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Full Length Article

Genetic Variation of Wheat for Salt Tolerance Based on Physiological and Agronomic Traits

Mutahhar Y. Al-Khaishany[1*], Fahad H. Al-Qurainy[1], Ibrahim A. Alaraidh[1], Mohamed Najeb Barakat[2], Adel Ahmed Elshafei[3], Manzer H. Siddiqui[1], Saud A. Alamri[1], Hayssam M. Ali[1], Abdulaziz A. Alsahli[1], Saud M. Alzahrani[1] and Muhammad Ishfaq[4]

[1]*Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 2455, Saudi Arabia*
[2]*Crop Science Department, Faculty of Agriculture, University of Alexandria, Alexandria, Egypt*
[3]*Genetic Engineering and Biotechnology Division, National Research Centre, Cairo, Egypt*
[4]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad-38040, Pakistan*
[*]For correspondence: muthr20@yahoo.com

Abstract

Two consecutive years experiments were conducted in pots to evaluate the performance of ten wheat genotypes under salt stress. On the basis of different physiological and agronomic traits we identified salt tolerant genotypes, and also found out reliable traits to be used as screening criteria for salt tolerance. To achieve the objectives of these experiments, the effects of different levels of salt stress (0, 50, 100, 150 and 200 m*M* NaCl) on the different physiological and agronomic characteristics of ten genotypes of wheat were studied following split plot arrangement under completely randomized design (CRD) with five replicates. The results reveal that salt stress significant affected all wheat genotypes and reduced physio-biochemical parameters and agronomic traits, whereas significant increased proline and Na^+ contents in leaves. Among the ten genotypes, Shebam8 genotype was marked as salt tolerant, while Yecora Rojo, KSU106, Samma, Sonalika and Gemmiza9 were found moderately tolerant and remaining genotypes (Maaya, Pawni, samra and Mesri) showed the lowest tolerance to salinity. Genotype Shebam8 exhibited better ionic homeostasis (higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in plant leaves), membrane stability index, leaf area and chlorophyll content. Thus, above-mentioned characteristics could be used as screening criteria for salt tolerance. © 2018 Friends Science Publishers

Keywords: Salinity; Genotypic variation; Physiological-agronomic traits; *Triticum aestivum*

Introduction

Salinity is considered a substantial factor persuading crop production and agricultural sustainability in the arid and semiarid regions of world, reducing soil value and its productivity (Schleiff, 2008; Ashraf, 2010). It is reported that over 6.5% of total worldwide land area and 19.5% of irrigated land are salt affected (FAO, 2018).

Plants growing in saline environments suffer from low osmotic potential of soil solution due to increased concentrations of mineral ions. Eventually, these ions enter into cellular sap, causing ionic imbalance. The negative effects of this disturbance are reflected on plant growth (Perez-Alfocea *et al*., 1994) due to dehydration of the shoot or leaves through the low water potential of soil solution; nutritional imbalance through interference of salt in uptake and translocation and specific toxicity because of high accumulation of salt ions in the cytoplasm (Greenway and Munns, 1980). Salinity also adversely affects the soil physical properties *i.e.*, soil structure, permeability (Szabolcs and Darab, 1982; Szabolcs, 1989).

Crop genotypes differ in their capability to grow and produce in salt affected soils. Growth and reproduction depend on the species ability to control the absorption of salt from the soil and effectively excluded it at cellular level (Munns and Tester, 2008). Salinity tolerance is a complex trait and also variations are present not only between species but also amongst varieties/genotypes in a species (Ashraf, 2002). As genotypes of plants are different in its response in salinity tolerance (Munns, 1993), where the salinity sensitive genotypes quickly accumulate ions greater than salinity tolerant genotypes, thus resulting in death of leaves and of the plant. Salt tolerant plants depend on: regulation of transpiration, ion transport, plasma membrane stability, and the presence of salt glands. However, the salt resistance is controlled by a various genes and cannot be attributed to a single gene (Munns, 2002).

A physiological approach may accompaniment empirical breeding and can improve yield potential of crops under salinity by identifying imperative physiological traits accompanying with salt resistance (Almeselmani, 2012). Genetic diversity is one of the crucial aspect for the

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improvement of numerous crop plants containing wheat. The advantage of assessing salt resistance for genotypes depends upon physiological criterion as rapid, easy, non-destructive approaches and assists in understanding the variances of physiological mechanisms of salt resistance amongst genotypes (Noble and Rogers, 1992).

The main objective of this study was to examine the extent of variation in wheat genotypes based on physiological and agronomic traits. The aim of the study was to find out trustable traits that can be used as screening criteria for salt tolerance.

Materials and Methods

Research studies were carried out in a greenhouse conditions at College of Food and Agriculture Sciences, King Saud University, from the middle of November to the middle of March during 2012/2013 and 2013/2014. Ten wheat genotypes were collected from five countries (Table 1).

Germination Test

For germination parameter, seeds of different genotypes were surface sterilized in 1% solution of sodium hypochlorite for 1-2 min; subsequently washed with distilled water and air-dried. Seeds were placed in covered sterilized petri dishes and germination test was carried out in growth chamber at 25 ± 2°C in complete darkness. The experiment was done by arranging petri dishes in complete randomized design. Seeds were germinated in distilled water (0 m*M* NaCl as a control), and in 40, 80, 120 and 160 m*M* NaCl solutions. For each treatment, four replicates with each 50 seeds were used. Germination percentage for each genotype was calculated after every 24 h for seven days using the formula adopted by Yildirim *et al.* (2015) as following:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 100$$

Crop Husbandry

For pot experiments, the surface sterilized seeds (20 seeds) were sown in each plastic pot (25 cm diameter, 25 cm height) filled with soil carried from the Agricultural Research Station (Dierab, 40 km south Riyadh), the physical and chemical properties of the soil used are reported in Table 2. The experiment was done by arranging pots according to split plot under completely randomized design (CRD), ten wheat genotypes and five salinity levels with five replicates. The main split were salinity levels (0, 50, 100, 150 and 200 m*M* NaCl), while subplot were ten wheat genotypes. After thinning, in each pot 12 plants were maintained after two weeks of seed sowing. Salt stress was applied gradually by adding 50 m*M* NaCI every alternate day with irrigation water to reach the final concentration *i.e.*, 100, 150 and 200 m*M* NaCI stress according to the required treatments. After achieving required levels of NaCl; crop was irrigated with distilled water. Hoagland and Arnon's nutrient solution was supplied (Hoagland and Arnon, 1950).

In the green house average air temperature was 27°C during the day and 14°C during the night. Relative humidity varied among 45 and 55% at day/night, with lighting regime of 14 h light and 10 h dark.

Table 1: Name and origin of the 10 wheat genotypes used in the study

Genotypes	Origin
Yecora Rojo	USA
KSU106	Plant Production – KSA
Samma	KSA
Maaya	KSA
Pawni	GOAR*-Yemen
Shebam8	GOAR -Yemen
Samra	GOAR -Yemen
Sonalika	GOAR -Yemen
Gemmiza9	GOAR -Yemen
Mesri	GOAR -Yemen

* GOAR: General Organization for Agricultural Research, Yemen

Table 2: The physical and chemical properties of the soil used in the experiments

Property	Unit	Value
pH	-	8.01
EC	dS m^{-1}	0.49
Organic matter	%	1.61
Na^{+}	meq L^{-1}	27.9
K^{+}	meq L^{-1}	5.3
Ca^{2+}	meq L^{-1}	24
Mg^{2+}	meq L^{-1}	2
Cl^{-}	meq L^{-1}	29.3
HCO_3^{-}	meq L^{-1}	26.5
SO_4^{-2}	meq L^{-1}	2.4

Morpho-physiological Traits Measured

After one month of salinity stress, plants were collected from each pot to measure the morpho-physiological traits and left minimum four plants in each pot to reach at maturity to determine the yield traits. Plant fresh weight, dry weight, and leaf area were determined. Photosynthetic pigments were extracted by N, N-Dimethyl formamide as described by Wellburn (1994). Free proline content was quantified with the help of spectrophotometer (SPEKOL®1500 UV/Vis) before preparing acid-ninhydrin as explained by Bates *et al.* (1973). Cell membrane stability index was calculated according to the procedure of Sairam *et al.* (1997) by using conductivity meter (AD3000 Conductivity-TDS-TEMP Meter), and mineral contents *i.e.*, sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{+2}), and magnesium (Mg^{+2}) were determined by Perkin Elmer AAS-300 Atomic Absorption Spectrophotometer (Katz and Jenniss, 1983). Chemical properties of soil were measured using the method as described by Jackson (1973).

At maturity stage; plant height, days to heading, spike length, kernel number/spike, 1000 kernel weight and grain yield (per pot) were determined.

Statistical Analysis

The experiment was laid out according to split plot under completely randomized design (CRD) with five replicates. The five salinity levels regimes were assigned to the main plots, while the wheat genotypes distributed randomly over the sub- plots. Analysis of variance of collected data was performed by using the SAS 9.1 program (SAS, 2007).

Results

Germination Percentage

Germination percentage was reduced consistently with application of additional increment of salinity. The average of germination percentage under control, 40, 80, 120 and 160 m*M* NaCl treatments was 85.12, 84.68, 77.72, 70.96 and 67.00%, respectively.

Under salinity, the maximum mean of germination percentage (84.08%) was recorded in Sonalika followed by Yecora Rojo (82.72%), Samma (80.16%), KSU106 (79.60%) and Shebam8 (79.12%); and minimum mean of germination percentage (57.84%) was from Maaya. At high salinity level (160 m*M* NaCl), the Mesri genotype showed a greater reduction in germination (*i.e.*, 34.65%) than genotypes of Sonalika, Samma, KSU106, Shebam8 and Yecora Rojo (*i.e.*, 12.90%, 13.15%, 13.33%, 15.49% and 16.59%, respectively) (Fig. 1).

Morpho-physiological Traits

The shoot fresh and dry weight, leaf area, chlorophyll content, carotenoid, proline, and membrane stability index in leaves were significantly *($P < 0.05$ or $P < 0.01$)* influenced by different salinity levels, however effect varied for various genotypes used (Table 3a, b, c and d). Plant growth was suppressed with increasing salinity but such reduction in plant growth also varied with respect to genotypes.

Maximum shoot fresh weight per plant was recorded for Shebam8 followed by KSU106, Maaya, Sonalika, Gemmiza9 and Yecora Rojo while minimum shoot fresh weight per plant was recorded with Pawni during both 2012 and 2013 seasons (Table 3a).

Shoot fresh weight was significantly (*$P < 0.05$ or 0.01*) decrease by increasing salinity level; the reduction in shoot fresh weight decreased by 9.02, 15.08, 23.26 and 29.68% with application of 50, 100, 150 and 200 m*M* NaCl treatments as compared with the control treatment respectively during 2012 season, while during 2013 season shoot fresh weight decreased by 16.85, 27.76, 41.09 and 47.07% with application of 50, 100, 150 and 200 m*M* NaCl treatments over control treatment, respectively, (Table 3b).

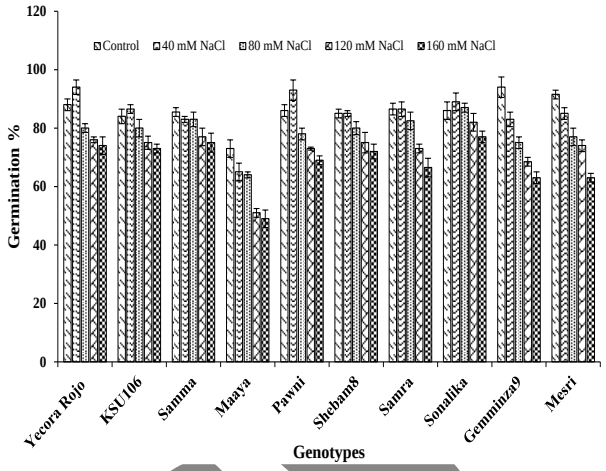

Fig. 1: Effect of different NaCl concentrations on germination of ten wheat genotypes. Column represents the mean values, while vertical bars above mean denote standard error of five replicates

Maximum average shoot dry weight per plant was recorded in Shebam8 followed by KSU106, Maaya, Gemmiza9 and Sonalika, minimum shoot dry weight per plant in Pawni during both years 2012 and 2013 seasons (Table 3a).

Application of NaCl significantly reduced the shoot dry weight. However, under 200 m*M* NaCl, Pawni and Maaya genotypes showed larger reduction in shoot dry weight (51.97 and 56.73%, respectively) than Sonalika and Shebam8 genotypes (33.41 and 34.30%, respectively) in 2012 season. While in 2013 season, genotypes Samra and Maaya genotypes also showed a greater reduction in shoot dry weight under 200 m*M* NaCl (58.67 and 60.53%, respectively) than Shebam8 and Sonalika genotypes (28.38 and 31.91%, respectively) (Table 3a and b).

Application of NaCl treatments significantly reduced the leaf area. The leaf area decreased with increasing levels of NaCl in both seasons. Among the genotypes of wheat, Shebam8 and Gemmiza9 proved best by giving the highest values for leave area, while genotypes Pawni and Mesri performed poorly by giving the least values for leaves area in both seasons (Table 3a).

A significant variance was found among wheat genotypes for chlorophyll (*a, b*) content under salinity. The highest values for chlorophyll *a* content were recorded in genotypes Shebam8 and Maaya, however, the lowest values was noticed in genotypes Pawni and Mesri under salt stress. Under NaCl stress, no significant difference was found among Yecora Rojo, KSU106 and Gemmiza9 genotypes for chlorophyll *a* content. The highest chlorophyll *b* content was recorded in genotype Maaya, but the slightest decrease was found in genotypes Pawni and Samra. The results showed that no significant variance was found among the genotypes Shebam8, Mesri and Gemmiza9 for chlorophyll *b* content (Table 3a and b).

Table 3a: Means of genotypes for shoot fresh weight, shoot dry weight, leaf area, chlorophyll *a*, chlorophyll *b*, carotenoids, proline, and membrane stability index in leaf under salinity stress at seasons 2012 and 2013

Genotypes	Shoot fresh weight(g plant^{1})		Shoot dry weight (g plant^{-1})		Leaf area (cm^{2})		Chlorophyll *a* (mg/g FW)		Chlorophyll *b* (mg/g FW)		Carotenoids (mg/g FW)		Proline (mg/g FW)		Membrane stability index %	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Yecora Rojo	1.421c	3.139b	0.292cd	0.624c	27.35b	62.84c	1.272bc	1.348b	0.607ef	0.641b	0.356bc	0.349abc	0.173de	0.162e	55.124a	55.99a
KSU106	1.557b	2.935c	0.372ab	0.679b	27.24b	59.39d	1.347ab	1.353b	0.658dce	0.606b	0.363b	0.375ab	0.173de	0.154e	48.77cd	42.69bc
Samma	1.270e	3.257b	0.265de	0.683b	22.77d	64.77c	1.187de	1.258b	0.640ed	0.598b	0.317de	0.343bc	0.178cd	0.241a	46.95de	42.72bc
Maaya	1.447c	2.913c	0.356b	0.636bc	24.86c	64.62c	1.379a	1.318b	0.859a	0.601b	0.347bcd	0.366abc	0.176cde	0.216ab	47.99cd	39.05d
Pawni	1.153f	1.513e	0.261e	0.278e	18.68e	24.58g	0.981f	0.955c	0.416g	0.458c	0.297ef	0.251d	0.181c	0.206b	37.93h	36.03e
Shebam8	1.672a	3.819a	0.399a	0.779a	30.30a	80.87a	1.374a	1.577a	0.686cbd	0.726a	0.417a	0.382a	0.175de	0.172cde	50.92b	44.22b
Samra	1.334d	2.252d	0.313c	0.442d	28.15b	52.04f	1.268bcd	1.061c	0.562f	0.492c	0.356bc	0.288d	0.187b	0.192bcd	43.52f	40.39cd
Sonalika	1.433c	3.227b	0.346b	0.759a	27.63b	72.61b	1.240cde	1.304b	0.628ed	0.635b	0.330cd	0.357abc	0.202a	0.169de	49.67bc	44.40b
Gemmiza9	1.431c	3.316b	0.349b	0.674b	30.46a	82.96a	1.302abc	1.261b	0.707bc	0.576b	0.321de	0.334c	0.166f	0.171cde	45.88e	43.84b
Mesri	1.247e	2.839c	0.288cde	0.610c	21.35d	55.98e	1.168e	1.284b	0.744b	0.587b	0.273f	0.365abc	0.177cde	0.200bc	40.43g	37.71de

Means followed by same letter(s) are not significantly different, according to LSD (shoot fresh weight 0.1215, 0.4528; shoot dry weight 0.0672, 0.1062; leaf area 4.5792, 6.4469; chlorophyll *a* 0.1836, 0.3522; chlorophyll *b* 0.1320, 0.1581; carotenoids 0.0688, 0.0859; proline 0.0124, 0.0662 and membrane stability index 4.3066, 6.0733 for 2012 and 2013 seasons, respectively) at 0.05 level of probability

Table 3b: Means effect of salinity levels on shoot fresh weight, shoot dry weight, leaf area, chlorophyll *a*, chlorophyll *b*, carotenoids, proline, and membrane stability index in leaf at seasons 2012 and 2013

Salinity (m*M* NaCl)	Shoot fresh weight (g plant^{-1})		Shoot dry weight (g plant^{-1})		Leaf area (cm^{2})		Chlorophyll *a* (mg/g FW)		Chlorophyll *b* (mg/g FW)		Carotenoids (mg/g FW)		Proline (mg/g FW)		Membrane stability index %	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
0	1.651a	3.977a	0.407a	0.810a	31.74a	73.24a	1.569a	1.461a	0.765a	0.686a	0.428a	0.380a	0.100e	0.114e	59.627a	52.876a
50	1.502b	3.307b	0.370b	0.683b	28.53b	68.19b	1.547a	1.399a	0.722b	0.646a	0.421a	0.369a	0.139d	0.147d	50.312b	45.837b
100	1.402c	2.873c	0.315c	0.602c	26.55c	61.61c	1.321b	1.292b	0.690c	0.600b	0.357b	0.354a	0.165c	0.182c	45.969c	43.400c
150	1.267d	2.343d	0.284c	0.522d	22.81d	56.18d	1.035c	1.179b	0.615d	0.547b	0.265c	0.325b	0.194b	0.221b	42.410d	38.504d
200	1.161e	2.105e	0.245d	0.465d	19.76e	51.13e	0.787d	1.028c	0.460e	0.481c	0.218d	0.277c	0.296a	0.277a	35.267e	32.908e

Means followed by same letter(s) are not significantly different, according to LSD (shoot fresh weight 0.1215, 0.4528; shoot dry weight 0.0672, 0.1062; leaf area 4.5792, 6.4469; chlorophyll *a* 0.1836, 0.3522; chlorophyll *b* 0.1320, 0.1581; carotenoids 0.0688, 0.0859; proline 0.0124, 0.0662 and membrane stability index 4.3066, 6.0733 for 2012 and 2013 seasons, respectively) at 0.05 level of probability

Table 3c: The analysis of variance for shoot fresh weight, shoot dry weight and leaf area traits of the ten genotypes from wheat under the five levels of salinity

S. O. V	D.F	Shoot fresh weight		Shoot dry weight		Leaf Area	
		2012	**2013**	**2012**	**2013**	**2012**	**2013**
Replicates	4	0.331	5.903	0.033	0.208	181.80	713.70
Salinity treatment (S)	4	1.857**	32.14**	0.223**	0.974**	1107.24**	3958.99**
Ea	16	0.011	0.235	0.006	0.025	15.32	26.64
Wheat genotypes (G)	9	0.580**	10.15**	0.055**	0.566**	377.33**	6848.92**
S × G	36	0.026**	0.340**	0.004NS	0.009NS	19.34NS	84.130**
Eb	180	0.010	0.132	0.003	0.007	13.48	26.72

NS: not significant at $p \leq 0.05$

*, **: significant at $p \leq 0.05$ and 0.01, respectively

Table 3d: The analysis of variance for chlorophyll *a*, chlorophyll *b*, carotenoids, proline, and membrane stability index traits in leaf of the ten genotypes from wheat under the five levels of salinity

S. O. V	D.F	Chlorophyll *a*		Chlorophyll *b*		Carotenoids		Proline		Membrane stability index %	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Replicates	4	0.054	0.627	0.001	0.177	0.010	0.055	0.004	0.106	19.94	1336.84
Salinity treatment (S)	4	5.344**	1.503**	0.668**	0.335**	0.412**	0.098**	0.276**	0.202**	4122.51**	2842.04**
Ea	16	0.024	0.086	0.002	0.023	0.001	0.005	0.0002	0.002	12.97	8.620
Wheat genotypes (G)	9	0.356**	0.706**	0.338**	0.142**	0.040**	0.043**	0.003**	0.019**	640.76**	753.77**
S × G	36	0.234**	0.136*	0.030**	0.026*	0.020**	0.012**	0.002**	0.003NS	29.18**	32.77NS
Eb	180	0.022	0.080	0.011	0.016	0.003	0.005	0.0001	0.003	11.92	23.71

NS: not significant at $p \leq 0.05$

*, **: significant at $p \leq 0.05$ and 0.01 respectively

The free proline content in leaves increased with increasing levels of NaCl (0 to 200 m*M*). Among the genotypes, the highest proline content was recorded in genotype Sonalika. However, the lowest proline content was observed in genotype Gemmiza9. No significant difference was found for proline content among the genotypes Yecora Rojo, KSU106, Samma, Maaya, Shebam8 and Mesri during 2012 season. In season 2013,

the highest proline content was recorded in genotype Samma, while the lowest proline content was found in genotypes KSU106 and Yecora Rojo (Table 3a). Appliaction of NaCl significantly affected the accumulation of proline in the leaves of wheat (Table 3b).

Data reveal that the highest values for membrane stability index (MSI) were recorded with genotypes Sonalika, Shebam8 and Yecora Rojo. However, the lowest values for MSI were recorded with genotypes Pawni, Mesri and Samra. For example, MSI increased by 49.67% in Sonalika, by 50.92% in Shebam8 and by 55.12% in Yecora Rojo and decreased by 37.93% in Pawni, by 40.43% in Mesri and by 43.52% in Samra under salinity in 2012 season. On the other hand, during 2013 season, the highest MSI percentage were found genotypes Shebam8 (44.22%), Sonalika (44.40%) and Yecora Rojo (55.99%), while the genotypes Pawni, Mesri and Maaya gave the least MSI percentage by giving 36.03, 37.71 and 39.05%, respectively (Table 3a). Membrane stability index of all genotypes was negatively influenced by salinity stress (Table 3b). Interaction among salinity and genotype was found significant ($P \leq 0.05$) with exception of shoot dry weight in both seasons (Table 3c and d).

Mineral Contents

Highly significant *($P < 0.01$)* differences among the different salinity levels and mineral (Na^+, K^+, K^+/Na^+ ratio, Ca^{2+} and Mg^{2+}) contents in leaves of all genotypes were observed (Table 4a, b and c) in both seasons experiments.

The content of Na^+ in the leaves of wheat plants increased significantly by increasing NaCl concentrations (Table 4b and c). Under salinity, the genotypes KSU106, Shebam8, Yecora Rojo, Sonalika, Samma and Gemmiza9 exhibited minimum values for Na^+ content in leaves as compared to genotypes Mesri, Maaya, Samra and Pawni in 2012 season. In 2013 season, the results of Na^+ content were found almost similar as described above Table 4a.

The content of K^+ in leaves significantly reduced with increasing salinity levels (Table 4b). K^+ content decreased by 9.56, 17.52, 39.67 and 56.54% with the application of 50, 100, 150 and 200 m*M* NaCl over the control, respectively during 2012 season. Whereas during 2013, K^+ content decreased by 4.77, 10.87, 17.99 and 23.77% with the application of 50, 100, 150 and 200 m*M* NaCl levels, respectively. Among the genotypes, Shebam8 had maximum content of K^+ as compared to other varieties of wheat in the season of 2012 (Table 4a).

The ratio K^+/Na^+ in leaves was significantly reduced with increasing salinity levels. The application 50, 100, 150 and 200 m*M* NaCl decreased ratio K^+/Na^+ by 39, 66, 81 and 90% over the control, respectively, during 2012 and by 49, 68, 84 and 90% over the control, respectively, during 2013 season (Table 4b). The maximum K^+/ Na^+ ratio was observed in Shebam8 followed by Yecora Rojo, Sonalika, KSU106 and Samma while the minimum was observed in Samra and Pawni (Table 4a).

The content of Ca^{2+} in leaves, decreased slightly with salinity. For example, at moderate and high salinity, it was reduced by 7.45 and 14.05%, respectively over the control (Table 4b). At low salinity levels (50 and 100 m*M* NaCl) no significant difference was found during 2012 season. In 2013 season, the Ca^{2+} concentration in leaves was also reduced slightly with increasing salinity levels. Ca^{2+} content decreased by 2.46, 5.32, 7.97 and 15.28%, with the application of 50, 100, 150 and 200 m*M* NaCl over the control, respectively. All the genotypes in this study had a different response in Ca^{2+} concentration with increasing salinity. The ratio of Ca^{2+} and Na^+ was found maximum in variety Shebam8 and Yecora Rojo in 2012 and in Sonalika, Shebam8 and Yecora Rojo in 2013 season (Table 4a).

The application of salinity reduced Mg^{2+} content in leaves of wheat plants, while the low levels of salinity (50 and 100 m*M*) effect on Mg content was found non-significant in season 2012 (Table 4c). However, in 2013, a sharp decreased in Mg^{2+} content in leaves was found. Application of 50, 100, 150 and 200 m*M* NaCl decreased Mg^{2+} content in leaves by 2.96, 7.44, 14.38 and 21.35%, respectively, during 2012, while in 2013, decreased by 7.28, 10.33, 13.43 and 23.47%, respectively. Among the genotypes, the highest Mg^{2+} content was recorded in genotypes Shebam8, Sonalika and Gemmiza9, but the lowest content was observed in genotype Pawni under salt stress. In season 2013, the highest Mg^{2+} content was recorded in genotype Shebam8 and the lowest content was observed again in genotype Pawni under salt stress (Table 4a).

Agronomic Traits

There were significant variations among wheat genotypes for plant height trait over two seasons (Table 5a, b and c). Shebam8 cultivar was found to be the tallest among all studied wheat genotypes under salinity levels. The data show that the plant height markedly reduced with increasing the salinity levels (Table 5b). The salinity level 200 m*M* NaCl gave the maximum inhibitory effect on plant height of wheat plants during 2012 and 2013 seasons. Among the all genotypes, Pawni gave the shortest plant, whereas the genotype Shebam8 gave the tallest plant at 200 m*M* NaCl.

In both year experiment, application NaCl decreased spike length with increasing levels of NaCl (Table 5b). Application of 200 m*M* NaCl significantly gave the shortest spike during 2012 and 2013. Among the cultivars, Yecora Rojo and Shebam8 exhibited maximum values for spike length as compared to other genotypes in 2012 season (Table 5a), and genotype Sonalika gave the highest value for this traits in 2013 (Table 5a). However, interaction among salinity and genotype was found significant ($p \leq 0.05$) only in plant height and grain yield traits in 2013 season (Table 5c).

Table 4a: Means of genotypes for Na^+, K^+, K^+/Na^+ ratio, Ca^{2+}, Ca^{2+}/Na^+ ratio, and Mg^{2+} under salinity stress at seasons 2012 and 2013

Genotypes	Na^+ (mg/g DW)		K^+ (mg/g DW)		K^+/Na^+ ratio		Ca^{2+} (mg/g DW)		Ca^{2+}/Na^+ ratio		Mg^{2+} (mg/g DW)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Yecora Rojo	2.560e	3.114e	13.99c	29.74ef	5.46b	9.55a	32.29b	35.02f	12.61a	11.25a	3.35c	2.29bc
KSU106	2.204f	3.694e	13.80c	28.65f	6.26a	7.76c	29.01d	37.02ed	13.16a	10.02b	3.20c	2.35ab
Samma	3.040cd	3.916de	14.76b	30.73cde	4.86c	7.85c	30.71c	36.34e	10.10b	9.28c	3.30c	2.26cd
Maaya	4.402b	5.866c	11.40e	34.11b	2.59e	5.81d	34.73a	40.73b	7.89d	6.94d	3.21c	2.21d
Pawni	7.378a	13.66a	11.50e	25.33g	1.56f	1.85f	34.59a	40.28b	4.69e	2.95f	3.05c	1.76f
Shebam8	2.549e	3.137e	15.47a	31.10cd	6.07a	9.91a	31.24c	34.59f	12.26a	11.03a	3.85ab	2.36a
Samra	7.277a	10.08b	12.47d	31.76c	1.71f	3.15e	30.61c	38.49c	4.21e	3.82e	3.49bc	1.93e
Sonalika	2.945cd	3.784e	14.16bc	30.36de	4.81c	8.02c	30.68c	42.78a	10.42b	11.31a	3.89ab	2.28c
Gemmiza9	3.162cd	4.016de	12.46d	35.72a	3.94d	8.89b	30.74c	37.94cd	9.72bc	9.45c	4.05a	2.27c
Mesri	3.432c	5.038cd	11.63e	28.63f	3.39d	5.68d	30.51c	37.96cd	8.89c	7.53d	3.27c	2.20d

Means followed by same letter(s) are not significantly different, according to LSD (Na^+ 0.6613, 2.7363; K^+ 1.5756, 2.5352; K^+/Na^+ 1.2934, 3.6680; Ca^{2+} 1.7181, 2.4946, Ca^{2+}/Na^+ 2.0170, 3.6432 and Mg^{2+} 1.0155, 0.1394 for 2012 and 2013 seasons, respectively) at 0.05 level of probability

Table 4b: Means effect of Salinity levels on contents of Na^+, K^+, Ca^{2+}, Mg^{2+}, K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio at seasons 2012 and 2013

Salinity m*M* NaCl	Na^+ (mg/g DW)		K^+ (mg/g DW)		K^+/Na^+ ratio		Ca^{2+} (mg/g DW)		Ca^{2+}/Na^+ ratio		Mg^{2+} (mg/g DW)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
0	1.588e	1.543d	17.47a	34.58a	11.00a	22.41a	33.16a	40.64a	20.88a	26.34a	3.817a	2.458a
50	2.373d	2.854cd	15.80ab	32.93b	6.66b	11.54b	32.39b	39.63b	13.65b	13.89b	3.704ab	2.279b
100	3.857c	4.260c	14.41b	30.82c	3.74c	7.23c	32.81a	38.48c	8.51c	9.03c	3.533ab	2.204c
150	4.982b	8.144b	10.54c	28.36d	2.12d	3.48d	30.69c	37.40d	6.16d	4.59d	3.268bc	2.128d
200	6.675a	11.35a	7.592d	26.36e	1.14d	2.32d	28.50d	34.43e	4.27e	3.03d	3.002c	1.881e

Means followed by same letter(s) are not significantly different, according to LSD (Na^+ 0.6613, 2.7363; K^+ 1.5756, 2.5352; K^+/Na^+ 1.2934, 3.6680; Ca^{2+} 1.7181, 2.4946, Ca^{2+}/Na^+ 2.0170, 3.6432 and Mg^{2+} 1.0155, 0.1394 for 2012 and 2013 seasons, respectively) at 0.05 level of probability

Table 4c: The analysis of variance for Na^+, K^+, Ca^{2+}, Mg^{2+}, K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio traits of the ten genotypes from wheat under the five levels of salinity

S. O. V	D.F	Na^+		K^+		Ca^{2+}		Mg^{2+}		K^+/Na^+ ratio		Ca^{2+}/Na^+ ratio	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Replicates	4	6.132	7.571	291.63	415.39	36.34	73.75	45.26	0.392	110.99	647.57	92.80	1558.4
Salinity treatment (S)	4	206.92**	816.68**	812.02**	554.22**	186.27**	286.05**	5.481**	2.242**	1116.8**	5200.3**	2889.9**	7171.7**
Ea	16	0.442	16.455	37.85	4.042	0.725	1.677	1.069	0.008	14.61	131.15	4.682	290.77
Wheat genotypes (G)	9	90.74**	305.34**	53.25**	212.52**	332.16**	166.38**	2.944**	0.943**	138.11**	411.20**	319.24**	560.21**
S × G	36	6.820**	45.39**	8.763**	17.02**	24.49**	16.12**	0.194NS	0.072**	21.80**	52.02**	42.10**	66.01**
Eb	180	0.281	4.813	1.596	4.132	1.898	4.001	0.663	0.013	1.076	8.649	2.615	8.533

NS: not significant at $p \leq 0.05$

*, **: significant at $p \leq 0.05$ and 0.01, respectively

Data reveal that a significant decreased in kernels/spike was found with increasing application of NaCl (Table 5b) in both seasons. Among the genotypes, Yecora Rojo and Shebam8 proved best by giving the highest values for kernels/ spike in both year experiments (Table 5a). However, the number of kernels/spike trait of Pawni cultivar had the lowest value for this parameter. The thousand-kernel weight trait markedly decreased with increasing the salinity levels during 2012 and 2013 seasons (Table 5b). Also, genotypes Yecora Rojo and Shebam8 proved best by giving the highest values for 1000- Kernel weight during 2012 and 2013 seasons (Table 5a).

Application of NaCl substantially decreased grain yield (Table 5b). Among the treatments, application of 200 m*M* NaCl was found more lethal dose for grain yield attribute than other NaCl treatments. In both years, genotype Shebam8 proved best for grain yield, but in 2013 Shebam8 followed by Yecora Rojo. However, the grain yield trait of Pawni (1.62 g per pot) and Samara genotypes were found the lowest in 2013 season.

Discussion

Salinity is a foremost restriction to crop production especially in arid and semiarid regions of the world, where high surface evaporation, low precipitation, usage of saline water for irrigation and poor irrigation practices accelerate level of soluble salts (Hollington, 1998).

Salinity might cause membrane damage, changed levels of growth regulators, nutrient imbalance, enzymatic inhibition and metabolic dysfunction, containing photosynthesis which eventually leads to plant death (Hasanuzzaman *et al.*, 2013).

Table 5a: Means of plant height, and spike length, kernel number/ spike, 1000- kernel weight, and grain yield traits as influenced by wheat genotypes over two seasons (2012 and 2013 seasons)

Genotypes	Plant height (cm)		Spike length (cm)		Kernel number/spike		1000- Kernel weight (g)		Grain yield (g pot^{-1})	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Yecora Rojo	33.68ef	51.32e	5.63a	7.41bc	14.80a	22.28a	31.37a	40.16cde	2.35a	4.98b
KSU106	39.00c	59.08c	4.79cd	6.84d	8.96cd	20.80a	32.26a	40.40bcd	1.43b	4.02c
Samma	32.44f	48.84f	5.18b	7.63b	10.64b	22.12a	27.41b	40.85bcd	1.54b	4.31c
Maaya	40.60b	58.44c	4.64d	6.64d	9.28cd	10.96c	14.02d	36.78de	0.77cd	2.10d
Pawni	28.64g	38.68g	4.43e	6.19e	4.28e	5.48d	18.03c	44.92ab	0.63d	1.62e
Shebam8	48.76a	70.68a	5.59a	7.17c	13.64a	21.70a	31.52a	48.90a	2.31a	5.39a
Samra	34.98d	58.48c	4.00f	5.89f	8.20d	10.12c	14.58cd	35.62e	0.76cd	2.00de
Sonalika	40.90b	67.76b	4.92c	8.08a	8.96cd	18.92b	31.68a	48.21a	1.55b	4.38c
Gemmiza9	38.10c	58.56c	5.11b	7.50b	9.52bc	21.44a	30.69ab	42.78bc	1.52b	4.19c
Mesri	34.28de	56.12d	4.42e	6.16e	8.08d	11.32c	17.56cd	38.24cde	0.85c	2.30d

Means followed by same letter(s) are not significantly different, according to LSD for 2012 and 2013 seasons, respectively) at 0.05 level of probability

Table 5b: Means of days to heading, plant height, spike length, kernel number/ spike, 1000- kernel weight, and grain yield traits as influenced by salinity levels over two seasons (2012 and 2013 seasons)

Salinity stress	Plant height (cm)		Spike length (cm)		Kernel number / spike		1000- Kernel weight (g)		Grain yield (g pot^{-1})	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Control	41.29a	66.50a	5.37a	7.69a	12.40a	19.66a	27.49a	48.34a	2.06a	5.21a
50 mM NaCl	39.10b	61.62b	5.08b	7.31b	10.92b	18.13a	26.45a	46.21a	1.66b	4.38b
100 mM NaCl	36.76c	57.20c	4.83c	7.02c	9.64b	16.33b	24.91ab	42.08b	1.31c	3.40c
150 mM NaCl	35.14d	52.30d	4.64d	6.56d	8.12c	14.74bc	23.05b	36.95c	1.01d	2.55d
200 mM NaCl	33.40e	46.36e	4.44e	6.19e	7.10c	13.73c	22.65b	34.85c	0.82e	2.10e

Means followed by same letter(s) are not significantly different, according to LSD at 0.05 level of probability

Table 5c: Analysis of variance for plant height, spike length, kernel number/spike, 1000- kernel weight and grain yield their interaction of the ten wheat genotypes under the five levels of salinity over two years (2012 and 2013 seasons)

S. O. V	D.F	Plant height		Spike length		Kernel number/ spike		1000- Kernel weight		Grain yield	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
Replicates	4	55.38	409.84	1.23	5.99	42.72	132.19	188.96	2151.26	2.17	19.87
Salinity treatment (S)	4	489.95**	3084.07**	6.68**	17.53**	225.22**	335.89**	220.24**	1679.58**	12.49**	86.61**
Ea	16	6.277	9.739	0.094	0.189	10.274	16.357	46.296	68.038	0.161	1.090
Wheat genotypes (G)	9	790.75**	2079.92**	6.84**	13.16**	216.48**	1004.16**	1531.49**	508.78**	9.68**	47.56**
S × G	36	3.630NS	15.883**	0.102NS	0.260NS	3.142NS	11.450NS	12.389NS	63.346NS	0.138NS	1.079**
Eb	180	5.232	6.744	0.101	0.201	4.968	10.415	44.438	66.660	0.099	0.497

NS: not significant at $p \leq 0.05$

*, **: significant at $p \leq 0.05$ and 0.01, respectively

The inhibitory consequence of NaCl stress on seed germination is owing to osmotic effect and/or ion toxicity and of course the germination of all genotypes was reduced under salt stress (>40 m*M* NaCl), nevertheless the reduction in germination was not consistent among genotypes. As salt tolerant genotypes have additional Na^+ exclusion (Munns and James, 2003) containing higher efficiency to exclude Na^+ from transport to the shoot (Zhu *et al.*, 2016). It is basic characteristics of salt tolerant genotypes to stand with salinity (Ahmad *et al.*, 2013).

There was gradual but significant reduction in physiological and agronomic traits with increase in salinity levels; however such diminishing pattern differs among wheat genotypes as reported by Saddiq *et al.* (2018). Those wheat genotypes that exhibited additional decline in biomass can be categorized as salt-susceptible genotypes and vice versa (Table 3a and b) such as salt stress in salt susceptible genotypes additionally declines the photosynthetic efficiency (Munns, 2002). Such genetic variation in wheat genotypes with respect to biomass under salt stress were also reported by Gurmani *et al.* (2014). Decreased shoot biomass and other related growth parameters can be credited to the decrease in water potential and growth allied with osmotic effects under salt stress (Munns *et al.*, 1995). In an earlier study, different levels of salinity in wheat genotypes significantly influenced growth traits by decreasing the length and fresh weight of root and shoot (Al-Ashkar and El-Kafafi, 2014). The salt stress had an influence on shoot and root fresh weight, shoot and root dry weight (Sourour *et al.*, 2014). Salinity reduces the water potential of the roots and this rapidly causes declines in growth rate, together with a suite of metabolic variations those initiated by water stress (Munns, 2002), and found in present study. Nevertheless, the decrease in growth parameters was minimum in Shebam8 and Gemmiza9 mentioning their ability to with stand against alt stress.

Proline content are increased with increasing salinity level to combat with osmotic stress (Ueda *et al.*, 2007), however genotypic variation exists to improve proline content.

In present study, genotypic variation was found in membrane stability index (MSI) as reported by Atiq-ur-Rahman *et al.* (2014) that MSI was negatively influenced by salinity, but this reduction was more visible in sensitive genotype than in salt-tolerant genotype. Other studies found that under salinity stress MSI of wheat genotypes was negatively influenced (Esfandiari *et al.*, 2011; Rao *et al.*, 2013). Therefore, MSI is a good indicator of salt resistance which was observed in this study and Shebam8, Yecora Rojo, KSU106, Samma, Sonalika and Gemmiza9 showed better MSI and ultimately grain yield as well (Table 3a and 5a).

Among other factors contributing to salinity resistance may be better Na^+ homeostasis to save the metabolic activities in cytosol. Shebam8, Yecora Rojo and KSU106 exclude more of the Na^+, while Mesri, Maaya, Samra and Pawni accumulate more Na^+ in the leaf. The control of Na^+ exclusion from the xylem and in that way from the leaves by *Nax1* and *Nax2* loci initiate additional leaf longevity and uninterrupted photosynthesis under salt stress (James *et al.*, 2012). The decreased uptake of K^+ is considered one of the important physiological mechanisms contributing to yield reduction under salt stress, however maintaining the K^+ concentration in plant tissue develop salt tolerance in the plants (Gupta and Srivastava, 1989). A strong correlation is reported between salinity tolerance and leaf K^+ contents in leaf (Garthwaite *et al.*, 2005). Shebam8, Yecora Rojo and KSU106 had the highest K^+ concentration with a lowest Na^+ concentration in the leaves under salt stress. The more increase of Na^+ content in Pawni, Samra, Maaya and Mesri may indicate more relative sensitivity towards salt stress condition. Chamekh *et al.* (2014) reported the highest ratio of K^+ to Na^+ in salt-tolerant and moderate-tolerant genotypes while the lowest in sensitive genotypes (Table 4a and b). It is also stated that if K^+/Na^+ ratio is wide then the genotype is considered salt tolerant, in contrast if its narrow then the genotype is considered salt sensitive (Tester and Davenport, 2003). Furthermore, Ca^{2+} concentration in the plant tissue also supressed with increasing salinity stress and improved Ca^{2+} concentration increases salt resistance in plants. Shebam8, Yecora Rojo, KSU106 and Sonalika could maintain better Ca^{2+}/Na^+ ratio in plant tissue indicating better salt resistance (Rengel, 1992; El-Hendawy, 2004; Wakeel, 2013).

Ultimately, Shebam8, Yecora Rojo, KSU106, Samma, Sonalika and Gemmiza9 produced better spike length, and grain yield as compared to other genotypes perhaps due to better compatible solutes production, MSI and ionic homeostasis under salt stress conditions. However, Maaya, Samra, Pawni and Mesri produce minimum grain yield and showed sensitivity to all other plant growth related parameters. Most salt tolerant was Shebam8, while Yecora Rojo, KSU106, Samma, Sonalika and Gemmiza9 can be considered as moderately salt tolerant genotypes.

Conclusion

Sufficient genetic variation was found in the wheat genotypes (Shebam8, Yecora Rojo, Ksu106, Samma, Sonalika, Gemmiza9, Pawni, Samra, Maaya and Mesri) for salinity tolerance. Tolerant and moderate genotypes categorized based on better agronomic performance containing better ionic homeostasis (higher K^+/Na^+ and Ca^{2+}/Na^+ ratios in leaves), membrane stability index, leaf area and chlorophyll content compared to non-tolerant ones. Shebam8 has been considered as most salt tolerant genotype, while Yecora Rojo, KSU106, Samma, Sonalika and Gemmiza9 were concluded as moderately tolerant to salt stress. Remaining four genotypes showed the lowest resistance to salinity. The above-discussed characteristics (morpho-physiological traits and ionic homeostasis) can be considered as important traits and could be used in breeding program for the development of salt tolerant wheat genotypes.

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