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Research Article

Evaluation of Some Physiological and Molecular Mechanisms of Wheat Cultivars Under Salt Stress

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Abstract: Salt stress is an important problem in the cultivation of crops in dry and semi-arid environments, which restricts crop production. Considering that soil salinity in Iran and neighboring Turkey is increasing with decreasing celestial precipitation, it is important to select genotypes and tolerant wheat varieties for cultivated in saline soils by breeding for future generations. The present research was conducted to evaluate *SOS2*, *SOS3,* and *SDH* genes in wheat leaves using QRT-PCR. This experiment was done as a factorial in the form of a completely randomized design in each plot with three replications for four varieties. Bread wheat seedlings (*Triticum aestivum* L.) varieties including Kavir, Roshan, Bam, and a native landrace (3623) were screened by 200 mM NaCl for 10 days, and physiological and molecular parameters analysis of chlorophyll contents, fluorescence, cations, and proline contents for *SOS2*, *SOS3,* and *SDH* genes expression. Generally, salt stress significantly enhanced ions and organic compounds content (Calcium and sodium concentration), chlorophyll and carotenoid pigment, and the amino acid concentration of proline and chlorophyll fluorescence indices in varieties. Analyses revealed that 3623 can be regarded as a relatively "tolerant" genotype compared with the Kavir. After studying its agricultural indice, it will be considered for breeding programs. Overall, NaCl treated wheat, inducing salt-tolerance genes, effectively facilitates deficiency tolerance. Considering the expression of relatively higher *TaSOS2* and *TaSOS3* in the root of 3623 under stress conditions, perhaps most of the sodium absorbed by the root is returned to the environment.

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1. Introduction

Triticum aestivum L., or common wheat, is an allohexaploid species that can be cultivated throughout the temperate regions of the world in both the southern and northern hemispheres, wheat is grown due to its high yield and resistance to cold temperatures. Wheat is classified first among cereals,

playing a significant role in human nutrition. Wheat reacts physiologically, morphologically, and molecularly to salinity stress. Wheat encounters a variety of environmental stresses during crop cultivation, such as the salinity of the soil. A foundation for sustainable wheat production may be established by the development of genotypes of wheat that are tolerant of salt (Saddiq et al., 2021; Matkovi´c Stojšin et al., 2022).

Abiotic stressors that cause a sharp decline in crop productivity include salt stress, which is one of the most significant factors. It interferes with transpiration, stomatal conductance, photosynthesis, germination, and plant development (Arif et al., 2020; Janczak-Pieniazek et al., 2022). Salinity affects over 954 million hectares of land worldwide, particularly in dry and semi-dry areas (Liu et al., 2020). Salinity is thought to be one of the most dangerous stresses from abiotic sources. Salinity affects over twenty percent of the world's cultivable land. It occupies about 954 million hectares of the total surface of the earth (Shu et al., 2012).

In basic terms, there are two ways to explain how plants react to salinity stress. In the first step, which occurs in the first minutes to a few days after the application of salt stress, ion-independent responses are perceived in the shoots and are believed to be associated with Na^+ sensing and signaling (Negrão et al., 2017). In the second step, responses related to sodium accumulation are detected, which occur during long-term stress and up to a few weeks after the onset of stress, and ultimately cause Na^+ accumulation in old leaves with reduced photosynthesis yield and plant death (Negrão et al., 2017). The three primary methods of tolerance to salinity stress in crops are the restriction of harmful sodium ions from the shoots, tissue tolerance, and shoot ion-independent endurance to maintain growth and absorb water regardless of the rate of Na^+ concentration in the shoot.

Instead, in the competition between Na^+ and K^+ to pass in the plant through transporters in saline soils, fewer potassium ions are present. Due to its role in the evolution and expansion of plants, potassium is needed in the plant system (Singh Yadav et al., 2012; Dissanyake et al., 2021). Conversely, the first recorded response to increasing sodium around the root is to increase cytosolic calcium. Calcium as a secondary messenger induces osmolyte synthesis like proline under stress conditions. Also, calcium may detract the destructive effect of brininess on herb growth through diverse paths, such as reducing the absorption and shift of sodium to the shoot, increasing potassium uptake and consequently increasing potassium to sodium ratios, preventing root cell membrane degradation, improving nitrogen metabolism and photosynthetic activity of the plant (Mohammadi et al., 2013). Since the cycle of photosynthesis is the basic process of herb growth and yield, the potential to maintain it steadily under environmental stresses is important for preserving functional stability (Xu et al., 2015). Stress from salinity reduces the photosynthesis process. Under salt stress, measurements are also made of the conductance of stomatal cells, the fluorescence of chlorophyll, and the content of chlorophyll (Faseela et al., 2020; Janczak-Pieniazek et al., 2022), which inhibits the flow of electrons, decreases the function of the photosystems, decreases the activity and frequency of RuBisco (Riboluse Bisphosphate carboxylase/oxygenase), changes in the ultrastructural organization of chloroplasts, the reduction of total chlorophyll contents, the ratio of leaf carotenoids and chlorophyll a/b (Mehta et al., 2011).

Considering the salinity of Iran's soils and its increasing expansion with decreasing celestial precipitation, it is necessary to select genotypes and tolerant cultivars to cultivate in saline soils. Knowledge of tolerance in plants is obtained by examining how to restore the ionic and osmotic hemostasis after placing them in salt stress conditions in laboratories or limited farms, and then the biochemical status and physiological requirements for plants to develop and achieve their life cycle in a different environment. A great deal of variation has been reported between bread wheat cultivars in terms of tolerance to salinity, suggesting that there are many opportunities to increase the tolerance to salinity in wheat by breeding and selecting. Previously, *several* wheat-tolerant varieties were provided by the Cereal sector of Iran, Seed, and Plant Improvement Institute's (SPII) gene bank. The primary purpose of this investigation was to peruse and compare reactions from four wheat varieties to salt stress and to approve the salt-tolerant genotypes for subsequent wheat programs of breeding.

2. Material and Methods

2.1. Plant culture and stress treatment

Seeds of three bread wheat varieties Kavir (a typical salt tolerant cultivar), Roshan (a typical salt-sensitive cultivar), Bam (a semi-salt tolerant cultivar), and a native landrace (3623) were provided by the Cereals sector Iran, Seed, and Plant Improvement Institute's (SPII) Gene Bank. Based on the findings of Barampuram et al. (2014), the seeds were cleaned and sterilized. Then, the seeds were treated with a fungicide, Benomil, for 15 minutes. After that, the seeds were placed on sterilized filter paper in Petri dishes and placed inside an incubator (4°C) to germinate. Germinated seedlings were transferred into plastic pots with a diameter of 7 cm and filled with sand and perlite at a ratio of 1:4. Each pot contained 5 seeds, which were transferred to phytotron growth chambers. A factorial experiment was carried out with three replicates in each plot as a completely randomized design for four varieties. Johnson's solution was used to irrigate the control group of untreated plants without the addition of salt. Until the three-leaf stage, the pots were watered once every two days with sterilized Johnson's solution. In this project, the main goal was to apply 200 mM salinity stress for ten days to plants. Therefore, in four stages, each lasting two days, the salt concentration in the nutrient solution was gradually increased from 50 to 200 mM so that the plants acclimated to salinity. In the fourth stage, it reached 200 mM, and in this stage, salinity stress continued for ten days. Then, seedling shoots and roots were swiftly chilled in liquid nitrogen and stored at -85$^{\circ C}$ for analysis.

2.2. The chlorophyll content assay

The chlorophyll content was calculated in SPAD units from the midpoints of the youngest leaves using the chlorophyll meter (SPAD, 502). The SPAD value of each sample was read twice and then averaged to reduce the error.

2.3. Photosynthetic pigments content assay

Using the Wellburn (1994) method, the amounts of carotenoids, and chlorophyll pigments were determined in FW mg g^{-1}.

2.4. Chlorophyll fluorescence factors assay

In the 4th to 5th leaf stage, chlorophyll fluorescence factors were calculated with a PAM-chlorophyll florometer on the youngest leaves, followed by Baker and Rosengvist (2004). The plants were first accustomed to dark conditions for 30 minutes, and then F0, Fm, and Fv/Fm factors were measured after adapting to lighting conditions for 30 minutes φPSII (Y).

2.5. Na+ and K+ content assay

0.1 grams of dehydrated root and/or shoot tissues were digested down using a 1:1 HNO_3/CH3COOH solution. After this, the specimens were placed in the centrifuge for 15 minutes at 9000 rpm. The top extract obtained was utilized to measure the concentrations of Na^+, K^+, and Ca^{2+} ions using a spectrophotometer with an atomic absorption device (Japan model AA-670) (Munns et al., 2006).

2.6. Proline contents

Proline content in root and shoot tissues was measured based on Bates (1973). for every 25 mg of plant dry tissue, 3% sulfosalicylic acid was added and centrifuged at 10000 rpm, for 10 minutes. A solution of proline, ninhydrin (Sigma Aldrich), and glacial acetic acid (Merck) in equal portions was added (1: 1: 1). The solution was boiled at 100°C in a water bath for 10 minutes and then immediately placed on ice until the reaction was terminated. Then, one milliliter of toluene was added to the Falcon tube and vortexed for 15-30 seconds. The chromium-containing toluene was removed and absorbed at 520 nm with a spectrophotometer (CE9500-9000 SERIES, UK) using toluene as a blank. Three replicates were used for each sample.

2.7. Water loss

Water loss was calculated using (Eq 1):

$$\text{Water loss} = [(FWC - DWC) - (FWS - DWS)] \quad (1)$$

FW: fresh weight; DW: dry weight; C: control; S: stress.

2.8. Primer designing and qPCR condition

The sequences of the genes in wheat and *Triticum monococcum* L., rice (*Oryza sativa* L.), and barley (*Hordeum vulgare* L.) were retrieved by referring to gene data banks, NCBI. Designing of primers was performed using the OLIGO software version 7 (Table 1). Following the manufacturer's instructions, the RNax Plus kit (Cinnagene, Iran) was used to extract total RNA from both the roots and leaves of wheat seedlings. To determine the expression levels of *SOS*$_2$, *SOS*$_3$, and *SDH* in wheat seedlings treated with or without NaCl, a quantitative analysis of QRT-PCR results was produced using 20 µL of SYBR Green Master Mix (Transgene Biotech) as per the directions provided by the manufacturer. *DNase I* RNAse free (5U) was injected into each sample following the manufacturer's instructions (Fermentase Malaysia) to remove any remaining DNA. cDNA synthesis was performed according to the manufacturer's instructions (Fermentase Malaysia). For each specimen and experiment, three biological and three technical replicates were operated.

Table 1. List of primers used for Q-RT-PCR

Name	Sequence 5'-3'	Fragment length bp	Tm °C
***SOS*$_2$F**	TTGAGGGCAGTTATGTAGCGG	126	58
***SOS*$_2$R**	CCTGTCGCCTGTCAAATAGTG		58
***SOS*$_3$F**	GGTCCTTAGGTGTCTTCCATC	214	58
***SOS*$_3$R**	GTCGTTTTTGCGGTCTGCTTG		58
***SDH*F**	TGCCACACCATCAAGAACTGC	152	58
***SDH*R**	GTCACCACGGAGCCAAAACAA		58
***GT*F**	TGGAGCACAAGAGCCCCGAG	157	63.5
***GT*R**	TCGCCTTCCCTCAGCAGGTC		63.5

2.9. QRT-PCR

Ten nanograms of cDNA and SYBR Green Master Mix were used to perform QRT-PCR. The temperature program consisted of heating at 95 °C for five minutes, then for thirty-five cycles of ten seconds at 95 °C, twenty seconds at 58 °C, and ten seconds at 72°C. Each gene expression was measured by normalizing to glutathione transferase, a housekeeping gene. The linear correlation between two sets of data was measured by the Pearson correlation coefficient (PCC).

2.10. Statistical analysis

All treatments were with three replications. The mean of the data from each measurement was calculated, and the comparison of the meanings and the significant difference between them was done by one-way ANOVA at the level of $p < 0.05$ and the means were grouped using the Duncan test in the SPSS version 21. The charts were drawn with the Excel 2013 software. The standard error (SE) was plotted as a vertical bar in the graphs. Schmittgen and Livak's (2008) description of the relative expression software tool was followed in the analysis of the QRT-PCR data. For every treatment, three technical and biological replications were conducted, and the relative levels of gene expression were assessed using the $2^{-\Delta\Delta CT}$ method.

3. Results

3.1. Ions and organic compounds content

The averages of sodium content in the shoot and root of varieties changed dramatically by applying NaCl. However, Na^+ accumulation in both treatments in roots was significantly more than in shoots (Figure 1). Sodium content increased significantly in both the root and shoots of all varieties (Figure 1A). Meanwhile, the Na^+ content of root in 3623 and bam showed the maximum and minimum levels under salt stress, correspondingly (Figure 1A, B).

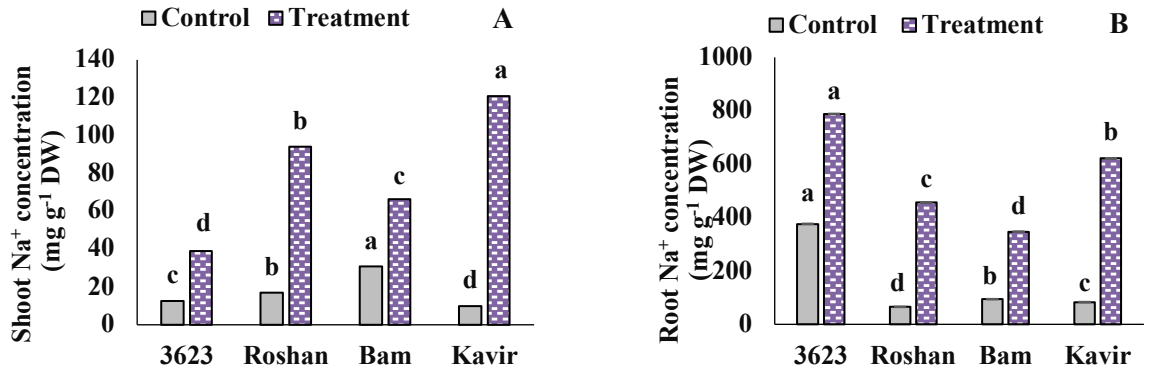

Figure 1. Comparison of the effect of 200 mM sodium chloride treatment on Na^+ content in shoot (A) and root (B) in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD). At the probability level of 0.05, unequal letters indicate a significant difference between the various treatments. Duncan's Multiple Range Test was used to group the mean of Na^+ content.

Although the potassium content in the control conditions was almost the same in both the root and the shoot of the samples, by salt stress, this amount decreased in the shoots, while all plant samples showed a significant increase in potassium accumulation in their roots. K^+ content was the highest in 3623 root in this regard (Figure 2A, B).

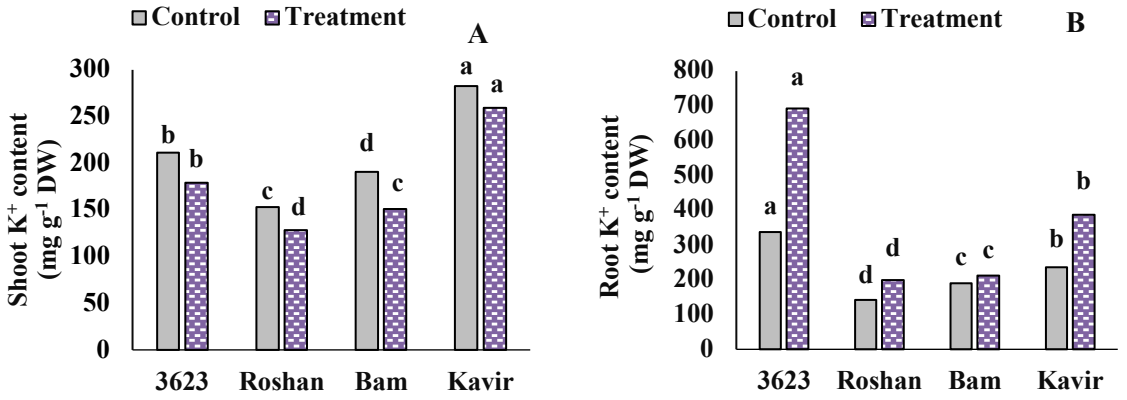

Figure 2. Comparison of the effect of 200 mM NaCl on K^+ content of aerial and terrestrial organs: shoot (A) and root (B) in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD). At the probability level of 0.05, unequal letters indicate a significant difference between the various treatments. K^+ content averages were grouped using Duncan's test method.

Despite the high K^+/Na^+ ratio of shoot and root of Kavir in control conditions, the ratio was significantly reduced in all varieties, especially Kavir, by applying salinity strain. However, the ratio in both shoot and root of 3623 was higher than others under salinity stress (Figure 3A, B).

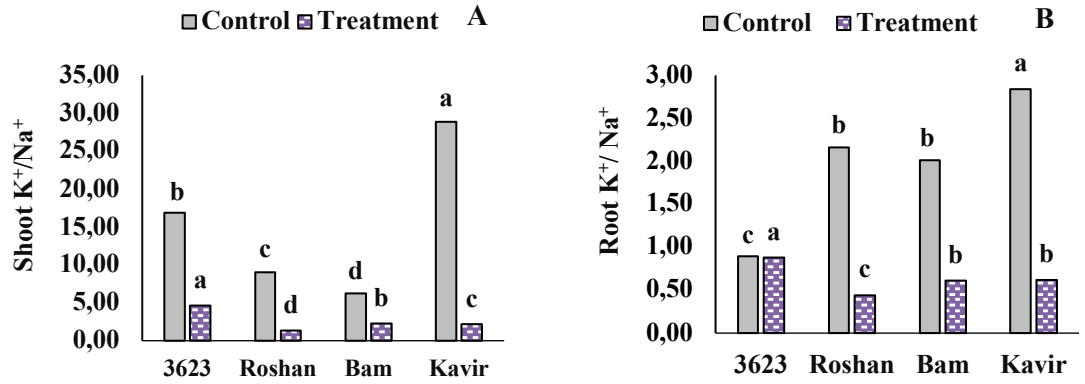

Figure 3. Comparison of the effect of 200 mM NaCl treatment on K^+/Na^+ ratio; shoot (A) and root (B) in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD). At the probability level of 0.05, unequal letters indicate a significant difference between the various treatments. The K^+/Na^+ ratio averages were categorized using Duncan's Multiple Range Test.

The calcium content in the roots of all varieties increased after applying salt stress. However, increasing the amount of Ca^{2+} in all varieties' roots under salt stress, especially in 3623, was significantly different (Figure 4B). Meanwhile, Ca^{2+} content decreased in the shoots of all varieties except for the CV. Roshan (Figure 4A, B).

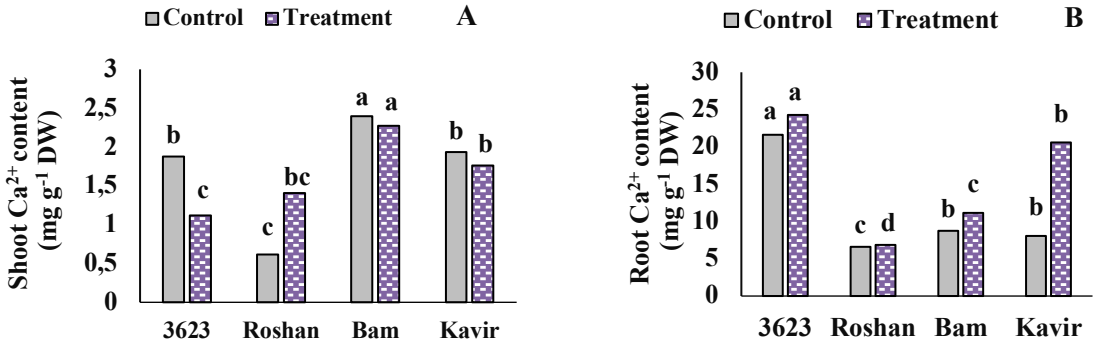

Figure 4. Comparison of the effect of 200 mM NaCl on Ca^{2+} content; shoot (A) and root (B) in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD).

At the probability level of 0.05, non-common letters between different treatments show a significant difference. The averages of Ca^{2+} content were categorized using Duncan's Multiple Range Test.

The content of proline in the root and shoot of varieties was significantly increased by applying NaCl. Meanwhile, proline content in the shoot and the roots of 3623 was higher (Figure 5A, B).

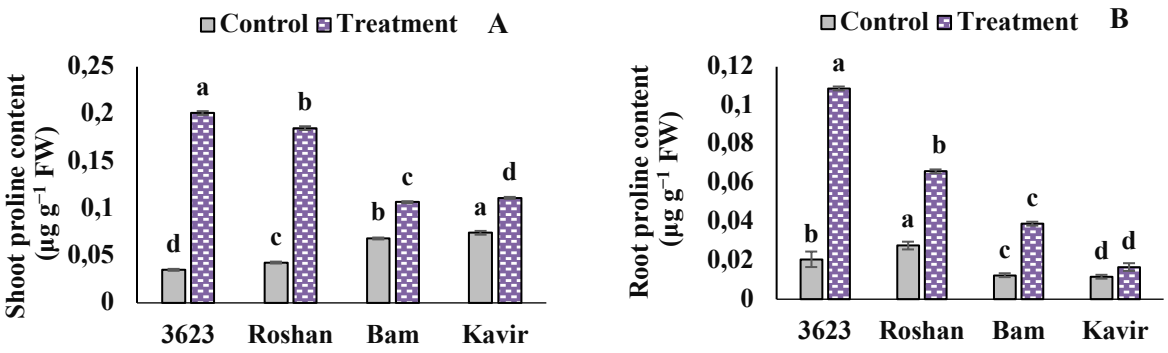

Figure 5. Comparison of the effect of 200 mM NaCl treatment on proline content in shoot (A) and root (B) in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD).

At the probability level of 0.05, non-common letters between different treatments show a significant difference. Proline content averages were grouped using Duncan's Multiple Range Test.

3.2. Photosynthesis pigment content

Compared to control conditions, the total amount of carotenoids enhanced when exposed to salt strain (Figure 6A). The highest amount of carotenoids was observed in Kavir, in both control and salt treatments (Figure 6A). Meanwhile, the amount of chlorophyll increased, except in the Kavir, which decreased (Figure 6B). The research indicated that there was a significant difference between varieties in terms of the photosynthesis pigments' content under the control conditions, so that the highest amount of chlorophyll was found in Kavir and the lowest in Roshan. While in all varieties, the amount of chlorophyll b increased by applying NaCl (Figure 6C), the lowest amount was perceived in 3623. The total amount of chlorophylls a and b increased in all varieties, except in Kavir (Figure 6D). The highest and the lowest total pigment content were detected in the control conditions in Kavir and Roshan, respectively (Figure 6D). The highest and lowest chlorophyll content (based on SPAD) under control was related to Bam and 3623, respectively. During salinity stress, this amount was higher in Bam and Kavir than in other varieties (Figure 6E). There was a direct correlation between photosynthesis pigment content and salinity stress (Table 2).

Pearson's correlation was calculated using the genotype average (3 wheat varieties and a landrace) between salt tolerance parameters characteristics (take the K^+/Na^+ content and ratios of aerial and terrestrial organs) and photosynthesis pigment content. Table 2 displays a significant correlation ($P \leq 0.01$) between the salt tolerance traits, with the highest correlations observed between SKNa and RKNa ($r = 0.725^{**}$). The correlation coefficients among photosynthesis pigments revealed a strong positive relationship between Chla and chla+b content ($r = 0.988^{**}$). Additionally, a significant opposite relationship was identified between RKNa and photosynthesis pigments content, with the highest belonging to the RKNa and chlb content (r=-.679**) (Table 2).

Table 2. Pearson's correlation between three wheat varieties and a landrace between the salt tolerance index traits, the content and ratio of K+ and Na+ in shoot and root, as well as the concentration of photosynthetic pigments

	S Na^+	S K^+	S K^+/Na^+	R K^+/Na^+	Chla	Chlb	Chlab	Car
S Na^+	1	-.105	-.704**	-.703**	.372**	.777**	.438**	.536**
S K^+	-.105	1	.641**	.415**	.597**	-.297*	.528**	.427**
SK^+/Na^+	-.704**	.641**	1	.725**	.273*	-.583**	.183	.034
RK^+/Na^+	-.703**	.415**	.725**	1	-.119	-.679**	-.210	-.415**
Chla	.372**	.597**	.273*	-.119	1	.327**	.988**	.938**
Chlb	.777**	-.297*	-.583**	-.679**	.327**	1	.452**	.542**
Chlab	.438**	.528**	.183	-.210	.988**	.452**	1	.966**
Car	.536**	.427**	.034	-.415**	.938**	.542**	.966**	1

* and ** indicate significant correlation at the 0.05 and 0.01 level (2 tails), respectively. R: Root, S: shoot, Car: carotenoid content.

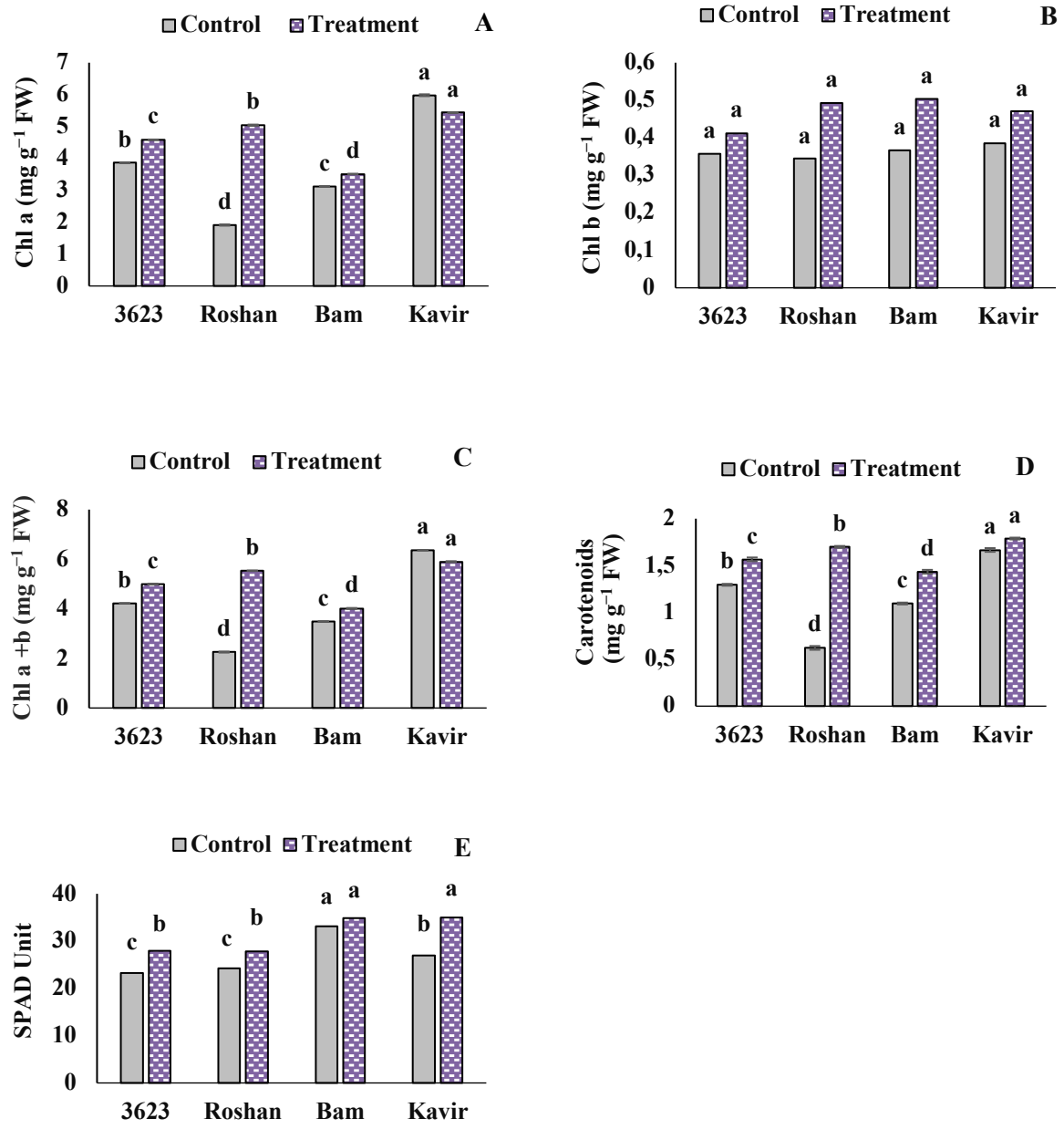

Figure 6. Comparison of the effect of 200 mM sodium chloride treatment on the carotenoids (A), Chl a+b (B), Chl b (C), Chla (D), and total Chl (E) contents in three wheat varieties and a landrace.

For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD). At the probability level of 0.05, non-common letters between different treatments show a significant difference. The averages were grouped using Duncan's Multiple Range Test

3.3. Chlorophyll fluorescence factors

The incremental or decreasing variation of the fluorescence parameters of cultivars was affected by salinity. The relationship between salt stress and F_0 value varied in varieties (Table 2), which decreased in Kavir and Roshan, meanwhile, it was increased by applying NaCl except for Roshan. On the other hand, in Bam and 6323, increasing the F_0 value was greater than that of control plants (Figure 7A). The highest F_m among varieties under control conditions was observed in Bam (Figure 7B). In control conditions, the lowest F_v/F_m was perceived in 3623. Under salt treatment, the F_v/F_m value only increased in 3623 (Figure 7C). The Y value was changed by applying NaCl in all cultivars except for

Roshan (Figure 7D) so that it increased in Kavir and 6323 and decreased in Bam. Under salt stress, the highest amount of Y value was found in 6323 (Figure 7D).

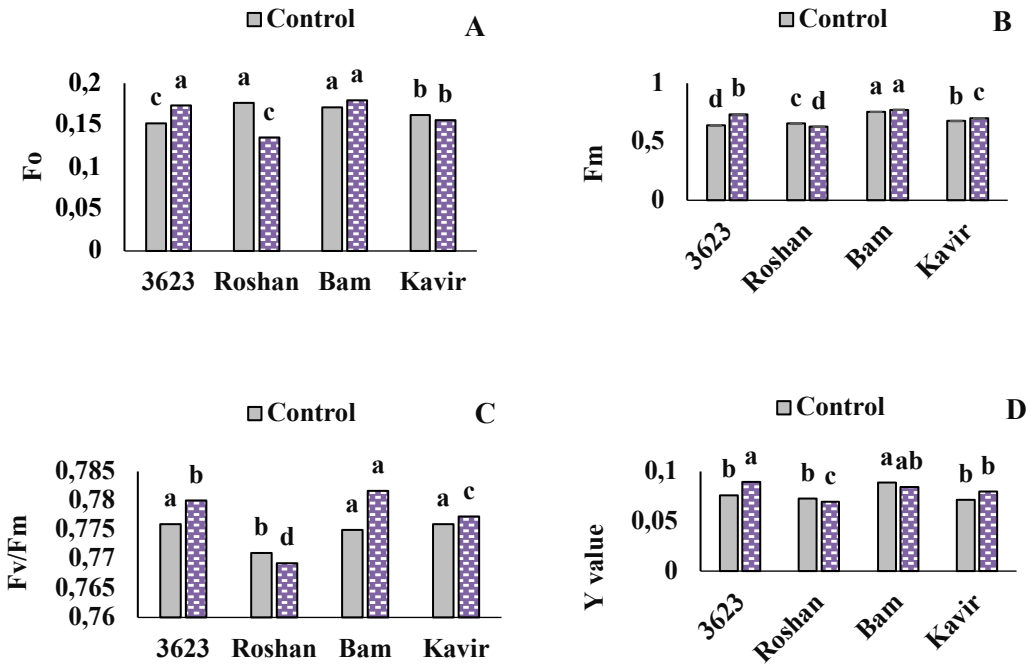

Figure 7. The Comparison of the effect of 200 mM sodium chloride treatment on the F_0 (A), F_m (B), F_v/F_m (C), and Y value (D) in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD). At the probability level of 0.05, non-common letters between different treatments show a significant difference. Values content averages were grouped according to Duncan's Multiple Range Test.

Pearson's correlation of salt tolerance indices traits using the genotype mean (3 wheat varieties and a landrace) (the content and ratio of K^+ and Na^+ in shoot and root) and the chlorophyll fluorescence factors was calculated. As mentioned above the salt tolerance characteristics were a positive and significant correlation ($p \leq 0.01$) observed between SKNa and RKNa ($r = .725^{**}$) (Table 3). Pearson's correlation did not indicate a significant correlation between salt tolerant indices and the chlorophyll fluorescence factors in almost all of the factors except for F_v/F_m and SNa, SKNa, and RKNa, in which the highest value belonged to the RKNa ($r=-.334^{**}$) (Table 3).

Table 3. Pearson's correlation between three wheat varieties and a landrace between the salt tolerance index traits, the content and ratio of K^+ and Na^+ in shoot and root, and the chlorophyll fluorescence factors

	S Na^+	S K^+	S K^+/Na^+	R K^+/Na^+	F_0	F_m	F_vF_m	Y
S Na^+	1	-.105	$-.704^{**}$	$-.703^{**}$	-.140	.070	$.304^{**}$	.227
S K^+	-.105	1	$.641^{**}$	$.415^{**}$	-.053	-.051	-.056	.150
SK^+/Na^+	$-.704^{**}$	$.641^{**}$	1	$.725^{**}$	-.043	$-.290^{*}$	$-.273^{*}$	-.122
RK^+/Na^+	$-.703^{**}$	$.415^{**}$	$.725^{**}$	1	.052	-.063	$-.334^{**}$	-.118
F_0	-.140	-.053	-.043	.052	1	.202	-.094	-.023
F_m	.070	-.051	$-.290^{*}$	-.063	.202	1	.093	$.253^{*}$
F_vF_m	$.304^{**}$	-.056	$-.273^{*}$	$-.334^{**}$	-.094	.093	1	-.036
Y	.227	.150	-.122	-.118	-.023	$.253^{*}$	-.036	1

* and ** indicate significant correlation at the 0.05 and 0.01 level (2 tails), respectively. R: Root, S: shoot, F0: minimum fluorescence, Fm: maximum fluorescence, Fv/Fm: quantum performance, Y: carotenoids content.

3.4. Gene expression analyses

The expression pattern of *TaSOS2*, *TaSOS3,* and *TaSDH* was compared in four varieties' roots and shoots in reaction to salt strain. Interestingly, *TaSOS2* expression was the same in both tissues of the varieties under salt stress except for 3623, which was decreased in roots (Figure 8A). In the meantime, we detected a different expression of *TaSOS3* in the root and shoot of the varieties. In this way, *TaSOS3* expression in roots increased in comparison to shoots, except for Kavir, which was decreased (Figure 8B). Also, the minimum expression of *TaSOS3* was detected in 3623 tissues (Figure 8B). *TaSDH* expression in Kavir and 3623 shoots in the control condition was higher; meanwhile, in Roshan root, it was much higher than others (Figure 8C).

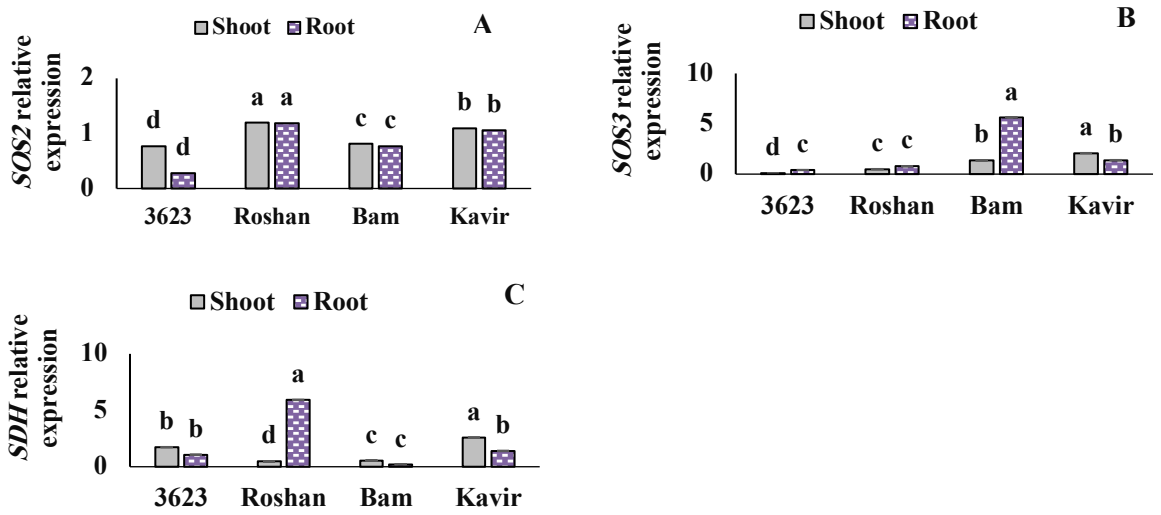

Figure 8. Comparison of the effect of 200 mM sodium chloride treatment on; *SOS2* (A), *SOS3* (B), and *SDH* (C) expression patterns in three wheat varieties and a landrace. For the histogram, analysis of variance and comparison of the mean of three replications have been used with vertical bars indicating standard deviation (SD). At the probability level of 0.05, unequal letters indicate a significant difference between the various treatments. The averages were grouped according to the Duncans Multiple Range Test.

4. Discussion

Three winter and well-known wheat varieties, as well as a landrace, were tested for salt resistance at the stage of seedling growth under control and salinity situations to determine genotypes that can be utilized in the breeding development and production of new varieties of wheat with enhanced and advantageous salt resistance, as well as for future genetic research. Incidentally, different physiological and molecular characteristics, including ion and organic compounds content, photosynthesis pigments, chlorophyll fluorescence factors, and three salt stress putative candidate genes' expression patterns were assayed. This observation can be used to introduce a potent salt-tolerant cultivar for future molecular breeding programs.

According to Borjigin et al. (2021), salt-tolerant germplasms retain a large K^+/Na^+ ratio under salt strain, which delays the collection of high sodium in the cell. İn general, the collection of sodium in both treatment conditions was much further in the roots than in the shoots. Although the sodium content of the shoots increased by salt, however, the amount of sodium entered into the shoots was low. Interestingly, in the landrace, 3623 we perceived less Na^+ entry into the shoot despite the high Na^+ accumulation in the root under salt stress (Figure 1A and B). Meanwhile, K^+ accumulation in aerial and terrestrial organs of the landrace was higher, so the higher K^+/Na^+ ratio was predictable (Figures 2 and 3).

Many plants, especially species with slight tolerance to salinity, retain potassium capability even at high salinity levels and prefer to have more potassium than sodium in their vacuoles under low to

moderate stress conditions (Up to 100 mM NaCl) (Almeida et al., 2017; Dissanyake, 2021). On other hand, osmotic adjustment is one mechanism adopted by plants to tolerate salt. Osmotic regulation is usually reported in many plants by raising the content of soluble sugars, amino acids, and inorganic ions, especially potassium, in reaction to water stress and salinity conditions. According to the present perusal, under 200 mM NaCl, 3623 absorbs more potassium ions through the root and accumulates it in shoot and root tissues. It seems that the more K^+ content in the tissues caused more tolerance to the salt stress in 3623. Moreover, some reports suggest that a large K^+/Na^+ ratio in tissue plants can be used as a suitable character for tolerance assay in wheat cultivars (Dissanyake, 2021). Therefore, according to the mentioned criteria, 3623 could be mentioned as a tolerant landrace.

Ca^{2+} is an important indicating aspect in plant environmental stress reactions, and when crops are subjected to salt, the amount of Ca^{2+} in the cytoplasm increases rapidly due to Ca^{2+} absorption from the apoplast and other compartments within the cell (Che Othman et al., 2017). Consequently, calcium, as a secondary messenger, causes sodium transfer and entry into the vacuoles by Na^+ transporters. It seems that in tolerant varieties, Kavir, Bam, and 3623, the increasing calcium accumulation in roots increases salinity tolerance. It seems that the higher amounts of calcium in the roots of 3623 both under control and salinity conditions cause more Na^+ compartmentalization in the vacuoles and facilitate K^+ entry into the root and thus into the shoot.

As mentioned, osmotic adjustment is one of the abiotic stress tolerance strategies the crop uses to deal with stress. Numerous organic and inorganic compounds have been identified in plants that interfere with osmotic regulation. In this regard, soluble carbohydrates and the amino acid proline may also provide osmolytes in many varieties that defend crops from salt stress. Proline is an essential component of the pathway mediated by PP, which manages the cellular redox effectiveness required to retain numerous antioxidants in their redox condition (Wani et al., 2019; Xie et al., 2020; Heydarzadeh et al., 2023). Proline probably protects proteins and enzymes from salt stress as a chaperone, or else it can assist plants in recovering from stress. Many researchers have shown that the external action of the amino acid proline in plants leads to a reduction in the damage caused by salt and drought stress (Foroutan et al., 2018; Wani et al., 2019; Raeisi Sadati et al., 2022). Increasing the activity of NADPH oxidase is generally done with the help of Ca^{2+}. It is also regulated by the process of phosphorylation of the explosion of homologous protein molecules, which may lead to an oxidative burst. Che-Othman et al. (2017) reported that reactive oxygen radicals are destroyed by metabolites such as ascorbic acid, tocopherol, glutathione, amino acid proline, flavonoids, and carotenoids, which are non-enzymatic antioxidant processes (Che Othman et al., 2017; Heydarzadeh et al., 2022). Generally, the time of increase of reactive oxygen species and calcium plays a key role in the type of response to salinity that leads to plant cell adaptation or death. Of course, the antioxidant role of proline in protecting biological membranes should not be ignored, and the amount of proline significantly increases with salinity (Xie et al., 2020). Considering that the content of proline in 3623 saline conditions is interestingly the highest in the root and shoot, it can be concluded that 3623 could be one of the tolerant candidate genotypes.

The current study found a beneficial impact on the ratio of chlorophyll a and chlorophyll b. Increased relative chlorophyll content of wheat leaves in saline conditions was also stated in several other research (Zhang et al., 2016). It seems that by decreasing the leaf area and increasing the thickness of the mesophilic cells, chlorophyll concentrates at a lower area of the leaves and therefore increases the SPAD number (Hamblin et al., 2014). In the desire for greater yields, a lower ratio of chlorophyll pigment concentration per unit leaf area of plants is probably beneficial. Possible explanations include improved glory dispensation in the plant tent and reduced photochemical destruction of leafage; they receive more light energy needed for the most powerful photosynthesis processes (Hamblin et al., 2014). In this study, a direct relation was found between the relative chlorophyll content (SPAD) and the collection of pigments in tolerant cultivars. The researchers concluded that increasing the amount of Na^+ in tolerant rice cultivars leads to an increase in the concentration of chlorophyll a and b pigments (Ma et al., 2018). Also, drought stress reduces chlorophyll a more than chlorophyll b, thereby increasing the chlorophyll a to b ratio (Guo et al., 2016). Also, short-term drought stress in wheat caused a complete stopping of photosynthesis and an enhancement in the chlorophyll a/b ratio but did not affect leaf chlorophyll content (Hamblin et al., 2014). Furthermore, it has previously been demonstrated that three wheat cultivars had a notable decline in their chlorophyll a and b content (Foroutan et al., 2018; Raeisi Sadati et al., 2022). Further, the process of photosynthesis is slowed down by salinity stress. Salt stress is also used to measure stomatal conductance, chlorophyll fluorescence, and chlorophyll content

(Faseela et al., 2020; Pan et al., 2021). Chlorophyll stability is an indicator of plant tolerance to environmental stresses. It seems that the chlorophyll reaction of plants to salinity is different among species and varieties. Chlorophyll content is reduced due to leaf damage as a result of saline conditions. Even other studies on some plants that are tolerant and resistant to stress show the ineffectiveness of stress on the amount of chlorophyll in the plant and the increase in the amount of chlorophyll molecules (Taïbi et al., 2016). Fluorescence assays are an excellent tool for quantifying damage to the photosynthetic apparatus and photosystem II (PSII) caused by salinity conditions. In this regard, it can be pointed out that the tolerant plants have a higher chlorophyll index. One of the reasons for increasing the number of chlorophyll molecules is to increase the performance of photosystem II (Abdeshahian et al., 2010). In the present study, the concentration of total chlorophyll was increased by salinity in all varieties. Incidentally, carotenoids also increased by salinity in all varieties. Carotenoids protect chlorophylls from photooxidation by rapidly quenching the excited chlorophylls (Ramel et al., 2012).

It has now been well established that photosystem II is not homogeneous in plants. Photosystem II is different in structure and function among plants, and this variation is known as the heterogeneity of photosystem II (Mehta et al., 2010). Due to an imbalance between light intake and consumption, photosynthetic activity is impaired under salt stress. The inappropriate regulation of photosystem II causes an inequity between the production and consumption of electrons. As a result, the lower the fluorescence of chlorophyll, the more tolerant plants are because they use the sun's light to the maximum and less reflects and waste it (Saddiq et al., 2021; Janczak-Pieniazek et al., 2022). This study observed heterogeneity in the performance of the photosystem II among the varieties (data is not shown). As shown, the highest F_0 level was observed in Bam and 3623 during salinity stress. The significance of F_0 indicates that there is a major difference in the efficiency of chlorophyll antenna among genotypes in saline conditions (Mathur et al., 2013). The increase and decrease of F_0 can respectively indicate less destruction and damage to PSII reaction centers under stress conditions (Abdeshahian et al., 2010; Saddiq et al., 2021). In this regard, Roshan and Kavir showed less F_0 quantity and probably the PSII fewer damages. Consequently, the increase in F_m is due to the damage to the PSII reaction centers. So, the comparison of the mean of F_m under salinity showed the highest and lowest values were related to Bam and Roshan, Kavir, and 3623, respectively. It seems that the Kavir photoprotection mechanism defends the photosystem II efficiently.

To prevent the PSII reaction centers from being damaged, plants have some strategies. For example, they can reduce the rate of electron transport by turning the excess light that is absorbed into thermal energy. The non-photochemical quenching of fluorescence from chlorophyll occurs when excessive excitation energy is dissipated as heat. A novel non-photochemical quenching (NPQ) of chlorophyll fluorescence occurs when excess aroused energy is wasted as heat. By opening a heat elimination channel, NPQ, a significant photoprotective reaction decreases the amount of chlorophyll excited states (Chl *) in PSII.

Since it has an extremely effective mechanism to protect it from photoinhibition, PSI, unlike PSII, is not often destroyed. Photodamage to PSI is created when the availability of electrons from PSII exceeds PSI's electron-accepting capacity, and once harmed, the restoration of PSI centers is significantly slow. In the present research, the F_v/F_m value was not elevated in comparison to the control, except for Kavir and 3623, which remained unchanged. Also, a comparison of the mean of F_v/F_m did not indicate a significant difference between cultivars under salinity. Salinity prevents the transportation of electrons from the primary early to the secondary plastoquinone receptors in the PSII receptor pathway, which results in a decrease in F_v/F_m. Given the significance of this index among the cultivars in control conditions, it appears that the quantum yield of the cultivars is different. Abdeshahian et al,. (2010) reported that drought-tolerant cultivars had a higher F_v/F_m than the sensitive cultivars, which affects the higher efficiency of the photosystem II in tolerant cultivars. In the present research, despite the different amounts of F_v/F_m, we did not perceive any significant changes among varieties under salt stress. ØPSII measures the efficiency of Photosystem II and is calculated as YII = $F_{m'}$-F\$F_{m'}$. This parameter indicates the fraction of light received by PSII that is utilized in the process of photochemistry. As a result, it can indicate overall photosynthesis by providing a scale of pace linear electron transport. Correspondingly, the Y value was the highest in 3623, and it showed a relatively healthy photosynthesis rate in 3623.

It is assumed that the plants can withstand salt stress better and can, in the long term, stabilize their main metabolisms, such as respiration and photosynthesis. The *TaSOS2* gene regulates sodium ion

equivalence by inducing *SOS1*, *NHX1,* and *HKT1* genes and regulating their activity. Therefore, by increasing sodium and calcium concentration, the expression of it is stimulated. The copy number of *AtSOS2* mRNA in Arabidopsis was upregulated in the roots with salinity (Dissanyake, 2021). The H^+/Na^+ antiporter activity of tonoplast by *SOS2* is independent of *SOS3*. *SOS2* is likely to play a role in regulating sodium (*SOS1*) and calcium ion transporters (*CAX1*). In the SOS pathway, when the *SOS3* regulatory protein is coupled to calcium ions, *SOS3* binds to *SOS2* and activates it to regulate the H^+/Na^+ antiporter (Singh Yadav et al., 2012; Dissanyake, 2021). The regulation of Na^+ categorization during salinity stress is largely dependent on transport mechanisms, including the K^+–Na^+ carrier (*HKT1*), the Na^+–H^+ antiporter *SOS1* (salt oversensitive 1) *AtNHX1*, and Ca^+-regulated transport proteins *SOS2/SOS3*. Moreover, the *SOS2-SOS3* complex down-regulates the *HKT1* gene or inactivates *HKT1* protein during salt stress to prevent Na^+ from entering the plant. On the other hand, this complex activates *NHX1* and thus plays a significant function in the compartmentation of Na^+ ions in intracellular sections, such as vacuoles, also by activating the *SOS1* protein in the removal of sodium from the plant (Asano et al., 2012; Deinlein et al., 2014). This increase inside the cytoplasm of Ca^{2+} causes *SOS3*, a Ca^{2+}-binding amino acid, to communicate with a protein kinase (*SOS2*) (Che Othman et al., 2017). It seems that under saline conditions, *SOS1* and *NHX1* genes play an important role in maintaining ion homeostasis in the cytoplasm. Recent research investigations have shown that overexpressing *Ta NHX* in *Nicotiana tabacum* (tobacco) improves the productivity of plants under stress caused by salinity (Che Othman et al., 2017).

One of the main cellular functions in crops that manifests an obvious reaction to salinity is mitochondrial function. Because operational mitochondria possess a negative membrane potential, Na^+ from the cytosol is drawn into the organelle and accumulates. A wide range of metabolic processes are involved in the mitochondrial adaptation response, which is likely a combination of enzymatic action in direct salt inhibition and changes in mitochondrial ATP to enable critical reactions in the remaining cell and prevent salt-induced cytotoxicity. These reactions become more important when the cell reaches the state of toxicity caused by sodium chloride and the occurrence of an "energy crisis" (Che Othman et al., 2017).

It is recommended that resistant species use more effective ATP manufacturing processes under conditions of salinity to supply one or both of these energy demands. *SDH* encoding the subunit of succinate dehydrogenase protein complex effective role in the Krebs cycle, the electron transfer chain, the respiration rate, and consequently, the supply of energy is a function of its expression. Previously, Che-Othman et al. (2017) reported the *TaSDH* increasing expression by salt in wheat. The increasing expression of *TaSDH* in wheat by salt stress, due to the impact of many growth processes and the development of increased respiratory efficiency, can provide the energy needed to absorb less sodium and more potassium despite the existing ion gradient. Given this aspect, it seems that sensitive varieties need more *SDH* to provide the required energy than tolerant plants.

5. Conclusion

Nowadays, due to climate changes and increasing soil salinity, the cultivation of crops is reducing gradually. Therefore, it is necessary to access new salt-tolerant genetic resources, especially in the case of wheat. Many attempts have been made to find and introduce wheat genotypes that are sensitive in this regard. However, considering that the plateau of Iran is the center of the genetic diversity of wheat, it is important to identify and evaluate the salt tolerance in cultivars introduced from this region. In the present study, despite the high absorption of sodium by the root of 3623, the amount of Na^+ in the shoot was less due to the inhibition of Na^+ transfer to the shoot or reabsorption. Although the expression of the genes in it was lower than in all plants. Considering the relatively higher expression of *TaSOS2* and *TaSOS3* in the root of 3623 under stress conditions, perhaps most of the sodium absorbed by the root is returned to the environment, which should be subjected to further examination.

Ethical Statement

Ethical approval is not required for this study because the researcher does not interfere with or manipulate the environment or the subjects' behaviors; they simply observe ongoing activities.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

SM; conducting experiments and collecting and analyzing data, SJ; thesis supervisor and MS reviewer, KR; thesis supervisor, project manager, grant applier, final data analyzer and final version of MS reviewer, SYR; MS reviewer, MA; data analyzre, final MS reviewer.

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