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# Enhancing wheat resistance to salinity: The role of gibberellic acid and β-carotene in morphological, yielding and ionic adaptations

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## ABSTRACT

Salinity is highly toxic to wheat growth. Both β-carotene and gibberellic acid can mitigate the saline toxicity in wheat, independently and in combined form, by positively impacting growth and yield under saline conditions. To explore our hypothesis two wheat varieties (Faisalabad-2008 and Galaxy-2013) were grown in the sand medium under two levels of salinity (0 mM and 150 mM) nourished with Hoagland's solution and sprayed with four different foliar approaches (water spray = $T_0$, 0.75 mM $GA_3$ = $T_1$, 0.25 mM β-carotene = $T_2$, 0.75 mM $GA_3$ + 0.25 mM β-carotene = $T_3$). Salinity comprehensively decreased the growth metrics of wheat. While foliar applications of 0.75 mM $GA_3$ 0.25 mM β-carotene significantly mitigate this toxicity by physiological alterations. Shoot fresh weight ($p < 0.001$) (maximum at $T_3$, root fresh weight ($p < 0.05$) maximum at $T_2$, Shoot dry weight ($p < 0.01$) maximum at $T_3$ reflect significant mitigation under salinity while root dry weight, shoot length, and root length non-significant response. Leaf calcium ($p < 0.01$) and root sodium ($p < 0.001$) readings showed much positive response at $T_3$. Flavonoids ($p < 0.05$) maximum at $T_3$, free proline ($p < 0.01$) maximum at $T_3$, ascorbic acid ($p < 0.05$) maximum at $T_{3,}$ and seeds weight ($p < 0.001$) Maximum at $T_1$. Overall Faisalabad-2008 performed better in saline conditions when treated with foliar application of $GA_3$ and β-carotene (water spray = $T_0$, 0.75 mM $GA_3$ = $T_1$, 0.25 mM β-carotene = $T_2$, 0.75 mM $GA_3$ + 0.25 mM β-carotene = $T_3$). This study is recommended for the growth of wheat cultivars by foliar applications of $GA_3$ and β-carotene under salt stress.

**Keywords:** salinity stress, wheat, growth regulators, gibberellic acid, β-carotene, ions profile.

## INTRODUCTION

Although wheat is essential for maintaining food and nutritional security, its production is seriously threatened worldwide by the rapidly increasing salt of the land and water (Ahmad et al., 2024). Global warming and climate change have recently increased the frequency and severity of several stresses, which has had a direct impact on crop output and quality. The most important staple crops in the world, wheat, rice, and maize, account for a sizable portion of daily protein and calorie intake (Yin et al., 2024). Due to its domestication and role as the world's main staple food crop, wheat is ranked top among these important cereals (Ulukan, 2024). With 137.7 and 26.4 million metric tons of wheat produced, respectively, Pakistan ranks seventh in the world (Filipenco, 2023). It is grown on around 9.17 million hectares in Pakistan, which satisfies 83.5% of the country's food grain requirement; the remaining 16.4% is imported from other nations (Zawar et al., 2024).

Numerous morphological, physiological, metabolic, and gene expression processes are impacted by salinity as an abiotic stressor (Dixit et al., 2024). Numerous parameters, including species, genotype, plant growth phase, ionic strength, duration and severity of salinity exposure, composition of salt solution, and which plant organ is subjected to stress, influence the extent of this effect (Parihar et al., 2015). Significant decreases in plant growth and biomasses, chlorophyll breakdown, water status disequilibrium, stomatal dysfunction, transpiration and respiration changes, and ion ratio disruptions were all brought on by salinity stress (Arif et al., 2020). Additionally, salt stress causes osmotic stress, ionic toxicity, and oxidative stress in addition to disrupting a variety of enzyme processes, photosynthesis, membrane structure, hormonal balance, water and nutrient uptake, and more (Arif et al., 2020). Gibberellins, of which $GA_3$ is the predominant kind, are significant plant hormones that are extensively employed to control plant growth throughout the whole crop plant life cycle. By encouraging cell growth and elongation, they facilitate plant growth and development, including fruit ripening, nutritional development, and seedling growth transformation. The hormones involved in sorghum growth, physiology, and molecular mechanisms under salt stress have advanced as a result of the rapid development of sorghum research in recent years (Liu et al., 2019).

Plants are shielded from reactive oxygen species and other damage induced by photooxidation by photosynthetic pigments like carotenoid (Foyer, 2018). Because of their antioxidant qualities, carotenoids can scavenge damaging radicals and reactive oxygen species (Alam et al., 2021). In all developing countries, 90% of fruits and vegetables contain carotenoids. In hypoxia, β-carotene primarily scavenges free oxygen radicals. The interaction between carotenoids and hydroperoxy radicals may be mediated by an imbalanced β-carotene. rotenoids are likely indicators that enable plants to implement certain protective strategies under stress condition (Mkindi et al., 2019). It is anticipated that β-carotene and $GA_3$ may have a significant impact in wheat in saline environments. The research aims to investigate the role of β-carotene on morphology, physiology, and biochemistry under saline conditions. The objective of the current study was to assess the effects of $GA_3$ and β-carotene foliar application, both alone and synergistically, on wheat plants subjected to salt stress.

## MATERIALS AND METHODS

Two separate experiments were executed in the net house of the Old Botanical Garden, University of Agriculture Faisalabad to examine the effect of salinity on wheat (*Triticum aestivum* L.) through exogenously application of gibberellic acid and β-carotene. These two trials were completed in year 2021 and 2022 between Novembers to April. Two wheat types, Faisalabad-08 and Galaxy-13, were stressed with 150 mM NaCl in addition to a control. Gibberellic acid (0.75 mM) and β-carotene (0.25 mM) concentrations were applied, along with their combined interaction by foliar treatments. After filling, plastic container holding 8 kg of sand were utilized for seeding. Ten seeds were planted in each pot, and after 25 days, six plants were retained following thinning. After 10 days, plants received full strength Hoagland's nutritional solution. Using four replicates, the trial was conducted using a completely randomized design (CRD. The very next day, foliar treatment was made following salinity stress. Gibberellic acid was collected and combined with 1000 milliliters of distilled water in a flask for foliar spray. Additionally, 0.1 milliliter of V/V% Tween 20 was added to the solution as a surfactant to guarantee optimum tissue saturation and uniform dispersion. In a similar manner, 1000 milliliters of distilled water were combined with β-carotene in a flask. Additionally, 1 milliliter of V/V% Tween 20 was added to the solution as a surfactant to guarantee optimum tissue saturation and uniform dispersion. Likewise, 1 application of both β-carotene and gibberellic acid was added. Similarly, for the combined applications of gibberellic acid + β-carotene were added in 1 L distilled water. Also, 1 ml V/V %Tween 20 was taken as a surfactant and added in the combined solution of gibberellic acid and β-carotene to ensure uniform distribution and maximum tissue saturation.

### Morphological parameters

After 82 days after sowing two out of six plants were uprooted then by using weighing balance machine, weights of shoots and roots of each plant were taken from each trial unit. These plants were then kept in oven at 70 °C until constant weight and dry completely. After that shoot and root dry weight were recorded. Measurements for plant shoot and root length performed on harvesting by using the measuring scale.

### Inorganic ions determination

#### *Digestion method*

Oven dried roots and shoots samples (0.1 g) were placed in 5 ml of sulphuric acid for a night. The next day, mixture was heated on the hot plate at 250 °C. Using the glass pipette, $H_2O_2$

was poured in each flask drop by drop until the material turned colorless. The solution was then cooled and filled with de-ionized water to make the volume up to 50 ml. After that, the mixture was filtered and sodium, calcium and potassium ions for leaf and root were measured using Sherwood flame photometer 410.

### Flavonoids

Kim et al. (1999) protocol was used for the examination of flavonoids concentration. Sample (0.1 g) fresh leaf was ground in 80% of 2 ml acetone in the pestle and mortar. Then the extract was centrifuged at 12000 rpm for fifteen minutes. Test tubes were taken and filled with 2 ml distilled water and 0.5 ml of supernatant. Then after 5 minutes, 5% of 0.6 ml sodium nitrite, 10% of 0.5 ml $AlCl_{3,}$ 2 ml of sodium hydroxide and 2.4 ml distilled water were poured in each test tube. In the end, absorbance was noted at 510 nm using spectrophotometer.

### Leaf free proline

Bates et al. (1973) method was employed for the determination of leaf free proline. Sample (0.5 g) of fresh leaf was ground in 3% of 10 ml sulfosalicylic acid in the pre chilled pestle and mortar. Then filter paper was used for the filtration of extract. A test tube was taken and filled with extract, 2 ml ninhydrin and glacial acetic acid. After that test tubes were placed in the water bath for 1 hour at 100 °C. Then 4 ml toluene was added in the test tubes and the mixture was vortex. In the end, absorbance was noted at 520 nm using spectrophotometer. As a blank reading, 3% sulfosalicylic acid was taken.

### Leaf ascorbic acid

Mukherjee and Chaudhuri (1983) method were employed for the determination of lead ascorbic acid. Almost 0.25 g fresh leaves were ground in 10 ml TCA of 6%. Then test tubes were filled with 2 ml dinitrophenyl hydrazine of 2% and 4 ml extracted solution. The mixture was then heated for 20 minutes. After that, 1 drop of 10% Thiourea was added, and the samples were stored at room temperature and 5 ml of 80% acetone was added in each sample. In the end, absorbance was taken at 530 nm using spectrophotometer.

### Yield attributes

#### *Spike length (cm)*

Spike length was measured from randomly selected two plants from each plot at maturity with the help of measuring scale and averaged thereof.

#### *Spikelet per spike*

Spikelet per spike was measured from randomly selected two plants from each pot at maturity, and their average was computed.

#### *Grains per spike*

The number of grains per spike was counted after threshing manually from randomly selected two plants that used for spikelet per spike, and their average was taken.

### Statistical analysis

Recorded data were investigated by using LSD through Statistic 8.1 software and treatment means were compared under CRD with three-factor factorials. The graphical presentation was done by Origin Pro 2024 and RStudio.

## RESULTS

Imposition of NaCl prominently reduced the shoot fresh weight in both wheat varieties Faisalabad-08 and Galaxy-13 as compared to control conditions. The exogenously applied of gibberellic acid ($GA_3$) and β-carotene showed positive response in mitigation of salinity (150 mM) in both varieties however maximum weight was observed in Faisalabad-08 when treated with combined level of $GA_3$+β-carotene (Fig. 1). Significant varietal difference was marked as Fsd-08 performed better over Galaxy-13 at 150 mM salinity stress. Overall, significant pattern recorded in the interaction of cultivars, salinity and treatments of $GA_3$+β-carotene the shoot fresh weight of wheat cultivars (Table 1; Fig. 1).

Root fresh weight exhibited non-significant effect under 150 mM of salinity as compared to control conditions (Fig. 1). Similarly, foliar treatments with $GA_3$ and β-carotene also showed non-significant behavior for root fresh weight. In contrast, the significant varietal difference was recorded as Faisalabad-08 exhibited much

**Table 1.** Means squares from analysis of variance of data for shoot fresh weight, root fresh, shoot dry, root dry weight, shoot length and root length of wheat (*Triticum aestivum* L.) when $GA_3$ and β-carotene were foliarly applied under salt stress conditions

| SOV | df | Shoot fresh weight | Root fresh weight | Shoot dry weight | Root dry weight | Shoot length | Root length |
|---|---|---|---|---|---|---|---|
| Varieties (V) | 1 | 79.767** | 2.8815*** | 0.1269ns | 0.4064*** | 40.322ns | 19.470ns |
| Salinity (S) | 3 | 144.27*** | 0.1871ns | 0.2569* | 0.2889*** | 1030.41*** | 160.33*** |
| Treatments ($GA_3$/β-carotene) | 3 | 124.8*** | 0.1917ns | 0.1311ns | 0.0239ns | 73.12ns | 23.763*** |
| V × S | 3 | 285.56*** | 0.0203ns | 1.6097*** | 0.0189ns | 8.122ns | 0.9264ns |
| V × $GA_3$/β-carotene | 3 | 79.033*** | 0.6374** | 0.0952ns | 0.0068ns | 69.72ns | 8.0639ns |
| S × $GA_3$/β-carotene | 9 | 112.7** | 0.4976* | 0.1929* | 0.0.118ns | 86.502ns | 6.5868ns |
| V × S ×$GA_3$/ β-carotene | 9 | 30.007* | 0.1307ns | 0.1784* | 0.0051ns | 28.086ns | 9.1784ns |
| Error | 96 | 8.7605 | 0.1509 | 0.0622 | 0.0092 | 31.936 | 4.6691 |

**Note:** ns – non-significant; *, **, *** – significant at 0.05, 0.01 and 0.001 levels, respectively.

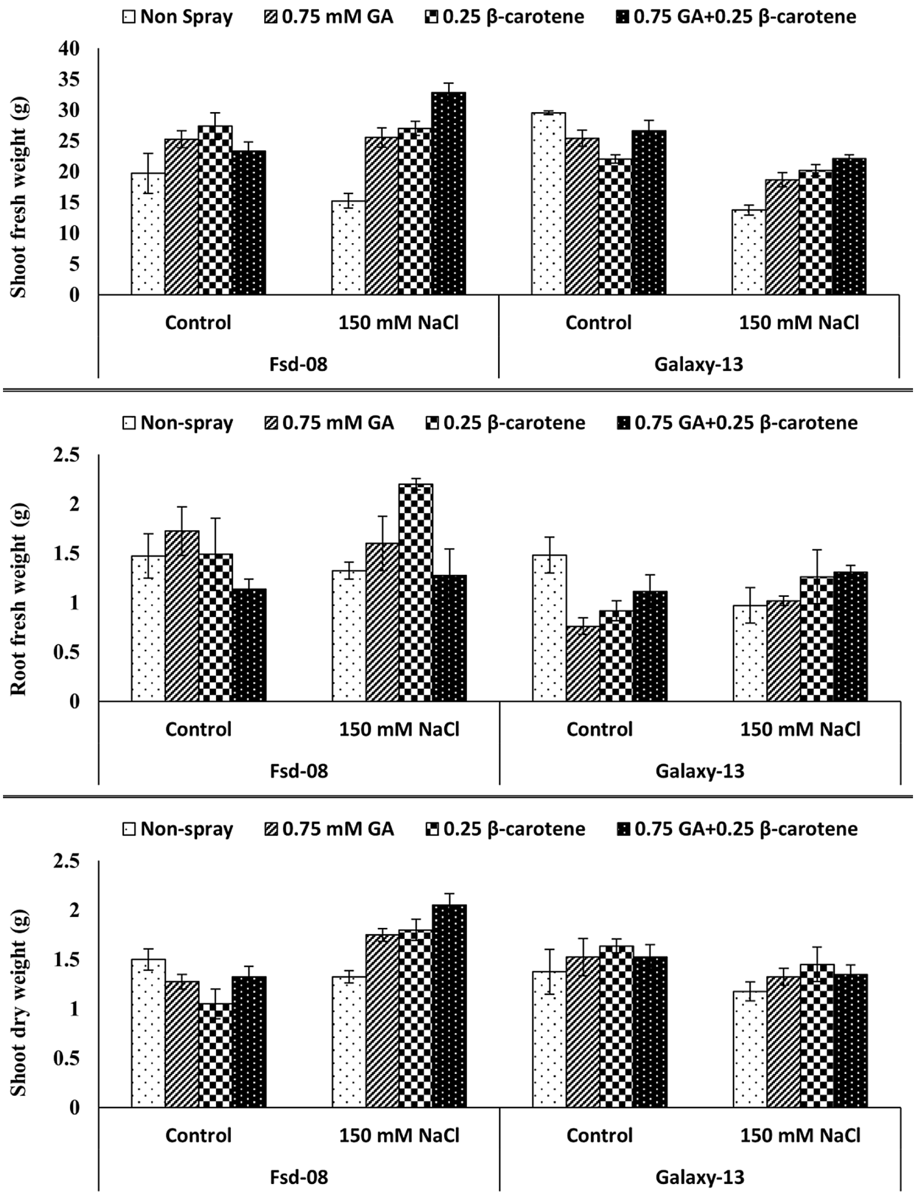


**Figure 1.** Shoot fresh, root fresh and shoot dry weight of wheat (*Triticum aestivum* L.) plants when $GA_3$ and β-carotene were foliarly applied under salt stress conditions

promising results under salinity (150 mM). Significant interaction between genotypes and $GA_3$/β-carotene recorded as maximum results marked for Faisalabad-08 when treated with β-carotene (0.25 mM). Root fresh weight of both varieties reflected significant difference in their mitigation, when imposed with 150 mM of NaCl, highest root fresh weight was marked in Faisalabad-08 when sprayed with β-carotene independently. Non-significant interaction between the stress, foliar treatments and varieties was observed for root fresh weight of wheat (Table 1; Fig. 1).

Shoot dry mass of two wheat varieties considerably decreased by the imposition of 150 mM salinity. (Fig. 1). Much reduction was recorded for Galaxy when treated with combined sprayed of $GA_3$+β-carotene (0.75 mM + 0.25 mM). Non-Significant varietal difference was observed while foliar treatments did significantly mitigate the adverse effects of salinity, much increase was observed in Faisalabad-08 when foliarly applied with combined $GA_3$+β-carotene. Overall, interaction between the varieties, salinity and foliar treatments was recorded slightly significant for shoot dry weight (Table 1; Fig. 1).

Analysis of variance for data regarding root dry weight showed considerable decrease, when exposed to 150 mM salt (Fig. 2). Foliar application of $GA_3$ (0.75 mM), β-carotene (0.25 mM) as well as their combination of $GA_3$+β-carotene

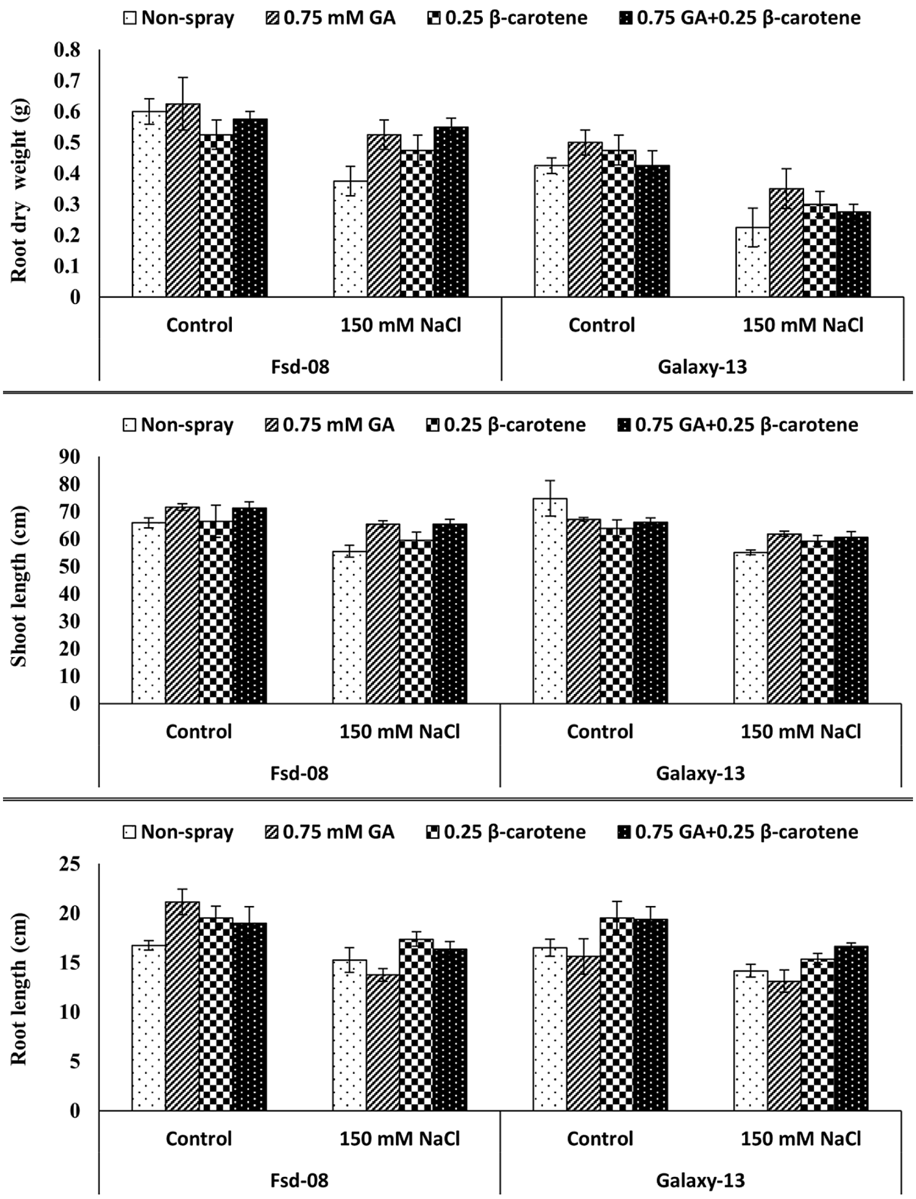


**Figure 2.** Root dry weight, shoot length and root length of wheat (*Triticum aestivum* L.) plants when $GA_3$ and β-carotene were foliarly applied under salt stress conditions

(0.75 mM + 0.25 mM) did not show significant effect in root dry mass of wheat varieties. Genotypic difference was non-significant for this attribute. Non-significant trend was recorded, for the overall interactions among stress, stress regulators and varieties (Table 1; Fig. 2).

Salt stress significantly reduced the shoot length of wheat varieties. Foliar treatments didn't affect this parameter significantly under saline conditions. Similar behavior was recorded for the varietal difference (Fig. 2). Parameters of wheat shoot length remarked the non-significant expression for the overall interaction between the salinity, foliar and the varieties. Foliar applications of $GA_3$, β-carotene and $GA_3$+β-carotene (0.75 mM+ 0.25 mM) didn't affect significantly to shoot length of wheat under salt stress (Table 1; Fig 2).

Root length significantly decreased by the exposure of salinity in wheat plants (Table 1). Varietal difference was non-significant for the root length parameter. Foliar applications of $GA_3$, β-carotene and $GA_3$+β-carotene (0.75 mM + 0.25 mM) did show significant response. Similarly, non-significant behavior recorded for the overall interaction among the varieties, salt stress and foliar treatments (Table 1; Fig 2).

Leaf $Ca^{2+}$ showed much considerable decrease under saline conditions (Table 2). At 150 mM salinity Galaxy-13 represented minimum contents of leaf calcium when not treated with any of the foliar treatments (Fig. 3). Foliar application significantly enhanced leaf $Ca^{2+}$ ions in both wheat varieties under saline on the other end overall interaction among the all the variables were non-significant (Table 2; Fig 3).

Leaf potassium in wheat significantly decreased in both varieties, much reduction was recorded in Faisalabad-08 when not treated with any foliar treatment (Fig. 2). High significant difference was observed among both wheat varieties for this attribute, as more leaf potassium ions recorded in Galaxy-13. Foliar applications of β-carotene (0.25 mM), gibberellic acid (0.75 mM), and the combination of $GA_3$+β-carotene (0.75 mM + 0.25 mM) showed positive contribution for both varieties but non-significant role for the salt toxicity. However, for the leaf potassium, the connection between the stress, foliar, and varieties was clearly not significant (Table 2; Fig 3).

Leaf $Na^+$ increased significantly in both wheat varieties when sodium chloride (150 mM) stress was imposed, with much destruction in Galaxy-13 when not provided with any of the foliar applications. Varietal difference was significant as Galaxy-13 showed high leaf $Na^+$ profile than Faisalabad-08 accessions. Conversely, there was no statistically significant interaction between any of the covariates. Application of foliar treatments of $GA_3$ (0.75 mM), β-carotene (0.25 mM) and $GA_3$+β-carotene (0.75 mM+ 0.25 mM) applied $GA_3$+β-carotene showed significant decrease for this attribute (Table 2; Fig. 3).

In both varieties, root $Ca^{2+}$ contents significantly dropped under stress (Table 2). The Galaxy-13 variety with non-spray showed the lowest root $Ca^{2+}$ concentration in saline treatment (Fig. 4). The foliar administration of β-carotene at a concentration of 0.25 mM, gibberellic acid at a concentration of 0.75 mM, and the combination of $GA_3$+β-carotene at a concentration of 0.75 + 0.25 mM showed a positive impact on both varieties. Other interactions among all the variables suggest that the results for this root calcium ions profile are not statistically significant. Similarly, inconsiderable varieties difference was recorded (Table 2; Fig 4).

**Table 2.** Means squares from analysis of variance of data for leaf $Ca^{2+}$, Leaf $K^+$, Leaf $Na^+$, root $Ca^{2+}$, root $K^+$, root $Na^+$ of wheat (*Triticum aestivum* L.) when $GA_3$ and β-carotene were foliarly applied under salt stress conditions

| SOV | df | Leaf $Ca^{2+}$ | Leaf $K^+$ | Leaf $Na^+$ | Root $Ca^{2+}$ | Root $K^+$ | Root $Na^+$ |
|---|---|---|---|---|---|---|---|
| Varieties (V) | 1 | 252.01*** | 234.47*** | 71.191*** | 0.0351ns | 17.5535** | 89.066*** |
| Salinity (S) | 3 | 129.39*** | 286.87*** | 197.75*** | 65.003*** | 326.25*** | 815.81*** |
| Treatments ($GA_3$/β-carotene) | 3 | 13.781*** | 40.201*** | 23.045*** | 8.6601*** | 20.378*** | 9.8372* |
| V × S | 3 | 0.5625ns | 1.4101ns | 0.0976ns | 0.4726ns | 1.1289ns | 30.941** |
| V × $GA_3$/β-carotene | 3 | 1.4635ns | 4.8684ns | 0.0768ns | 1.8580ns | 1.3268ns | 34.16*** |
| S × $GA_3$/β-carotene | 9 | 4.9427** | 0.7539ns | 1.5559ns | 0.3898ns | 2.983ns | 22.555*** |
| V × S × $GA_3$/ β-carotene | 9 | 0.2604ns | 0.4518ns | 3.1080ns | 0.3372ns | 3.3997ns | 8.9934* |
| Error | 96 | 1.1380 | 1.9361 | 2.6966 | 0.6731 | 1.621 | 2.608 |

**Note:** ns – non-significant; *, **, *** – significant at 0.05, 0.01 and 0.001 levels, respectively.

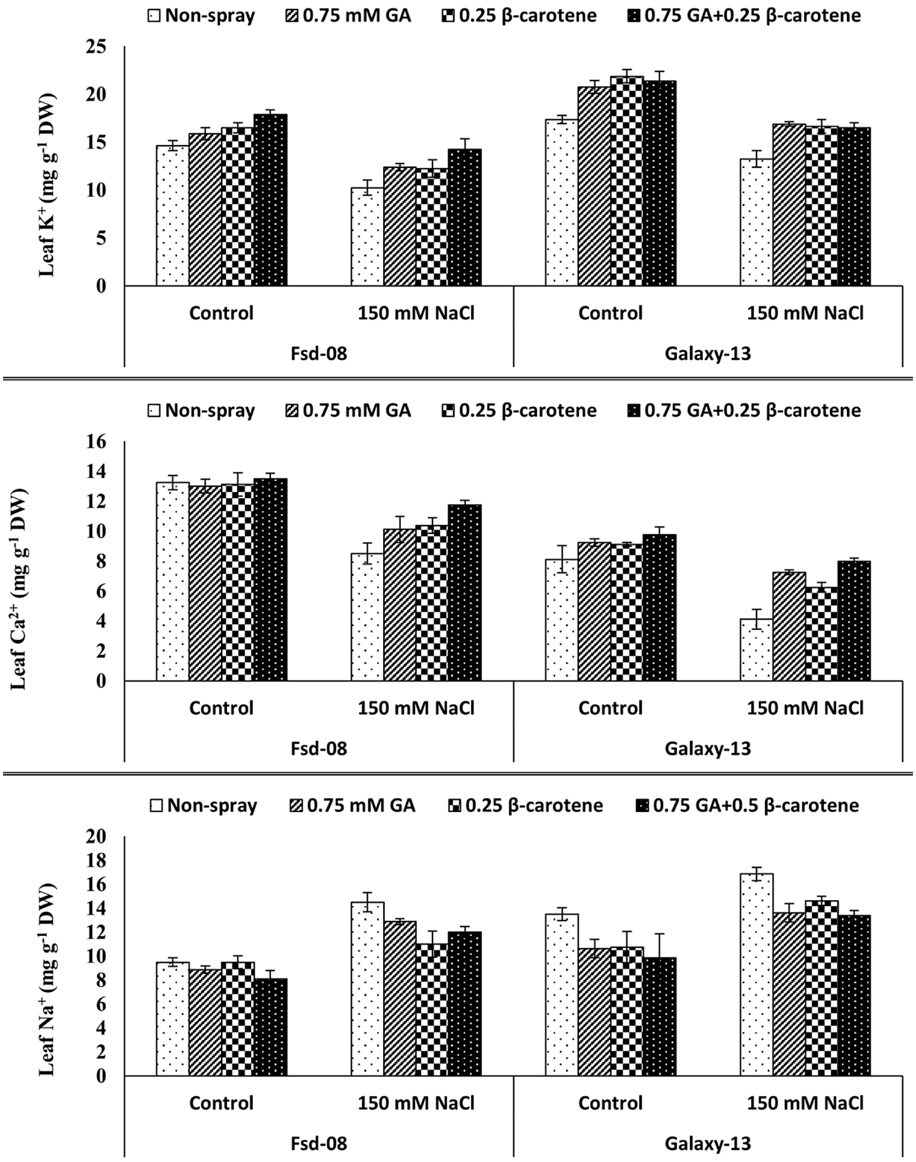


**Figure 3.** Leaf $Ca^{2+}$, leaf $K^+$ and leaf $Na^+$ of wheat (*Triticum aestivum* L.) plants when $GA_3$/β-carotene were foliarly applied under salt stress conditions

The application of sodium chloride resulted in a notable reduction in the potassium ($K^+$) levels in the roots of both wheat varieties Faisalabad-08 and Galaxy-13. The greatest decrease was reported in Galaxy-13 when it was not subjected to any of the foliar spray treatments (Fig. 4). The wheat varieties exhibited a highly significant response when subjected to foliar treatment of $GA_3$ (0.75 mM), β-carotene (0.25 mM), and $GA_3$+β-carotene (0.75 mM + 0.25 mM). Nevertheless, the interaction between stress, foliar factors, and cultivars was observed to be statistically significant. Similarly, there was no significant variation in genotype for the potassium contents of the roots (Table 2; Fig 4). A notable disparity in root sodium ions was noted when subjected to 150 mM salinity stress, with the Galaxy-13 variety exhibiting the highest root $Na^+$ ions. The application of salinity resulted in a substantial increase in root sodium $Na^+$ levels. A little variance was observed, with the Faisalabad-08 variety showing a lower concentration of $Na^+$ in its roots. The application of exogenous foliar treatments also demonstrated a beneficial response for these parameters. The Faisalabad-08 variety exhibited the lowest increase when treated with a combination spray of gibberellic acid and beta carotene (Table 2; Fig. 4).

The data analysis revealed a significant rise in leaf flavonoid content under salinized conditions, with Galaxy-13 exhibiting higher levels of flavonoid content (Table 5). Figure 10 showed that Galaxy-13 had significantly higher varietal expression

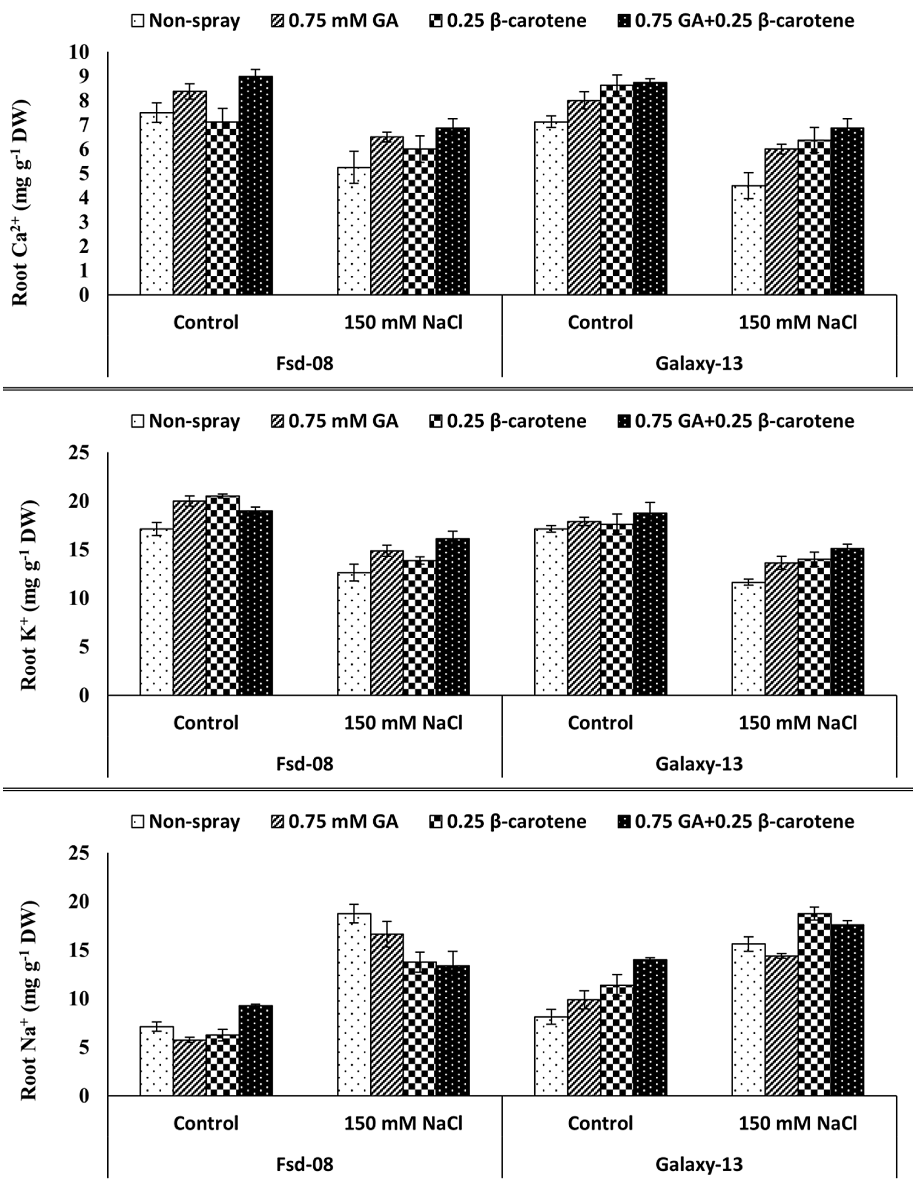


**Figure 4.** Root $Ca^{2+}$, $K^+$ and $Na^+$ of wheat (*Triticum aestivum* L.) plants when $GA_3$/β-carotene were foliarly applied under salt stress conditions

indicated for flavonoids than the Faisalabad-13 variety. Foliar treatments had a considerable impact on the flavonoids in leaves of wheat varieties, as Galaxy-13 represented more flavonoids contents when $GA_3$+β-carotene (0.75+ 0.25 mM) collectively applied under salt treatments. Conversely, there was no significant interaction overall between any of the covariates (Table 5; Fig 10).

Free proline concentrations were remarkable increased in wheat cultivars when administrated with 150 mM of salinity, much rise was recorded in Faisalabad-08 when treated with β-carotene (Fig. 5). Highly significant difference was observed among both varieties for this attribute, as more for proline was recorded in Faisalabad-08. Foliar applications of β-carotene (0.25 mM), gibberellic acid (0.75 mM) and the combination of $GA_3$+β-carotene (0.75 + 0.25 mM) showed notable increase in free proline for both varieties. Similarly, for the leaf free proline, the interaction between the stress, foliar treatments and varieties was significant (Table 3; Fig 5). Application of foliar treatments of $GA_3$ (0.75 mM), β-carotene (0.25 mM) and $GA_3$+β-carotene (0.75 mM + 0.25 mM) showed significant effect on leaf ascorbic acid, as maximum ascorbic acid was recorded in Faisalabad-13 when sprayed with β-carotene (0.25 mM) under salinity. Ascorbic acid increased highly significantly in both wheat varieties when sodium chloride (150 mM) stress was imposed (Fig. 5).

**Table 3.** Mean squares from analysis of variance of data for flavonoids, free proline, ascorbic acid, seeds weight, spikelets per spike and spike length of wheat (*Triticum aestivum* L.) when $GA_3$+β-carotene were foliarly applied under salt stress conditions

| SOV | df | Flavonoids | Free proline | Ascorbic acid | Seeds weight | Spikelet per spike | Spike length |
|---|---|---|---|---|---|---|---|
| Varieties (V) | 1 | 1.7895*** | 1.3618** | 0.2245*** | 454.48*** | 74.39*** | 0.4389ns |
| Salinity (S) | 3 | 3.3791*** | 21.874*** | 0.1498*** | 15.347*** | 66.015*** | 1.8564** |
| Treatments ($GA_3$/β-carotene) | 3 | 1.1223*** | 8.9356*** | 0.0129*** | 455.74*** | 10.098* | 0.2343ns |
| V × S | 3 | 0.2482* | 0.6158* | 0.0014ns | 0.0558ns | 3.5156ns | 0.4064ns |
| V × $GA_3$/β-carotene | 3 | 0.0184ns | 0.3312ns | 0.0034ns | 117.64** | 2.3906ns | 0.1639ns |
| S× $GA_3$/β-carotene | 9 | 0.2043* | 0.6097** | 0.0059* | 221.92*** | 4.9322ns | 0.1855ns |
| V × S × $GA_3$/β-carotene | 9 | 0.0827ns | 0.4077* | 0.0031ns | 6.9646ns | 2.0756ns | 0.2955ns |
| Error | 96 | 0.0601 | 0.1185 | 0.0071 | 18.667 | 2.4513 | 0.1939ns |

**Note:** ns – non-significant; *, **, *** – significant at 0.05, 0.01 and 0.001 levels, respectively.

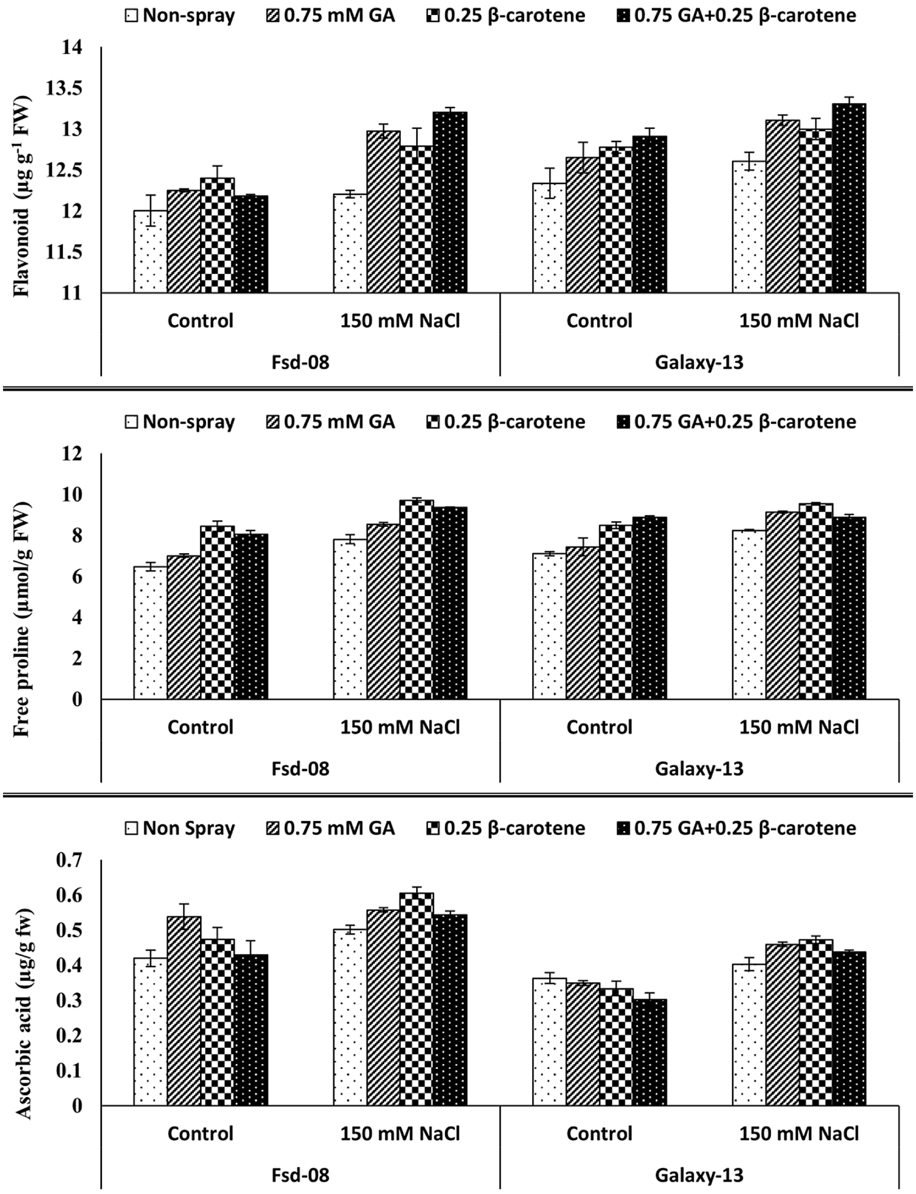


**Figure 5.** Leaf flavonoids, free proline and ascorbic acid of wheat (*Triticum aestivum* L.) plants when $GA_3$/β-carotene were foliarly applied under salt stress conditions

Varietal difference was significant as Faisalabad-08 showed more ascorbic acid profile than Galaxy-13 accessions. Overall, interaction was non-significant among the salinity, foliar treatments and varieties for the ascorbic acid (Table 3; Fig. 5).

In both varieties of wheat salinity (150 mM) stress exposure notably decreased the grain yield (Fig. 6). A strong varietal difference was observed as Faisalabad-08 performed better for this attribute. Foliar applications of $GA_3$ (0.75 mM), β-carotene (0.25 mM) and $GA_3$+β-carotene (0.75 mM + 0.25 mM) markedly mitigated the toxic impact of 150 mM salt stress. Highest grain yield observed in Faisalabad-08 under saline conditions, when sprayed with independent $GA_3$ (0.75 mM). Overall interactions among all the variables suggest that the results for grain weights are not statistically significant (Table 3; Fig. 6). Data analysis

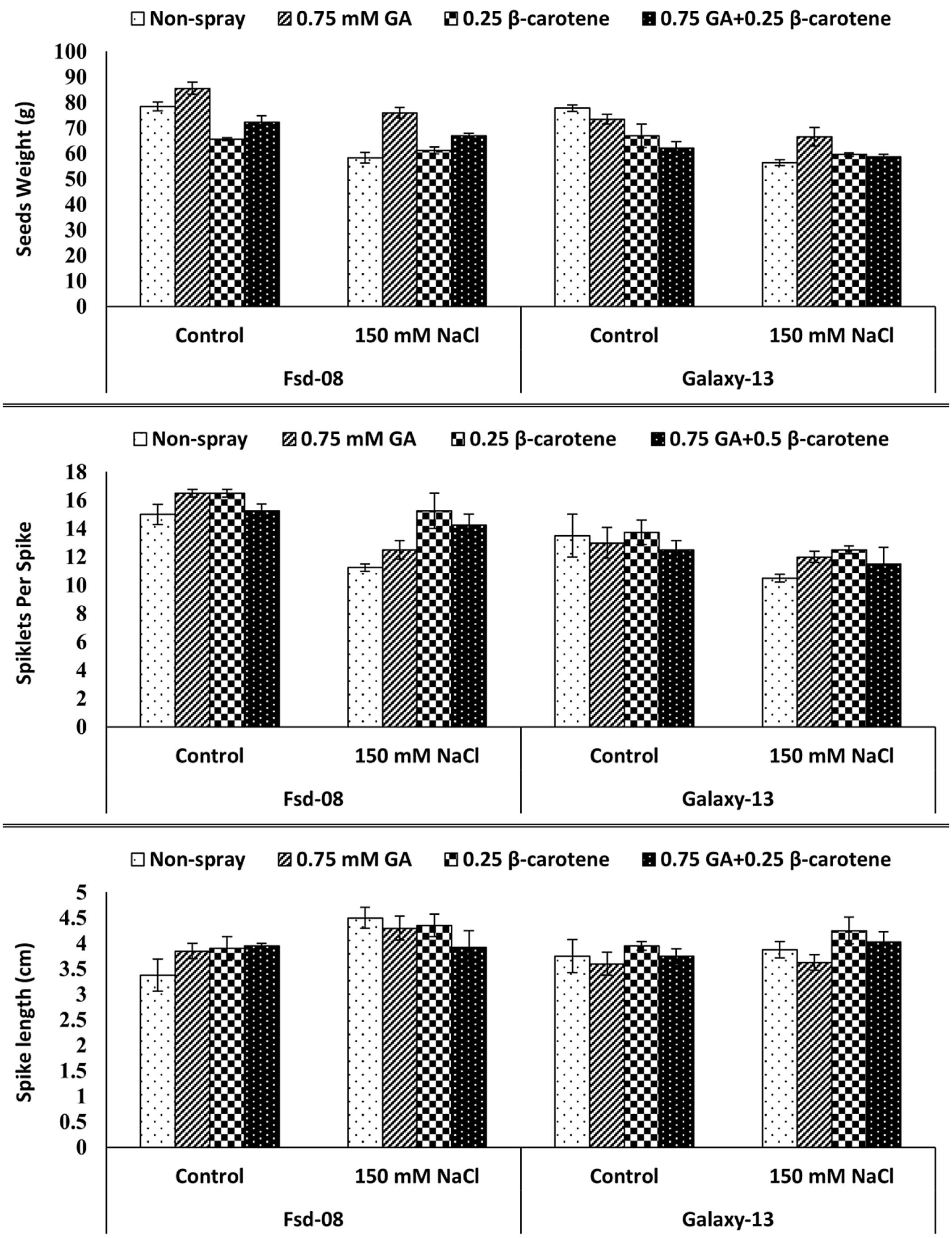


**Figure 6.** Seed weight, spikelets per spike and spike length of wheat (*Triticum aestivum* L.) plants when $GA_3$/β-carotene were foliarly applied under salt stress conditions

for the Spikelets per spike showed that 150 mM salt stress comprehensively decreased this yielding parameter. Highest reduction was observed in Galaxy-13 variety under saline conditions when not treated with any of the foliar treatments (Fig. 6). A notable positive impact was observed by foliar applications of $GA_3$ (0.75 mM), β-carotene (0.25 mM) and $GA_3$+β-carotene (0.75 mM + 0.25 mM) in mitigation of saline toxicity for this parameter. Data revealed noteworthy difference among both varieties related to spikelets per spike of the wheat cultivars (Faisalabad-08 and Galaxy-13). Conversely, there was no significant interaction overall between any of the covariates for the spikelets per spike of wheat varieties (Table 3; Fig. 6).

Non-significant data for exogenously applied foliar treatments recorded in the spike length of wheat varieties under saline and non-saline conditions (Table 3). Similarly, non-significant relationship recorded for the varietal difference. Imposition of salinity (150 mM) remarkably suppressed the spike length in both varieties of wheat. Overall interaction was also non-significant among the salt stress (150 mM), foliar treatments of $GA_3$ (0.75 mM), β-carotene (0.25 mM) $GA_3$+β-carotene (0.75 mM + 0.25 mM) and wheat cultivars (Faisalabad-08 and Galaxy-13) for this attribute (Table 3; Fig. 6).

## Analysis of correlation among the wheat pre-sowing vs. foliar applications

The correlation heatmaps visually represent the relationships between different plant growth and physiological parameters for pre-sowing and foliar applications. The colors indicate the strength and direction of the correlation: The correlation analysis presented in Figure 7 illustrate the relationships between various morphological and ionic traits of wheat under saline stress conditions. The study investigates the role of gibberellic acid ($GA_3$) and β-Carotene in mitigating salinity effects, focusing on shoot and root growth parameters, ionic composition, and physiological traits. A critical observation is the positive correlation of Ca and K with shoot and root growth, suggesting their protective role in counteracting sodium toxicity. Potassium (K) maintains osmotic balance and enzyme activation, which are vital for plant growth under salinity stress. The strong positive correlation between proline content and stress indicators such as root sodium (root Na) highlights its role as an Osmo protectant under saline conditions. Moreover, the spike length (spike L) and spikelets per spike (SPS) exhibit significant correlations with root and shoot parameters, indicating the impact of early growth and nutrient uptake on reproductive success. The foliar application heatmap suggests that the exogenous application of $GA_3$ and β-carotene influences these parameters by promoting nutrient uptake and stress tolerance. In conclusion, the correlation analysis underscores the importance of calcium and potassium homeostasis, root vigor, and osmolyte accumulation in mitigating salinity stress in wheat. The positive effects of foliar-applied $GA_3$ and β-carotene highlight their potential as effective agronomic strategies to enhance wheat resilience under saline environments.

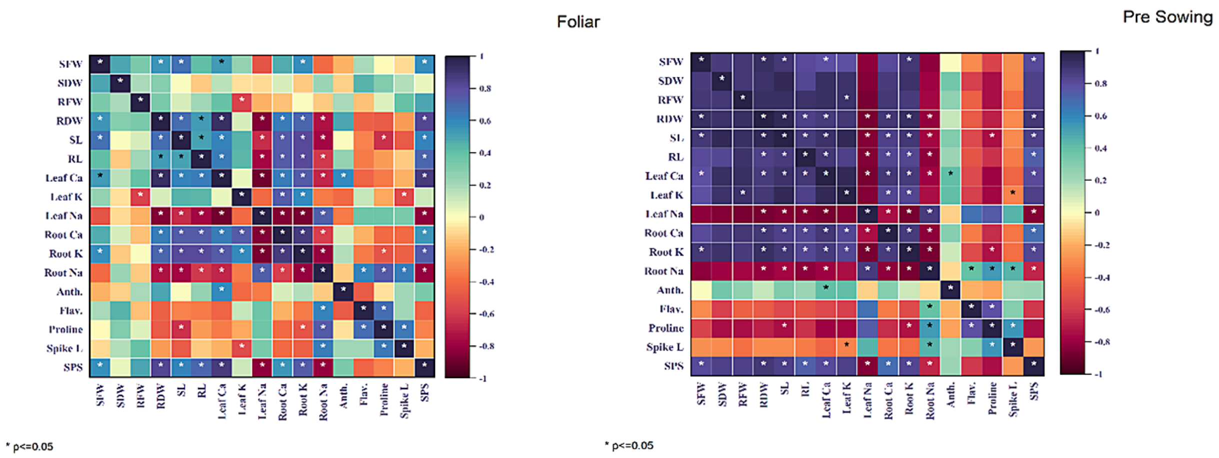


**Figure 7.** Analysis of correlation among the pre-sowing vs. foliar applications, shoot fresh weight (SWF), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), shoot length (SL), root length (RL), calcium (Ca), potassium (K), sodium (Na), anthocyanin (Anth.), flavonoids, proline, spike length, spikelets per spike

### Chord graph among the wheat pre-sowing vs. foliar applications

The chord diagrams illustrate the relationships between different variables in pre-sowing and foliar applications. Each segment of the circle represents a variable, and the connections between them show correlations or interactions. Pre-sowing chord diagram shows relationships between various physiological and biochemical traits before sowing. Strong connections are observed between different plant growth parameters such as shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW). Calcium (Ca), potassium (K), and sodium (Na) exhibit strong correlations with other traits, particularly those related to root and leaf health. Biochemical traits like anthocyanin (Anth.), Flavonoids, and Proline show linkages with physiological traits, indicating their role in stress response and growth.

Foliar chord diagram represents the relationships between the same set of traits after foliar application. The pattern of linkages shifts slightly compared to pre-sowing, indicating the effect of foliar treatments on plant growth and biochemical composition. Traits such as shoot and root length (SL, RL) show different interaction patterns, suggesting the influence of foliar application on elongation and biomass accumulation. Nutrients like Ca, K, and Na continue to show strong associations with other plant traits, but the strength and number of connections might differ compared to the pre-sowing stage. Comparison of pre-sowing vs. foliar applications remain consistent across both diagrams, showing stable relationships between traits. Changes in linkages highlight the impact of foliar application on plant development, potentially enhancing or modifying trait interactions. The chord diagrams help visualize how foliar treatments influence nutrient uptake, biomass distribution, and biochemical responses (Fig. 8).

### Heat-map among the wheat pre-sowing vs. foliar applications

Heatmaps for pre-sowing and foliar applications provide a comprehensive visual representation in (Fig. 9) of how different physiological and biochemical traits respond to treatments with $GA_3$ and β-carotene under salinity stress. These heatmaps allow us to observe patterns of similarity and divergence among traits across different treatments. Pre-sowing heatmap analysis shows the hierarchical clustering groups traits based on their response similarity. Biomass-related traits (SFW, SDW, RFW, RDW) cluster together, indicating a shared response to pre-sowing treatment. Sodium (Na) is distinctly separated from beneficial elements like potassium (K) and calcium (Ca), highlighting $Na^+$ antagonistic role in plant stress physiology. Higher leaf and root $K^+$ concentrations are associated with better shoot and root growth, showing the importance of potassium homeostasis under stress. Proline, an Osmo protectant, clusters near stress-related traits, reinforcing its protective role in mitigating salinity stress. Anthocyanins and flavonoids, known for their antioxidant properties, are actively involved in salinity stress resistance, forming

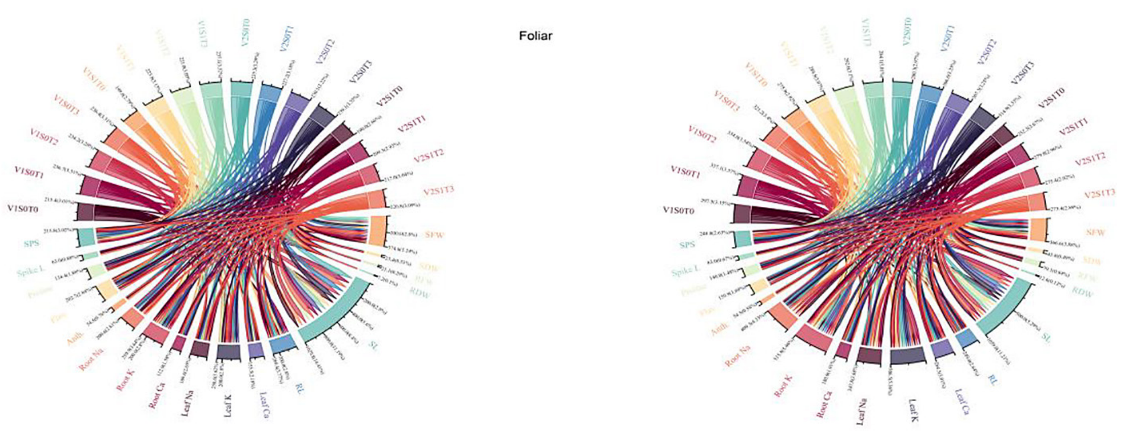


**Figure 8.** Chord graph among the pre-sowing vs. Foliar applications, shoot fresh weight (SWF), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RFW), shoot length (SL), root length (RL), calcium (Ca), potassium (K), sodium (Na), anthocyanin (Anth.), flavonoids, proline, spike length, spikelets per spike

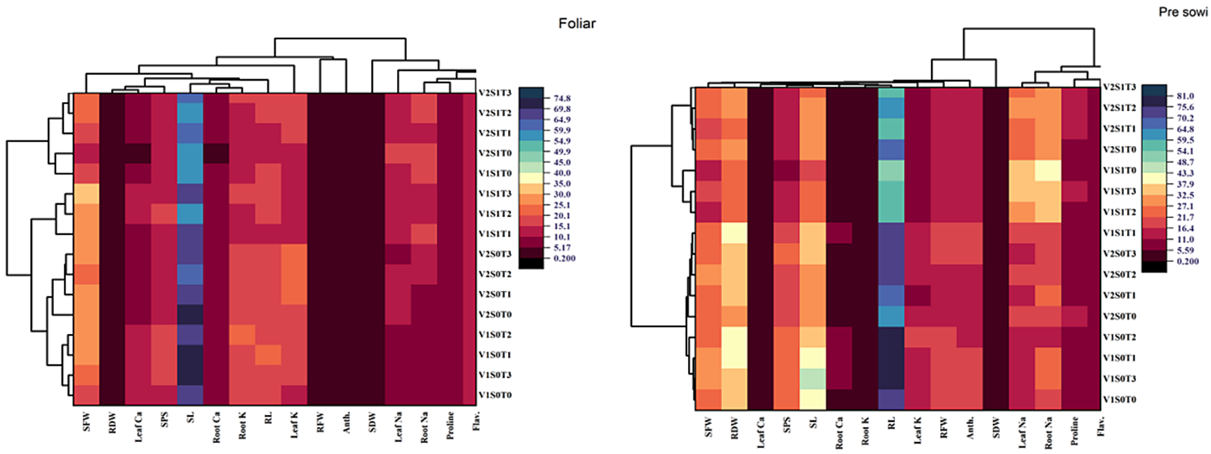

**Figure 9.** Heat-map among the pre-sowing vs. foliar applications, shoot fresh weight (SWF), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RFW), shoot length (SL), root length (RL), calcium (Ca), potassium (K), sodium (Na), anthocyanin (Anth.), flavonoids, proline, spike length, spikelets per spike

unique clusters. Foliar application heatmap analysis biomass and growth correlations, unlike pre-sowing treatment, shoot length (SL) and spike length (SPS) are more pronounced in foliar application, suggesting foliar $GA_3$ and β-carotene enhance shoot development post-germination. Root-related traits (root Ca, root K, root Na) show similar clustering patterns but with a slightly different intensity distribution compared to pre-sowing. Sodium influence and treatment effectiveness. Foliar-applied treatments result in a reduced accumulation of $Na^+$ in shoots and roots, implying an improved ionic balance compared to pre-sowing. Potassium (K) clustering with shoot biomass traits (SFW, SDW, SL) suggests its enhanced uptake with foliar $GA_3$ and β-carotene application. Proline and secondary metabolites. Proline shows higher expression in foliar-treated plants, indicating its role in stress adaptation when applied at later growth stages. Anthocyanins and flavonoids are actively clustered near $Na^+$, suggesting their involvement in oxidative stress mitigation. Pre-sowing treatment is more effective for root development and early-stage stress adaptation by regulating ionic balance. Foliar application enhances shoot elongation, spike length, and overall biomass retention, making it a stronger post-germination strategy. The heatmap suggests both treatments contribute uniquely to mitigating salinity stress in wheat, with foliar applications having a stronger impact on shoot-related traits and stress resistance mechanisms.

### Principal component analysis among the wheat pre-sowing vs. foliar applications

Pre-sowing PCA interpretation – the first principal component (PC1) accounts for 48.3% of the variation, while the second principal component (PC2) contributes 16.6%, explaining a significant proportion of the dataset's variability. Variables like shoot fresh weight (SFW), shoot dry weight (SDW), root length (RL), and calcium (Ca) are positively correlated and positioned towards the right, suggesting their strong influence under pre-sowing conditions. Sodium (Na) in root and leaves, along with Proline and Flavonoids, are grouped on the left side, indicating their inverse relation to growth-promoting traits. Two distinct clusters are observed, representing V1 (black points) and V2 (red points), with 95% confidence ellipses, suggesting significant differentiation between the two groups in response to treatments.

Foliar PCA interpretation – the overall variance explained remains the same (PC1: 48.3%, PC2: 16.6%), indicating that the major factors influencing growth remain consistent across pre-sowing and foliar treatments. Traits such as shoot length (SL), root K, and root Ca show a positive correlation in the right quadrant, while leaf Na, root Na, and Proline cluster on the left, implying stress-related responses. Spike length and spikelets per spike (SPS) appear closely associated with overall plant vigor, particularly under foliar treatments. The clustering pattern remains similar, but a slight shift in loadings suggests that foliar application might exert a different level of influence on specific traits compared to pre-sowing.

Both PCA plots indicate that growth-related parameters (SFW, SDW, RL, SL, and K) are positively correlated, whereas Na accumulation and Proline are inversely related to these traits. Foliar application appears to exert a slightly distinct effect, particularly on spike-related traits (spike L, SPS), suggesting a potential enhancement in reproductive growth. These findings highlight the importance of GA3 and β-carotene applications in mitigating salinity stress and promoting plant vigor through improved morphological and ionic balance (Fig. 10).

## DISCUSSION

The application of salt stress at a concentration of 150 mM through the rooting media resulted in detrimental effects on the morphological attributes of wheat varieties in the present investigation. The current experimental findings align with the results obtained from various crops like rice and rapeseed by Adamski et al. (2020) and Rahnama et al. (2019). Salinity stress hampers the growth of shoot and root masses due to the interference of salt concentrations in the soil with water absorption by the roots. This disruption results in diminished cell expansion and elongation (Bhat et al., 2020). Consequently, both shoot and root growth are inhibited (Munns & Gilliham, 2015). Our analysis revealed a decrease in salinity in both the shoots and roots when measured in terms of fresh weight. Similar findings were reported by Ahmed et al. (2022) in okra, Ahmed et al. (2022) in wheat, and Parvez et al. (2020) in quinoa. The decrease in plant growth is mainly by osmotic stress, ion toxicity, and secondary consequences such as nutritional imbalance and oxidative damage (El Sabagh et al., 2021). Plants under salt stress prioritize osmotic adjustment and ion exclusion mechanisms above biomass production, leading to a decrease in fresh weight (Balasubramaniam et al., 2023). Salinity stress frequently results in a reduction in leaf area. This decline can be attributed to many mechanisms, such as diminished cellular expansion, accelerated ageing of leaves, and decreased leaf formation (Yadav et al., 2011). In addition, the presence of high salt levels can negatively impact the process of photosynthesis, resulting in a reduction in the size of leaves as the plant allocates less resources to photosynthetic tissues (Munns et al., 2020). Our research revealed that the leaf area of both wheat varieties reduced when treated to a salt concentration of 150 mM in the rooting of plant. This is consistent with the findings of Zeeshan et al. (2020) in maize and Breś et al. (2022) in lettuce. The current work has demonstrated that the administration of $GA_3$ and β-carotene stimulates the elongation of shoot and root growth under salt conditions. Ahanger et al. (2017) reported that antioxidants can assist plants in expanding their search for water and nutrients in a greater area of soil, potentially reducing the negative impact of salinity stress on root growth (Seleiman et al., 2022). According to Egamberdieva et al. (2017), treating plants with $GA_3$ has been showed to boost leaf expansion and increase leaf area. This can improve the plant's ability to capture light and promote

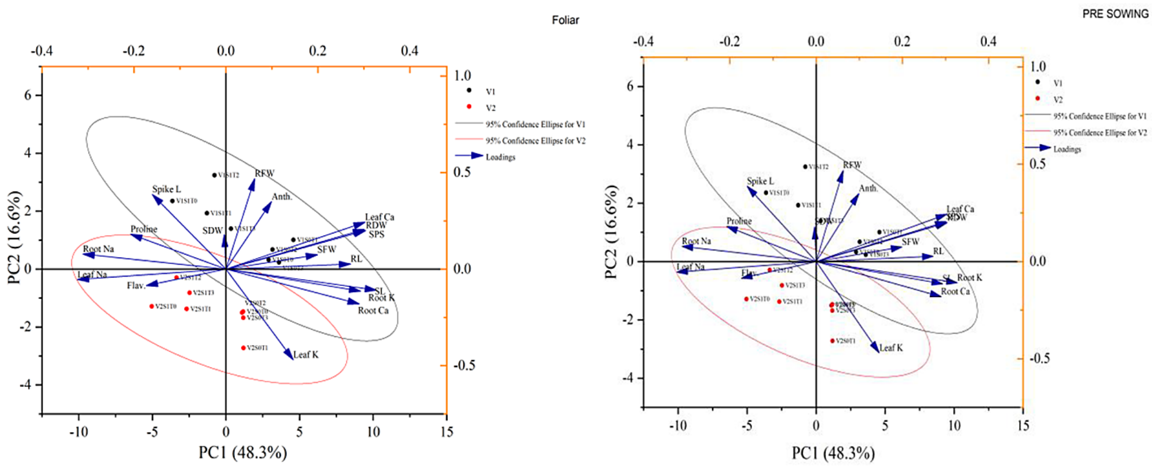


**Figure 10.** Principal component analysis among the pre-sowing vs. foliar applications**,** shoot fresh weight (SWF), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RFW), shoot length (SL), root length (RL), calcium (Ca), potassium (K), sodium (Na), anthocyanin (Anth.), flavonoids, proline, spike length, spikelets per spike

photosynthetic efficiency, which in turn helps to counteract the detrimental impact of salinity stress on leaf growth. In this experiment, the application of $GA_3$ and β-carotene mitigated the deleterious effects of salt on the shoot and root mass of wheat cultivars, as previously reported Rauf et al. (2022) in wheat plants. Beta-carotene mitigates oxidative stress, whilst $GA_3$ promotes growth processes. Collectively, they alleviate the detrimental consequences of salt, resulting in enhanced shoot and root biomass Voytas et al. (2023).

Salinity stress disrupts the ionic concentration by causing the absorption of inorganic ions such as $Cl^-$ and $Na^+$ from the growth media (Djanaguiraman and Prasad, 2012). The ongoing analysis has revealed that salinity stress lead enhancement of NaCl, while causing a decrease in the accumulation of calcium and potassium in both roots and shoots. Similarly, multiple investigations, including those conducted by Elkarim et al. (2010), Farooq et al. (2015), Yadav et al. (2011) have reported the same results. Excessive accumulation of the inorganic ions sodium and chloride in leaves can be hazardous to plants and disrupts important cellular processes such as protein synthesis and photosynthetic reactions (Arif et al., 2020). The presence of high concentrations of $Na^+$ and $Cl^-$ in soil disrupts the uptake of vital ions such as $K^+$ and $Ca^{2+}$ and $NO_3$ from the soil (Hussain et al., 2016; Osman and Osman, 2013). A high concentration of salts in the soil increases the osmotic pressure, which hinders the absorption of water and essential nutrients (such as potassium and calcium) by plant cells. This has been demonstrated in studies by Sardans and Peñuelas (2021), Wang et al. (2013), Nieves-Cordones et al. (2016). Potassium ($K^+$) is an essential macronutrient that plays a crucial role in plant growth and metabolism. The level of potassium in plants serves as a reliable measure of their ability to with stand against to salinity. According to Sardans and Peñuelas (2021), the ability to withstand physiological stress is mentioned. Prior studies (Karthika et al., 2018; Malvi, 2011) have shown that reduced $Na^+$ absorption led to enhanced levels of potassium ($K^+$) and calcium ($Ca^{+2}$) in both the shoot and root. $GA_3$ plays a crucial role in higher plants by regulating various important biological functions, including enhancing nutrient uptake and decreasing $Na^+$ concentrations, under conditions of salt. In a study conducted by Iqbal et al. (2022), it was observed that using a concentration of 0.75 mM of $GA_3$ can reduce the harmful effects of salt on the concentrations of $Na^+$ and $K^+$ in wheat leaves. Additionally, it has been revealed that this concentration of $GA_3$ can also decrease the $Na^+$ content in wheat leaves when exposed to high levels of NaCl (150 mM). The study revealed that applying foliar $GA_3$ supplementation at a concentration of 0.75 mM resulted in a decrease in the uptake of sodium ions by the plants, while simultaneously enhancing the uptake of nutrients in wheat types grown in a solution with a concentration of 150 mM. The NaCl findings are consistent with the research conducted by Iqbal et al. (2022). In addition, our results are consistent with del Cordovilla et al. (2023) findings that the injection of hormones led to an increase in $K^+$ and $Ca^{2+}$ buildup in the shoots of plants, both under non-saline and saline stress conditions.

Plants respond to salt by accumulating osmolytes such as free proline and ascorbic acid. This helps them to maintain cell turgor and counteract the harmful effects of salt (Hasanuzzaman et al., 2013). Total free proline levels were high with the inclusion of β-carotene. Massange-Sánchez et al. (2021) found similar outcomes in wheat, suggesting that increased levels of β-carotene, an organic compound/osmolyte, are linked to an initial defensive response in a saline environment. In order to elevate proline levels, salt stress hampers the functioning of proline oxidase while stimulating the activity of proline enzymes in living organisms, specifically glutamyl kinase and pyrroline-5-carboxylate reductase (Ahmad et al., 2014; Wani et al., 2016).

The experiment's findings demonstrated that salinity had detrimental effects on nearly all growth and yield measures. The impact of elevated salinity on the number of spikelets per spike and the weight of 1000 grains was more pronounced compared to non-saline circumstances. Our findings align with the research conducted by Kalhoro et al. (2016). In another study, Singh et al. (2010) observed a significant decline in yield attributes as saline levels increased. Xie et al. (2017) found that salt stress leads to a reduction in grain and straw yield, as well as the harvest index. The current study found that the grain yield per plant of wheat decreased under saline conditions as a result of a fall in the number of spikes per plant, grains per spike, and the weight of 1000 grains. The results align with the studies conducted by Kalhoro et al. (2016), which demonstrated that salinity significantly decreases wheat yield by causing a substantial decline in spike number, grain number, and 1000 grain weight. Zeng and Shannon (2000) showed a significant loss in grain production per plant due to a greater fall in the quantity of grains and the average grain size in salty circumstances. Application of synthetic plant growth regulators such as $GA_3$ enhanced the ability of wheat cultivars to tolerate and adapt to high salt conditions. The salt tolerance of these two wheat cultivars can be achieved through osmoregulation, which increases water flow and water status by utilizing organic solutes such as saccharides and proteins. This, in turn, leads to an increase in the photosynthetic area and crop yield of the wheat cultivars. The activation occurred simultaneously with the expansion of the photosynthetic area and the increase in crop production of two wheat varieties. This supports the perspective held by other authors (Fischer, 2011; Morales et al., 2020). The application of $GA_3$ not only mitigated the negative impact of salt stress on crop yield in both wheat cultivars, but also enhanced the crop yield of the wheat cultivars. This finding is consistent with the study conducted by Shahzad et al. (2021). However, the exogenous application of $GA_3$ did not improve the spike length under salinity conditions. β-carotene, when applied externally, also enhances the antioxidant status and productivity of wheat in the presence of abiotic stress factors (Farooq et al., 2009).

## CONCLUSIONS

The wheat plant's growth and yield were reduced by salt stress levels (150 mM NaCl). Furthermore, the wheat plant's growth and yield were enhanced by the exogenous application of $GA_3$, β-carotene, and their combos, $GA_3$+β-carotene (0.75 + 0.25 mM). Salinity (NaCl) increased the non-enzymatic antioxidants in wheat plants. The levels of foliar applications gibberellic acid (0.75 mM), β-carotene (0.25 mM) and their combinations ($GA_3$+β-carotene) played a main role in improvement of morpho-physiological parameters and yield under saline and non-saline environment. $GA_3$, β-carotene and $GA_3$+β-carotene treated plants showed more increase in activities of catalase, superoxide dismutase, leaf ascorbic acid and total phenolics in salt stressed wheat plants. Increased yield in wheat plants is directly related to improved attributes due to exogenous application of $GA_3$, β-carotene and $GA_3$+β-carotene. Out of two tested wheat genotypes, Faisalabad-08 proved to be more tolerant against salinity stress as compared to Galaxy-13.

## Acknowledgements

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