

Original Research

Evaluation of Ten Bread Wheat (*Triticum aestivum*) Cultivars and Five of Their Hybrids under Salinity Stress at Germination and Seedling Stage

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Abstract

Salinity poses a danger to food security; hence, it is necessary to create crop types that can withstand salt to meet the growing demand for food. The goal of the current study was to examine the morphological and biochemical responses of wheat (*Triticum aestivum* L.) genotypes under salt stress. Salt tolerance in ten wheat cultivars and five selected F1 hybrids was assessed at the germination and seedling stage. Genotypes were assessed for seven traits under control (0 mM NaCl) and salinity stress (150, 200, and 250 mM NaCl). According to the findings, salt stress significantly impacted every wheat genotype. However, according to the STI value at 250 mM NaCl stress, Kharchia-65 was the most tolerant cultivar among the tested ones, followed by Pasban-90 and Krl-1-4. Also, Misr-3 is a promising Egyptian cultivar with moderate susceptibility. H5 (Pasban-90 × Kharachia-65) recorded the highest STI value among the tested hybrids. SDS-PAGE showed the appearance of some new bands



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under salinity conditions (200 and 250 mM NaCl). Protein bands with molecular weights 37.5, 40, and 48.6 kDa appeared in salt-treated cultivars at both concentrations. The current study's findings may aid in developing salinity-tolerant wheat varieties. Ultimately, Kharachia-65 and H5 (Pasban-90 × Kharachia-65) recorded higher tolerance to salt stress. This data can be used in wheat breeding programs for salt-affected areas.

Keywords

Wheat; *Triticum aestivum* L.; salinity; tolerance index; F1; SDS-PAGE

1. Introduction

Egypt is currently facing a scarcity of water resources, in addition to the rapid increase in population size. These factors contribute to widening the gap between food production and consumption, necessitating the expansion of the agricultural area to close this gap, which raises the water requirements [1]. Due to the buildup of salts in the soil, salinity stress causes a decline in agricultural output in arid and semi-arid regions of the world [2]. In the recent period, wheat area in Egypt has declined due to poor irrigation water and high levels of soil salinity [3]. Wheat (*Triticum aestivum* L.) is one of the most nutrient-dense grains and is essential to feed the world's population. Egypt is regarded as one of the world's top importers of wheat because native production covers less than half of the demand. Raising wheat output to close the gap between production and consumption is the main challenge facing wheat breeders [4]. To solve this problem and guarantee the sustainability and effectiveness of wheat production systems within the current climatic trend, it is imperative to develop new salt-tolerant wheat cultivars using genetic alterations and wheat breeding procedures [5].

Several investigations reported the effects of salt stress on wheat cultivars and genotypes. Ahmad et al. [6] tested salinity stress on 172 wheat genotypes at germination and early seedling stage at 200, 250, and 300 mM NaCl stress. At 200 mM NaCl stress, 18 accessions were recorded as salt-tolerant, and two accessions at 300 mM. Gawayed and Abd-El-Moneim [7] tested the response of the seedlings of 14 Egyptian wheat genotypes to salt stress (0, 50, 150, and 250 mM NaCl). They reported four salt-tolerant cultivars. The combined analysis of biochemical and morphological data is important for understanding the impact of salt stress on plants [8]. Several morphological characters, such as root length, root fresh and dry weights, shoot fresh and dry weights, are associated with salt tolerance and could be used as selection criteria. Using morphological and SSR markers, Ahmad et al. [6] showed that Egyptian accession 11466 and the Pakistani accession 11299 were the most salt-tolerant wheat genotypes as compared with other genotypes. Amro et al. [9] studied seawater tolerance of 80 wheat genotypes collected from several countries at the seedling stage. They reported 8 highly tolerant genotypes depending on several growth parameters (fresh weight (FW), number of radicles (NOR), lengths of epicotyl (EL), and hypocotyl (HL)). NOR proved to be the most effective character in salinity tolerance as it recorded the strongest correlation with the other traits [9]. Uzair et al. [10] used morpho-physiological traits to assess salinity tolerance among 81 wheat genotypes. They recognized two tolerant genotypes at 150 mM NaCl treatment. Shoot length and weight, total seedling fresh

weight, and relative growth rate for weight were the most useful traits in evaluating tolerance and early screening for genotypes. Junaid et al. [11] studied the salt tolerance of 16 lines and eight genotypes by analyzing 13 agro-morphological traits under both control and high salinity levels (15 dSm⁻¹) in real field conditions, and the study identified several traits, some of which are associated with SSR markers influencing the salt tolerance of wheat genotypes. Results revealed significant statistical differences between the genotypes and salinity levels for all assessed traits.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a popular technique for protein separation and analysis according to molecular weight [12]. A study of changes in protein profiles by electrophoretic analysis under salinity stress is useful for understanding the salinity tolerance of genotypes and identifying proteins associated with salt stress. El-Seidy et al. [13] examined the electrophoretic banding patterns of proteins under normal and salinity conditions in five wheat parents. Eight bands with molecular weights of 25, 30, 75, 80, 110, 114, 150, and 215 kDa, respectively, had appeared in all parents under all conditions, which means that these bands were common bands in these cultivars. On the other hand, four bands with molecular weights of 15, 20, 35, and 40 kDa appeared in two genotypes under salinity conditions only, and this may be due to the manufacturing of specific proteins; modification of gene expression, and these genes might have a crucial role in response to different stresses. Sobhanian et al. [14] examined the electrophoretic pattern of protein in 4 wheat cultivars with different degrees of tolerance under controlled and salinity stress (0, 70, 140, 210 mM NaCl). The results showed fundamental similarities among cultivars and no polypeptide bands belonging to specific cultivars or to one of the salinity treatments were observed. They showed that the polypeptide bands with 54 and 56 kDa at high salinity were reduced for all cultivars. Increasing salinity caused a strong reduction in the level of the 55 kDa polypeptide band, which is related to the Rubisco enzyme, and according to the results of different studies, abiotic stresses have adverse effects on Rubisco enzyme activity. El-Saber [15] showed that patterns of proteins using SDS-PAGE have been used in discriminating between 7 salt-tolerant and salt-sensitive wheat lines. The number of bands ranged from 13 to 14, with molecular weights ranging from 6 to 130 kDa under saline conditions. The bands of 6, 7, 10, 12, 15, 25, 34, 50, 60, 82, and 130 kDa appeared in all lines, while bands of molecular weights 8, 18, and 29 kDa were absent in 5 lines, and unique bands of molecular weights 17 and 20 kDa appeared only in two lines. Gawayed and Abd-El-Moneim [7] used SDS-PAGE to characterize the protein patterns involved in the salinity response. The result showed that eighteen protein bands with molecular weights ranging from 21 to 197 kDa appeared, most of which were not affected by salinity compared with the control, and specific bands were rare. Singh and Singh [16] studied the changes in protein profiles using SDS-PAGE between two wheat cultivars (KRL 1-4 and UP 2338) under different salinity levels. They reported the appearance of new bands and an increase in the intensity of other bands under salinity stress.

Some studies tried to fully understand the physiological, biochemical, and molecular changes accompanying salinity-stressed wheat cultivars; however, more studies are required to characterize changes in Egyptian cultivars as well. To support Egypt in achieving self-sufficiency in this vital strategic crop, it is essential to develop new wheat varieties with improved tolerance to extreme salinity conditions. In this work, an integrated laboratory screening for ten potential wheat parents and five selected F1 hybrids from germination to the seedling stage was tested for their salt tolerance. In addition, parents were compared for their protein profiles in normal and stressed conditions.

2. Materials and Methods

2.1 Plant material

Grains of ten potential wheat (*Triticum aestivum* L.) parents were used in this study. In addition to five selected F1 hybrids developed by the Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Giza, Egypt (Table 1).

Table 1 Parents and hybrid names, localities, sources and pedigrees. CIMMYT and ARC stand for the International Maize and Wheat Improvement Center and the Agriculture Research Center, respectively.

S.	Cultivars	Code	Locality	Source	Pedigree
1	Lu-26	P1	Pakistan	CIMMYT	BLUESILVER/KHUSHAL-69[613][628]
2	Sakha-8	P2	Egypt	ARC	CIANO67(SIB)//SONORA-64/KLEIN-RENDIDOR/3/II8156[114][144]; INDUS66/(SIB)NORTENO[3396][4259]
3	Krl-1-4	P3	India	CIMMYT	KHARCHIA-LOCAL/WL-711[1324][3320]
4	Oasis-86	P4	Mexico	CIMMYT	AGATHA/3*YECORA-70[144][3054]
5	Shorawaki	P5	India	CIMMYT	LV-IND[2393]
6	Pasban-90	P6	Pakistan	CIMMYT	INIA-66/AG.DI//INIA-66/3/GENARO-81[1388][2857]
7	Kharchia-65	P7	India	CIMMYT	EG593/KHARCHIA[39][1322]; KHARCHIA-LOCAL*5/EG-953[1324][4077][4314]; UP-2441/K-9330[4281]
8	Giza 171	P8	Egypt	ARC	SAKHA93/GEMMEIZA9S.6-1GZ-4GZ-1GZ-2GZ-0S
9	Sids 14	P9	Egypt	ARC	BOW"S"/VEE"S"/BOW"S"/TSI/3/BANISEWEF1SD293-1SD-2SD-4SD-0SD
10	Misr 3	P10	Egypt	ARC	ATTILA*2/PBW65*2/KACHUCMSS06Y00582T-099TOPM-099Y-099ZTM-099Y-099M-10WGY-0B-0EGY
S.	Hybrid type	Hybrid code		Developed by	Parents
1	F1 hybrid	H1		ARC	Krl-1-4 × Misr-3 (P3 × P10)
2	F1 hybrid	H2		ARC	Oasis-86 × Misr-3 (P4 × P10)
3	F1 hybrid	H3		ARC	Pasban-90 × Misr-3 (LUP6 × P10)
4	F1 hybrid	H4		ARC	Kharchia-65 × Misr-3 (P7 × P10)
5	F1 hybrid	H5		ARC	Pasban-90, Kharachia-65 (P6 × P7)

2.2 Methods

2.2.1 Salinity Tolerance Experiment

Ten grains of each accession were surface sterilized and sown following the method described by Tlig et al. [17]. Grains were surface sterilized in 15% Clorox solution for 10 minutes; subsequently, they were washed 4-5 times with distilled water and air dried for germination experiments. In the experiment, all accessions were tested under four treatments of distilled water as a control and (150 mM, 200 mM, 250 mM) salt (NaCl) stress. The same sterilization procedures were applied to the 5 selected hybrids that were subjected only to (0 and 250 mM) salt (NaCl) stress after the parental screening. Gains of each accession were germinated on filter paper placed in petri dishes at room temperature (approximately 25°C during the day and 14°C at night). All petri-dishes were kept in the dark for one day, then subjected to 12 h of daylight and 12 h of darkness. Each treatment was replicated thrice, as each replicate consisted of 10 seeds, following a completely randomized design. Ten ml of NaCl solution was added for each replicate, which was daily replenished. When the radicle was at least as long as the grain and the plumule was more than half the length of the grain, the grain was said to have germinated. Germination rates were calculated through the experiment, and after 8 days, six morphological characters were recorded for each individual: percentage of germination, plumule and radical length, total seedling fresh and dry weights, and number of radicals. Germinating grains were counted daily until a constant number was reached. Germination rate (GR) was calculated as: $GR = X_i/Y_i + X_{ii}/Y_{ii} + \dots + X_n/Y_n$ [18] where X is the number of seeds germinated for the day, Y is the number of days from the first seed germination, and i, ii ... n are numbers of days. Eight-day-old seedlings of wheat, which emerged under control and (150mM, 200mM, 250mM) NaCl stress, were weighed and then oven-dried at 70°C for 3 days, following which dry weights were recorded. Salt tolerance trait index (STTI) at germination was calculated according to the formula of [19]:

$$STTI = (Value\ of\ trait\ under\ stress\ condition / Value\ of\ trait\ under\ controlled\ condition) * 100$$

The salt tolerance index (STI) was calculated as the mean of salt tolerance trait indices (STTIs).

2.2.2 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE technique was applied to analyze the total soluble proteins in wheat seedlings of the ten potential parents under control conditions and under 200 mM NaCl treatment. Total soluble proteins were extracted from wheat seedlings according to Laemmli [12]. A Hamilton syringe was used to load equal amounts of protein (40 µl), and electrophoresis was carried out at about 140 volts in 1× Tris/glycine- SDS running buffer.

2.2.3 Data Analysis

From the resulting patterns, only the strongest and most distinct Protein bands were manually rated as present (1) or absent (0) to be considered for analysis. The experiment's outcomes were statistically analyzed using SPSS version 25. A one-way ANOVA was performed to test significant differences in morphological traits between salinity treatments and genotypes. Following that,

post hoc comparisons between the means of the different outcomes were conducted using Duncan's test. Senaweera and Weaver [20] defined statistical significance as a p-value of less than 0.05.

3. Results

3.1 Effect of Salinity (NaCl) Stress on Growth Traits

Early stages of seedling growth, such as germination, are critical because they affect subsequent stages. The diverse response of genotypes starts with seed germination. Therefore, the effects of different concentrations of salinity stress on 15 genotypes of *Triticum aestivum* (represented by ten parent cultivars and five of their hybrids) were examined at this stage. Plant growth was determined by measuring 7 quantitative morphological traits, including germination rate and percentage, number of roots, shoot and root lengths, total fresh and dry weights. The measurements of these traits are shown in Figure 1.

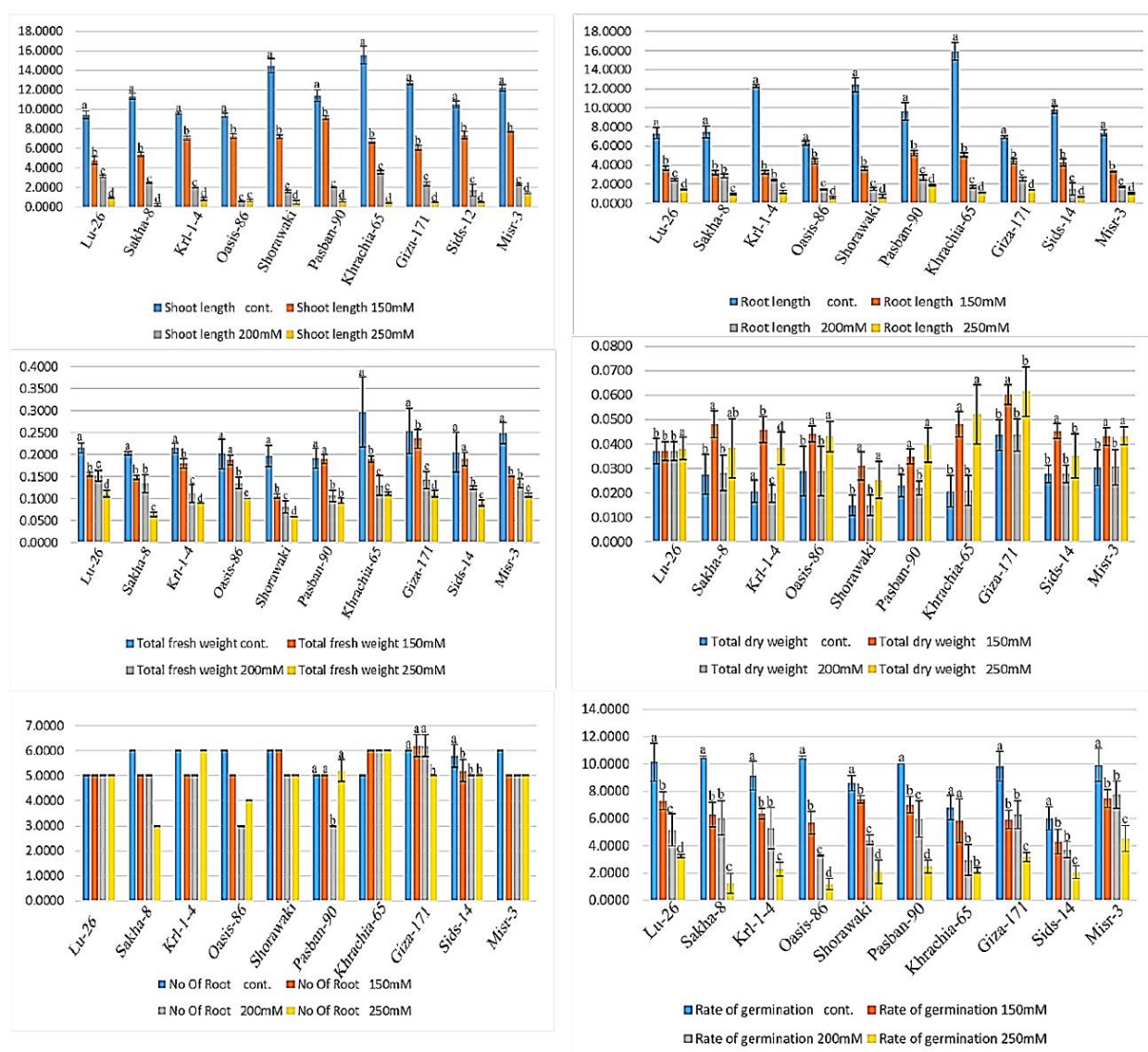


Figure 1 Effects of salinity stress on different morphological parameters (shoot length, root length, total fresh weight, total dry weight, no of roots, rate of germination)

under different levels of salinity stress (0, 150, 200, 250 mM) in the 10 wheat cultivars. Values are presented as means $\pm$ SD, with significant differences at $p \leq 0.05$. Different letters (a-d) indicate significant differences by Duncan's multiple range test.

Under normal conditions, cultivar Kharchia-65 showed the longest shoot (15.56 ± 0.92 cm) and the longest roots (15.94 ± 0.71 cm). In addition, this cultivar showed the largest fresh weight (0.29 ± 0.07 g). The cultivars Lu-26 and Oasis-86 have the shortest shoots (9.44 ± 0.39 cm and 9.36 ± 0.23 cm, respectively), and cultivar Oasis-86 has the shortest roots (6.32 ± 0.37 cm). On the other hand, the highest percentage of germination ($100 \pm 0.00\%$) and the highest germination rate (10.50 ± 0.09) were recorded for cultivar Sakha-8. In contrast, the lowest rate of germination (6.01 ± 0.85) and the lowest percentage of germination ($60 \pm 0.00\%$) were recorded in cultivar Sids-14.

The current study found that the examined cultivars exhibit a wide range of responses to salinity stress. The results show that applying NaCl at different concentrations significantly inhibited all growth attributes of wheat cultivars. Salinity has a direct negative impact on morphological traits. All seedling traits decreased proportionally with increasing NaCl concentration. Growth inhibition was expressed as reductions in shoot or root length; fresh and dry weight are major symptomatic effects under salinity conditions.

The results obtained after treating the ten cultivars with three NaCl concentrations (150, 200, and 250 mM) showed that all morphological criteria were significantly affected by salinity stress compared with control plants. Measurements of these traits are illustrated in the histograms (Figure 1). All 10 cultivars showed a highly significant decrease in shoot and root length under salinity stress as compared to the control. Kharchia-65 that reported the tallest plant shoot and root under normal conditions (15.56 ± 0.92 cm and 15.94 ± 0.71 cm respectively) was reduced under the applied salinity stress (150 mM NaCl) to 6.76 ± 0.23 cm and 5.06 ± 0.37 cm, reduced to 3.56 ± 0.16 cm and 1.70 ± 0.14 cm under 200 mM NaCl and to 0.40 ± 0.00 cm and 1.10 ± 0.10 cm under 250 mM NaCl respectively. Cultivars Lu-26 and Oasis-86 showed the lowest shoot lengths (9.44 ± 0.39 and 9.36 ± 0.23 cm) under normal conditions, these lengths were reduced to (4.78 ± 0.41 and 7.24 ± 0.25 cm) under 150 mM NaCl, to (3.14 ± 0.13 and 0.54 ± 0.05 cm) under 200 mM NaCl, to (0.92 ± 0.04 and 0.68 ± 0.10 cm) under 250 mM NaCl. On the other hand, their root lengths were reduced from (7.34 ± 0.58 and 6.32 ± 0.37 cm) under normal conditions to (3.64 ± 0.21 and 5.00 ± 0.00 cm) under 150 mM NaCl, to (2.48 ± 0.10 and 1.42 ± 0.10 cm) under 200 mM NaCl, to (1.42 ± 0.08 and 0.62 ± 0.17 cm) under 250 mM NaCl.

Based on the results obtained from the ten parents of *Triticum aestivum* cultivars, five F1 hybrids were selected to study their response to salinity stress. Kharchia-65 and Misr-3 are parents of most hybrids. Kharchia-6 was the most tolerant cultivar among the tested ones, and Misr-3 showed moderate tolerance but more consistency in some growth parameters under stress treatment. The effect of salinity stress (250 mM NaCl) on the 5 hybrids is shown in Figure 2. Under normal conditions, the two hybrids H2 and H3 recorded the highest values of most traits studied as compared with other hybrids. H3 recorded the highest values in shoot (9.37 ± 1.40) and root lengths (10.70 ± 0), followed by H2 with shoot length of 8.17 ± 0.31 and root length recorded (9.13 ± 0.99), and H5, which recorded shoot length of (6.70 ± 1.39) and root length (4.73 ± 1.50).

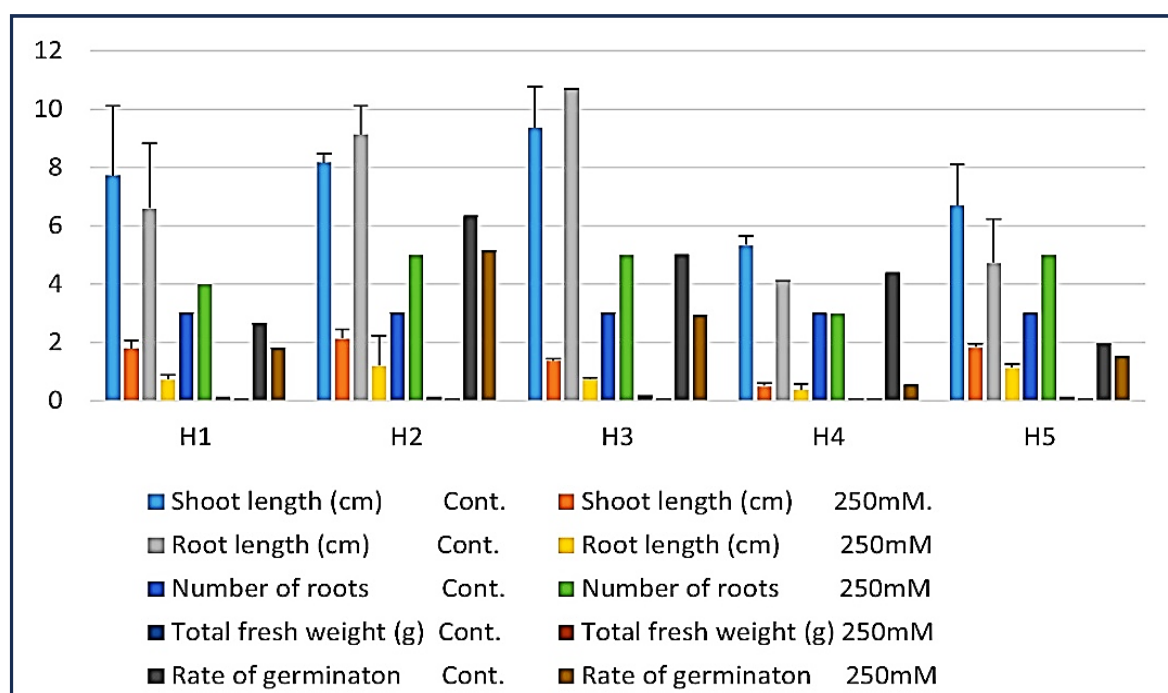


Figure 2 Histogram illustrates variation in quantitative traits in five *Triticum aestivum* hybrids (H1-H5) as listed in Table 1 under normal conditions and under salinity stress (250 mM NaCl). N.B. Hybrids are only subjected to (0 and 250 mM) salt (NaCl) stress after parental screening.

Under salinity stress (250 mM NaCl), the shoot length of hybrids H2 and H3 was reduced from 8.17 ± 0.31 and 9.37 ± 1.40 cm under control conditions to 2.13 ± 0.32 and 1.37 ± 0.06 cm, respectively. The root length of H2 and H3 was reduced from (9.13 ± 0.99 and 10.70 ± 0 cm) under normal water supply to (1.20 ± 1.04 and 0.73 ± 0.06 cm) (Figure 2).

All the cultivars and the 5 hybrids showed a significant decrease in response to salinity stress compared to the control. Only the number of roots increased under salinity stress in all hybrids except H4 (Figure 2). None of the hybrids under salinity stress (250 mM NaCl) has shoot or root length and rate of germination as high as their parents. Generally, the two hybrids H2 (Misr-3 $\times$ Oasis-86) and H3 (Pasban-90 $\times$ Misr-3) exhibit higher values for most growth traits than other hybrids. Results indicated that the two hybrids H2 and H3 show improved vegetative traits, while hybrids H1 and H4 revealed reduced productivity parameters under normal conditions. It can be concluded that the results of H2, H3, and H5 are consistent with the results of parent cultivars (Misr-3, Pasban-90, and Kharchia-65), which recorded higher shoot and root lengths, higher rates, and percentage of germination.

Under salinity stress (250 mM NaCl) and based on salt tolerance index (STI) values (listed in Table 2), the 15 wheat genotypes examined in this study can be classified into four tolerance groups according to [6]. Namely, salt tolerant (STI = above 70%), moderately salt tolerant (STI = 70 to 60%), moderately salt susceptible (STI = 60 to 50%), and salt susceptible (STI = below 50%). Only Kharachia-65 and H5 hybrid fell in the tolerant class; three genotypes were moderately salt tolerant, seven were moderately salt susceptible, and three were reported as salt susceptible (Table 3).

Table 2 Mean measurements of salt tolerance index (STI) in ten parents of *Triticum aestivum* cultivars under salinity conditions (150, 200, and 250 mM NaCl).

Cultivars	STI 150	STI 200	STI 250
Lu-26	73.2	67.7	53
Sakha-8	79.3	68	44
Krl-1-4	87.5	60.4	61.2
Oasis-86	88.3	53.1	41
Shorawaki	90.2	51.9	54
Pasban-90	94.3	62.1	63
Kharchia-65	94.4	66.7	72
Giza-171	81.7	60.4	54
Sids-14	94.3	61.3	60
Misr-3	76.8	66.6	59.7
Hybrids			
H1 (Krl-1-4 × Misr-3)			58.02
H2 (Oasis-86 × Misr-3)			66.81
H3 (Pasban-90 × Misr3)			55.39
H4 (Kharachia-65 × Misr-3)			41.17
H5 (Pasban-90 × Kharachia-65)			72.31

N.B. Hybrids are only subjected to (0 and 250 mM) salt (NaCl) stress after parental screening.

Table 3 Salt tolerance categories for wheat cultivars based on salt tolerance index (STI) at 250 mM salt stress.

Salt tolerance category	Range of STI	Name of wheat lines
Tolerant	70% and above	Kharachia-65 H5
Moderately tolerant	70-60%	Pasban-90 Krl-1-4 H2
Moderately susceptible	60-50%	Misr 3 Giza 171 Shorawaki Lu-26 Sids 14 H1, H3
Susceptible	Below 50%	Sakha-8 Oasis-86 H4

3.2 Impact of Salinity on Protein Using SDS-PAGE Assay

Obvious variations in the total protein banding patterns were detected through the SDS-PAGE technique among the ten wheat cultivars in normal conditions on one hand and 200 mM or 250

mM salt-treated cultivars on the other hand. In comparison of wheat cultivars under normal and 200 mM NaCl treatment, it was found that the produced protein molecular weights (MWs) ranged from 15 to 132.8 kDa. Some of the key proteins present under control conditions disappeared under salinity stress, while new protein bands appeared. Upon salt treatment, eleven protein bands appeared in the Lu-26 cultivar with molecular weights ranging from 23 to 132.8 kDa, and eight protein bands with molecular sizes ranging from 23 to 104.3 kDa, while only one band disappeared at 44.5 kDa. In Krl-1-4, 13 protein bands emerged in the range of 23 to 132.8 kDa, while in Oasis-86, six and 2 bands appeared in the ranges 70-126 kDa and 23-39 kDa, respectively. In addition, Shorawaki showed seven protein bands with molecular weights ranging from 23 to 126 kDa, and three bands disappeared at 73.9, 104.3, and 132.6 kDa when compared to its control (Figure 3A). Figure 3B showed the appearance of bands for the salt-treated Paspan-90 cultivar at Mws ranging from 37.6-66.5, 75.3-104.3 kDa, and the disappearance of two bands at 45 and 55.4 kDa while kharchia-65 has five new protein bands that were not in the control with molecular sizes of (46.8, 66.5, 75.3, 94 and 104.3 kDa) and only one band missed with molecular size (55.4 kDa) while Giza 171 has nine protein bands emerged at salinity stress with molecular weights ranging from 37.2 to 132.8 kDa and only one protein band with molecular size of 37.6 kDa vanished under salinity stress. The appearance of six protein bands with molecular weights of (37.2, 46.8, 75.3, 94, 96 and 104.3 kDa) and the disappearance of two bands with molecular sizes (45, 55.4 kDa) were reported for Sids14 while for Misr 3, one protein band was missed with molecular weight of 55.4 kDa and five protein bands were detected with molecular weights of (37.6, 66.5, 75.3, 94 and 104.3 kDa).

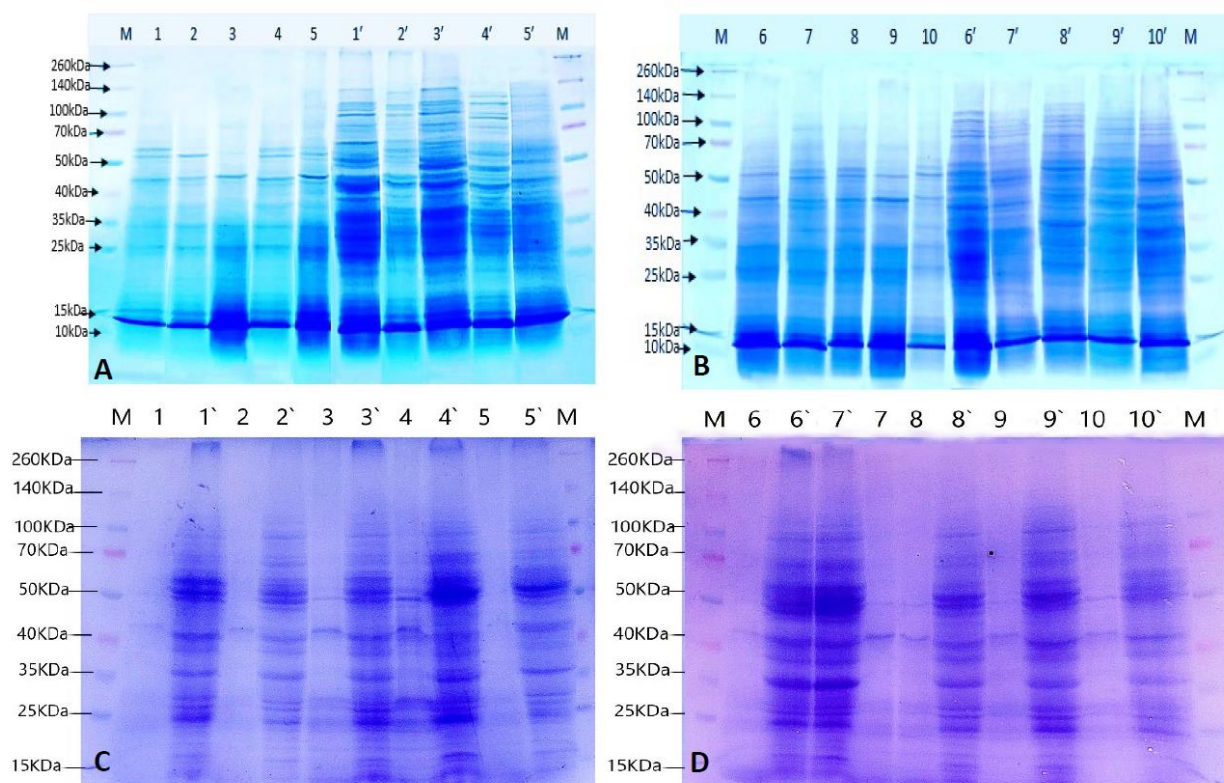


Figure 3 Protein profile of 10 parents of wheat (*Triticum aestivum* L.) based on SDS-PAGE analysis. M refers to the protein ladder, and wheat cultivars are numbered as

listed in Table 1. Numbers from 1 to 10 are cultivars grown in normal conditions, and 1' to 10' are cultivars grown under NaCl stress. A and B photos comparing control to 200 mM salt concentration, C and D comparing control to 250 mM salt concentration.

On the other hand, in comparison of wheat cultivars under normal and 250 mM NaCl treatment, it was found that the produced protein molecular weights (MWs) ranged from 14.4 to 97.6 kDa. Some of the key proteins present under control conditions disappeared under salinity stress, while new protein bands appeared. Upon salt treatment protein pattern analysis of Lu-26 and Shorawaki showed ten protein bands with MWs ranging from (46.2-97.6 kDa) and (Sakha-8, Pasban-90) had eight protein bands with the same range except two bands not found (59 and 75.8 kDa) moreover the six protein bands with MWs ranging from 50-97.6 kDa at the cultivars (Kharachia-65, Giza-171, Sids-14) and Misr-3 showed five protein bands with MWs ranging from (56.3-97.6 kDa) appeared under 250 mM salinity treatment and the three protein bands with MWs (35, 37.5, 37.90) appeared at cultivars (Lu-26, Sakha-8, Shorawaki, Pasban-90, Sids14, Misr-3) while the four protein bands with MWs ranging from (35-37.9 kDa) appeared at cultivars (Krl-1-4, Oasis-86, Kharachia-65, Giza-171).

4. Discussion

Enhancing salt tolerance in wheat crops is an important objective in areas with limited water supply and those affected by soil salinity. Wheat productivity can be increased by improving tolerant genotypes [21, 22]. It is essential to gain a comprehensive understanding of how various traits respond to salinity stress across different genotypes and stages [23]. The present study was conducted to demonstrate the diverse response of ten wheat cultivars (represented by 4 Egyptian and 6 exotics from CIMMYT) and 5 selected F1 hybrids against salinity stress based on monitoring the changes of 7 quantitative growth parameters (root and shoot lengths, total fresh and dry weights, percentage and rate of germination, and number of roots). In addition to evaluating the variations in band profiles of total soluble proteins using SDS-PAGE assay at the early seedling stage, we exposed them to different salinity levels. Morphological traits can be used as a selection criterion for salt tolerance at the seedling stage [24-26]. Results showed that all seedling traits decreased proportionally with increasing NaCl concentration. Similar results were recorded by the work of Ragab and Taha [27], who showed that increasing salt concentrations decreased seedling root length, shoot length, and shoot and root dry weights in nine Egyptian wheat cultivars. Also, Amro et al. [9] showed that seedling traits of 48 wheat genotypes decreased proportionally with increasing seawater concentrations (10, 40, and 50%). Ashraf et al. [28] and Radi et al. [25] attributed the decrease in morphological parameters to lower levels of photosynthetic pigments, transpiration rate, and the synthesis of carbohydrates and proteins in plants.

The ten cultivars and the five hybrids showed a highly significant decrease in shoot and root lengths under salinity stress as compared with the control. Cultivar Kharchia-65 recorded the tallest plant shoot and the tallest root under normal conditions (16.4 and 14.7, respectively), which were reduced to 6.35 and 4.56, respectively, under the applied salinity stress (150 mM NaCl), reduced to 2.94 and 1.72, respectively, under (200 mM NaCl) and to 0.52 and 1.32 respectively under (250 mM NaCl respectively). The results of the present study about Kharchia-65 is in agreement with the work of Munns and Tester [23] who showed that Kharchia-65 was the tallest cultivar among the tested genotypes in normal conditions and revealed decline in plant

shoot height under salt stress because the accumulation of Na⁺ and/or Cl⁻ at toxic levels affects the photosynthetic capacity, resulting in less supply of carbohydrates to the young leaves and further reduce the shoot growth rate.

Early stages of seedling growth, such as germination, are critical because they influence subsequent stages, including grain yield. It was reported that poor germination and weak seedling growth are major problems that lead to significant yield losses [29-31]. In this study, cultivars Sakha-8, Pasban-90, and Misr-3 recorded the highest germination percentage and rate. The accumulation of Na in the seeds may adversely affect seed germination, delaying the mean germination time. Salinity causes undesirable effects on plant growth due to low soil solution osmotic potential (osmotic stress), ion effects (salt stress), or a combination of these factors. The main reason for germination failure was the inhibition of seed water uptake due to a high salt concentration, whereas others have suggested that germination was affected by salt toxicity [32]. The study by Mahfouz and Rayan [33] showed that *Allium cepa* meristematic cells treated with NaCl (100 and 200 mM) exhibited inhibition of cell division, and this inhibition was accompanied by a reduction in the germination rate of the seedling.

In the present study, salt tolerance index (STI) values decreased with increasing NaCl concentration. According to STI values, cultivar Kharchia-65 recorded the highest value and fell in the tolerance category, followed by Pasban-90 and Krl-1-4, which are moderately tolerant. In addition, cultivars Giza 171 and Sids 14 are moderately susceptible. In this respect, El-Hendawy et al. [34] supported the study results as they showed that the Indian genotype Kharchia-65 was recorded as the most tolerant cultivar to salinity stress. Also, the study of Mujeeb-Kazi and Diaz de Leon [35] was conducted to assess the genetic variation for salinity tolerance in 172 spring wheat genotypes based on morphological and DNA markers; the data showed that the cultivar 'Pasban-90' (from Pakistan) is salt-tolerant and could be used in breeding programs for improving salt tolerance in future wheat cultivars. In accordance with the present study results, Darwish et al. [36], Hagars et al. [37], and Abd-El-Hamid et al. [38] reported that Giza 171 had moderate soil salinity tolerance. Khatab et al. [39] reported Sakha 8 as a tolerant cultivar and Giza 171 with moderate salinity tolerance among the tested cultivars. Their experiment was conducted using 10.50 dsm⁻¹ irrigation water. This agrees to some extent with the results of the present study, as these cultivars recorded very high STI values at 150 mM NaCl stress, followed by decreases at higher stress concentrations. Elkot et al. [40] studied the effect of soil salinity on different wheat cultivars and the results showed that cultivars Giza 171 and sids-14 were the best genotypes at saline soil conditions and Giza 171 was considered as tolerant wheat cultivars among the studied genotypes. Results of the present study showed that there was no clear phenotypic advantage among the five crosses tested under stress conditions. This may be attributed to the fact that the more divergent the parents are, the stronger the hybrids will be. Parents used to produce these hybrids were found to be genetically close to each other after evaluation using biochemical, SSR, and ScoT markers [41]. The genetic similarity between Krl-14 and Misr-3 is 0.775, Oasis-86 and Misr-3 0.752, Pasban-90 and Misr-3 0.783, Kharchia-65 and Misr-3 0.757, Pasban-90 and Kharchia-65 0.761. The genetic similarity between the whole 10 tested cultivars ranged from 0.74 to 0.83. The cross H5 reported the highest STI value among the tested hybrids, followed by H2 at 250 mM. This suggests they are promising for cultivation under high salinity conditions. In this regard, Khatab et al. [39] reported that 5 F1 wheat crosses exhibited higher salinity tolerance than the control in their evaluation of salinity tolerance indices.

Total soluble proteins that were extracted from seedlings of the 10 wheat cultivars under normal and saline conditions were separated through SDS-PAGE. This has been used as an additional tool for genotype tolerance testing, alongside the other assays. By inspecting the gel comparing the ten wheat cultivars under control and 200 mM salt concentration, it is clear that bands with molecular weights of 15.7, 16.5, 23, 25, 42, 50, 54.5 kDa were common between the control and treated cultivars. While 102 and 104.3 kDa bands were present in most salt (200 mM) treated cultivars. On the other hand, 14.4, 15, 22.3, 23.7, 24.4, 25.6, 26.3 were common bands between control and 250 mM NaCl-treated plants, and (35, 37.9, 56.3, 62.8, 66.2, 93.3, 97.6 kDa) bands appeared only in cultivars treated with 250 mM NaCl. Some distinguished bands were observed in either 200 or 250 mM salt-treated cultivars, with Mws of 37.5, 40, and 48.6 kDa. Thus, it is concluded that storage protein banding patterns differed across alinity concentrations. In this regard, El-Seidy et al. [13], El-Saber [15], and Gowayed and Abd-El-Moneim [7] reported the appearance of a number of new bands in salt-treated wheat cultivars. However, Sobhanian et al. [14] did not recognize the emergence of new bands. Instead, they documented variation in the intensity of some bands. Singh and Singh [16] reported a 17 kDa band in a tolerant wheat cultivar. In addition to another band at 87 kDa in the 100 mM NaCl-treated cultivar. They also recorded a decrease in the intensity of some bands.

5. Conclusions

Tolerance evaluation of wheat genotypes to NaCl stress at the seedling stage effectively reduces the effort required in field screening. Indeed, as salt stress increases, the inhibitory impact on growth parameters increases. However, different genotypes respond differently to stress based on their genetic makeup. The tested characters were able to detect differences in responses among wheat genotypes. According to STI values, all cultivars at 150 mM NaCl stress fell in the tolerance category. At 200 mM, Oasis-86 and Shorawaki cultivars were classified as moderately susceptible, and the rest of the cultivars were moderately tolerant. Finally, the responses of genotypes at 250 mM NaCl stress proved the outstanding performance of Kharachia-65 and hybrid 5 (Pasban-90 × Kharachia-65) to be ranked as tolerant genotypes, Pasban-90, Krl-1-4, and H2 as moderately tolerant, Misr 3, Giza 171, Shorawaki, Lu-26, Sids 14, H1, and H3 as moderately susceptible. Sakha-8, Oasis-86, and H4 are susceptible. SDS-PAGE showed the appearance of some new bands in salt-treated cultivars at both concentrations. The appearance of these protein bands may be attributed to salinity stress; however, this hypothesis can be confirmed by further molecular studies. Finally, Kharachia-65 and hybrid 5 showed the highest salt tolerance, along with the Egyptian cultivar Misr 3, which is a promising salt-tolerant cultivar. These genotypes may act as candidates for parental materials for salt-tolerance breeding or as potential screening materials for salt-affected areas at early stages.

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Author Contributions

Doaa Mokhtar: Methodology, writing – original draft, formal analysis. Amina Abdel-Hamid: Conceptualization, funding acquisition, resources, supervision. Ahmed Mohamed Mostafa: Conceptualization, resources. Ahmed M.S. Elfanah: Methodology, resources. Mohamed A Badawi: investigation, manuscript review and editing. Abdullah A. Saber: funding acquisition, manuscript review and editing. Hoda S. Barakat: Conceptualization, resources, supervision, manuscript review and editing. Sara Aly: Conceptualization, supervision, writing – original draft, manuscript review and editing. All authors reviewed and approved the final manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

Data Availability Statement

The data generated from this study is available upon reasonable request from the corresponding author.

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