we hypothesized that higher levels of SA priming enhance wheat's salinity tolerance compared with lower SA levels. This study endeavors to explore the effects of priming seeds with different concentrations of SA on wheat's growth, yield, and physio-biochemical responses under saline conditions. Additionally, this study aims to deepen our understanding of salt-stressed wheat's oxidative and antioxidative activities.

## 2. Results

### *2.1. Changes in Plant Growth*

The growth of both varieties of wheat grown in NaCl-spiked soils was significantly ($p < 0.001$) reduced as indicated by the reduction in shoot and root length, and fresh and dry weight. The length of root and shoot, fresh weight, and dry weight decreased by 17, 34, 40, and 35% in V1, and 13, 28, 41, and 35% in V2, respectively, as compared with the control. Pretreatment with water, 10- and 20-mM SA improved plant growth under both stressed and stress-free plants (Figure 1).

Under unstressed conditions, the root length of V2 showed the highest increase of 16% after hydropriming treatment. Meanwhile, the shoot fresh weight of V1 experienced a boost of 30% after priming with 10 mM SA, and a further increase of 45% with 20 mM SA. The combination of salt stress and priming with water, 10 mM, and 20 mM SA showed maximum improvement in root length (13.8%) and fresh root weight (43% and 71%) in V1, respectively.

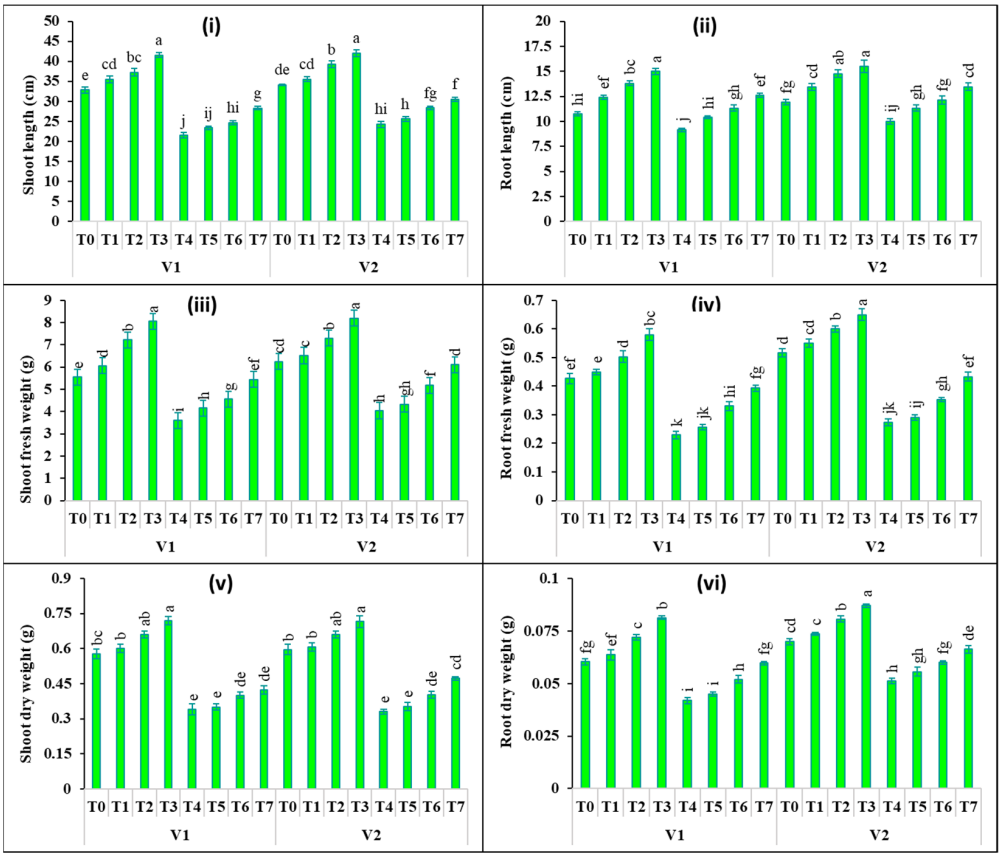


**Figure 1.** Influence of salicylic acid priming on (**i**) shoot and (**ii**) root length, (**iii**) shoot and (**iv**) root fresh weight, and (**v**) shoot and (**vi**) root dry weight under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl + hydropriming), T6 (170 mM NaCl + 10 mM SA priming), and T7 (170 mM NaCl + 20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

### *2.2. Changes in Photosynthetic Pigments and Gas Exchange Attributes*

The salt stress caused a significant ($p > 0.001$) decrease in photosynthetic pigments in both wheat varieties under salt stress. The reduction in pigments was 43 and 37% for chlorophyll *a*, 44 and 35% for chlorophyll *b*, and 49 and 46% for carotenoids in V1 and V2, respectively, in comparison with the control. However, seed priming with water, 10 mM SA, and 20 mM SA helped to overcome the decrease in photosynthetic pigments. The highest improvement in Chl. A, Chl. B, and total chlorophyll was observed in V2 with 20 mM SA priming with an increase of 45, 65, and 60%, respectively. The highest improvement in carotenoids was seen in V1 under 20 mM SA priming, with an increase of 60%. The least improvement was observed in water-primed seed plants for chlorophyll *a* (8%) in V2, chlorophyll *b* (6%), and carotenoids (11%) in V1 compared with T4 (Figure 2).

Salinity significantly reduced the gas exchange attributes of the wheat plants, except for water use efficiency (A/E). The highest reduction in transpiration rate (E) and stomatal conductance (Gs) was 46 and 44% in V2 and V1, respectively. The lowest levels of sub-stomatal $CO_2$ (Ci) and net $CO_2$ assimilation (A) were recorded in V1 by 13 and 34%, respectively, under 170 mM NaCl compared with the control. On the other hand, A/E increased by 4% in V1 and 31% in V2 under salt stress. Priming treatments showed an improvement in gas exchange attributes with the order of water <10 mM SA < 20 mM SA under salinity. The least effect on A/E under 20 mM SA priming was recorded as 0.3% in V1, while the maximum effectiveness against salt stress under hydropriming was recorded in E (11.8%) in V1 (Figure 3).

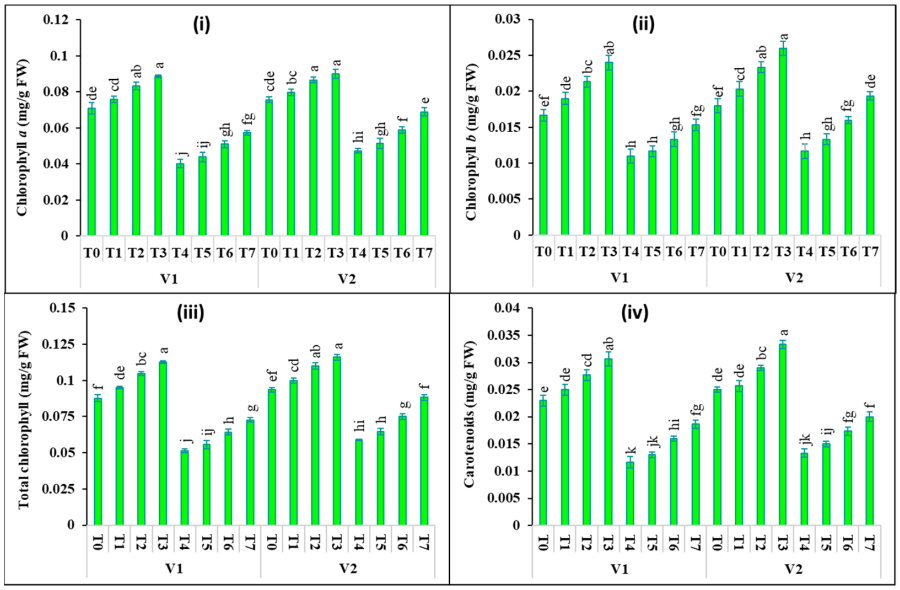


**Figure 2.** Influence of salicylic acid priming on (**i**) chlorophyll *a*, (**ii**) chlorophyll *b*, (**iii**) total Chlorophyll, and (**iv**) carotenoids weight under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl + hydropriming), T6 (170 mM NaCl + 10 mM SA priming), and T7 (170 mM NaCl + 20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

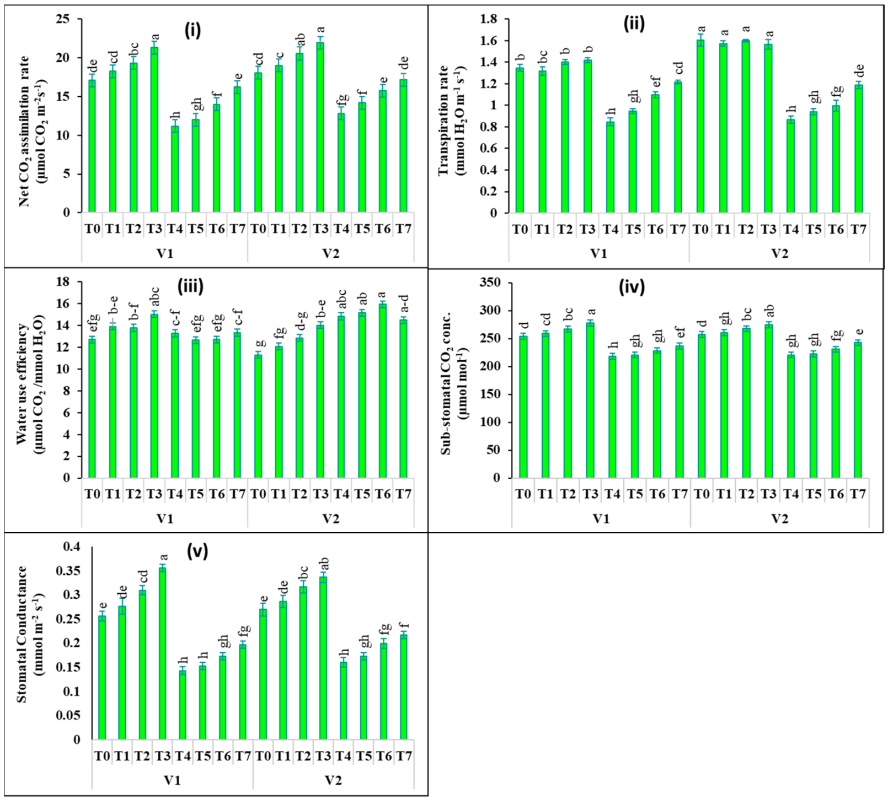


**Figure 3.** Influence of salicylic acid priming on (**i**) net $CO_2$ Assimilation rate, (**ii**) transpiration, (**iii**) water use efficiency, (**iv**) sub-stomatal $CO_2$ concentration, and (**v**) stomatal conductance under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl + hydropriming), T6 (170 mM NaCl + 10 mM SA priming), and T7 (170 mM NaCl + 20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

### *2.3. Changes in Oxidants and Antioxidants Activities*

The results showed a significant increase in the oxidant activities of the wheat plants under salt stress, as indicated by a rise in malondialdehyde (MDA) and $H_2O_2$ levels ($p < 0.001$). The highest increase in MDA was recorded in V1, at 60% higher than the control. Meanwhile, V2 showed the highest $H_2O_2$ levels at 56% compared with those of the control plants under salinity. However, priming treatment caused a considerable reduction in oxidative damage. The highest reductions in MDA and $H_2O_2$ were recorded under 20 mM SA priming, with a decrease of 17% and 22% in V1 and 37% and 31% in V2, respectively, compared with those of T4. Salt stress resulted in a significant increase in the activities of catalases (CAT), peroxidases (POD), and superoxide dismutase (SOD) compared with those of the control. SOD content was slightly ($p < 0.05$) influenced by salinity and variety. NaCl toxicity caused an increase of 41 and 48% POD, 46 and 42% SOD, and 34 and 31% CAT activity in V1 and V2, respectively. Priming treatments further increased the antioxidant activities in the wheat plants. The highest POD (40%) and CAT (26%) activity were recorded in V1 under 20 mM SA priming compared with those of the control. Meanwhile, the highest SOD activity (38%) was recorded in V1 under a combined treatment of 20 mM SA priming and salt stress (Figure 4).

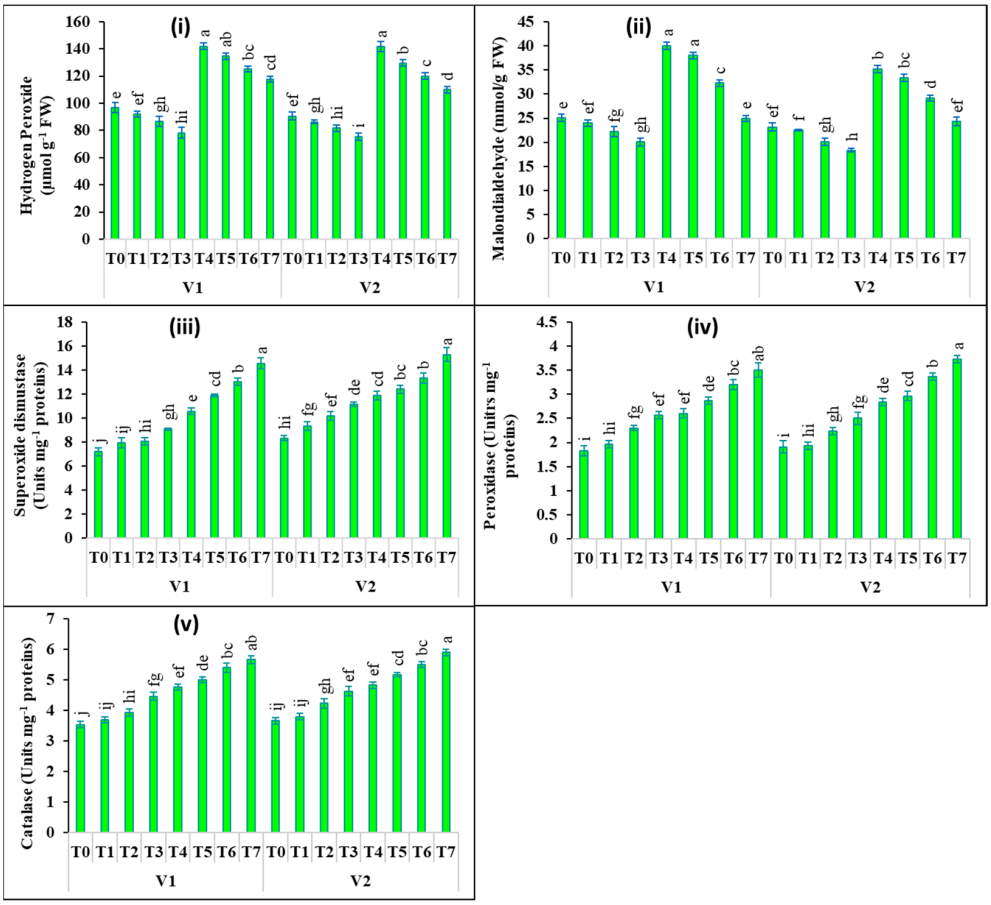


**Figure 4.** Influence of salicylic acid priming on (**i**) hydrogen peroxide/$H_2O_2$, (**ii**) malondialdehyde/MDA, (**iii**) superoxide dismutase/SOD, (**iv**) peroxidase/POD, and (**v**) catalases/CAT under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl + hydropriming), T6 (170 mM NaCl + 10 mM SA priming), and T7 (170 mM NaCl + 20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

### *2.4. Changes in Biochemical Attributes*

Salinity and varieties interactions significantly ($p > 0.01$) affected the glycine-betaine (GB) content, whereas salinity and priming had a slightly significant effect on total soluble sugars (TSS) ($p < 0.05$). Results showed that 170 mM NaCl-spiked soil enhanced the GB, AsA, TSS, total soluble proteins (TSP), phenolics, and proline contents of V1 by 18, 19, 12, 33, 33, and 29%, and those of V2 by 19, 24, 11, 33, 32 and 29%, respectively, compared with the stress-free plants. Interestingly, priming treatments further increased the biochemical attributes of both wheat varieties. The highest increase was recorded under pre-treatment of 20 mM SA for all the biochemical attributes. Hydropriming had the greatest impact on AsA content (13%) of V2, and 10 mM SA priming had the greatest impact on phenolics content (26%) of V1. The maximum contents observed under 20 mM SA priming were as follows: GB 30%, AsA 30%, TSS 27%, TSP 42%, phenolics 38% in V1, and 35% proline in V2 compared with the plants under salt stress (Figure 5).

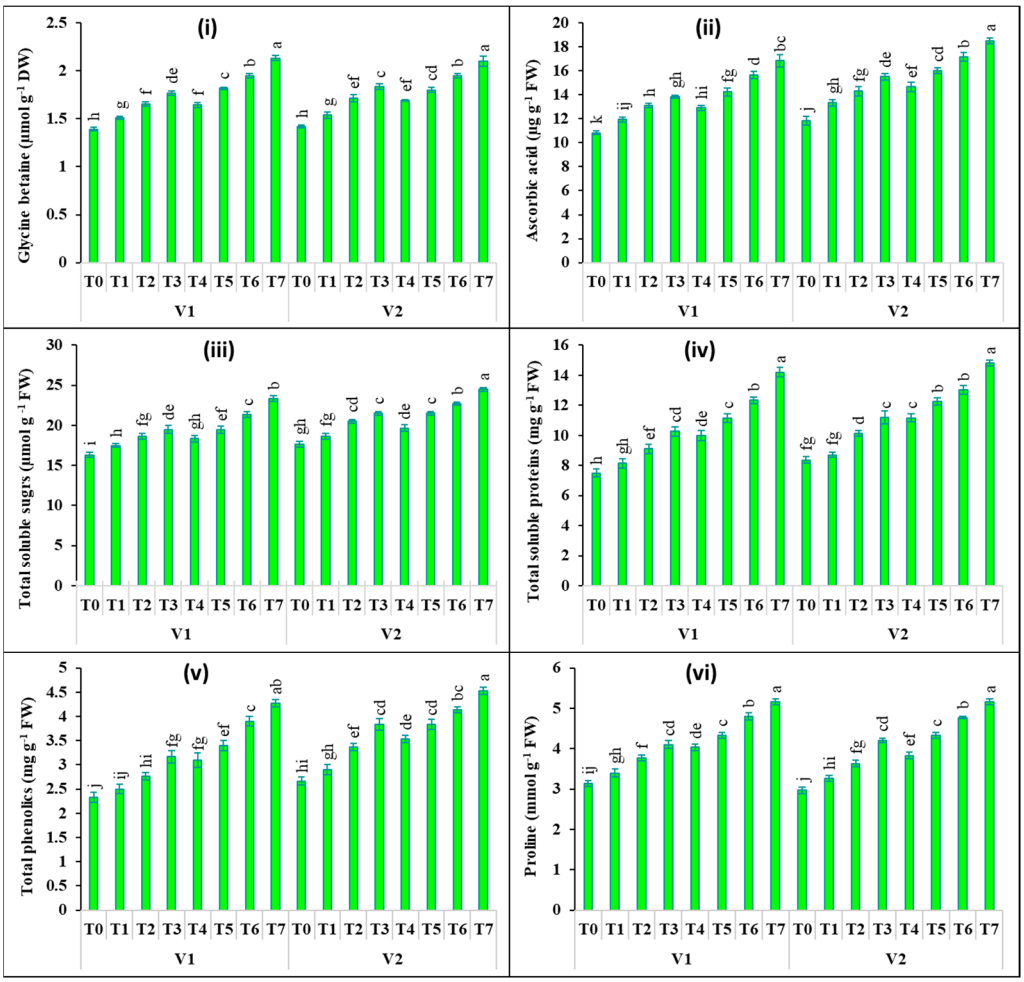


**Figure 5.** Influence of salicylic acid priming on (**i**) glycine betaine/GB, (**ii**) ascorbic acid/AsA, (**iii**) total soluble sugars/TSS, (**iv**) total soluble proteins/TSP, (**v**) total phenolics, and (**vi**) proline content under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl + hydropriming), T6 (170 mM NaCl + 10 mM SA priming), and T7 (170 mM NaCl + 20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

### *2.5. Changes in the Concentration of Mineral Ions*

The data recorded for the effect of salt stress and priming on ionic concentration ($Na^+$, $K^+$, and $Ca^{2+}$) in wheat plants showed varying results. Under salinity, there was a significant increase in $Na^+$ concentration and a significant decrease in $K^+$ and $Ca^{2+}$ content

in wheat. The highest increase in $Na^+$ concentration was observed in V2, with 50% in the root and 43% in the shoot compared with control plants. Pre-treatment with water and SA was found to decrease $Na^+$ concentration in both roots and shoots. The largest decrease was recorded in shoot $Na^+$ concentration (27%) of V2 under 20 mM SA compared with stressed plants.

The results indicate that salt stress had a negative effect on the concentration of $Ca^{2+}$ in the roots and shoots of both V1 and V2 wheat plants, with reductions ranging from 32 to 39%. However, pre-treatment with water and SA (10 mM) helped to mitigate the losses of $Ca^{2+}$ under salt stress. The highest $Ca^{2+}$ concentration was recorded in the roots of V2 and shoots of V1 under 20 mM SA priming, at 49 and 57%, respectively. The interaction of priming, salinity, and variety had a significant effect ($p > 0.05$) on root K+ concentration, with shoots having higher K+ concentration compared with roots in both varieties throughout all treatments. Salt stress caused a decrease in root and shoot K+ concentration in V1 by 36 and 38% and in V2 by 30 and 29%, respectively. Each priming treatment was beneficial for the K+ concentration in both roots and shoots, with the highest improvement observed in shoots after 10- and 20-mM SA priming, at 17%, 35%, and 59%, respectively (Figure 6).

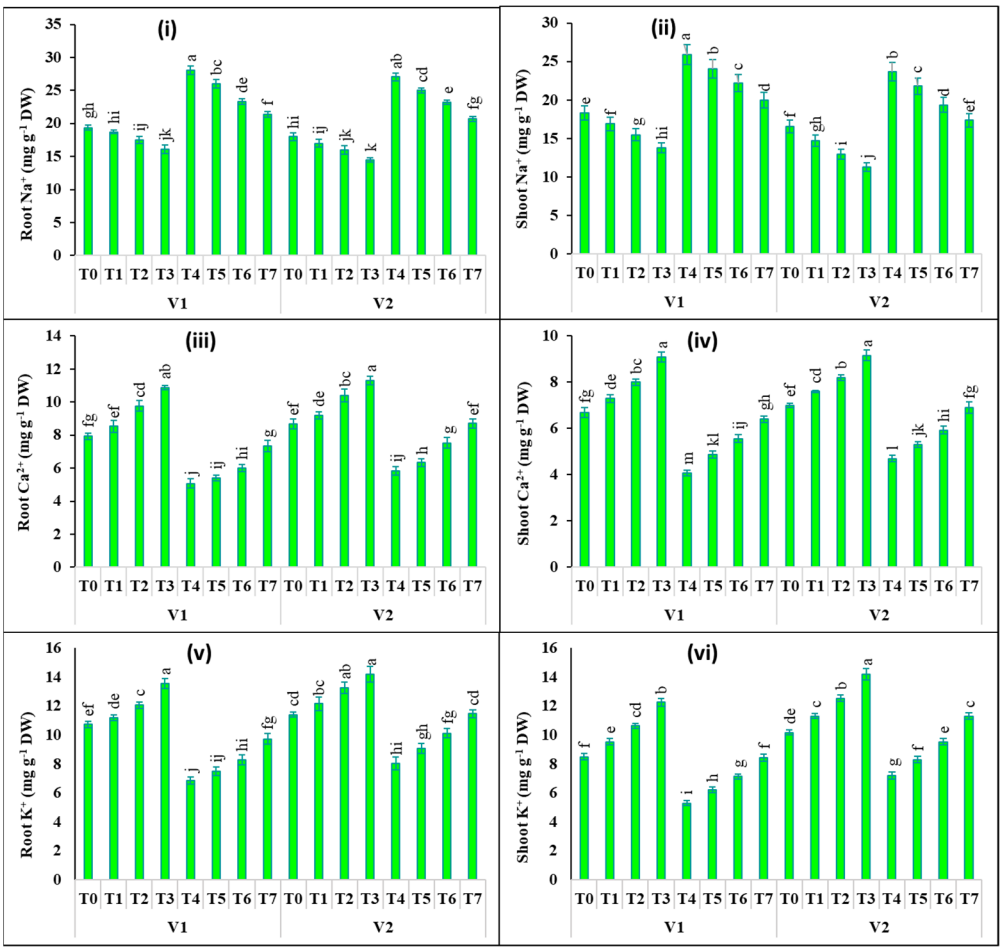


**Figure 6.** Influence of salicylic acid priming on (**i**) root Na+, (**ii**) shoot Na+, (**iii**) root Ca2+, (**iv**) shoot Ca2+, (**v**) root K+, and (**vi**) shoot K+ content under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl + hydropriming), T6 (170 mM NaCl+10 mM SA priming), and T7 (170 mM NaCl+20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

### *2.6. Changes in Yield Attributes*

The soil NaCl had a negative impact on the overall yield of wheat plants. The data showed that the 1000-grain weight and spike length of wheat plants were greatly affected ($p < 0.001$) by priming treatments. Under salt stress, the highest decrease in 1000-grain weight and spike length was observed in V1 (40%) and V2 (39%) compared with unstressed plants. The pre-treatment of 20 mM seeds showed the highest increase in 1000-grain weight (32%) and spike length (33%) in V2 among unstressed plants. All priming treatments, even under stress, improved yield further. The water, 10 mM, and 20 mM SA priming showed the maximum increase in 1000-grain weight (6%, 21%, and 45%) and spike length (14%, 31%, and 47%) in V1 and V2, respectively, compared with T4 (Figure 7).

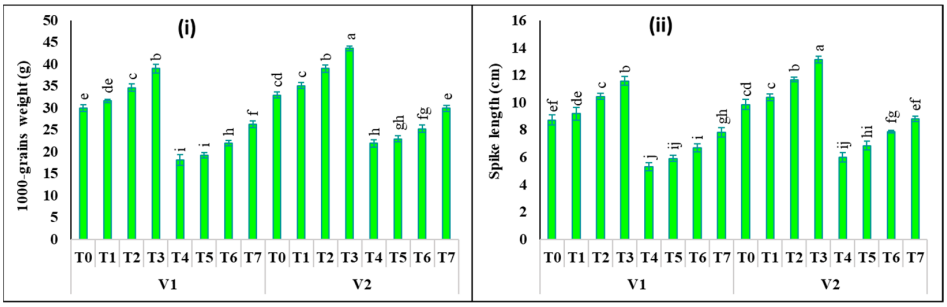


**Figure 7.** Influence of salicylic acid priming on (**i**) 1000-grains weight and (**ii**) spike length under salt stress; T0 (control), T1 (hydropriming), T2 (10 mM SA priming), T3 (20 mM SA), T4 (170 mM NaCl), T5 (170 mM NaCl+ hydropriming), T6 (170 mM NaCl+10 mM SA priming), and T7 (170 mM NaCl+20 mM SA priming); bars sharing same case letter or without lettering, for a parameter, do not significantly differ ($p \leq 0.05$) by the LSD test.

## 3. Discussion

The widespread use of fertilizers to enhance crop production has led to an increase in the soluble salt in soil and groundwater, which ultimately increases soil salinity [24]. This increased soil salinity impedes the growth, physiology, and yield of crops. Different parts of a plant have varying levels of tolerance against environmental stresses [25]. In the present study, two different wheat varieties were grown in saline soil to investigate their tolerance to salt stress with pre-treatment of water and 10- and 20-mM SA. The results supported the hypothesis that higher levels of SA improved the physio-biochemical attributes of wheat under salt stress.

In this study, salt stress resulted in a significant reduction in the length and fresh and dry weight of roots and shoots in both wheat varieties. This decrease in physical growth is due to the combined effects of osmotic and ionic stress produced as a result of restricted water and nutrient uptake, reduced turgor pressure [26,27], minimized photosynthetic activity, excessive $Na^+$ and $Cl^-$ accumulation [28,29], and cell membrane damages under salt stress [30]. However, the results indicated that SA priming improved the growth of saline-stressed plants by improving water and nutrient uptake, controlling the membrane Na+ pumps, and enhancing secondary metabolites and nutrient uptake. These findings are consistent with previous studies [31–37], which have shown that SA can improve the tolerance of plants to salt stress.

Our study found that elevated salt levels in the soil reduced the photosynthetic pigments and gas exchange parameters of wheat plants. The reduction in the photosynthetic pigments can be attributed to the high salinity damaging the thylakoid membrane, causing reduced $CO_2$ uptake, rapid stomatal closure, and inactivation of enzymes of dark reactions of photosynthesis [28,38]. Similar results were also suggested by [3,39]. The decreased gas exchange was linked to reduced xylem pressure resulting from salt-induced water stress [40], which led to stomatal closure and decreased rate of photosynthesis [41,42]. In contrast, pre-treatment with SA improved wheat plants' total chlorophyll and carotenoid content, even under stress. SA lowers the concentration of $Na^+$ in the leaves, promoting the

activation of photosynthetic processes [43]. It also increases antioxidant levels, improves photosynthetic capacity [44,45], and regulates stomatal opening to reduce water loss and increase photosynthesis [46,47].

Under high NaCl conditions, the activities of $H_2O_2$ and MDA in wheat plants increased. Salinity affects chloroplasts and generates more reactive oxygen species (ROS) [48,49]. The rapid stomatal closure in leaves also results in the production of ROS in leaves as stress signals [50]. Our study showed that SA priming improved oxidative damage in salt-stress plants. This reduction in oxidants can be attributed to the increase in antioxidants such as POD, SOD, and CAT produced under SA treatment in stressed plants [51–53]. These antioxidant enzymes are known for plant defense by scavenging ROS [54]. SOD scavenges the ROS by converting them into $O_2$ and $H_2O_2$, which activates the sensitive signaling activities [55,56]. The CAT further catalyzes the $H_2O_2$ into $O_2$ and water, protecting the plants from various abiotic stress [57,58], whereas MDA scavenging is performed by a non-enzymatic antioxidant such as proline [59,60].

Our study found that higher salt concentrations in the soil resulted in increased levels of osmoprotectants, non-enzymatic antioxidants, and secondary metabolites in wheat varieties. These findings align with previous research that has shown that salt stress increases the accumulation of these substances [56,61]. Our results also showed that pre-treatment with SA (systemic acquired resistance) resulted in further enhancement of osmoprotectants such as glycine-betaine, proline and soluble sugars, etc. [62], similar to what has been observed in cotton and tomato under salt stress [63,64]. Pre-sowing SA treatment triggers early production of proline and ABA [65,66], which are well known for mitigating salt stress by increasing biochemical content [67]. Additionally, our findings illustrated that salt stress increased $Na^+$ and decreased $Ca^{2+}$ and $K^+$ in the soil. Higher Na+ levels in soil disturb the osmotic pressure of soil as well as plant cells. This disturbance of osmotic pressure in the soil and plant cells prevents the plant from absorbing $K^+$, $Ca^{2+}$, and water [61,68,69]. However, our results indicate that SA pre-treatment significantly increased the uptake of K and $Ca^{2+}$, while reducing the $Na^+$ concentrations, due to the shielding effect of SA on the roots cell membrane and $H^+$-ATPase activities in plant roots [70,71].

Salt stress significantly reduced the wheat yield, but SA seed priming markedly improved the yield under both control and stressed conditions as environmental stresses such as salinity during early developmental stages negatively affect the yield qualitatively and quantitatively [72]. It has also been suggested that the effects of salinity on plant growth, phytochemicals, mineral uptake, and water availability contribute to yield loss [73,74]. Multiple studies have shown that the application of SA alone and in combination with other polyamines positively affects the yield attributes of rice (*Oryza sativa*) [75] and wheat [76,77] under severe salt stress conditions. SA application improves salt stress tolerance by decreasing oxidative stress, promoting plant growth, photosynthesis, nutrient uptake, and ultimately enhancing yield [78,79].

## 4. Materials and Methods

### *4.1. Chemicals*

All the chemicals (SA, acetone, trichloroacetic acid, phosphate buffer, Bradford reagent, phenol reagent, etc.) and apparatus (spectrophotometer, flame photometer, hot plate, vortex machine, centrifuge machine, glass wares, etc.) utilized in this study were supplied by the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

### *4.2. Experimental Design and Treatments*

For this study, the two high-yielding wheat varieties (*Triticum aestivum* L.), Ujala (V1) (4777*2/5/CIANO67/8150//TOBARI66/CIANO67/4/NOROESTE66/3/12300//LEMAROJO64/8156/6/ (SIB)TRIFON) and AARI (V2) (SH-88/90A-204//MH97) were grown in a pot experiment at the Old Botanical Garden of the University of Agriculture, Faisalabad, Pakistan, during November 2020 to March 2021. Eight treatment combinations (T0 = dry seeds + no NaCl (control/ no priming or NaCl stress), T1 = hydropriming + no NaCl, T2 = 10 mM SA priming + no NaCl, T3 = 20 mM SA priming + no NaCl, T4 = no priming + 170 mM NaCl, T5 = hydropriming + 170 mM NaCl, T6 = 10 mM SA priming +

170 mM NaCl and T7 = 20 mM SA priming + 170 mM NaCl) were applied. The experiment was conducted employing a completely randomized design (CRD) in which three plants of each variety received a similar treatment combination (3 × 2 × 8 = 48 pots). An amount of 170 mM NaCl was maintained in three successive intervals ((i) 60 mM, (ii) 60 mM, and (iii) 50 mM) to avoid sudden salt injury. For all the water and SA priming treatments, the seeds were soaked in 1000 mL distilled water and 10-and 20-mM SA solution for 15 h. Later, these seeds were dried in laboratory conditions (29–31 °C) for the next two days and then subjected to sowing. Primed seeds were dried under shade in laboratory conditions until the original weight of seeds was achieved.

### *4.3. Estimation of Plant Growth and Biomass*

Three fully grown and healthy plants from each pot were harvested after 50 days of sowing, i.e., growth stage 49 according to the BBCH scale for crops [80]. Various morphological attributes of each sample were measured to analyze the growth of the salinity-based and SA-treated plants. Plant height was calculated by measuring the lengths of the root and shoot with the help of a common inch tape. Plant biomass of freshly harvested plants was recorded using a digital weighing balance, and then the dry biomass of the same plants was recorded after five days of oven drying at 70 °C [81].

### *4.4. Estimation of Photosynthetic Pigments and Gas Exchange Properties in Leaves*

The method of Arnon [82] was followed with slight modifications to measure the photosynthetic pigments of the plants after 50 days of seeding. An amount of 10 mg of the fully developed leaf of wheat plant was mixed in 5 mL acetone solution (80%) and left in the dark for 12 h. Later on, these samples were observed at 663 nm, 645 nm, and 480 nm with the help of a spectrophotometer (IRMECO U2020, IRMECO gmbh, Schwarzenbek, Germany). Changes in substomatal $CO_2$ concentration, stomatal conductance, Net $CO_2$ assimilation rate, and transpiration rate were measured in a fully expanded leaf using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, UK). The water use efficiency was calculated by dividing every treatment's photosynthetic and transpiration rates.

### *4.5. Estimation of Oxidants and Antioxidative Enzymes Activities in Leaves*

Activities of oxidants ($H_2O_2$ and MDA) and antioxidative enzymes (SOD, POD, and CAT) of wheat leaves were analyzed. For $H_2O_2$ determination, 0.25 of the healthy fresh leaf of wheat mixed in 5 mL of 0.5 % Trichloroacetic acid (TCA) were centrifuged for 12 min at 12000 rpm. The supernatant was collected and added to phosphate buffer and potassium iodide solution [83]. Absorbance was recorded at 390 nm with the help of a spectrophotometer. To determine MDA, 0.3 g of fresh leaf mixed in 3 mL of 0.5% TCA solution was centrifuged. A supernatant mixed with 0.5% thiobarbituric acid (TBA) was set for incubation at 95 °C [84]. The absorbance for these samples was recorded at 532 nm and 600 nm with the help of a spectrophotometer. To determine the activities of enzymatic antioxidants, a leaf sample was prepared by homogenizing 0.25 g of fresh, fully-grown wheat leaves with phosphate buffer (5 mL) under chilled conditions. These mixtures were subjected to centrifugation at 12,000 rpm for 15 min. The supernatant was collected for further analysis. An amount of 1 mL of collected supernatant was mixed in 1 mL $H_2O_2$ to measure the CAT activity on 0 s, 30 s, 60 s, 90 s, and 120 s at 240 nm with the help of a spectrophotometer [85]. The method of Spitz and Oberley [86] was followed to determine the SOD activity. The supernatant was added in 400 µL distilled water, 250 µL phosphate buffer, 100 µL methionine, 100 µL Triton-X, 50 µL NBT solution, 50 µL riboflavin, and 50 µL supernatant-containing quartz cuvettes. Samples were placed under a lamp for 0.5 h, and then absorbance was recorded at 560 nm with the help of a spectrophotometer. For measuring the POD activities, 750 µL buffer, 100 µL guaiacol, 100 µL $H_2O_2$, and supernatant [84] were mixed, and absorbance was recorded at 470 nm on 0 s, 30 s, 60 s, 90 s, and 120 s with the help of a spectrophotometer.

### *4.6. Estimation of Biochemical Activities*

The method proposed by Grieve and Grattan [87] was followed to measure the GB content. The filtrate obtained after mixing 10 g leaf in 25 mL for 24 h was added into 2 mL of 2 N $NH_2SO_4$. These samples were bathed in ice water for 60 min. Chilled KI-I reagent was added to these samples and centrifuged at 12,000 rpm for 15 min. The supernatant was mixed in periodide crystals dissolved in 5 mL of 1,2-dichloroethane and stored at 4 °C for 2 h. The absorbance was recorded at 365 nm. Supernatant collected after centrifugation of 0.1 g fresh wheat leaf samples homogenized with 60% TCA solution was mixed with 2% DNPH and only a drop of thiourea followed by incubation at 50 °C for 15 min. After that, 2 mL of diluted $H_2SO_4$ was added to each sample to measure the ascorbic acid content [88] by observance at 530 nm with the help of a spectrophotometer.

To determine the total soluble sugars (TSS), test tubes containing 10 mg of the leaf and 10 mL of distilled water were placed in a hot water bath at 90 °C for 60 min. The mixture was diluted with distilled water until 50 mL was obtained. An amount of 1.5 mL of this dilution was mixed in 5 mL anthrone reagent and again water bath for 20 min. The samples were allowed to cool, and absorbance was checked via a spectrophotometer at 620 nm [89]. Total soluble proteins (TSP) content was measured by adding a supernatant of 0.25 g fresh fully grown wheat leaf mixed in phosphate buffer into 5 mL of Bradford reagent for each sample. The sample was mixed by vortex and absorbance was recorded at 595 nm with the help of a spectrophotometer [90].

The total phenolic content of the plants under salinity and priming treatments was determined according to the method by McCue [91]. An amount of 50 mg of each sample was mixed with 2.5 mL of 95% ethanol and frozen for 48–72 h. Samples were homogenized and centrifuged at 12,000 rpm for 5 min. An amount of 1 mL supernatant was mixed in 1 mL ethanol, 1 mL of 50% Folic–Ciocalteu phenol reagent and distilled water was subjected to incubation. After 5 min, 1 mL of 5% sodium was added and briefly vortexed. This mixture was incubated for 60 min before the absorbance at 725 nm was determined. To estimate the changes in proline content, the supernatant was collected after mixing plant extract and 3% sulphosalicylic acid solution. An amount of 100 µL of the supernatant was mixed in glacial acetic acid and ninhydrin and placed in the water bath for 1 h at 100 °C. The reaction was terminated in the ice bath. The reaction mixture was mixed with toluene, and optical density was measured at 520 nm [92].

### *4.7. Estimation of Mineral Ion Contents*

Dried plant samples were used to analyze the wheat plants' ion concentrations ($Na^+$, $Ca^{2+}$, $K^+$). An amount of 100 mg of dried samples was mixed with 2 mL pure $H_2SO_4$ and left in the dark overnight. Later, these samples were placed on the hot plate, and $H_2O_2$ was gradually added until the sample turned colorless. Samples were diluted with distilled water, and analytical measurement was performed using a flame photometer (Model-410, Sherwood Scientific Ltd. Cambridge, UK) [93].

### *4.8. Estimation of Yield-Related Traits*

To estimate the effects of SA priming on the yield of wheat plants under stress and control conditions, 1000-grain weight and spike length was recorded. The association of these traits with overall yield was found according to the method suggested by [94].

### *4.9. Statistical Analysis*

This experiment was conducted through a completely randomized design (CRD) with three replicates of each treatment. The data collected for different attributes were analyzed using 3-way ANOVA on USA Statistix (8.1 version) software [95]. The mean treatments were compared at a 5% level of significance. For the graphical work, Microsoft Excel (Version 365) was used.

## 5. Conclusions

The current study aimed to assess the impact of SA seed priming on the morpho-physiological and biochemical attributes of wheat grown in a salinized environment. The results showed that both 10 and 20 mM SA priming treatments were effective and produced positive outcomes compared with those of hydropriming and control treatment. However, 20 mM SA pre-treatment was found to have a more pronounced effect on the growth, photosynthetic pigments, proline, and phenolic content of salt-stressed wheat plants, leading to an improved yield. These findings provide valuable insights into the effectiveness of priming treatments for salt resistance in wheat and can serve as a basis for future research. Further studies should be conducted in open-field settings to gain a better understanding of the combined effects of water and SA priming treatments on wheat and other crops.

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