Evaluation of morphophysiological characteristics of wheat genotypes in different foliar spraying treatments under normal conditions and salinity stress

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Abstract

Salt stress is one of the major factors that decreases wheat yield. The aim of this study is to examine the effects of K, SA, and GABA application on yield components, grain yield, and nutrient uptake in high-salinity in wheat genotypes. The research was conducted as a split plot based on a randomized complete block design with three replications under normal as control, and salinity (8 dS/m) conditions. The main plots included foliar application with growth stimulants (K, SA, GABA, and control), and subplots encompassed seven wheat genotypes. The salinity caused a 49.95% decrease in grain yield compared to normal conditions. The Mihan genotype showed the highest grain yield when treated with potassium (10970.6 kg/ha), GABA (11370.1 kg/ha), and salicylic acid (10650.1 kg/ha) under non-stress conditions. Furthermore, under salinity conditions, the Mihan genotype sprayed with potassium (7036.1 kg/ha) and GABA (5070.1 kg/ha) produced the maximum grain yield. Foliar spraying with potassium and GABA in both conditions improved the Fe, Cu, Zn, and Mn content in grains compared to control. Exposure to salt stress caused a decrease in iron (50.05%), copper (27.86%), and magnesium (18.86%) content in the seeds. Treatments with potassium and GABA in both conditions increased the Fe, Cu, Zn, and Mn content in grain. The highest nitrogen and potassium content and lowest sodium content in leaves were observed in Mihan genotype sprayed with K. Therefore, foliar application of K and GABA can moderate the effects of salinity on wheat.

Keywords

GABA; grain elements; salicylic acid; yield, zinc

Introduction

Wheat (Triticum aestivum) is considered as the most significant grain crop among all the cereals and ranked first globally among grain-producing crops, especially for human consumption (1). Salinity stress impacts 6% of the world’s land, including 20% of arable land and 33% of irrigated land, affecting global wheat yield significantly. By 2050, providing food to 2.3 billion people while dealing with the loss of agricultural land due to salinity stress will be challenging (2). Salinity stress causes osmotic stress and ion toxicity by increasing...
the assimilation of Na⁺ ions and decreasing the Na⁺/K⁺ ratio due to lower osmotic potential within the plant roots. Furthermore, these ionic imbalance affects the uptake and transport of other important essential mineral nutrients in target cells and hamper the crucial plant processes and functions (3). Salinity in soil can impact plant properties such as nutrient absorption, growth, and yield (4). It also increases reactive oxygen species production, causing oxidative stress, lipid peroxidation, and nucleic acid damage, ultimately reducing grain yield quality and quantity (5).

Cultivating salt-tolerant wheat cultivars through phytomelioration is a cost-effective strategy to enhance wheat productivity in salt-affected areas. The high intensity of salt stress severely affects membrane stability, chlorophyll, and biomass in the early vegetative stages. This, in turn, reduces tillering and grain yield during the reproductive stages (6).

Various studies have reported a decrease in wheat grain yield caused by salt stress (7–9). Conversely, to achieve global food security and sustainability, it is necessary to identify salt-resistant genotypes and utilize them to develop salt-tolerant wheat cultivars (10). Researchers have used various physiological traits to screen for salt tolerance in wheat crops. These traits include chlorophyll reduction, relative water content, plant height, fresh and dry weight of shoots and roots, photosynthesis rate, and levels of Na⁺, K⁺, and Ca²⁺ in the plant (11, 12). Shoot Na⁺, shoot K⁺, root Ca²⁺, shoot K⁺/Na⁺, and root Na⁺/Ca²⁺ ratio are the most crucial morphological markers to identify salt tolerance in bread wheat (13).

Gamma-aminobutyric acid (GABA) is a four-carbon non-protein amino acid produced by glutamate decarboxylase from glutamic acid. GABA often accumulates rapidly in plants in response to living and non-living stresses, including drought, salinity, wounds, oxygen deprivation, heat shock, and pathogen contamination (14). The positive effect of GABA on increasing tolerance to environmental stresses in plants has been documented in previous studies (15, 16).

Potassium (K) reduces water loss in plant tissues by increasing the opening and closing of pores, keeping cells from inflammation, increasing water use efficiency, and reducing the effect of stress (17). Potassium plays important roles in plant physiology, including photosynthesis, carbohydrate and protein formation, nutrient and water transport, nitrogen utilization, and early growth stimulation. Potassium promotes NO³⁻ absorption and amino acid conversion to protein, increasing plant grain protein content (18).

Salicylic acid is a molecule that elicits specific responses in response to biotic and abiotic stressors. This phytohormone plays an important role in opening and closing pores, nutrient uptake, protein and chlorophyll synthesis, inhibiting ethylene biosynthesis, evapotranspiration, photosynthesis, and osmotic regulation (19). There are several reports on the role of salicylic acid in reducing the effect of biological and non-biological stresses (20, 21).

The study of the combined effect of salinity and K has shown that K deficiency increases salinity’s negative effects in photosynthesis (22). Hamid et al. (23) reported that the pretreatment of wheat seeds with salicylic acid under salinity stress produced stronger and larger seedlings and increased the plant’s chlorophyll, soluble sugar, and protein contents.

Paying attention to the increase in salinity in agricultural fields, it is necessary to identify salinity-tolerant cultivars with acceptable grain yield and find solutions to adjust the effect of salinity stress on crops, such as different foliar treatments. Therefore, the present study aimed to evaluate the morpho-physiological characteristics of wheat genotypes in different foliar spraying treatments under normal and salinity stress conditions.

**Materials and Methods**

**Experimental treatments and design**

The study was conducted on a farm located in an agricultural research station in Mianoob (at 36°58′ N and 46°6′ E and altitude of 1314 m above sea level) in North West Iran during the two cropping years 2017-18 and 2018-19.

**Experimental design**

The research was conducted as a split plot based on a randomized complete block design with three replications. The main plots was salinity conditions (normal and soil salinity (8 dS/m)). The sub plots consisted of foliar application with growth stimulants (K, GABA, SA, and control), and the sub-sub plots included seven genotypes (i.e., Orum, Zare, Mihan, Heydari, MS-89-12, MS-89-13, and MS-91-14).

**Field experiment**

The land was first irrigated in late summer and plowed in early autumn. Before planting, the soil of the test site was sampled and analyzed from a depth of 0–30 cm. The required fertilizers were mixed according to the soil test and placed 5–7 cm below the seeds in a row. The seeds were disinfected with tebuconazole toxin (d5 2% Semiran Co.) before sowing, and their density was considered for sowing 450 seeds per square meter according to the region's custom.

The amount of the applied fertilizers was based on soil test results. The urea fertilizer (46%) in the amount of 160 kg ha⁻¹ was applied in three stages (a 20 kg urea fertilizer at the same time as preparing the land and before planting as the basic fertilizer, 70 kg as a road fertilizer in the tillering stage, and 70 kg in the shooting stage). In addition, potassium sulfate fertilizers (45%) and diammonium phosphate at the rates of 80 and 100 kg ha⁻¹ were simultaneously used with planting, respectively.

The electrical conductivity (EC) and acidity (pH) of irrigation water were measured by an electric conductivity meter (JENWAY model 4310) and a pH meter (6991 Metrohm), respectively. Carbonate and bicarbonate were also estimated by the sulfuric acid titration method and using reagents (phenolphthalein and methyl), respectively. Eventually, irrigation water and sodium (Na) were
measured by complexometry and direct reading with the CORNING M410 flame photometer.

Irrigation was performed in autumn for seed germination. After favorable environmental conditions, chemical control was conducted against weeds using 2,4-D and Fenoxaprop-p-ethyl (GOLSAM CHEMICALS Co., Gorgan Iran) herbicides.

**Application of treatments**

At the end of stem elongation and beginning of spike emergence, foliar spraying with potassium (2 per thousand \( K_2O \) (28%), according to the manufacturer’s instructions), salicylic acid (1 mM), and GABA (2.5 mM) were done. The potassium used under the trade name Hygro Kaforce was a product of the Tarim Ecological Company of Turkey, which was dissolved and used at 40 cc in 20 L of water. The salicylic acid used was a product of Merck, Germany, which, after dissolving in pure ethanol, 0.138 g of it was dissolved in a liter of distilled water and was sprayed at the appointed time.

Gamma-aminobutyric acid (GABA) was obtained in powder form from the Faculty of Sciences of Kharazmi University (Tehran, Iran). In order to use GABA in the amount of 2.5 mM, after dissolving it in distilled water in the amount of 0.1128 g/L, it was used for spraying. Foliar application was carried out once at the end of stem elongation and the beginning of spike emergence.

**Sampling and data collection**

To measure the grain yield, whole experimental plots were harvested, and after drying for four days, the grains in all harvested spikes were weighed and recorded as grain yield. The kernel number per spike was determined by counting the grains on ten plants and ten spikes during harvesting. The thousand-grain weight was also determined by hand-shelling ten random spikes from each plot.

**Content of grain elements**

To measure the concentration of grain nutrients after physiological maturity and harvest, 30 g of the sample was selected from each plot, and the amount of the grain material was measured using the atomic absorption spectroscopy method.

**Content of leaf elements**

To determine levels of \( Na^+ \) and \( K^+ \), young, fully expanded leaves were detached from plants, and their fresh and oven-dried weights were recorded. The dried leaf samples were dissolved in a 1% solution of \( HNO_3 \) for digestion. An aliquot of 25 mL solution was taken from 1% \( HNO_3 \) solution in falcon tubes and digested on a hot plate for 4 h at 85 °C. After digestion, the mixture was diluted with distilled water to a 10-fold concentration and run through a flame photometer (Sherwood, UK, Model 360) (24). The amount of leaf nitrogen was measured by the Micro-Kjeldahl Method.

**Statistical analysis**

Before analyzing variance, its assumptions were checked. The data were analyzed using SAS 9.4, and mean comparisons were performed with Duncan’s method (LSR).

**Results**

The results of saline and normal soil analyses are presented in Table 1. In addition, Table 2 lists the chemical properties of water used for irrigation.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Texture Class</th>
<th>pH</th>
<th>EC (dS/m)</th>
<th>Total N (%)</th>
<th>Available P (mg/kg)</th>
<th>Available K (mg/kg)</th>
<th>Available Fe (mg/kg)</th>
<th>Available Zn (mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Silt</td>
<td>8</td>
<td>0.81</td>
<td>0.12</td>
<td>11.2</td>
<td>250</td>
<td>6.24</td>
<td>0.74</td>
</tr>
<tr>
<td>Saline</td>
<td>Silt</td>
<td>9</td>
<td>8.1</td>
<td>0.09</td>
<td>8.4</td>
<td>180</td>
<td>3.3</td>
<td>0.51</td>
</tr>
</tbody>
</table>

**Table 2.** Chemical properties of irrigation water in experiments.

<table>
<thead>
<tr>
<th>Water Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC (mmhos/cm)</td>
<td>1.8</td>
</tr>
<tr>
<td>( CO_3^{2-} ) (meq/L)</td>
<td>0</td>
</tr>
<tr>
<td>( HCO_3^- ) (meq/L)</td>
<td>8.4</td>
</tr>
<tr>
<td>Cl (meq/L)</td>
<td>11.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
</tr>
<tr>
<td>( Ca^{2+} ) (meq/L)</td>
<td>4.8</td>
</tr>
<tr>
<td>( Mg^{2+} ) (meq/L)</td>
<td>5.5</td>
</tr>
<tr>
<td>( Na^+ ) (meq/L)</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**Grain yield and its component**

The combined ANOVA revealed that the number of spikelets, number of grains, 1000 kernel weight, grain yield, and harvest index were affected by Year, Salinity, Year × Salinity, Foliar spray, Salinity × Foliar spray, Genotype, Salinity × Genotype, Foliar spray × Genotype and Salinity × Foliar spray × Genotype (Table 3).

**Number of spikelets**

Under normal conditions, the MS89-13 and Mihan genotypes produced the highest number of spikelets per spike, with an average of 34.1 and 32.2 grains, respectively, in response to foliar potassium application. In contrast, the Zare genotype under the salicylic acid foliar treatment had the lowest spikelets per spike, with an average of 14.8 spikelets (Table 4).

Under salt stress, MS89-12 genotype treated with GABA had the highest number of spikelets per spike (30.5 spikelets), followed by Orum, MS89-13, MS91-14 genotypes sprayed with potassium and GABA, and Mihan and MS91-14 genotypes treated with salicylic acid; The differences were not significant. The Zare variety sprayed with GABA had the lowest spikelet count (9.3 spikelets) (Table 4).

**Number of grains**

The research findings indicate that the Mihan genotype had the highest number of seeds per spike, with an average of 64.2, 61.7, and 7.54 seeds for GABA and potassium.
folar application and non-folar application treatments. Heydari genotypes treated with GABA and Zare treated with salicylic acid had the lowest number of seeds under these conditions, with 28.5 and 27.5 grains, respectively (Table 4).

Under salinity stress, the Mihan genotype sprayed with GABA (40.7 grains) and salicylic acid (39.2 grains) had a significantly higher number of seeds than other genotypes. The Zare genotype sprayed with GABA (17.02 grains) had the lowest number of grains per spike (Table 4).

**Thousand kernel weight**

The study found that MS89-12 and MS89-13 genotypes treated with potassium (66.0 and 65.0 g, respectively), GABA (65.3 and 70.0 g), and salicylic acid (69.2 and 64.8 g, respectively) had the highest thousand kernel weight. There was no significant difference between the mentioned treatments and the control treatment of folar spraying in genotypes MS89-12 and MS89-13 regarding thousand seed weight. The minimum thousand kernel weight was related to the Mihan genotype sprayed with GABA and salicylic acid (45.0 and 46.2 g, respectively) (Table 4).

Under salt stress conditions, the highest thousand kernel weight was found in MS89-12 genotype folar-sprayed with potassium and GABA and MS89-13 genotype folar-sprayed with GABA with an average of 62.3, 62.5, and 60.7 g, respectively, while the unsprayed Zare genotype produced the lowest thousand kernel weight (42.8 g) (Table 4).

**Grain yield**

In this study, folar application of potassium, GABA, and salicylic acid in the Mihan genotype produced the highest Grain yield with an average of 10970.6, 11370.1, and 10650.1 kg/ha, respectively. The difference between the mentioned treatments with potassium folar spraying in the Zare genotype was non-significant. The lowest grain yield in non-stress conditions was related to the Orum genotype in the control treatment of folar application with an average of 5537.6 kg/ha, which was followed by folar treatments with potassium, GABA, and salicylic acid in the Orum genotype. The results showed that under normal conditions, potassium folar application in the Zare genotype and GABA in the Mihan genotype significantly increased grain yield compared to the corresponding control (Table 4).

Under the conditions of salinity stress, the Mihan cultivar sprayed with potassium produced the maximum grain yield with an average of 7036.1 kg/ha, and the Orum cultivar in the treatment of no folar application with an average of 1877.3 kg/ha showed the minimum grain yield. The results revealed that Orum and Zare genotypes filated with GABA and Mihan with potassium and GABA increased grain yield compared to the corresponding control treatment (Table 4).

**Harvest index**

In this study, the highest harvest index under non-stress conditions with an average of 50.50% was assigned to the MS89-13 genotype under potassium treatment. In comparison, the minimum of this trait was obtained with an
average of 38.17 and 37.50%, respectively, for the Heydari genotype treated with potassium and GABA (Table 4).

Under salinity stress conditions, the highest harvest index was assigned to the Orum genotype under salicylic acid treatment (56.00%). While Heydari, MS89-12, and MS89-13 genotypes showed the lowest harvest index under foliar control treatment with an average of 28.67, 29.33, and 28.83, respectively (Table 4).

Concentration of grain elements and protein

Fe, Cu, Zn, Mg, Mn, and grain protein content were significantly affected by the Year, Salinity, Year × Salinity interaction, Foliar spray, Genotype, Salinity × Genotype, and Foliar spray × Genotype. The two-way interactions between Salinity × Foliar spray and the three-way interactions between Salinity × Foliar spray × Genotype were significant on Fe, Cu, Zn, Mg, Mn, and grain protein content (Table 5).

Concentration of Fe

Under normal conditions, the genotype of Mihan sprayed with salicylic acid had the highest grain Fe content with an average of 181.0 mg. In these circumstances, the Heydari and MS89-12 genotypes treated with salicylic acid and the Zare genotype in the foliar spray control treatment had the least Fe content in their grain, with an average of 161.5 mg. Under normal conditions, potassium and GABA foliar application on Zare, Mihan, MS89-12, and MS89-13 genotypes could significantly increase Fe content compared to the corresponding control.

Under salinity stress, MS89-12 and MS91-14 genotypes sprayed with GABA produced the highest and lowest Fe content in seeds with values of 132.3 and 115.7 mg, respectively.

Under stress conditions, the foliar application of GABA in genotypes MS89-12 and MS89-13 and the foliar application of genotype MS91-14 with potassium and
salicylic acid significantly increased the Fe content of grain compared to the corresponding control treatment (Table 6).

### Concentration of Cu

Under normal conditions, the highest grain copper content was assigned to the Orum genotype sprayed with GABA with an average of 30.18 mg, which was followed by MS89-13 and Mihan genotypes sprayed with potassium and Heydari genotype sprayed with GABA, and the difference between them was not statistically significant. In contrast, the Zare genotype sprayed with salicylic acid had the lowest copper content in seeds, with an average of 13.42 mg. The findings indicated that the copper content in the grain of all the examined genotypes treated with potassium was significantly greater than in the control treatment (Table 6).

Under salinity conditions, foliar application of potassium in the Orum genotype with an average of 16.70 mg had the maximum, and foliar application of genotype MS89-13 with MS91-14 with an average of 10.78 mg obtained the minimum amount of grain copper content. The application of potassium through the leaves significantly increased the copper content in the grain of the Orum genotype under stress conditions, compared to the control treatment (Table 6).

### Concentration of Zn

According to the results, Heydari sprayed with salicylic acid had the highest Zn content in the grain under normal conditions, with an average of 24.17 mg. This treatment was statistically at the same level as Heydari and MS89-12 genotypes sprayed with GABA, and the difference between them was insignificant. The lowest grain Zn content, with an average of 17.33 mg, was assigned to the Orum genotype sprayed with potassium. The Zn content of grains in Mihan, MS89-12, MS89-13, and MS91-14 genotypes was significantly increased by foliar spraying with GABA under normal conditions compared to the corresponding control (Table 6).

Under salinity stress conditions, the grain's maximum and minimum Zn content was assigned to genotype MS89-12, sprayed with salicylic acid with an average of 41.67 mg, and genotype MS91-14 sprayed with potassium with an average of 25.33 mg. Foliar spraying with potassium in Orum, Zare, and Mihan genotypes caused a significant increase in the Zn content of grains compared to the corresponding control treatment under salinity stress conditions (Table 6).

### Concentration of Mg

Under no stress, genotype MS91-14 sprayed with GABA had the highest Mg content of grains with an average of 0.2268 mg/g dry wt. The difference between the mentioned treatment and the Zare genotype cultivated in the control treatment was negligible. The lowest grain Mg content was produced by Orum and Zare genotypes treated with potassium (with an average of 0.1407 mg/g dry wt). Mean comparisons also showed that under normal conditions, there was no significant difference between foliar treatments and corresponding control treatments in all genotypes (Table 6).

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**Table 5.** Combine analysis of variance for the effect of salinity and foliar spray on grain nutrient and protein content of wheat genotypes.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Mean of Squares</th>
<th>DF</th>
<th>Coefficient</th>
<th>Coefficient</th>
<th>Coefficient</th>
<th>Coefficient</th>
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<td>S.O.V</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Year (Y)</td>
<td>1</td>
<td>180144.04**</td>
<td>180144.04**</td>
<td>10960.1**</td>
<td>10960.1**</td>
<td>43361029.96**</td>
<td>43361029.96**</td>
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<tr>
<td></td>
<td>Salinity (S)</td>
<td>1</td>
<td>53353.44**</td>
<td>53353.44**</td>
<td>14204.76**</td>
<td>14204.76**</td>
<td>245575.13**</td>
<td>245575.13**</td>
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<tr>
<td></td>
<td>F × G</td>
<td>3</td>
<td>53353.44**</td>
<td>53353.44**</td>
<td>14204.76**</td>
<td>14204.76**</td>
<td>245575.13**</td>
<td>245575.13**</td>
</tr>
<tr>
<td></td>
<td>S × G</td>
<td>6</td>
<td>53353.44**</td>
<td>53353.44**</td>
<td>14204.76**</td>
<td>14204.76**</td>
<td>245575.13**</td>
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<td></td>
<td>Y × G × S</td>
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<td>53353.44**</td>
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<td>S × F × G</td>
<td>18</td>
<td>53353.44**</td>
<td>53353.44**</td>
<td>14204.76**</td>
<td>14204.76**</td>
<td>245575.13**</td>
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<td>Y × S × F × G</td>
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<td>14204.76**</td>
<td>245575.13**</td>
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</tr>
</tbody>
</table>

**Note:** ns, not significant, * and ** significant at the 5% and 1% levels of probability, respectively.
The Zare genotype under salinity stress conditions showed the highest Mg content of seeds with an average of 0.1808 mg/g dry wt. The minimum Mg content was achieved for the Orum genotype treated with salicylic acid at an average of 0.1330 mg/g dry wt. The Mg content of the examined genotypes was not improved by foliar spraying under salinity conditions compared to the corresponding control (Table 6).

**Concentration of Mn**

The highest Mn content in grains was found in genotype MS89-12 grown under potassium foliar treatment (171.7 mg/g dry wt) and Mihan grown under salicylic acid foliar treatment (169.5 mg/g dry wt) under normal conditions. Under the above conditions, the foliar application on the Heydari genotype with salicylic acid (145.7 mg/g dry wt) registered the lowest Mn concentration. The manganese content of grains in all genotypes evaluated was significantly increased by potassium foliar application under normal conditions compared to the corresponding control (Table 6).

Foliar application with GABA and salicylic acid (202.3 and 204.1 mg/g dry wt, respectively) of the Zare genotype was able to achieve the highest manganese content under salinity stress.

The Heydari genotype sprayed with GABA (142.8 mg/g dry wt) recorded the lowest content of the mentioned element. Under stress conditions, foliar spraying with GABA, and salicylic acid on the Zare genotype and
potassium on the MS89-12 genotype had significantly higher grain manganese content than the control treatment (Table 6).

**Protein content**

According to the mean comparison results, salicylic acid foliar application in the Orum genotype had the highest protein content, with an average of 13.02%, which was followed by potassium foliar spraying in MS89-12 genotype and foliar spraying of the Zare genotype with salicylic acid. Foliar application of potassium in the Heydari variety, with an average of 8.37%, had the lowest grain protein percentage (Fig. 1).

**Concentration of leaves elements**

The combined ANOVA revealed that the effect of the Year, Salinity, Year × Salinity, Foliar spray, Genotype, Salinity × Genotype, Foliar spray × Genotype, and Salinity × Foliar spray × Genotype were significant for N, Na, and K content of the leaf. The two-way interactions between Salinity × Foliar spray were significant on Na and K content (Table 7).

**Leaf nitrogen content**

Under normal conditions, foliar application of the Mihan genotype with GABA (3.00 mg/g dry wt) and foliar application of the Heydari genotype with potassium (1.36 mg/g dry wt) obtained the leaf's highest and lowest nitrogen content, respectively. In this research, foliar spraying of Orum, Zare, and Heydari genotypes with salicylic acid significantly increased the leaf nitrogen content compared to the corresponding control treatment (Table 8).

Under salinity stress, the Mihan genotype treated with potassium (2.00 mg/g dry wt) had the highest leaf nitrogen content, while the MS89-13 genotype in the foliar control treatment (1.27 mg/g dry wt) had the lowest leaf nitrogen content. The leaf nitrogen content in Orum, Mihan, and MS89-13 genotypes sprayed with potassium was significantly higher than the corresponding control treatment.

**Potassium content**

The results showed that the Mihan genotype sprayed with potassium and GABA had the highest leaf potassium content with an average of 56.33 and 56.17 mg/g dry wt, respectively, and the Orum genotype sprayed with salicylic acid had the lowest leaf potassium content with an average of 42.00 mg/g dry wt. Under these conditions, foliar application of Orum, Mihan, Heydari, MS89-12, and MS91-14 genotypes with potassium significantly increased the potassium content of leaves compared to the control treatment.

Under salinity stress conditions, foliar application of the Mihan genotype with potassium had the highest (30.00 mg/g dry wt) leaf potassium content. Zare and Mihan genotypes (22.83 and 22.67 mg/g dry wt) had the lowest leaf potassium content. Under salinity conditions, foliar

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**Table 7.** Combine analysis of variance for the effect of salinity and foliar spray on leaf nutrient content of wheat genotypes.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>DF</th>
<th>Nitrogen (N)</th>
<th>Sodium (Na)</th>
<th>Potassium (K)</th>
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<td>80.81**</td>
<td>36708.76**</td>
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<td>Y × S</td>
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<td>2.82**</td>
<td>7.69**</td>
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<td>R (Y × S)</td>
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<td>Foliar spray (F)</td>
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<td>0.52**</td>
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<tr>
<td>S × F</td>
<td>3</td>
<td>0.129**</td>
<td>0.355**</td>
<td>65.69**</td>
</tr>
<tr>
<td>Y × S × F</td>
<td>3</td>
<td>0.001ns</td>
<td>0.004ns</td>
<td>0.071ns</td>
</tr>
<tr>
<td>Error 1</td>
<td>24</td>
<td>0.001</td>
<td>0.004</td>
<td>0.439**</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>6</td>
<td>0.29**</td>
<td>0.466**</td>
<td>67.28**</td>
</tr>
<tr>
<td>Y × G</td>
<td>6</td>
<td>0.002ns</td>
<td>0.004</td>
<td>0.248ns</td>
</tr>
<tr>
<td>S × G</td>
<td>6</td>
<td>0.177**</td>
<td>0.268**</td>
<td>42.77**</td>
</tr>
<tr>
<td>Y × S × G</td>
<td>6</td>
<td>0.001ns</td>
<td>0.002ns</td>
<td>0.153ns</td>
</tr>
<tr>
<td>F × G</td>
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<td>0.451**</td>
<td>0.191**</td>
<td>77.78**</td>
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<tr>
<td>Y × F × G</td>
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<td>0.004</td>
<td>0.002ns</td>
<td>0.515ns</td>
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<tr>
<td>S × F × G</td>
<td>18</td>
<td>0.394**</td>
<td>0.284**</td>
<td>72.07**</td>
</tr>
<tr>
<td>Y × S × F × G</td>
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<td>0.004ns</td>
<td>0.002ns</td>
<td>0.409ns</td>
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<tr>
<td>Error 2</td>
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<td>0.002</td>
<td>0.356**</td>
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<tr>
<td>CV (%)</td>
<td></td>
<td>2.63</td>
<td>1.21</td>
<td>1.64</td>
</tr>
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</table>

**ns**, not significant, * and ** significant at the 5% and 1% levels of probability, respectively.
spraying with potassium significantly increased the content of this element in the leaves of all studied genotypes (Table 8).

**Sodium content**

The results of the mean comparison showed that under non-stressed conditions, Heydari foliar spraying salicylic acid (3.31 mg) and genotype MS89-13 foliar spraying (3.24 mg) had the highest, and Mihan cultivar foliar sprayed with potassium (2.47 mg) had the lowest sodium content. Under normal conditions, potassium foliar application in Mihan, Heydari, and MS89-12 genotypes significantly reduced sodium content in leaves compared to the corresponding control (Table 8).

Under salt stress conditions, the leaves’ maximum and minimum sodium content was observed with MS91-14 genotype under control treatment, and the Mihan genotype foliated with potassium had the lowest sodium content in the leaves (Table 8).

**Discussion**

**Yield and yield component**

The present study showed that the foliar spraying of potassium, GABA, and salicylic acid could significantly increase the grain yield in the Mihan genotype under non-stress conditions compared to the corresponding control treatment. Under salt stress conditions, grain yield decreased in all genotypes. Orum and Zare genotypes were sprayed with GABA, and Mihan treated with potassium increased grain yield compared to the corresponding control treatment.

The decrease in grain yield in the examined geno-
types under salinity conditions in the current research can be due to the negative effect of this stress on the components of grain yield. In this study, salinity reduced the number of spikelets per spike, the number of grains per spike, and the thousand kernel weight by 21.47, 26.81, and 8.25%, respectively, compared to normal conditions, which finally led to a decrease of 49.95% in grain yield.

One of the most concerning abiotic stresses on crop plants is salinity. It can significantly impact their physiological, morphological, and biochemical characteristics, affecting how they absorb water and nutrients, their ability to germinate and grow, photosynthesis, enzyme actions, and, ultimately, their yield (4). In addition, the increase in reactive oxygen species (ROS) production under salinity stress causes oxidative stress, leading to lipid peroxidation and damage to nucleic acids, ultimately decreasing the quality and yield of grain (5).

Previous research has shown that the reproductive phase of crops is highly vulnerable to abiotic stresses such as salinity (25, 26), resulting in significant reductions in yield for crucial crops like wheat (26). Additionally, salinity conditions can decrease the rate of photosynthesis, biomass accumulation, and the source sinks activity, ultimately leading to premature aging of reproductive organs and a negative impact on yield response factors (27).

Exposure to high levels of salt in the soil can lead to a decrease in the number of fertile tillers, a reduction in the number of spikelets per spike, a decrease in kernel weight, and a negative impact on grain yield (7). According to Hasan et al. (8), exposure to salinity stress at a rate of 15 dSm⁻¹ significantly reduces the number of grains per spike, 1,000-grain weight, and seed yield in tolerant and sensitive wheat cultivars. Additionally, saline stress can lead to a decrease in grain weight. This stress is caused by pollen sterility, lower production of assimilates and reduced allocation towards the economic parts of the plant (the grains). A study on 151 synthetic wheat-breeding lines revealed that salt stress is related to Na⁺ toxicity, resulting in a 20% reduction in total kernel weight and a 6% decrease in starch content (9).

Also, the positive effect of potassium and GABA foliar spraying treatments under normal conditions and salt stress could be attributed to the positive effect of these treatments in improving yield components.

The effect of salt stress is mitigated by various physiochemical and biological mechanisms due to the availability of nutrients. Applying potassium to the leaves could enhance photosynthesis, increase the effectiveness of antioxidant enzymes, boost potassium uptake in plants, and improve their ability to absorb sodium and survive in saline conditions (28). The accumulation or transportation of photosynthetic products might cause a difference in the response of varieties to potassium fertilizer (29). The potassium fertilizer was beneficial to the transportation and accumulation of photosynthetic products for Mihan and ultimately increased the grain yield.

Stress tolerance can be improved by using exogenous GABA (30). GABA can function as a signaling molecule and a metabolite. The application of GABA has been proven to alleviate stress by regulating sugar and proline metabolism, reducing photosynthesis and mitochondrial activity inhibition, and maintaining chloroplast integrity under stress (31).

Application of GABA reduces Na⁺ concentration, increases K⁺ concentration, amino acid, and organic acid accumulation, promotes PAs production, inhibits their metabolism (15), and increases unsaturated fatty acid content (γ-linolenic acid) while reducing saturated fatty acid content (32). Under the treatment of GABA, all these processes enhance plant stress tolerance. In wheat, studies have shown that GABA promotes salt tolerance by improving nitrogen and carbon assimilation (33), enhancing photosynthesis, antioxidant enzyme activity (32), and signal transduction pathways. Studies have shown that GABA can induce phenolic accumulation under NaCl stress and enhance antioxidant activity in barley seedlings (34). Kumar et al. (35) experimented with examining the effect of GABA on morphological characters and yielding attributes of salt-stressed blackgram (Vigna mungo). Also, the positive effect of GABA foliar application on improving the growth characteristics and economic performance of blackgram (Vigna mungo) and lentil cultivars (Lens culinaris Medik.), respectively, in the studies of Al-Quraan and Al-Omari (36) and Kumar et al. (35) was documented.

Our research has revealed that the salicylic acid foliar application can increased grain yield for certain genotypes in non-stressful conditions. Research has demonstrated that applying SA externally to wheat can act as a signaling molecule, prompting the plant’s internal radical detoxification system to activate (20). When SA is used on wheat externally, it helps produce antioxidants by utilizing ROS as a secondary messenger (21).

Our research found that applying salicylic acid to wheat leaves did not improve the yield of grains in salinity conditions. This phenomenon could be due to the different reactions of genotypes, dosage, and time of foliar spraying of this substance.

**Concentration of grain elements and protein**

Results revealed that the content of Fe, Cu, and Mg decreased by 27.86, 50.05 and 18.86%, respectively, in the studied genotypes under salinity stress versus normal conditions. While, the content of Zn and Mn elements increased by 27.86, 50.05 and 18.86%, respectively, in the studies of Al-Quraan and Al-Omari (36) and Kumar et al. (35) was documented.

Salinity stress significantly affects grain quality traits. The wheat (cv. Shatabdi) crop exposed to 200 mM NaCl showed a 155, 10, and 20 increase in Na⁺, K⁺, and Ca²⁺ content under stress (37). According to Nadeem et al. (38), salinity had a negative impact on the yield of wheat crops, including the length of grains, test weight, and overall grain yield. The mineral nutrient content, including K, Ca, Fe, P, Zn, and Mg, was also negatively affected.

Excessive accumulation of salt ions such as Na⁺, Cl⁻, Mg²⁺, and SO₄²⁻ alters the composition of soil solution, severely disturbing ionic harmony. Excessive accumulation
of sodium disrupts the uptake of cationic nutrients such as potassium or calcium, leading to a nutrient imbalance (39). Furthermore, high sodium concentration reduces potassium absorption, decreasing shoot growth. Similarly, high levels of Cl− can impair nutrient uptake by disrupting anion uptake (40).

The response of genotypes to foliar spraying treatments varied depending on the content of grain elements in this study. The Mihan genotype, which received salicylic acid, had the highest Fe content under normal conditions; the highest Cu, Zn, and Mg content was achieved in the Mihan genotype treated with potassium. The Zare genotype grown in the foliar spraying control treatment had the highest grain magnesium content. Under salinity stress conditions, the MS89-12 genotype treated with potassium had the highest Fe content, the Mihan genotype sprayed with GABA showed the highest Cu and Mn content, the Heydari genotype treated with salicylic acid had the highest Zn content, and Zare treated with GABA achieved the highest Mn in the grains.

The present study showed that foliar spraying with potassium and GABA in both conditions improved the Fe, Cu, Zn, and Mn content in grains compared to the corresponding control.

Fe is a major nutrient that influences the formation of chlorophyll in plants. As a result, a decrease in nutrient uptake will likely cause a reduction in the uptake of Fe, leading to less iron being stored in the shoot, particularly in the grain. Copper is important for plant growth and stress adaptability. It is needed for antioxidants, photosynthesis, and nitrogen fixation (41). In this study on creeping bentgrass, GABA application enhanced N, P, and Cu content in leaves under heat stress, suggesting that GABA may enhance those elements (42).

Judaki et al. (43) found that when plants were exposed to a salinity level of 8 dS/m, feeding them with K between 1 to 2 mM significantly increased the uptake of Fe and Cu in the shoots. Another report indicated that the use of different levels of K in safflower under different salinity levels increased the concentration of Cu (44).

It has been reported that GABA application increased the levels of minerals, including potassium (K), phosphorus (P), calcium (Ca), iron (Fe), manganese (Mn), and zinc (Zn) in grains (45). In another study on rice, Xie et al. (46) showed that GABA250 increased Na, Mn, Zn, and Fe contents by 10.95%, 25.70%, 11.14%, and 43.30%, respectively compared with control treatment. The present research showed that the protein content of different genotypes showed different reactions to foliar spraying treatments, but in general, foliar spraying with salicylic acid led to the improvement of grain protein. According to reports, salicylic acid can increase grain protein levels by reducing the amount of starch in the grain (47).

**Concentration of leaf elements**

The findings revealed that in leaves under salt stress, the nitrogen and potassium content decreased by 29.56% and 44.69%, respectively, compared to normal conditions, while salt stress increased the sodium content of leaves by 33.79% compared to normal conditions.

Under normal conditions, the Mihan genotype sprayed with GABA had the highest nitrogen and potassium content in the leaves. Under salt stress conditions, the Mihan genotype displayed the highest leaf nitrogen and potassium content when potassium foliar was applied. It should be noted that the lower sodium content of the leaves under both conditions was attributed to the Mihan genotype under K treatment. The present study revealed that potassium foliar application increased the nitrogen and potassium content of leaves and decreased the sodium content compared to other treatments.

Genotypes that accumulated low sodium levels in their leaves showed greater tolerance by controlling the sodium fluxes and maintaining a high ratio of potassium to sodium (48). This low accumulation of sodium ions has been linked to either the exclusion of sodium ions from the leaves or a reduced uptake of sodium ions from the roots. Munns and Tester (6) reported that the removal of Na+ from the cytoplasm into the apoplast is due to a salt-inducible Na+/H+ antiporter located at the plasma membrane. Researchers suggest that glycophytes tolerate salt stress by maintaining an appropriate K+/Na+ ratio (49). It is also reported that if the K+/Na+ ratio is high, a variety could be categorized as salt-tolerant (50). It is demonstrated from the results of this study that genotypes with low Na+ accumulation are more tolerant to salt stress, which aligns with previous research findings (49). One possible reason for the tolerance of the Mihan genotype in this research may be its ability to absorb potassium and excrete sodium effectively.

**Conclusion**

According to the findings, the Mihan cultivar had a higher grain yield in non-stressful and stressful conditions than the other cultivars examined. Additionally, applying potassium, GABA, and salicylic acid to the Mihan cultivar in non-stress conditions and applying potassium under salinity stress conditions increased grain yield compared to the control. In addition to producing a high grain yield, the mentioned cultivar has also shown a good response to foliar spraying treatments. The current study found that salt stress decreased the levels of Fe, Cu, and Mg elements in the grains. However, applying potassium and GABA in both conditions improved the content of Fe, Cu, Zn, and Mn. So, improving nutrient absorption and balance in plants through potassium and GABA treatment is a viable method for mitigating the effects of salinity stress. Out of the cultivars studied, the Mihan cultivar displayed higher levels of leaf potassium and lower levels of leaf sodium compared to other genotypes. These results suggest that the Mihan cultivar had a greater salt stress tolerance than other cultivars. Finally, to improve grain yield under salt stress conditions, appropriate varieties (such as Mihan) and the application of foliar spraying of potassium and GABA can be effective solutions.
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Authors’ contributions

VS and SS carried out the experiment. MR and MK wrote the manuscript with support from NA and MR.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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