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# Introduction of Morpho-physiological and Biochemical Markers to Select Salt-tolerant Wheat (*Triticum aestivum*) Genotypes under Salinity Stress

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## ABSTRACT

As a strategic crop, wheat is important to the world's food supply. Salinity is a major threat to the food supply in the whole world and Iran. The research was carried out for identifying morpho-physiological and biochemical markers to select salt-tolerant wheat genotypes. The research was done on two salt-tolerant (Pishgam) and susceptible (Shahryar) bread wheat cultivars under saline conditions (250 mM NaCl). The results showed a high genetic diversity for most traits. The traits of harvest index, root-to-shoot dry weight ratio, root dry weight, plant height, potassium-to-sodium ion ratio, total protein, superoxide dismutase, catalase, peroxidase, and total carbohydrates showed the highest alignment with increasing grain yield under salt stress conditions. The traits of potassium ion accumulation ($K^+$), superoxidase dismutase (SOD), proline (Pr), and relative water content (RWC) were entered into the regression model, as the most important traits affecting grain yield under salinity conditions, respectively. According to the results, it is possible to suggest two groups of markers to select salt-tolerant genotypes. The first group includes some morpho-physiological markers namely a high amount of harvest index, main spike weight, relative water content, water consumption, root-to-shoot dry weight ratio, total carbohydrates, proline, and root dry weight; and the second group includes the ionic and biochemical markers namely a high amount of $K^+$ accumulation, $K^+/Na^+$ accumulation ratio, SOD, catalase, peroxidase, and a low amount of $Na^+$ accumulation, malondialdehyde, and hydrogen peroxide. Therefore, the above-introduced markers can be useful indicators to select salt-tolerance genotypes in future wheat breeding programs.

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## 1. Introduction

Salinity stress is one of the most important abiotic stresses that significantly decrees the quality of arable lands (Ahmad and Prasad, 2012). This stress involves changes in various physiological and metabolic processes, depending on severity, duration of the stress, and crop growth stage (James *et al.,* 2011). Salinity stress is one of the most important factors limiting the production of crops in the world and in Iran (Dadashi *et al.,* 2007). Iran, as a primary center of origin of wheat, has valuable genotypes adapted to adverse conditions including drought and salinity (Nazari *et al.,* 2018; Moosavi *et al.,* 2022). These genotypes have different morpho-physiological, biochemical, and ionic characteristics, to help plant breeders to identify salt-tolerant cultivars (Harb and Pereira, 2013). Using different plant breeding methods is the best sustainable and economic solution to deal with salt stress (James *et al.,* 2011). To achieve this goal, introducing different morpho-physiological, and biochemical indicators to select genotypes tolerant to abiotic stress will significantly contribute to future breeding programs in this valuable plant (Rozema and Flowers, 2008; Moosavi *et al.,* 2017).

Due to the growing expansion of saline areas in the world, therefore, the future of agricultural production is increasingly dependent on our ability to develop plant growth in saline and marginal lands, using saline waters. Based on this, the most effective way to manage salinity stress is to identify and select salinity-tolerant cultivars and use these genotypes in saline areas. Therefore, it is necessary to use appropriate indicators to select salt-tolerant genotypes (Rozema and Flowers, 2008).

Sodium is known as a beneficial element in some plants. The presence of sodium in the growth environment of halophyte plants and C4 plants is essential and for such plants, it can be considered as an essential element of low consumption. On the other hand, sodium, especially for glycophyte plants, is not considered an essential element and can cause plant toxicity in high concentrations (Munns *et al.,* 2006).

In high concentrations of salt, sodium enters the plant through non-selective cation channels. This time, due to the same physicochemical similarities, sodium will compete with potassium, and by occupying binding sites related to potassium, it will disrupt all processes related to it. Therefore, observing an optimum ratio of these two elements is necessary for the health of the plant (Irshad *et al.,* 2022).

The production of reactive oxygen species (ROS) in the mitochondria and chloroplasts of plants under the influence of environmental stresses is one of the most important adverse effects of these stresses, which can cause disturbances in cell metabolism, damage to nucleic acids, and finally cell death through peroxidation of membrane lipids, oxidation of proteins (Tanou *et al.,* 2009).

In order to reduce the adverse effects of environmental stresses and control the level of ROS to protect cells, plants have various protective mechanisms, including enzymatic and non-enzymatic antioxidant systems, which are effective at different levels of environmental stress (Shi *et al.,* 2007). Peroxidase, superoxide dismutase, and catalase enzymes are among the most prominent protective mechanisms to remove ROS, which convert free radicals of superoxide ($O_2^{*-}$) into hydrogen peroxide ($H_2O_2$) and finally hydrogen peroxide was transformed into oxygen and water (Tanou *et al.,* 2009).

Wheat as a strategic plant with high genetic diversity in Iran has different drought and salt-tolerance genotypes (Moosavi *et al.,* 2016). Native Iranian genotypes, due to their high adaptability to adverse environmental conditions, are an important gene pool for genes that respond to abiotic stresses (Moosavi *et al.,* 2022). According to previous research (Tattini *et al.,* 1997; Dadashi *et al.,* 2007; Rozema and Flowers, 2008; Moosavi *et al.,* 2016), morpho-physiological, ionic, and biochemical indicators can be among the appropriate and useful selection criteria to evaluate the salinity tolerance of plant genotypes. Therefore, the present

research was done to introduce three different markers of morpho-physiological, biochemical, and ionic to identify salt-tolerant wheat genotypes.

## 2. Material and methods

### *2.1. Plant materials, statistical design, and salinity treatment*

The present research was carried out in two salt-tolerant (Pishgam) and sensitive (Shahryar) wheat cultivars (Table 1), based on a randomized complete block design with three replications.

**Table 1.** Plant material information in the present study

| Cultivar code | Cultivar name | Scientific name | Explanations |
|---|---|---|---|
| G1 (At1) | Pishgam | *Triticum aestivum* | Relative salt-tolerant |
| G2 (At2) | Shahryar | *Triticum aestivum* | Relative salt- susceptible |

For vernalizing, the seeds were kept moist at a temperature of 4°C for 35 days. Then the seeds were planted in plastic pots with a diameter of 20 cm and a height of 40 cm. The weight of the dry soil in each of the pots was about 9 kg, which included a mixture of agricultural soil, windblown sand, and rotted animal manure in equal proportions. Before applying the salinity treatment (at the 10-leaf stage), pots were irrigated with distilled water to the extent of field capacity. Then, at the 10-leaf stage of the plants, salinity treatment was applied in the form of 250 mM sodium chloride per liter for 30 days.

### *2.2. Traits measurement*

In this research, the following traits (Table 2) were measured. After applying salinity treatment, to measure biochemical traits, leaf samples were placed in liquid nitrogen and transferred to the laboratory. Then, the samples were transferred to a -80°C freezer until these traits were measured. To measure relative leaf water content (RWC), in the central part of the canopy, several leaves were selected and their fresh weight (FW) was measured. Then, to calculate the turgor weight (TW), these leaf samples were immediately put in distilled water for 6 hours in a refrigerator at a temperature of 4 °C. To measure the dry weight (DW), the samples were placed in the oven at 70°C for 48 hours and their dry weight was measured.

The relative water content of each sample was calculated with the following equation:

RWC= [(FW-DW)/ (TW-DW)] ×100 (Pierzynski *et al.*, 2013)

To measure $K^+$ and $Na^+$, after collecting the leaf samples, the samples were washed with distilled water to remove external salts from the leaf surface. Then, they were dried for 48 hours at 70°C. In the end, it was measured by flame photometry (Allen *et al.*, 1985).

To extract total protein, 1 gram of plant tissue was crushed by a mortar and then homogenized with 5 ml of extraction buffer (50 mM phosphate buffer, pH 7.0, 0.1 mM EDTA, 4% polyvinyl pyrrolidone). Then, the homogenous solution was transferred to a 1.5 ml centrifuge tube and centrifuged for 20 minutes at 4°C at 13,000 rpm. The supernatant was used as a solution containing protein. The Bradford method measured total protein content using bovine serum albumin (Sigma) as a standard. The reaction mixture containing 20 mM phosphate buffer (pH =7) and 16 mM $H_2O_2$ was prepared, and incubated at 30°C for 3 minutes to measure catalase. Then, 5 microliters of protein extract were added to the 200 microliters reaction solution, and the decrease in absorption in the 240 nm spectrum was read for 2 minutes at 330-second intervals. In the end, catalase activity was calculated as the absorbance difference of 240 nm/mg protein/min (ΔA240/mg protein/min).

**Table 2.** Trait's information in the present study

| Trait name | Abbreviati | Trait name | Abbreviatio |
|---|---|---|---|
| Day to fifteen leaves | DTFL | Biological yield per plant (g) | BY |
| Days to heading | DTH | Harvest index (%) | HI |
| Days to maturity | DTM | Water use (l) | WU |
| Plant height (cm) | PH | Relative water content (%) | RWC |
| Root length (cm) | RL | Relative water loss (%) | RWL |
| Root dry weight (g) | RDW | Water use efficiency (g/l) | WUE |
| Leaves number per plant | LN | Root-to-shoot dry weight ratio | RDW/SDW |
| Tiller number per plant | TN | Sodium ion accumulation (mg/g dry weight) | $Na^+$ |
| Number of fertile spikes per plant | NFS | Potassium ion accumulation (mg/g dry weight) | $K^+$ |
| Number of spikelets per spike | NSS | Potassium to sodium ion ratio | $K^+/Na^+$ |
| Grain number per main spike | GNMS | Catalase (μ mol/g fresh weight) | CAT |
| Grain number per plant | GNP | Superoxide dismutase (μ mol/g fresh weight) | SOD |
| Main spike weight (g) | MSW | Peroxidase (μ mol/g fresh weight) | Pro |
| Grain weight per main spike (g) | GWMS | Hydrogen peroxide (μ mol/g fresh weight) | ProH |
| Peduncle length (cm) | PL | Proline (μ mol/g fresh weight) | Pr |
| Peduncle weight (g) | PW | Total carbohydrate (mg/g fresh weight) | TCar |
| Thousand-grain weight (g) | TGW | Total protein (mg/g fresh weight) | TP |
| Economic yield per plant (g) | EY | Malondialdehyde (μ mol/g fresh weight) | MDA |

Superoxide dismutase (SOD) activity was measured by measuring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) using a spectrophotometer at 560 nm. A 3 ml reaction mixture contained 50 mM phosphate buffer (pH = 7.8), 13 mM methionine, 63 μM nitro blue tetrazolium, 1.3 μL riboflavin, and 100 μL of enzyme extract. The tubes were placed for 10 minutes under two 15-watt fluorescent moonlights, at a distance of 30 cm, and were shaken. One unit of SOD activity was defined as the amount of enzyme needed to inhibit a 50% reduction of nitrobuterazolium activity (Kang and Saltiveit, 2002).

To start the peroxidase activity (POD), one gram of green leaves was crushed and mixed with 0.1 gram of phosphate buffer. Then it was centrifuged at a speed of 4000 rpm for 15 minutes and measured in the spectrum of 470 nm with a spectrophotometer. The reaction cuvette contained 0.1 ml of crude plant extract, 2.9 ml of sodium phosphate buffer, 1 ml of 1% guaiacol, and 1 ml of 0.1 M $H_2O_2$. One unit of POD activity was defined as units of POD per minute in fresh weight (Kang and Saltiveit, 2002).

To measure the concentration of hydrogen peroxide[1], 0.5 g of fresh leaf tissue was homogenized with 5 ml of 0.1% (w/v) trichloroacetic acid in a pre-cooled mortar. This homogenate was then centrifuged at 12,000 rpm for 15 minutes. 0.5 ml of supernatant, 0.5 ml of potassium phosphate buffer (pH = 7), and 1 ml of potassium iodide were added. Then the mixture was homogenized and its absorbance was read in the 390 nm spectrum with a spectrophotometer (Kang and Saltiveit, 2002).

To measure proline, 1 gram of fresh leaves was ground in 5 ml of 3% sulfosalicylic acid, and the extract was filtered. 100 μl of the extract was treated with 2 ml of glacial acetic acid, 2 ml of ninhydrin, and 20 ml of 6 M phosphoric acid for 1 minute at 100 degrees Celsius. After cooling, the mixture was treated with 1 ml of toluene. The optical density of the toluene-containing chromophore was read at 520 nm with a spectrophotometer. Proline concentration

[1] $H_2O_2$

was calculated by the following equation:

µmoles proline per gram tissue = [(µg proline per ml) x ml of toluene)/115.5 µg/µmole] / [(g sample)/5]

Where 115.5 is the molecular weight of proline (Nawaz *et al*., 2020)

To measure the content of malondialdehyde (MDA), 1 gram of fresh leaf was rubbed with 2.5 ml of 10% trichloroacetic acid and then centrifuged for 20 minutes at a speed of 15,000 rpm. Then an equal volume of the above solution and 0.5% thiobarbituric acid in 20% trichloroacetic acid was transferred into the test tube and treated for 30 minutes at 96 degrees Centigrade and placed in ice water for 5 minutes. The obtained solution was centrifuged at 10000 rpm for 5 minutes. The absorbance of the obtained solution was measured in the spectrum of 532 and 600 nm with a spectrophotometer and was calculated with the following equation (Extinction coefficient $155 mM^{-1} cm^{-1}$): The amount of malondialdehyde (mmol) = (OD reduction per minute ×volume of the test mixture) / extinction coefficient (Heath and Packer, 1968).

To measure the amount of soluble sugars, 0.2 grams of fresh leaves were ground in a mortar containing liquid nitrogen in 1 ml of purified laboratory water. Then, the samples were incubated for 10 minutes at 95°C to inactivate sugar-degrading enzymes and centrifuged for 30 minutes at 15,000 rpm at 4°C. The same amount of acetonitrile was added to the supernatant and centrifuged for 15 minutes at 15,000 rpm at 4°C, and then the supernatant was filtered through filter paper and then through a 0.45 µm filter. Then the amount of soluble sugars was read by HPLC.

During the growing season, traits such as days to 15 leaves, days to heading, and days to maturity, RWC, and ELWR were measured. At the end of the growing season, other morphological parameters such as height, dry weight of aerial organs, root weight, etc. were measured.

### *2.3. Statistical Analysis*

Duncan's multiple tests, Pearson's correlation coefficient, step-wise regression (forward method), and principal component analysis were performed using SAS 9.3 and Minitab ver. 16. Programs.

## 3. Results

### *3.1. Mean comparison results*

For all traits, except for tiller number per plant, number of fertile spikes per plant, and number of spikelets per spike, there was a statistically significant difference ($p \leq 0.05$) between cultivars. According to the results (Table 3), the salt-tolerant cultivar (Pishgam) has higher values of the traits of days to heading, day to maturity, plant height, root length, root dry weight, leaves number per plant, grain number per main spike, grain number per plant, main spike weight, grain weight per main spike, peduncle weight, thousand-grain weight, economic yield per plant, biological yield per plant, harvest index, water consumption, relative water content (RWC), water use efficiency, root-to-shoot dry weight ratio, $K^+$, catalase, superoxide dismutase, peroxidase, $K^+/Na^+$ ratio, proline, and total carbohydrates. However, the Shahryar salt-susceptible cultivar had higher values of peduncle length, RWL, $Na^+$, hydrogen peroxide, and malondialdehyde (Table 3).

### *3.2. Correlation analysis results*

Economic yield per plant showed the highest positive and significant correlation with the traits of $K^+$, $K^+/Na^+$ ratio, total protein, root dry weight, root-to-shoot dry weight ratio, SOD, peroxidase, catalase, RWC, root length, harvest index, water consumption, plant height, main spike weight, grain weight per main spike and total carbohydrate, respectively (Table 4). Nevertheless, the economic yield per plant had the most negative and significant ($p \leq 0.05$) correlation with malondialdehyde, relative water loss (RWL), hydrogen peroxide, and $Na^+$, respectively.

**Table 3.** Results of t-test mean comparison for different traits of two salt-tolerant (G1) and susceptible (G2) wheat cultivars under salt stress conditions

| Trait (unit) | G1 (Pishgam) | G2 (Shahryar) | Trait (unit) | G1 (Pishgam) | G2 (Shahryar) |
|---|---|---|---|---|---|
| DTFL | 82.33[a] | 82.50[a] | By (g) | 11.11[a] | 9.63[b] |
| DTH | 96.33[a] | 91.00[b] | HI | 16.07[a] | 10.46[b] |
| DTM | 121.33[a] | 111.66[b] | WU (l) | 9699.3[a] | 9005.3[b] |
| PH (cm) | 62.30[a] | 55.90[b] | RWC (%) | 87.50[a] | 80.13[b] |
| RL (cm) | 38.60[a] | 31.73[b] | RWL (%) | 289.33[b] | 391.33[a] |
| RDW(g) | 4.88[a] | 2.99[b] | WUE | 0.0011[a] | 0.0010[b] |
| LN | 17.33[a] | 16.00[b] | RDW/SDW | 0.44[a] | 0.31[b] |
| TN | 3.66[a] | 3.33[a] | $Na^+$ (mg/g dry weight) | 39.43[b] | 41.66[a] |
| NFS | 1.66[a] | 1.66[a] | $K^+$ (mg/g dry weight) | 62.36[a] | 46.66[b] |
| NSS | 20.33[a] | 19.66[a] | $K^+/Na^+$ | 1.58[a] | 1.12[b] |
| GNMS | 24.66[a] | 20.00[b] | CAT (µ mol/g fresh weight) | 227.33[a] | 188.66[b] |
| GNP | 35.66[a] | 27.33[b] | SOD (µ mol/g fresh weight) | 790.00[a] | 555.66[b] |
| MSW(g) | 1.84[a] | 1.36[b] | Pro (µ mol/g fresh weight) | 736.00[a] | 524.66[b] |
| GWMS (g) | 1.58[a] | 1.24[b] | ProH (µ mol/g fresh weight) | 38.00[b] | 46.66[a] |
| PL (cm) | 21.56[b] | 23.65[a] | Pr (µ mol/g fresh weight) | 39.33[a] | 30.66[b] |
| PW(g) | 0.58[a] | 0.39[b] | Tcar (mg/g fresh weight) | 42.13[a] | 36.00[b] |
| TGW (g) | 49.41[a] | 37.14[b] | TP (mg/g fresh weight) | 3.15[a] | 2.71[b] |
| EY(g) | 1.77[a] | 1.00[b] | MDA (µ mol/g fresh weight) | 9.35[b] | 11.04[a] |

The full names of the traits and their abbreviations are given in Table 2.

**Table 4.** Results of correlation analysis between economic yield per plant and different traits of two salt-tolerant (G1) and susceptible (G2) wheat cultivars under salt stress conditions

| Trait | Economical yield per plant | Trait | Economical yield per plant |
|---|---|---|---|
| DTFL | -0.20 | BY | 0.69* |
| DTH | 0.53 | HI | 0.96** |
| DTM | 0.82** | WU | 0.85** |
| PH | 0.88** | RWC | 0.88** |
| RL | 0.86** | RWL | -0.94** |
| RDW | 0.98** | WUE | 0.45 |
| LN | 0.47 | RDW/SDW | 0.96** |
| TN | 0.03 | $Na^+$ | 0.68* |
| NFS | -0.06 | $K^+$ | 0.99** |
| NSS | 0.25 | $K^+/Na^+$ | 0.99** |
| GNMS | 0.65* | CAT | 0.95** |
| GNP | 0.61* | SOD | 0.96** |
| MSW | 0.86** | Pro | 0.96** |
| GWMS | 0.80** | ProH | -0.75* |
| PL | -0.65* | Pr | 0.74* |
| PW | 0.95** | Tcar | 0.82** |
| TGW | 0.93** | TP | 0.98** |
| EY | 1.00 | MDA | -0.97** |

The full names of the traits and their abbreviations are given in Table 2.

*3.3. Stepwise regression results*

According to the stepwise regression results (Table 5), the traits of $K^+$, SOD, proline, and RWC were entered into the regression model, as the most important traits affecting the economic yield per plant under salt conditions.

*3.4. Principal component analysis results*

The values of eigenvectors (Table 6) indicated a high value of the first component and a low value of the second component is suitable for improving economic yield under salt conditions. Therefore, the best area of the biplot diagram is the 4$^{th}$ area of the biplot, based on which, the Pishgam variety was identified as the most favorable salt-tolerant genotype. The traits of harvest index, root-to-shoot dry weight ratio, root dry weight, plant height, $K^+/Na^+$ ratio, total protein, SOD, catalase, peroxidase, and total carbohydrate showed the highest alignment with increasing grain yield and they were identified as the most desirable traits to select favorable genotypes under salt conditions (Figure 1) .

**Table 5.** Results of stepwise regression for economic yield, as a dependent variable, in two salt-tolerant (G1) and susceptible (G2) wheat cultivars under salt stress conditions

| Regression step | Intercept | Regression coefficients for entered different traits in 4 steps in the regression model | | | | Cumulative detection coefficient |
|---|---|---|---|---|---|---|
| | | $K^+$ | SOD | Pr | RWC | |
| 1 | -1.33 | 0.050 | - | - | - | 78.03** |
| 2 | -1.65 | 0.087 | 0.002 | - | - | 89.72** |
| 3 | -1.99 | 0.109 | 0.004 | 0.013 | - | 95.91** |
| 4 | -1.41 | 0.119 | 0.005 | 0.022 | 0.012 | 99.00** |

The full names of the traits and their abbreviations are given in Table 2.

**Table 6.** The results of eigenvalues and eigenvectors of the first and second components for different traits of two salt-tolerant (G1) and susceptible (G2) wheat cultivars under salt stress conditions

| Trait | PC1 | PC2 | Trait | PC1 | PC2 |
|---|---|---|---|---|---|
| DTFL | 0.01 | 0.37 | BY | 0.17 | 0.20 |
| DTH | 0.15 | 0.26 | HI | 0.16 | -0.26 |
| DTM | 0.18 | 0.07 | WU | 0.18 | 0.02 |
| PH | 0.15 | -0.23 | RWC | 0.19 | 0.02 |
| RL | 0.18 | 0.02 | RWL | -0.18 | 0.07 |
| RDW | 0.19 | -0.11 | WUE | 0.138 | 0.310 |
| LN | 0.09 | -0.10 | RDW/SDW | 0.16 | -0.23 |
| TN | 0.05 | 0.32 | $Na^+$ | -0.10 | 0.28 |
| NFS | 0.04 | 0.07 | $K^+$ | 0.19 | -0.10 |
| NSS | 0.06 | 0.03 | $K^+/Na^+$ | 0.18 | -0.15 |
| GNMS | 0.16 | 0.10 | CAT | 0.19 | -0.03 |
| GNP | 0.16 | 0.24 | SOD | 0.19 | -0.03 |
| MSW | 0.18 | 0.04 | Pro | 0.19 | -0.03 |
| GWMS | 0.17 | 0.09 | ProH | -0.18 | -0.10 |
| PL | -0.16 | -0.20 | Pr | 0.18 | 0.17 |
| PW | 0.19 | -0.03 | Tcar | 0.17 | -0.02 |
| TGW | 0.19 | -0.02 | TP | 0.19 | -0.09 |
| EY | 0.18 | -0.15 | MDA | -0.19 | 0.07 |
| Eigenvalue | 25.66 | 4.42 | Cumulative variance | 0.71 | 0.84 |

The full names of the traits and their abbreviations are given in Table 2.

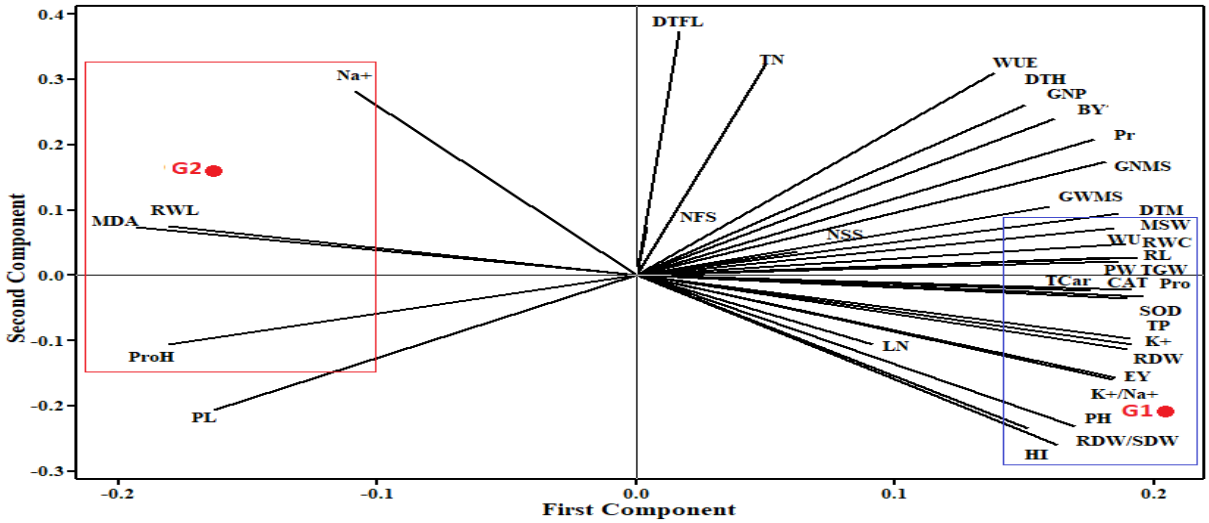


**Fig. 1.** Results of principal component analysis for two salt-tolerant (G1) and susceptible (G2) wheat cultivars under salt stress conditions (The full names of the traits and their abbreviations are given in Table 2; G1: Pishgam and G2: Shahryar).

## 4. Discussion

The results of the mean comparison for different traits indicated the existence of a high genetic diversity between the cultivars for most of the traits (Table 3). The requirement for selection to tolerate any environmental stress, including salinity stress, is the existence of a wide range of genetic diversity among genotypes, which fortunately, in this research, significant diversity was observed for most of the traits. In previous studies, (Moosavi *et al.*, 2022; Harb and Pereira, 2013) a high genetic diversity between planted cultivars and wild ecotypes of wheat has been reported under abiotic stress conditions. According to the results (Table 3), Pishgam, a salt-tolerant cultivar, showed a high value of phenological traits, including days to heading and days to maturity, and a lower value of day to fifteen leaves. In reality, this result shows that reducing the time to reach fifteen leaves and increasing the heading and maturity period, causes the faster establishment of the plant and more exploitation of the potential of the growing season. So that the results of the correlation analysis (Table 4) confirmed this issue. Indeed, the traits of day-to-heading and day-to-maturity had a positive correlation with economic yield, while days to fifteen leaves had a negative correlation with grain yield. One of the mechanisms to absorb more water and deal with salt stress in the plant is to increase the length and weight of the roots and root-to-shoot dry weight, which was observed in the salt-tolerant variety in this research. So, the results of this research also showed a strong and significant ($P \leq 0.01$) correlation between root characters with economic yield (Table 4). In a research (Chaurasia *et al.*, 2022) was reported that root length and root dry weight showed a strong positive correlation with salt tolerance index. They proposed these traits for the selection of salt tolerance lines in wheat breeding programs.

The improvement of the yield components including grain number per main spike, grain number per plant, main spike weight, grain weight per main spike, peduncle weight, thousand-grain weight, and harvest index in the salt-tolerant cultivar, led to an increase in its grain yield under saline conditions. The correlation results also confirm this issue. Irshad *et al.*, (2022) reported a higher $K^+/Na^+$ ratio, free amino acids, proline, and total chlorophyll in the salt-tolerant genotype. Therefore, $K^+$ has a major role in the maintenance of the overall shoot $K^+/Na^+$ ratio in salt-tolerant wheat genotypes (Chaurasia *et al.*, 2022). Previously, Shabala *et al.*, (2010) also reported that salt-tolerant wheat genotypes maintained higher $K^+/Na^+$ accumulation ratios

under saline conditions. In our study, salt-tolerant wheat genotypes also maintain a relatively lower level of $Na^+$ accumulation content and a higher level of $K^+/Na^+$ accumulation ratios, which can provide valuable genetic resources to understand the genetic mechanism of salt tolerance in wheat and donors for the improvement program.

The higher number of leaves in the plant and increasing the plant height will lead to improved photosynthesis and increased biological performance, which will ultimately increase economic yield. More absorption capacity and more water use, high relative water content, more water use efficiency along with higher photosynthetic ability (through having a higher amount of traits of leaf number, plant height, and biological performance) in the Pishgam cultivar, leading to improved salt tolerance and increased grain yield. Reduced growth in saline conditions is the result of several physiological responses, the most important of which is reduced photosynthesis. The reduction of photosynthesis caused by salinity stress can be due to lower stomatal conduction, and reduction of metabolic processes (Jamil *et al.*, 2007).

The major negative effects of salinity on plant growth and development include inhibition of photosynthesis (Sharma., *et al.*, 2005), water deficiency, and ion toxicity related to the increase of chlorine and sodium ions (Patel and Pandey, 2008), which this issue leads to nutrient imbalance (Misra *et al.*, 1997).

The salinity-tolerant cultivar of Pishgam has high potassium absorption and sodium excretion, which has led to an improvement in the ratio of potassium to sodium absorption in this genotype. However, the Shahryar salt-susceptible cultivar had higher values of relative water loss (RWL) and sodium ion accumulation (Table 3). The photosynthesis process is adversely affected by the accumulation of $Na^+$ in plants (Jiang *et al.*, 2017). The growth and development of the plant depend on potassium, so potassium plays key roles such as the activation of more than fifty types of enzymes, and the osmotic regulation of the plant (Jiang *et al*., 2017). Several types of research (Jiang *et al.*, 2017; Hamamoto *et al.*, 2015) that have been conducted about the mechanisms of dealing with salinity in plants show the high importance of the $K^+/Na^+$ ratio in these mechanisms. It has been shown in various studies that the high $K^+/Na^+$ ratio has a direct relationship with the salinity tolerance capacity of the plant.

The high concentration of potassium in the plant will moderate the negative effects caused by the accumulation of sodium in the plant. By activating channels in the cell membrane, potassium causes sodium to leave the xylem vessels and store them in the vacuole (Irshad *et al.*, 2022). Sodium is known as a beneficial element in some plants. The presence of sodium in the growth environment of Halophyte plants and C4 plants is essential and for such plants, it can be considered as an essential element of low consumption. On the other hand, sodium, especially for Glycophyte plants, is not considered an essential element and can cause plant toxicity in high concentrations.

Excessive amounts of neutral soluble salts (including the sulfates and chlorides of Mg, K, Ca, and Na) in soil or water cause adverse effects on the growth of plants by disrupting the uptake of essential macro-or micro-nutrients (Athar and Ashraf, 2009). Accumulation of $Na^+$ is one of the harmful effects of salinity (Irshad *et al.*, 2022). The accumulation of $Na^+$ in the plant inhibits the intake of other essential macronutrients such as $K^+$ and $Ca^+$ from the soil (Brini *et al.*, 2009). Maintenance of the $K^+$ /$Na^+$ ratio in the shoot is one of the main strategies to control stress in plants (Hamamoto *et al.*, 2015).

While, the Pishgam cultivar had higher amounts of catalase, superoxide dismutase, peroxidase, proline, and total carbohydrates, the Shahryar cultivar had higher amounts of sodium ion accumulation, hydrogen peroxide, and malondialdehyde. Proline plays an important role in osmotic adjustment and the stability of the cell membrane structure during stress in plants (Munns, 2002; Romero-Aranda *et al.*, 2006).

Salt-tolerant genotypes with relatively high total soluble proteins, provide information on the total proteins in a salt-tolerant variety (Ashraf, 2004). In plants, shoots and roots showed differential responses to the monovalent and divalent cations about accumulation/ distribution. Roots absorb more $Na^+$ because it has direct contact with the soil as compared to shoots under control condition. However, in stress conditions, $Na^+$ is transported and executed from leaves to maintain the optimum level as observed in Pishgam. Salt-tolerant genotypes possess exclusion mechanisms that control the entry of Na+ into roots (Munns *et al.,* 2020), and exclusion of the excessive $Na^+$ from photosynthetic tissues (Tabassum *et al.,* 2021). The adverse effect of salt stress on $K^+$ uptake could be seen in the wheat genotypes where the maximum reduction in absorption/transport was observed.

To reduce the adverse effects of environmental stresses and control the level of ROS to protect cells, plants have various protective mechanisms, including enzymatic and non-enzymatic antioxidant systems, which are effective at different levels of environmental stress (Shi *et al.,* 2007). Peroxidase, superoxide dismutase, and catalase enzymes are among the most prominent protective mechanisms to remove ROS, which convert free radicals of superoxide ($O_2^{*-}$) into hydrogen peroxide ($H_2O_2$) and finally hydrogen peroxide was transformed into oxygen and water (Tanou *et al.,* 2009).

## 5. Conclusion

According to the results, it is possible to suggest two groups of markers to select salt-tolerant genotypes. The first group includes some morpho-physiological markers namely a high amount of harvest index, main spike weight, relative water content, water consumption, root-to-shoot dry weight ratio, total carbohydrates, proline, and root dry weight, and the second group includes the ionic and biochemical markers namely a high amount of $K^+$ accumulation, $K^+/Na^+$ accumulation ratio, SOD, catalase, peroxidase, and a low amount of $Na^+$ accumulation, malondialdehyde, and hydrogen peroxide. Of course, to ensure the obtained results and practical use of the above-suggested indicators, it is necessary to repeat the experiment under similar salinity conditions.

Based on the comprehensive analysis of morpho-physiological, and biochemical traits, we identified a salt-tolerant cultivar. The previous research (Chaurasia *et al.,* 2022) reported that six parameters, i.e., plant biomass, RWC, shoot $K^+$ content, root $Ca^{2+}$, shoot $K^+/Na^+$ ratio, and root $Na^+/Ca^{2+}$ ratio, are the most suitable parameters for salinity stress tolerance screening in bread wheat. According to our results, a high amount of harvest index, main spike weight, relative water content, water consumption, root-to-shoot dry weight ratio, total carbohydrates, proline, root dry weight, $K^+$, $K^+/Na^+$ ratio, SOD, catalase, peroxidase, and a low amount of $Na^+$, malondialdehyde, and hydrogen peroxide, were proposed as selective indicators for screening wheat salt-tolerant genotypes. Moreover, the accumulation of total free amino acids plays a role in changes in osmotic potential but did not play a role in differential salt tolerance.

## Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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