Response of bread wheat cultivars to salinity stress trend under greenhouse conditions

Mojgan Barani Biranvand, Omidali Akbarpour*, Hamidreza Eisvand

Department of Plant Production and Genetic Engineering, Faculty of Agriculture, Lorestan University, Khorramabad, Lorestan, Iran

Abstract

Salinity has emerged as a major threat to crop production worldwide and particularly in Iran. This study aimed to assess the salinity tolerance of selected Iranian wheat cultivars under greenhouse conditions. Twelve wheat cultivars were exposed to various salt concentrations, including both freshwater and saline water with electrical conductivities of 3, 6, 9, 12, and 15 dS/m. Yield, yield components, and some physiological traits were subjected to analysis of variance and supplementary analyses. The ANOVA results revealed that all the characteristics demonstrated different responses, and their interactions with cultivar and treatment were statistically significant ($P<0.01$). In response to increasing salinity levels, yield and yield components were negatively impacted while Na^+ in leaves and electrolyte leakage increased. The cultivars Bam, Kuhdasht, Pishtaz, and Aflak outperformed the others in terms of grain yield and electrolyte leakage, indicating their tolerance to increased Na^+ concentration. However, the results also suggested that an increase in Na^+ or a decrease in K^+ ions led to a decreased yield in all cultivars. Therefore, Na^+ and K^+ cannot exclusively describe the yield response to stress conditions, and their behavior is beyond Na^+ and K^+ processes or their ratio. Overall, this study demonstrated that the evaluated cultivars are significantly diverse, and they can be used in crossing methods to extend the range of salinity tolerance in wheat germplasm.

Keywords: grain yield, ion content; physiological stress, tolerance, tissue

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Introduction

Salinity is a major obstacle for economic production of wheat, which is a strategically important commodity for food security worldwide. Every year, thousands of irrigated acres of land are lost in countries such as Iran, Iraq, Pakistan, and Australia due to soil salinization (AbdelRahman, 2023). The ability to cultivate plants in soil affected by salinity depends on the degree of salinity and the plant tolerance to it. While the reclamation of saline soils is sometimes recommended, it can have environmental consequences such as the loss of quality water. Sustainable and cost-effective solutions can be found in the discovery of new genetic resources and the study of genes that control mechanisms for coping with salinity (Colmer et al., 2006).

The main constraints in breeding programs for wheat salinity tolerance are a limited gene pool, insufficient priority given to breeding against

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** Corresponding Author*
E-mail Address: akbarpour.oa@lu.ac.ir
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salinity, negative linkage between high-salinity tolerance and high-performance genes, and a lack of knowledge about the complexity of salt-tolerance mechanisms (Akbarpour et al., 2015; Ashraf and Akram, 2009; Shannon and Qualset, 1984). The effect of salinity on crop yield reduction has spurred interest in studying the genetics of plant tolerance mechanisms (Chinnusamy et al., 2005). Different cultivars of wheat, barley, and rice have been reported with varying degrees of salinity stress tolerance (Flowers, 2004). Despite the tremendous challenges, breeding for salinity tolerance remains one of the most effective ways to sustainably produce crop yields in saline environments (Genc et al., 2010). Salinity tolerance allows genotypes to grow in a relatively saline environment and produce acceptable yields (Munns, 2002).

There are three mechanisms by which crops can cope with salinity stress tolerance: osmotic tolerance, ion exclusion, and tissue tolerance, which are used in breeding and screening programs for a wide range of plant genetic materials, especially wheat. Despite debates on screening methods, choosing target genotypes via conventional breeding methods for these mechanisms is a reliable approach for screening wheat under salinity stress (Li et al., 2017; Munns and Tester, 2008). However, studying wheat grain yield in salinity stress conditions is complex and is directly and indirectly influenced by the expression of multiple genes related to physiological, biochemical, and molecular factors (Läuchli and Grattan, 2007).

Despite the limitations in breeding under saline conditions, new promising genetic resources have been reported for salt tolerance in wheat. Dadshani et al. (2019) found that Z86 was a potentially strong wheat population that can improve desirable morphological, physiological, and agronomic traits in different stages of plant growth under salinity stress. Akbarpour et al. (2015) showed that the Iranian wheat germplasm exhibits great variety in salt tolerance under field conditions and suggested that most morphological and agronomic characteristics are suitable for classifying wheat genotypes in salinity stress. Additionally, a diallel mating system in Iranian wheat cultivars was found to have additive and non-additive effects on controlling traits, and recurrent selection followed by pedigree breeding was recommended as a useful method for improving salinity tolerance (Dehdari et al., 2005).

Production of high-yielding cultivars is limited by different abiotic factors such as soil salinity stress, especially in arid and semi-arid countries. Therefore, evaluating the cultivated plants in their origin leads to improving the cultivars with superior genes and offers additional opportunities to select and introduce promising candidate varieties to other locations that are affected by salinity matter. This research aims to investigate and identify the salinity tolerance of some new releases of Iranian bread wheat cultivars with high diversity to salt tolerance and compare their responses to increasing salinity treatments under greenhouse conditions.

Materials and Methods

Genotype materials and experimental design

This study was conducted in a greenhouse at the Department of Plant Production and Genetics, Lorestan University in Khorramabad, Iran during 2019. Twelve wheat cultivars of Iranian germplasm were used with distinct genetic makeups and origins including Aflak, Arg, Auhadi, Bam, Backcross BC_Rasad, Chamran, Ghabus, Karim, Kuhdasht, Pishtaz, Pougari, and Sardari collected from various sources. The average day and night temperatures during sowing until the tillering time were 16 ± 8 ℃ and 10 ± 6 ℃, respectively, with an average relative humidity ranging from 35% to 71% and a photoperiod of 9 to 11.5 hours.

The experiment was carried out in a completely randomized block design with three replications of 12 wheat cultivars and five salt treatments, with freshwater serving as the control treatment. Seeds were directly sown in pots with dimensions of 22 cm wide by 22 cm deep equaling an area of 0.007 m^2 with well-mixed loam soil that contained 25% well-rotted manure, 25% sand, and 50% soil.

Prior to seed cultivation, physical and chemical properties including electrical conductivity (EC) of the pot soil, were assessed by taking common samples. The analyzed soil samples contained organic matter (11.5 mg/g), total N (0.94 mg/g), available N (122.2 mg kg^{-1}), effective P (52.4 mg kg^{-1}), and available K (222.4 mg kg^{-1}). For each pot, 8 seeds were planted 3-4 cm below the soil surface.

Six irrigation water treatments with salt concentrations ranging from 0 to 15 dS/m (0, 3, 6, 9, 12, and 15 dS/m) were applied for each cultivar in three replications. Salt concentration treatments were achieved by adding NaCl to freshwater until each treatment reached the target concentration level. All pots were initially watered with freshwater until the three-leaf stage. The freshwater lacked NaCl and had an EC of 0.7. The initial EC of irrigated water for salinity treatments of 3 and 6 dS/m was applied at the start of the treatments after the three-leaf stage and was then increased according to Villalobos et al. (2016). The final concentration of saline water (15 dS/m) was applied in the second irrigation after the start of the salt treatments. To prevent excessive salt accumulation and to maintain the salt balance in the soil during irrigation with saline water, 20% water was added to the initial field capacity of the soil or absorbed water by the root system as a leaching fraction. The estimated EC in the saturated soil (EC_e) was calculated using the following equation.

$$\mathrm{EC_e} = -0.5\mathrm{EC_w}\frac{\ln(\mathrm{LF})}{1-\mathrm{LF}}$$

where LF is the leaching fraction. Excess water was applied to avoid salt concentration accumulation in the soil during the plant lifetime.

Physiological measurements

Ions

After exposing the plants to the highest level of saltwater concentration (15 dS/m) twice, leaf samples were gathered from each pot for a series of physiological measurements. The treatment involving the highest level of NaCl was administered twice, and three leaves were collected from each replication (pot). The leaves were then digested in 10-20ml of 1% HNO_3 at 85°C for 4 hours using a Teflon hot-block. Finally, the concentrations of Na^+ and K^+ were determined via flame photometry.

Electrolyte leakage

Electrolyte leakage is a test used to determine the permeability of leaf cell membranes. To carry out this test, fresh leaf samples were obtained, following the method stated in Lutts et al. (1996), by cutting pieces of approximately 1 cm in size, which were then washed three times with distilled water to remove any surface contamination. Next, the samples were placed in sealed glass tubes, each containing 10 ml of distilled water, for two hours at room temperature (25 ℃). The initial electrical conductivity measurement of the solution was recorded (EC_1) using a conductivity meter. The samples were then taken and autoclaved for 20 minutes at 121 ℃ to release all electrolytes. After cooling down to room temperature, the electrical conductivity of the resulting solution was measured once again (EC_2). Finally, the percentage of electrolyte leakage (EL) was calculated using the following equation:

$EL = (EC_1/EC_2) \times 100$ (%).

Chlorophyll content

To determine the chlorophyll content, a SPAD meter (SPAD 502, Minolta Japan) was used to take measurements at two different intervals: 45 and 50 days after cultivation. The average of five SPAD chlorophyll readings was used to determine the final chlorophyll content measurement.

Yield-related traits

The final harvest was conducted when at least 50% of all treatment pots reached full maturity, with five plants per pot. The aboveground biomass of the wheat plants was harvested and measured accurately. Threshing was done with great care to ensure that all grains were retained, and other characteristics (not reported) were estimated, including the harvest index.

Statistical Analysis

To analyze the data, SAS Version 9.1 (SAS, 2004) was used to perform ANOVA. Moreover, to examine the impact of water salinity levels on the characteristics of grain yield for various cultivars, a simple linear regression analysis was carried out and visually represented using the R software with

Table 1
Analysis of variance for wheat characteristics in factorial design arrangement

S. O. V	Df	Biological Yield	Chlorophyll Contents	Grain Yield	Harvest Index	Electrolyte Leakage	K^+	K^+/Na^+	Na^+
Replication	2	71.27^{**}	35.16^{**}	0.02^{ns}	0.33^{**}	4.85^{ns}	13443756^{**}	13^{ns}	75197^{**}
Treatment	11	925.48^{**}	3869.50^{**}	128.2^{**}	7.79^{**}	1857.04^{**}	59406367^{**}	11395^{**}	23337597^{**}
Cultivar	5	270.16^{**}	95.10^{**}	26.2^{**}	3.06^{**}	96.98^{**}	392747525^{**}	877^{**}	949307^{**}
Cultivar × Treatment	55	45.24^{**}	36.67^{**}	1.7^{**}	0.25^{**}	22.06^{**}	35280150^{**}	184^{**}	150239^{**}
Error	142	7.09	4.73	0.17	0.05	9.28	1868920	19	13727

ns and ** indicating non-significant and significant at 0.01 probability level, respectively

packages such as *rlang*, *ggplot2*, *reshape2*, *lattice*, and *RColorBrewer* (R core team, 2017). Descriptive statistics were also obtained to provide a summary of the data. Additionally, the relationship between the dependent variables and cultivars and treatments were displayed graphically in a biplot using the *factoextra* package in R (R Core Team, 2017) after performing principal component analysis (PCA).

Results

Electrical conductivity in water and soil

The correlation between the electrical conductivity of saturated soil (ECe) and the salinity of applied water (EC_w) yielded an R^2 value of 0.93 (as shown in Fig. I). Moreover, even with an increase in EC_w from 0.7 to 15 dS/m, EC_e remained below 15. The regression line had a slope of 0.7, indicating that for every unit increase in EC_w, EC_e increased by 0.7. Failure to apply a leaching fraction of 20% would have resulted in elevated salinity levels in saturated soil. Numerous factors contributed to the variances observed in EC_e values under various salinity treatments in Fig. (I), including cultivar, soil water-holding capacity, soil physical and mineral characteristics, soil heterogeneity, and other unidentified factors that differed in individual pots.

Yield-related traits

The study analyzed the effects of cultivars and increasing salinity treatments on various yield-related characteristics. The interaction between cultivars and treatments was significant, making the selection of superior cultivars based on mean comparison challenging. Regression analysis was used to identify high saltwater tolerant cultivars for yield stability (Fig. II). Results indicated a negative impact of salinity on grain yield, suggesting varying cultivar salinity tolerance (Fig. II).

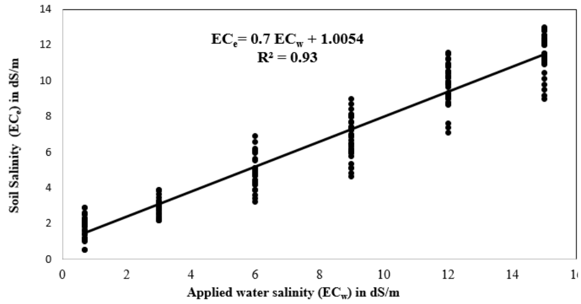

Fig. I. Graphical regression that relates the salinity of saturated soil to the applied salinity levels of water treatments

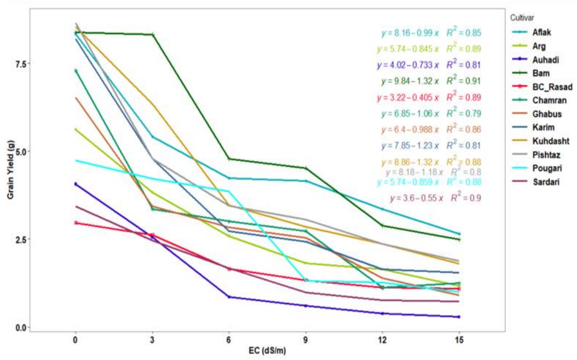

Fig. II. Yield reduction trend of evaluated wheat cultivars at different salinity levels, based on regression parameters

Sardari, BC_Rasad, Auhadi, and Pougari were identified as the most salt-sensitive cultivars while Aflak and Bam had the lowest coefficient of variation (CV) and highest yield stability. The best cultivars should be selected based on mean yield, intercept, slope of regression, and R^2, rather than relying solely on the yield stability index. Bam, Aflak, Pishtaz, and Kuhdasht cultivars showed the

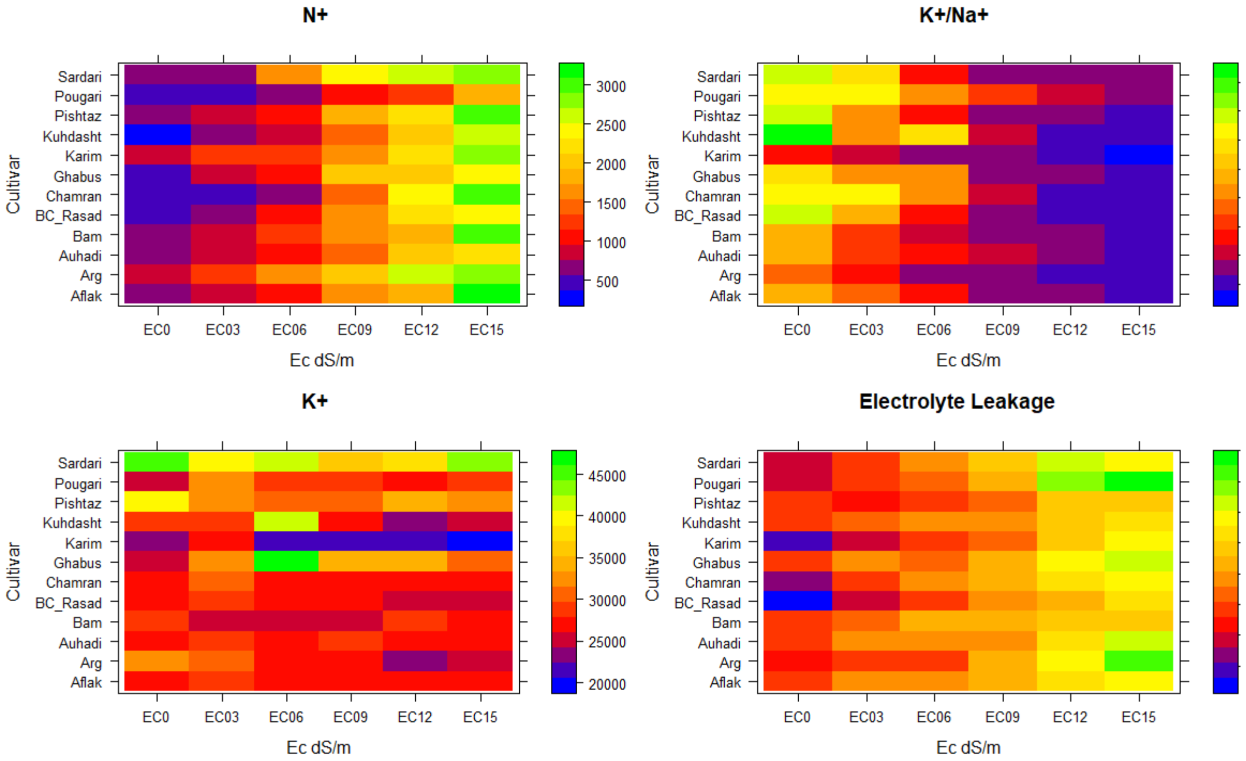

Fig. III (a) The heatmap displays the pattern of responses observed in grain yield, biological yield, harvest index, and chlorophyll content for each cultivar under varying treatments. (b) The heatmap depicts the responses observed in electrolyte leakage, Na^+ and K^+ ion concentrations, and their ratio for each cultivar under different treatments.

highest yield across salinity treatments and had the highest harvest index and biological yield. The harvest index had a high correlation with grain yield while the Aflak, Pishtaz, Kuhdasht, Arg, and Bam cultivars had the lowest CV and maximum values of it.

In sum, cultivar selection based on their adequate performance or adaptation to saline conditions is crucial in alleviating salinity effects rather than only relying on the yield stability index.

Physiological characteristics

Fig. (III, a) showed how the cultivars differed in terms of their chlorophyll contents when compared to their yield and biological yield. Although all cultivars had a decreasing trend in their chlorophyll contents, their reactions were not the same. For example, among all cultivars, Chamran showed the lowest and a linear decreasing chlorophyll content, whereas Karim, Pishtaz, Pougari, Bam, and B_Rasad showed linear decreasing chlorophyll contents. Additionally, some cultivars such as Sardari, Kuhdasht, Ghabus, Auhadi, Arg, and Aflak had varied rankings and inconsistency in their chlorophyll contents from EC 3 to EC 15, bearing salinity levels.

Table 2 shows that Aflak and Sardari had the lowest (0.24) and the highest (0.43) values, respectively, for the CV parameters, which indicate the high and low stability of chlorophyll contents across treatments.

Conversely, all cultivars showed an increase in electrolyte leakage (EL) with growing NaCl levels when compared to non-saline conditions as shown in Fig. (III, b). Bam, Kuhdasht, Aflak, and Pishtaz had the lowest CV of EL (Table 2). However, Karim and B_Rasad had the maximum variation for EL, indicating instability in the treatment area. Additionally, the cultivars' reaction to EL under different stress treatments increased as salinity rose and varied from one cultivar to another. Lastly, high-yielding cultivars tended to have less EL, especially at higher salinity stress levels.

Table 2
Variations in wheat cultivar characteristics mean, standard error, and coefficient across various salinity levels

Cultivar	Biological yield		Chlorophyll content		Grain yield		Harvest Index	
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV
Aflak	23.1±2.57	0.27	29.6±2.93	0.24	4.69±0.82	0.43	0.2±0.013	0.16
Arg	17.5±1.73	0.24	32.6±3.55	0.27	2.78±0.68	0.60	0.15±0.024	0.38
Auhadi	19.1±3.23	0.41	30.2±3.48	0.28	1.46±0.62	1.05	0.06±0.018	0.70
Bam	26.9±1.74	0.16	29.2±3.4	0.29	5.23±1.05	0.49	0.19±0.03	0.38
BC_Rasad	25.2±2.08	0.20	35.1±5.42	0.38	1.8±0.33	0.45	0.07±0.011	0.39
Chamran	23.1±2.34	0.25	35.5±4.95	0.34	3.13±0.92	0.72	0.13±0.026	0.50
Ghabus	21.4±1.47	0.17	32.3±5.54	0.42	2.94±0.81	0.68	0.13±0.029	0.53
Karim	26.2±3.18	0.30	33.5±4.37	0.32	3.56±1.04	0.72	0.13±0.022	0.43
Kuhdasht	29.2±4.07	0.34	29.3±4.06	0.34	4.23±1.08	0.63	0.14±0.019	0.33
Pishtaz	24.6±3.54	0.35	31.1±3.54	0.28	4.03±1.01	0.61	0.16±0.017	0.26
Pougari	24±1.9	0.19	34.3±4.85	0.35	2.73±0.7	0.63	0.11±0.027	0.58
Sardari	16.3±1.07	0.16	34.1±6.03	0.43	1.67±0.44	0.65	0.1±0.022	0.54

Cultivar	Electrolyte Leakage		K^+		K^+/Na^+		NA^+	
	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV	Mean ± SE	CV
Aflak	36.42±2.29	0.15	27046±311	0.03	25.16±6.08	0.59	1479±376	0.62
Arg	36.07±4.18	0.28	27655±1272	0.11	18.4±4.35	0.58	1854±311	0.41
Auhadi	36.65±2.51	0.17	27532±255	0.02	25.05±5.41	0.53	1376±268	0.48
Bam	35.18±1.52	0.11	26760±581	0.05	22.91±5.36	0.57	1504±333	0.54
BC_Rasad	30.7±3.46	0.28	26868±470	0.04	30.76±9.54	0.76	1355±332	0.60
Chamran	33.97±3.21	0.23	27777±563	0.05	34.08±10.12	0.73	1431±452	0.77
Ghabus	37.7±2.78	0.18	33764±2834	0.21	31.21±7.58	0.59	1489±337	0.55
Karim	31.01±3.79	0.30	22597±883	0.10	16.52±3.07	0.46	1626±287	0.43
Kuhdasht	35.14±2.07	0.14	29141±2748	0.23	36.98±11.68	0.77	1301±357	0.67
Pishtaz	32.92±2.24	0.17	33085±1548	0.11	29.41±8.42	0.70	1575±364	0.57
Pougari	37.36±4.57	0.30	28412±1026	0.09	38.41±8.2	0.52	972±224	0.56
Sardari	35.87±3.29	0.22	40894±1546	0.09	33.15±9.82	0.73	1780±385	0.53

The analysis of Na^+, K^+, and K^+/Na^+ ratio of the wheat leaves showed that the cultivar × treatment effects were significant, as shown in Table 1. The trend of the ions accumulated in the leaves for K^+ differed entirely from Na^+ as presented in Fig. (III, b). The accumulated Na^+ in the leaves increased in all cultivars across different treatments, without exception. However, the process was not equivalent for all cultivars. Similarly, the accumulation of K^+ in the leaves of cultivars showed different trends. For example, the maximum K^+ was observed at the lowest and highest water salinity concentration for Sardari cultivar, whereas other treatments had intermediate values. Moreover, there were no significant differences in leaves' K^+ among cultivars of Aflak, Auhadi, Bam, B_Rasad, and Chamran, indicating the narrowest variety for this characteristic, as shown in Fig. (III, a) and Table 2. Conversely, in other cultivars such as Ghabus, Karim, Kuhdasht, Arg, and Pishtaz, there were comparatively significant differences, indicating complex responses to applied saline treatments, as shown in Table 2. Lastly, the K^+ accumulated in the leaves of some cultivars decreased as salinity levels increased. Moreover, in some cultivars, linear models were not observed, indicating the complex response of K^+ accumulation to salt treatment.

Biplot Analysis

The biplot analysis depicts how the independent variable, which includes a combination of salinity treatment levels and cultivars, relates to the dependent variables as shown in Fig. (IV). The figure displays the "which-won-where" pattern, revealing the winning cultivar in each condition for the target variable. The first two principal components explained a total variation of 75.9%. The cosine of angle vectors and Euclidean distance can help determine the correlation between cultivars, treatments, and other variables. For example, grain yield showed a positive correlation

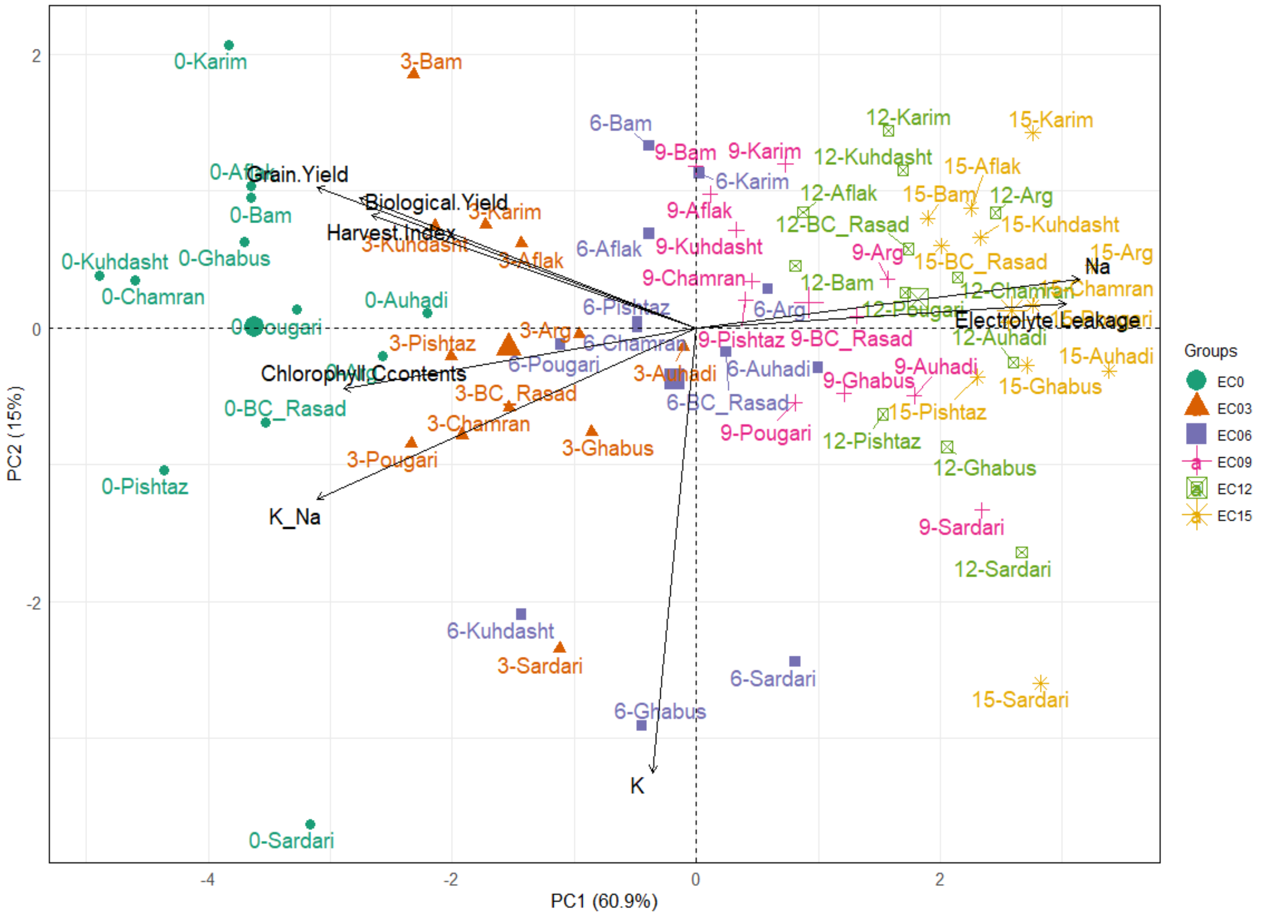

Fig. IV. Visualization of the biplot pattern and interrelationships among 12 wheat cultivars in various salinity levels against eight variables; the preceding numeral before the dash signifies the EC_w treatment level in the combination of treatment-cultivars.

with normal conditions, with Aflak, Arg, and Bam having the minimum Euclidean distance from the yield position in the biplot layout (Fig. IV). The position of each subject, such as cultivar, treatment, and evaluated characteristic, can be determined by its distance from others. A close Euclidean distance indicates a coincidence and positive correlation of the subject with others.

Moving from left to right in the plot, the salinity treatments are represented by different colors, and they show a more significant Euclidean distance from grain yield, biological yield, harvest index, chlorophyll contents, and K^+/Na^+, indicating a decrease in these variables with increasing salinity levels. On the other hand, Na^+ and electrolyte leakage increased with growing salinity levels or stress salinity levels. Ghabus, Sardari, and Kuhdasht had the most K^+ values in EC 6 treatment while Sardari had the most K^+ values in EC 3, 0, and 15.

Discussion

EC_e levels in soil can vary depending on the cultivar response to different levels of EC_w in irrigation water. In this study, varying responses to different salinity treatments were observed in terms of biomass production and shoot growth relative to root growth and leaf area. These responses impact the cultivars' water uptake, causing modifications in soil moisture and salt concentration. The intricate relationship between plant growth and soil moisture is underscored, with robust plants exhibiting enhanced water uptake. Additionally, observations revealed genotype-dependent alterations in soil component solubility. This highlights the dynamic, reciprocal connection between plant growth and soil moisture, emphasizing the impact of cultivar responses to salinity in both irrigation water and soil while recognizing the genotype-dependent complexity in the plant-soil system. This finding is consistent with previous research (Munns and Tester, 2008). The control of the leaching fraction could help to maintain the balance of salt levels in the soil, as suggested by Villalobos et al. (2016). Cultivar type mainly determines the different ECe values, and different salinity treatments lead to varying soil salinity levels (Cavalcante et al., 2010).

As soil salinity is adversely affecting crop yield globally, investigations regarding the salt tolerance of wheat cultivars are of great importance (Fageria et al., 2012). The main focus of breeding programs should be on discovering new variation sources to expand the gene pool of modern wheat varieties tolerant to salinity. Akbarpour et al. (2015) discovered considerable variation in grain yield and yield components of salt-stressed Iranian wheat germplasm in a field study. Additionally, Jafari-Shabestari et al. (1995) conducted a field experiment and found that Iranian landrace accessions of hexaploid wheat displayed substantial genetic variability in response to salinity treatments. This research confirms the existence of genetic variations in Iranian wheat cultivars' response to salinity treatments.

It is crucial to understand the interaction effects between cultivar and treatment for practical breeding strategies. Regression models or graphical representation can identify the type of interaction (Singh et al., 1999). Maas and Hoffman (1977) proposed a curve-fitting model consisting of two-line segments to quantify salt tolerance. However, this model did not apply to all cultivars due to their specific trends and relative responses. The pattern of two-piece functions in rice under salinity conditions varied across genotypes and variables (Radanielson et al., 2018). Environmental factors also affect the genotype response to salinity stress, and crop plants may have different tolerance levels to lower salinity stress (McFarlane et al., 2016). Bam cultivar, derived from the heat and drought-tolerant Mexican wheat line "Vee's/Nac//1-66-22" and also the hybrid wheat strain "T. Aest/5/Ti/4/La/3/Fr//Kal/Gb" exhibited exceptional performance under lower salinity stress (Vahabzadeh et al., 2009; Izadi et al., 2014). Bam cultivar was also found to have the highest level of POD activity among tested cultivars, further indicating its tolerance properties.

The biological yield for certain cultivars including Bam, Karim, Sardari, Arg and Pougari showed a slight increase from EC9 to EC12. However, the yield decreased again at EC15. The fluctuation in the biological yield may be attributed to unequal seed germination in each plot, along with uncontrolled burning on the leaves or spikes of cultivars prior to harvest. This may result in varied and often unpredictable biological yield values, particularly during high salinity stress. Salinity stress can lead to wheat leaf and spike burning when the plants are exposed to salt levels higher than their normal threshold values. Previous studies (Barrera et al., 2019) have reported on this phenomenon.

The accumulation of Na^+ in leaves due to ionic stress or ion toxicity occurs when Na^+ accumulates in the plant leaves, causing damage to them and increasing the number of damaged cells in transpiring leaves, which eventually leads to the leaf death. If the rate of new leaf formation exceeds the death rate, the plant can create enough photosynthetic leaves for flower and seed production even in small quantities. However, if the leaf death rate outpaces that of new leaf formation, the plant may not survive until it produces seeds (Munns, 1993). Effectively managing the transport of sodium ions (Na^+) to the plant shoots and avoiding their accumulation in the leaves is a function of the Na^+ exit process. This process encompasses the regulation of the initial entry of Na^+ into the root epidermis and surface cells, maintaining a balance between Na^+ entry and exit at the epidermal surface of roots, regulating the loading of Na^+ into the vascular system, removing Na^+ from the vascular system before it reaches the shoot, adjusting Na^+ distribution within specific parts of the shoot, and overseeing Na^+ storage (Tester and Davenport, 2003).

For cereals such as wheat, these processes help keep Na^+ out of the transpiration pathway, preventing it from reaching the leaves. Nonetheless, for bread wheat, Na^+ outflow does not always correlate with salinity tolerance (Genc et al., 2007). However, other species show a strong link to salt tolerance (Walker et al., 1999). There are also reports that genotypes with lower Na^+ concentrations show higher dry matter accumulation (Munns and James, 2003), fewer injured leaves, and a greater proportion of surviving leaves relative to dead ones. This result could be due to the impact of lower sodium on growth, improving carbon balance in the genotypes. Our findings indicate that, in addition to Na^+ concentration, there may be other properties that could aid in improving salinity tolerance, which seems to be a significant contributing factor in this experiment. Low Na^+ concentrations in wheat and barley have been previously reported as a tolerance property (Byrt et al., 2014; Shelden et al., 2016) while in some experiments, tissue tolerance is considered an indicator of salinity tolerance (Genc et al., 2007).

K^+ is a crucial element for plant growth. A comparison of K^+ trends in the leaves of different cultivars showed significant superiority of the Sardari cultivar in salinity treatments except for the Kuhdasht cultivar in EC6 treatment. In general, Kuhdasht, Ghabus, Pishtaz, and Karim had the lowest amount of K^+ followed by Sardari in salinity treatments. The process of K^+ absorption appears to be different from other traits such as yield or Na^+. An increase in salinity and Na^+ ions in the plant environment can reduce the absorption of K^+ ions, and consequently, plants may face a deficiency in K^+ (He and Yang, 2007). When there is a high concentration of Na^+ ions, the plant absorbs more Na^+ ions than K^+ and Ca^{2+} ions, causing a depletion of their ions and reducing plant growth (Meloni et al., 2003). Due to the competition of Na^+ to bind to K^+ sites, potassium-dependent metabolic processes get inhibited in the cytoplasm. Therefore, it is essential to keep the Na^+ level at its minimum within cells (Ke et al., 2007). The competitive position of site-binding of Na^+ and K^+ may be one of the main causes of lowering the high level of K^+ in Sardari cultivar, which additionally had the maximum Na^+ in different salinity levels. K^+ plays a significant role in controlling vacuoles, leaf development, and plant growth. Hence, plant biomass depends profoundly on the accumulation of plant K^+ (Guo et al., 2004).

K^+/Na^+ ratio has a particular advantage and disadvantage as an indicator characteristic because it does not significantly correlate with other traits. Therefore, the selection process must be made with more caution, and its success mostly depends on the genetics of the selected cultivar. N^+, K^+, and K^+/Na^+ ratio are the best-suggested traits for early screening in salinity conditions (Munns and Tester, 2008; Munns and James, 2003). While an elevated K^+ content or reduced Na^+ content in certain cultivars may result in an increased K^+/Na^+ ratio, our analysis did not reveal significant differences among the cultivars for K^+/Na^+ ratio. It is notable that the K^+/Na^+ ratio, essentially a computational index derived from the ratio of these two ion traits, poses challenges in pinpointing specific genes that control this ratio. The complexities inherent in identifying these genes add to the intricacy of unraveling the genetic basis of the K^+/Na^+ ratio. This intricacy makes the quest for genes regulating the K^+/Na^+ ratio more challenging and potentially misleading when compared to the comparatively straightforward identification of individual genes associated with the distinct processes of K^+ and Na^+. The K^+/Na^+ ratio was not an appropriate criterion for assessing salt stress tolerance, unlike previous researches (Munns et al., 2012; Munns and Tester, 2008) and in agreement with the results of (Chhipa and Lal, 1995). Previous research suggests that the ratio of K^+/Na^+ in the cytosol of shoot tissue cells is more appropriate than entire shoot parts to assess salinity tolerance because some accumulated Na^+ in plant tissues can be stored in the vacuoles (Munns et al., 2016). This ratio can be applied to the selection in salinity stress when the correlation between Na^+ and K^+ concentrations in shoots is negative. This happens when the genotypes are exposed to high levels of salinity (Genc et al., 2010). These results showed that the variation of the K^+/Na^+ ratio at higher salinity levels in the cultivars significantly reduced compared to normal and lower salinity stress, indicating the trait low capacity for classifying cultivars at intensified salinity levels.

Along with salinity stress, the levels of oxidative stress indices increased with Na^+ ion accumulation. A high salinity level induces oxidative stress in different plants and tissues

(Chawla et al., 2013). Furthermore, the EL characteristic indicates the tissue tolerance and the amount of Na^+ ion tolerance in the plant. In this regard, research has been reported on reducing the membrane stability index due to salinity (Farooq and Azam, 2006). Under salinity stress, the level of ROS (reactive oxygen species) increases in the plant tissues, which is reflected in damaged membranes and elevated EL levels (Chawla et al., 2013; Sharma et al., 2012).

SPAD values, representing chlorophyll content, decreased in all cultivars as the salinity stress increased. Husain et al. (2003) found that lines with higher Na^+ content lost their chlorophyll faster. Over time, the Na^+ ions accumulated in the leaves under the EC15 treatment in all cultivars, leading to a reduction in chlorophyll content. The decrease in chlorophyll concentration may be attributed to the increase in the activity of chlorophyllase enzyme (Reddy and Vora, 1986). Salinity-sensitive plants like rice have a limited control over Na^+ entering the transpiration pathway (Flowers et al., 1991). Studies have reported higher concentrations of Na^+ in susceptible varieties, with older leaves having more Na^+ compared to the young ones (Shannon et al., 1998).

The biological yield may serve as a useful criterion for screening different genotypes under salinity stress (Flowers and Hajibagheri, 2001). The results suggested that the grain yield was positively affected by the biological yield while it was negatively impacted by Na^+ concentration and EL in leaves under salinity stress. These findings are consistent with those of Akbarpour et al. (2015). The observed differences in final performance between tolerant and sensitive cultivars in wheat are not limited to exposure time to salt conditions but are also a function of physiological age, leaf area index (LAI), and biomass growth rate, which are dependent on daily solar radiation and a radiation use efficiency (RUE) parameter. The early and strong cultivars under saline conditions are capable of producing more LAI, which allows them to capture more solar energy. The competition among cultivars starts in the initial life stages, where tolerant cultivars with more LAI can trap more energy than susceptible cultivars, and their LAI is relatively higher under salinity stress. This process continues dynamically over time, leading to a significant gap between sensitive and tolerant cultivars in plant size, ultimately resulting in divergent yields. We employed biplot analysis to identify the highest and lowest values for each trait under different conditions. Thus, we found biplot analysis to be the best method to visualize the variability in relationships between traits and independent effects (Fig. IV).

Conclusion

The study found that increasing salinity levels decreased the yield and yield components of wheat while increasing Na^+ and electrolyte leakage from the leaves. A negative correlation between Na^+ and chlorophyll content was observed, and the traits had significant, treatment-dependent interaction effects. Some cultivars displayed significant diversity with respect to certain traits and could indicate salinity tolerance useful in breeding programs. Among the cultivars tested, Bam, Kuhdasht, Pishtaz, and Aflak had superior yield and biological traits and tissue tolerance for increasing Na^+. However, the amount of Na^+ present in these cultivars was still not very low. The relationship between Na^+ in leaves and yield was negative, but depended on the cultivar. The increase or decrease of K^+ did not have a significant relationship with grain yield. However, the K^+/Na^+ ratio played an important role in classifying better cultivars for tolerance and high yield, although it was not always the most robust trait. Ultimately, factors beyond Na^+ and K^+ alone affect grain yield response under stress conditions. Tissue tolerance and electrolyte leakage can be useful screening criteria for wheat cultivars more than ion distribution.

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