



SCREENING OF IRAQI WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES AGAINST SALINITY AT EARLY GROWTH STAGE: STUDIES IN HYDROPONIC CULTURE

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ABSTRACT

Soil salinity has a very pronounced and adverse effect on most crops. Salinity is a major limiting factor in middle and southern regions of Iraq. Influence of salt stress on some growth parameters and pattern of ion accumulation was observed in 11 Iraqi wheat genotypes, Buhooth10, Buhooth158, Furat, Fath, Iraq, Hashimia, Saberbeg, Iba99, AbuGraib, Uruk and Axad9 at the seedling Stage. The wheat genotypes were grown in hydroponic culture under saline (100, 150 and 200 mM NaCl) and non-saline regimes (0 mM). Seedling growth parameters (*e.g.* fresh weight, dry weight, plant area, plant height, K⁺ and Na⁺ content and K⁺/Na⁺ ratio) were determined at day 30 after sowing. Analysis of variance showed that salinity had positive and significant effect on Na⁺ concentration and negative effect on K⁺ concentration and ratio of K⁺/Na⁺. Increase of salinity levels decreased fresh weight, dry weight, and plant area and plant height. Based on K⁺/Na⁺ ratio, Uruk and Furat were the most tolerant genotypes. On the basis of above plant growth parameters, K⁺ and Na⁺ contents and K⁺/Na⁺ ratio measured at the seedling stage can be considered for screening wheat genotypes at high salinity concentrations.

KEYWORDS: Salinity, crops, wheat genotypes, fresh weight, dry weight.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most valuable cereal foods among crops globally. It is the main source for human consumption and industries, and it contains more protein than maize and rice (Memory and Mrinal, 2011). It provides around 21 percent of the protein and 19 percent of the food calories for the world's population (Ortiz, 2011). Screening method for wheat genotypes at seedling stage using a hydroponic system is a useful technique for distinguish tolerant wheat genotypes, because screening a large number of genotypes in field for salt tolerance is difficult because of soil heterogeneity in salinity concentration and organic carbon (OC), in addition to releases of carbon dioxide (CO₂) from soil (Richards, 1983; Setia *et al.*, 2012). Although, hydroponic is a modern technique to overcome the environmental perturbations for screening seedling for salt tolerance, it has been used on commercial markets for about 40 years (Sheikh, 2006; Bado *et al.*, 2016b). Salt tolerance of crops may vary with their growth stage (Mass and Grieve, 1994). However, a difference in the salt tolerance among genotypes may also occur at different growth stages. Kingsbury and Epstein (1984) found that individual lines from 5000 accessions of spring wheat showed differing tolerance during their life cycle. Therefore, the salt tolerance of different wheat genotypes must be evaluated at different growth stages. A number of researches have suggested that evaluating salt tolerance of genotypes at one growth stage could be effective if the evaluation was done at the sensitive growth stage of crops (Ferdose *et al.*, 2009, Mohammadi-Nejad *et al.*, 2010; El-Hendawy *et al.*, 2011). In wheat, the seedling or early vegetative growth

stage is known to be more sensitive to salt stress compared with later growth stages (Bhutta and Hanif, 2010; Khayatnezhad and Gholamin, 2010). Therefore, evaluating salt tolerance at early vegetative growth stages will be possible to screen salt tolerance of wheat genotypes, and simple and quick for evaluating salt tolerance of genotypes with a minimum investment of cost and time (El-Hendawy *et al.*, 2011). Salinity has inhibitory effects on wheat phenological aspects such as leaf number, leaf rate expansion, root growth rate root/shoot ratio, plant height and total dry matter yield, increase of Na⁺ and Cl⁻ and decrease of K⁺ concentrations (El-Hendawy *et al.*, 2011; Akbari ghogdi *et al.*, 2012; Asgari *et al.*, 2012; Kanwal *et al.*, 2013; Keshavarz *et al.*, 2013; Sharbatkhari *et al.*, 2013; AL-Jobori and Salim, 2014). Sodium chlorid ion considers as the most soluble and common salt in soil. Therefore, all plants have developed mechanisms to regulate the accumulation of ions and increasing other nutrients present generally in low concentrations such as Nitrate (NO₃⁻) and Potassium (K⁺) (Jaiswal *et al.*, 2016). The maintenance of ionic homeostasis is an important for plants salt tolerance at high salinity. Houmani and Corpas (2016) indicated that plant cells maintain high K⁺/Na⁺ ratio by compartmentization of sodium ions in vacuoles and also the mechanism of sharply differentiate between sodium (Na⁺) and potassium (K⁺) in cells are the most important strategy to overcome salt stress.

In Iraq, wheat production facing two important difficulties. Firstly, rainfall during wheat growing is low from northern to southern of Iraq. Secondly, high level of salinity is appeared in water irrigation and soil in southern of Iraq lands. For example, increasing the productivity of wheat

crop was doubled in 2005th than any year from 2000s to 2009th due to good rainfall than other years (Christen and Saliem ,2012). Therefore, salinity has reduced the Iraqi land productivity by 70% of the total irrigated area with more than 30% completely disappeared from production (FAO, 2011). Unfortunately, the mechanisms of salinity tolerance at germination and early seedling were not a guarantee that tolerant genotypes will be tolerant at later stages (Munns *et al.*, 2000; Tsegay and Gebreslassie, 2014). Therefore, eleven genotypes were nominated for experiment including sensitive and tolerant genotypes to evaluate the K^+/Na^+ ratio in addition to other growth traits for further genetic analysis.

MATERIALS & METHODS

Plant Materials

From our previous study, eleven genotypes were selected phenotypically for determination which are tolerance or susceptible genotypes to salt stress. Six genotypes were selected as salt tolerance included Fath, Hashimia, Axad9, Buhooth10, Buhooth158 and Furat . And five genotypes as susceptible to salinity stress included Uruk, AbuGraib, Saberbeg, Iraq and Iba99. .

Deep Water Culture (DWC) Hydroponic Systems

Equipments of DWC hydroponic system were included four reservoirs with a capacity of 1 liter connected with heavy duty air pump , eleven pots had prepared with a diameter about 9mm and 15mm height in each reservoir. In addition, artificial soil (Paralit, AGRi LITE, SAK) was used to support plant growth.

Nutrient Solution and NaCl treatment

Nutrient solution was used in the experiment, which contains all the elements necessary for the growth of wheat plants perfectly. Wheat plant were supplied with nutrients solution containing micronutrients such as $0.2 \text{ mol m}^{-3} \text{ NH}_4\text{NO}_3$, $5.0 \text{ mol m}^{-3} \text{ KNO}_3$, $2.0 \text{ mol m}^{-3} \text{ Ca}(\text{NO}_3)_2$, $2.0 \text{ mol m}^{-3} \text{ MgSO}_4$, $0.1 \text{ mol m}^{-3} \text{ KH}_2\text{PO}_4$, and $0.05 \text{ mol m}^{-3} \text{ NaFe}(\text{III})\text{EDTA}$. Whereas, micronutrients containing $0.5 \text{ mmol m}^{-3} \text{ CuSO}_4$, $10 \text{ mmol m}^{-3} \text{ ZnSO}_4$, $0.1 \text{ mmol m}^{-3} \text{ Na}_2\text{MoO}_4$, $50 \text{ mmol m}^{-3} \text{ H}_3\text{BO}_3$, $5 \text{ mmol m}^{-3} \text{ MnCl}_2$. PH is between 6.5 – 7.0. Nutrient solution represent 10% from total solution .

Sowing Methods and Measurements

Seeds surface were sterilized by washing with 70% ethanol (v/v) for one minute and then soaking with Sodium hypochlorite 3% (v/v) for five minutes (Sauer and Burroughs, 1986; Berger *et al.*, 2012). Seeds were placed in petri dishes lined with filter paper soaked in distilled water. After 72 hour, uniform germinated seeds were selected to transplanted in pots at 3 cm depth. wheat genotypes were sowed in four containers each one contained eleven pots with five replicates in an artificial soil (Paralit, AGRi LITE, SAK) .500 mL nutrient solution was used in each container which contains all the elements necessary for the growth of wheat plants . pH

level was checked two times a day with replacement the solution every 24 hour. Plants were growing in normal solution until the third leaf emergence of approximately 80% of the all plants . After that, seedlings were treated with NaCl concentrations 100, 150 and 200 mM .Control was grew in nutrient solution only. To avoid salt shock stress, each salt concentration was added three times daily over 3 days to reach a final concentration for each treatment (Munns, 2005). Seedling Fresh and dry weight were measured. Image J software (Schneider *et al.*, 2012) was used to measure the plants height and area. Determination of K^+ , Na^+ ions and K^+/Na^+ ratio were carried out according to the method of Munns *et al.* (2010)

.Statistical Analysis

The results were subjected to ANOVA analysis (Steel and Dickey ,1997). Mean comparisons were performed using least significant difference (LSD) test ($P < 0.05$).

RESULTS

Agronomic traits

In the present study, 11 wheat (*Triticum aestivum* L.) genotypes were assessed for salt tolerance on the basis of some growth traits and ionic contents at the seedling stage sowing in hydroponic culture. The genotypes were significantly ($P = 0.05$) different from each other for seedling fresh weight, dry weight, plant area and plant height (Table 1). The genotype Buhooth10 had higher fresh weight, dry weight, and plant area reached to 947.5, 402.5 mg and 27.177 cm^2 , respectively. The minimum mean of fresh weight and plant area were 487.5 mg and 14.385 cm^2 recorded by Uruk genotype and dry weight 197.5 mg recorded by AbuGraib genotype. The maximum length value of 40.273 cm was shown by genotype Saberbeg . In contrast, Uruk genotype had length of only 23.618 cm. While the other genotypes were intermediate in their relative performance (Table 1).

Salinity treatments demonstrated significant ($P = 0.05$) when increased salt concentration to 100, 150 and 200 mM NaCl (Table 2). Control treatment showed highest fresh weight 725.64 mg, reduced clearly to 647.45, 618.91 and 557.27 mg with the increase of salinity. Statistical analysis revealed that dry weight decreased significantly ($P = 0.05$) to 277.272 and 266.363 with the increasing of salt concentrations to 150 and 200 mM, whilst the concentration 100 mM gave maximum mean of dry weight was 298.181 mg and not differ significantly from control treatment which gave 295.181 mg. According to analysis of variance, salinity levels showed significant difference for plant area and plant height, the concentrations 100 and 200 mM NaCl gave minimum plant area (18.802 and 18.257 cm^2) and plant height (29.986 and 29.831 cm), respectively. While the concentration 150mM NaCl gave Plant area of 20.118 cm^2 and plant height 30.618 cm , which not differ significantly from control which gave 20.680 cm^2 and 31.381 cm , respectively (Table 2).

TABLE 1. Some growth traits of 11 wheat genotypes

Cultivars	Fresh weight/mg	Dry weight/mg	Plant Area/cm	Plant Height/cm	Concentration of K ion/ umole g ⁻¹	Concentration of Na ion / umole g ⁻¹	K:Na Ratio/ umole g ⁻¹
Hashimia	674.5 ±25.05	262.5 ±4.79	18.795 ±0.73	28.13 ±0.76	1268951.27 ±41642.24	1280302.3 ±412208.64	6.9935821
AboGraib	487.5 ±13.67	197.5 ±8.54	17.940 ±1.49	28.36 ±0.87	1277533.29 ±70280.14	1012506.59 ±254865.04	7.7376524
Buhooth158	713.0 ±27.01	330.0 ±9.13	21.867 ±0.56	28.965 ±1.15	1314402.70 ±84713.01	1207255.41 ±309995.99	6.408258
Uruk	461.5 ±40.42	230.0 ±12.25	14.385 ±0.81	23.717 ±0.77	1149161.52 ±76068.73	838481.87 ±233938.65	8.7500145
Ibag9	650.5 ±73.20	270.0 ±34.88	16.967 ±1.73	29.875 ±2.24	1248340.11 ±61269.01	1196565.06 ±386523.34	7.3334504
Fateh	542.5 ±29.07	245.0 ±11.90	17.862 ±1.20	28.522 ±0.47	1323747.56 ±28382.26	1322686.21 ±421600.24	6.506227
Iraq	690.0 ±56.40	257.5 ±12.50	18.812 ±1.30	32.295 ±0.92	1273097.11 ±88564.98	1164800.54 ±373702.14	6.934236
Saberbeq	638.5 ±23.41	342.5 ±21.75	19.672 ±0.70	40.272 ±1.48	1263001.86 ±30522.30	1377973.31 ±440843.91	5.9700072
Furat	620.5 ±38.44	287.5 ±13.15	23.495 ±0.66	30.965 ±0.66	1391080.39 ±57482.62	1292199.1 ±413725.85	8.1072661
Buhooth10	947.5 ±72.35	402.5 ±28.69	27.177 ±0.15	32.160 ±0.28	1271147.27 ±68788.93	1335499.28 ±424610.06	4.3266805
Axad9	584.5 ±48.77	302.5 ±19.74	17.132 ±1.48	31.730 ±1.14	1265667.05 ±71279.18	1266816.66 ±415190.29	7.0857739
LSD	224.48	107.07	7.769	3.995	72444.13	31679.85	0.8842

TABLE 2. effect of salt concentrations of some growth traits of 11 wheat genotypes

NaCl (mM)	Fresh weight /mg	Dry weight / mg	Plant Area / cm ²	Plant Height /cm	Concentration of K ion/ umole g ⁻¹	Concentration of Na ion / umole g ⁻¹	K:Na Ratio/ umole g ⁻¹
control	725.64 ±0.051	295.454 ±21.292	20.68 ±0.904	31.381 ±1.083	1419441.68 ±29571.47	57559.42 ±2996.74	25.336109
100	647.45 ±0.044	298.181 ±18.281	18.802 ±1.273	29.986 ±1.350	1223519.29 ±25973.33	1585546.64 ±52009.00	0.7762385
150	618.91 ±0.033	277.272 ±18.978	20.117 ±1.142	30.618 ±1.562	1232199.04 ±35372.46	1572571.23 ±81104.89	0.7979434
200	557.27 ±0.037	266.363 ±19.875	18.257 ±1.352	29.831 ±1.379	1232523.67 ±27525.48	1618899.56 ±87328.80	0.7817634
LSD	43.5649	20.77938	1.507807	0.7677046	14059.04	6148.024	0.1715897

There was a significant difference between genotypes for fresh weight, dry weight, plant area and plant height under stress and control condition. Buhoth10 showed maximum fresh weight at all salt treatments and was 816 mg at 200 mM NaCl. Uruk genotype gave minimum fresh weight reduced to 362 mg at 200mM NaCl (Figure 1). Also Buhoth 10 gave dry weight of 400 mg at 200 mM NaCl, whereas Saberbeg showed dry weight 400 mg at 150 mM salt treatment. The minimum means of dry weight was 180 mg recorded by AboGraib genotype at 200 mM NaCl concentration (Figure2). The genotype

Buhoth 10 had the maximum plant area of 27.590cm² at the salinity concentration of 100mM, at other concentrations 150 and 200mM were closely with control 26.900 and 27.190 cm², respectively. The plant area of Uruk, Iba99 and Axad9 were more affected by NaCl concentrations than the other genotypes. Uruk genotype had lower plant area of 12.710 cm² at 150 mM NaCl (Figure3). Based on the results the genotype Saberbeg had longer height among all genotypes reached to 41.450 cm at 200 mM NaCl. Whereas Uruk genotype had shorter height of 24.640 cm at 200 mM NaCl (Figure 4).

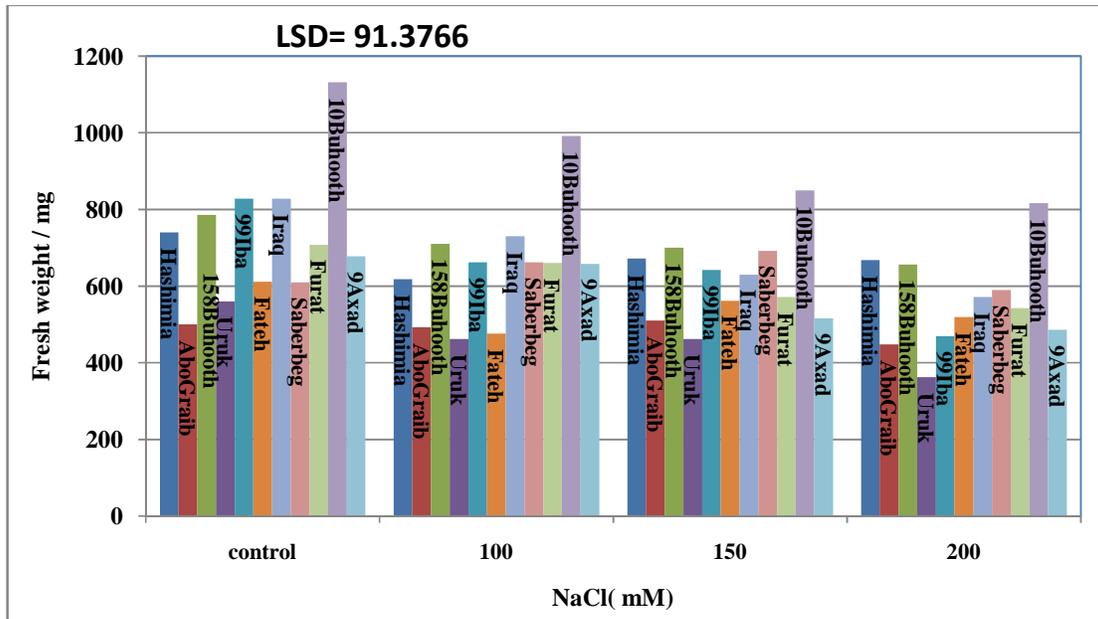


FIGURE.1. Effect of the interaction between genotypes and salinity on plant fresh weight

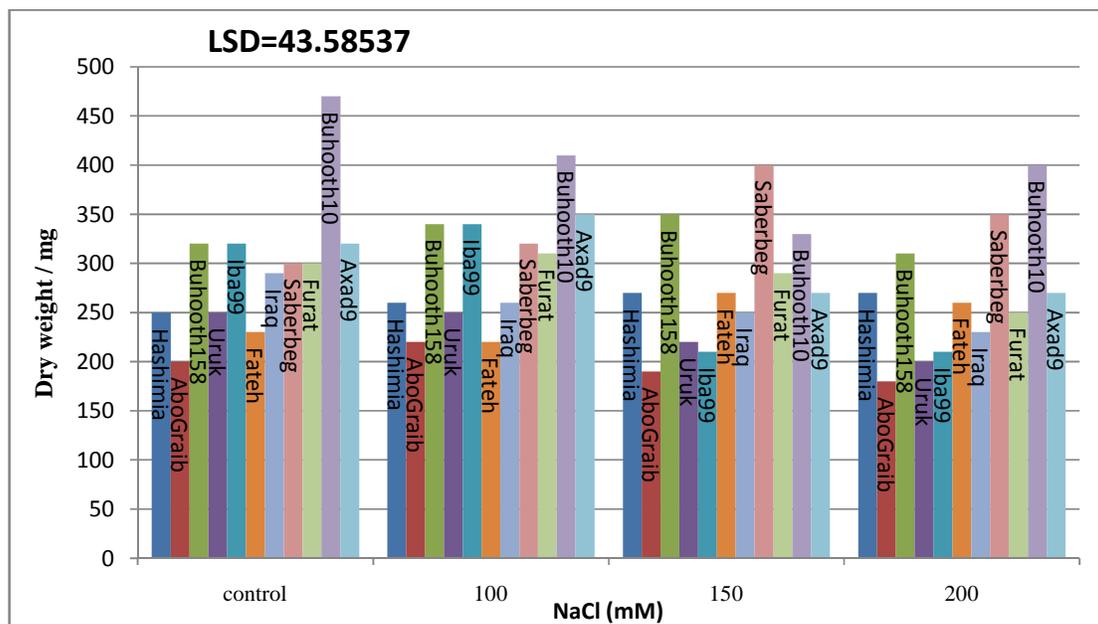


FIGURE 2. Effect of the interaction between genotypes and salinity on plant dry weight

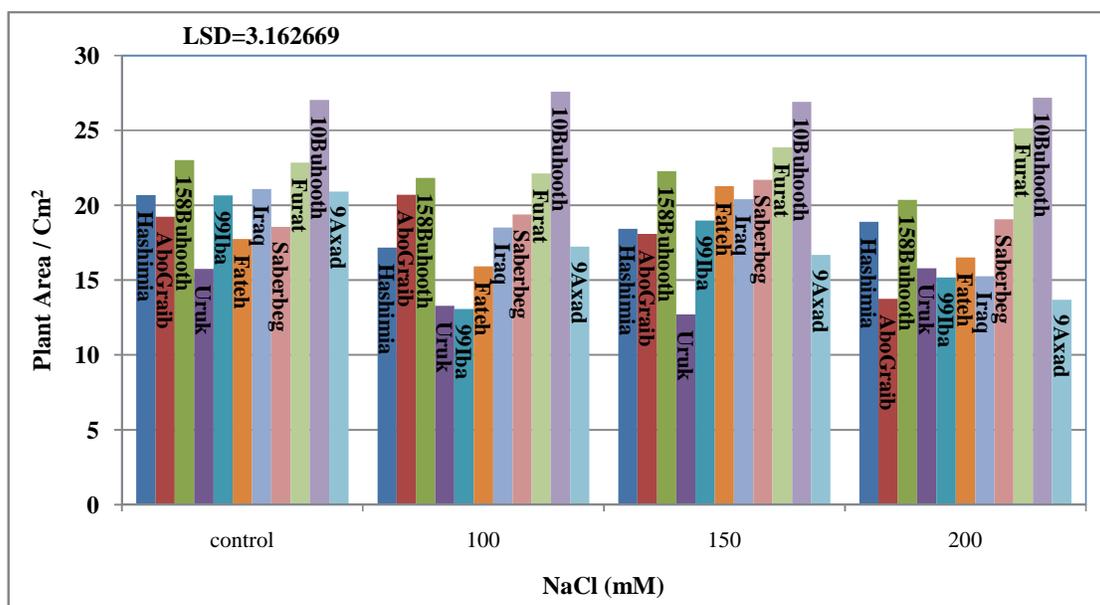


FIGURE 3. Effect of the interaction between genotypes and salinity on plant area

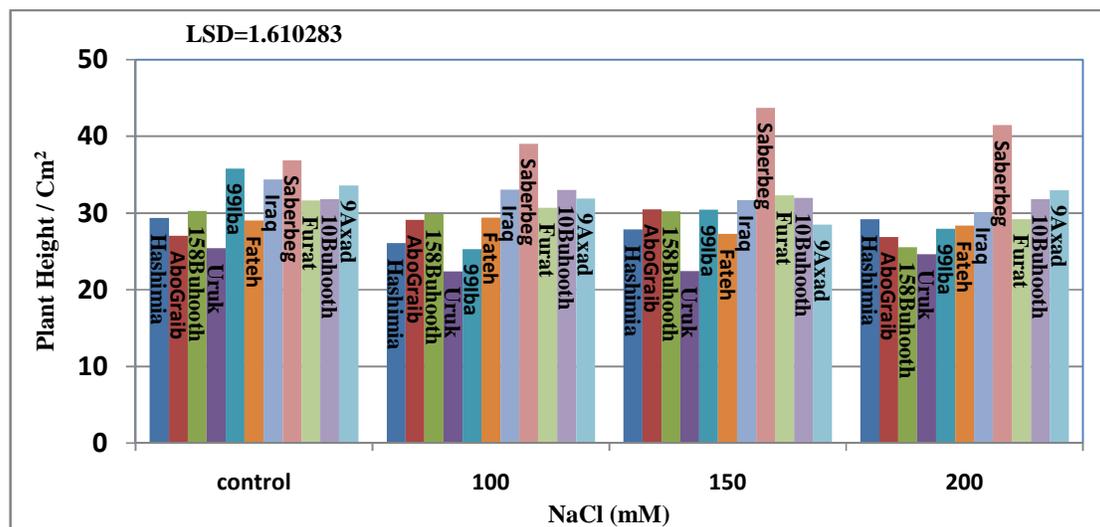


FIGURE 4. Effect of the interaction between genotypes and salinity on plant height

K⁺ and Na⁺ content and K⁺/Na⁺ ratio

According to analysis of variance, all the genotypes differed significantly ($P < 0.05$) from each other for K⁺ and Na⁺ content and K⁺/Na⁺ ratio depending on genetic variation under salinity stress (Table 1). The genotype Furat showed maximum content of K⁺ ions 1391080.39 $\mu\text{mole g}^{-1}$ followed by Fateh, and Buhooth158 which had K⁺ content 132374.56 and 1314402.70 $\mu\text{mole g}^{-1}$. In contrast, minimum K⁺ content was shown by the Uruk with 1149161.52 $\mu\text{mole g}^{-1}$. The genotype Saberbeg was ranked first in content of Na⁺ ions 1377973.31 $\mu\text{mole g}^{-1}$, followed by Buhooth10, Furat and Hashimia which accumulated 1335499.28, 1292199.10 and 1280302.30 $\mu\text{mole g}^{-1}$, respectively. On other hand, the genotype Uruk significantly ($P > 0.05$) maintained lowest Na⁺ concentrations 838481.81 $\mu\text{mole g}^{-1}$ followed by AbuGraib 1012506.59 $\mu\text{mole g}^{-1}$ compared to the other

genotypes under all salinity treatments. The maximum K⁺/Na⁺ ratio was belonged to the genotypes Uruk, Furat and AbuGraib, reached to 8.750, 8.107 and 7.738, respectively. And the lowest K⁺/Na⁺ ratio was observed in the genotypes Buhooth10 and Saberbeg were 4.327 and 5.970, respectively, while the other genotypes were intermediate in this range (Table 1).

The comparison of the means in three NaCl concentrations showed that salt treatment significantly ($P < 0.05$) decreased K⁺ concentration compared with control treatment (Table 2). Maximum K⁺ concentration was 1419441.68 $\mu\text{mole g}^{-1}$ recorded at control (fresh water) reduced to 1223519.29, 1232199.04 and 1232523.67 $\mu\text{mole g}^{-1}$ at 100, 150 and 200 mM NaCl, respectively. Growth medium salinity significantly ($P > 0.05$) increased Na⁺ content. However, 100, 150 and 200 mM concentration maintained significantly higher Na⁺ values

than control. The maximum increase in Na⁺ content 1618899.56 μmole g⁻¹ was observed at 200mM. K/Na ratio at control treatment was 25.336 represent normal level of K/Na ratio depending on the genetic variation of genotypes. Other treatment showed significant differences, maximum K/Na ratio 0.798 was observed at 150 mM NaCl followed by 0.782 and 0.776 at 200 and 100 mM NaCl concentrations, respectively (Table 2).

In the present investigation all genotypes subjected to increasing levels of NaCl were analyzed for K⁺ and Na⁺ content and K⁺/Na⁺ ratio. The interaction between salinity and genotypes was significant (P<0.05). Buhooth158 and Furat had maximum content of K⁺ 1349641.802 and 1345627.746 μmole g⁻¹ at 200 mM, respectively (Figure 5). The figure also demonstrated that minimum concentration of K⁺ ions exhibited by the genotypes Uruk 1108433.213 and 1016552.913 μmole g⁻¹ at 100 and 150, and Iraq 1094748.913 μmole g⁻¹ at 200 mM NaCl. When

the genotypes were subjected to increasing external NaCl concentrations they accumulated varying concentration of Na⁺ ions, and had an increase in concentration of Na⁺ in the tissues. The comparison of the means at 200mM NaCl concentration showed that Buhooth158 genotype had accumulated the maximum Na⁺ ions, which was followed by Hashimia and Axad9 with Na⁺ concentration of 1938284.797, 182334.797 and 1817357.779 μmole g⁻¹. On the other hand, Uruk genotype had minimum Na⁺ ion concentrations of 971867.190 μmole g⁻¹ (Figure 6). Based on their K⁺/Na⁺ ion uptake ratio. Uruk genotype was ranked first at 200 mM with the value of 1.135 followed by AbuGraib with the ratio of 0.916. Also AbuGraib had K⁺/Na⁺ of 1.010 at 150 mM NaCl. The minimum K⁺/Na⁺ ratio was shown by Saberbeg genotype followed by Buhooth158 with the values of 0.680 and 0.696, respectively (Figure 7).

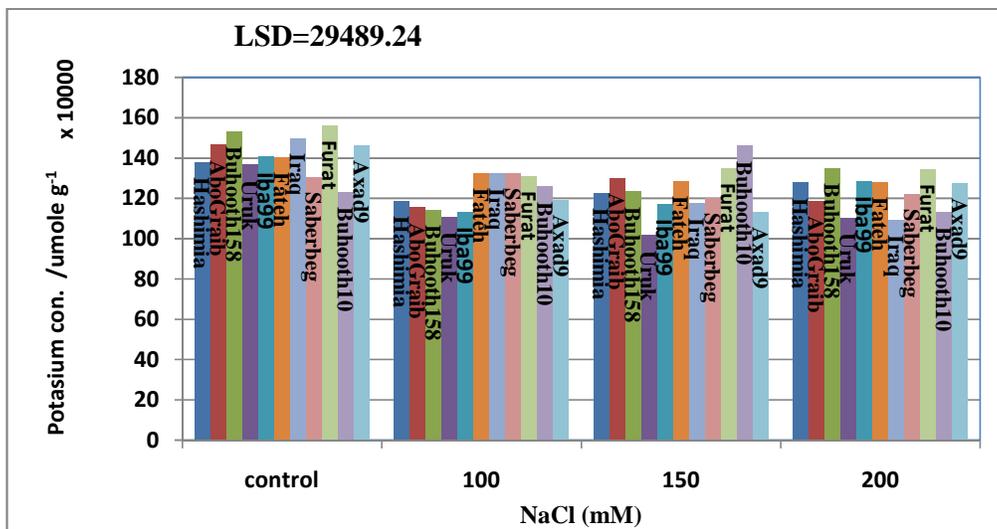


FIGURE 5. Effect of the interaction between genotypes and salinity on K⁺ concentration in plant tissues

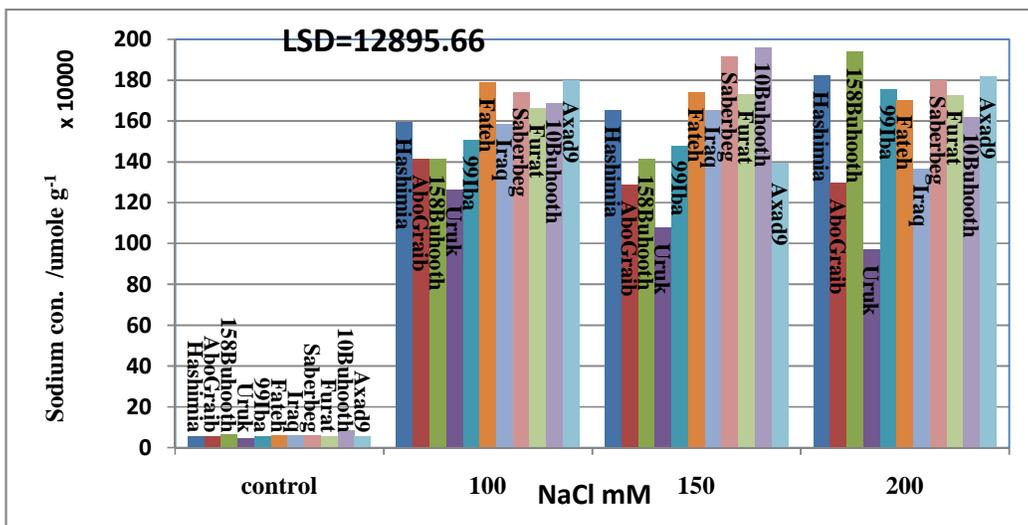


FIGURE 6. Effect of the interaction between genotypes and salinity on Na⁺ concentration in plant tissues

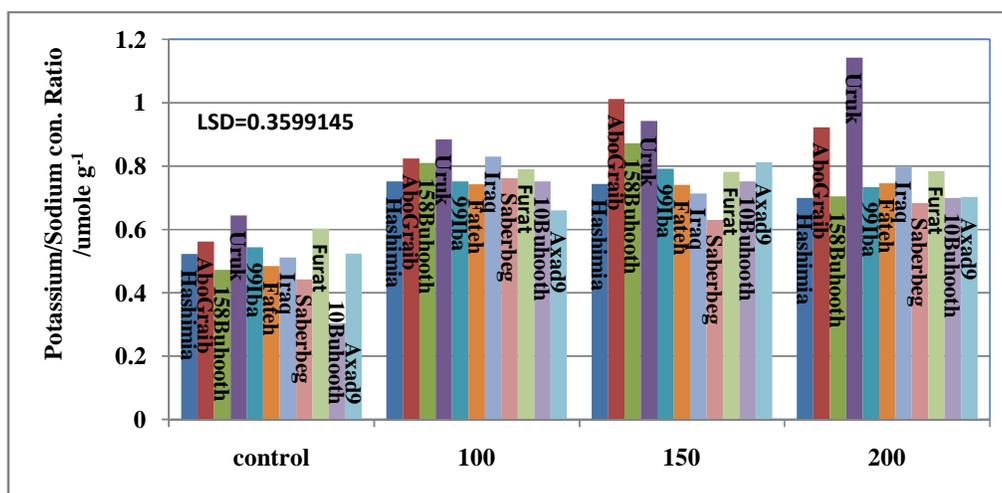


FIGURE 7. Effect of the interaction between genotypes and salinity on K⁺/Na⁺ ratio in plant tissues

DISCUSSION

Sodium is a harmful ion to plants because it is widespread in most saline soils and it accumulates in shoot and leaves at toxicity levels (Bado *et al.*, 2016a). Sodium chloride reduces all growth plant at high salinity (Lucini *et al.*, 2015). The seedlings were irrigated with fresh water for two weeks then irrigation with salt water for two weeks. The plants exposed to increasing levels of salinity experience of severe reduction in fresh and dry weight. Wheat genotypes showed significant differences for fresh weight (Table 1). The differences between of genotypes could be attributed to the differences in gene frequencies and their interaction with the environment (Maas, 1986). Overall, fresh weight was affected by increasing salinity concentrations (Table 2). The reduction in fresh weight occurs normally due to the disorder of physiological and chemical function of plant cells under salinity stress. The results of this study illustrates that though increasing NaCl concentrations significantly affected the fresh weight of all genotypes to a varying degree under NaCl concentrations (Figure 1). Under saline condition, the function of cells responds naturally by the reservation of water in optimal pressure and maintaining the turgor volume (Negrão *et al.*, 2016). The genotype Buhooth10 showed high salinity tolerance. Conversely, Uruk genotype has the minimum mean of fresh weight under salt concentrations.

The differences among genotypes in dry weight were clear. The genotypes Buhooth 10, Saberberg, and Buhooth 158 has maximum mean of dry weight, reduced to the lowest value for the genotype AbuGraib (Table 1). Several researches indicated that salinity reduces the dry weight due to the decrease of physiological and chemical traits such as photosynthesis, chlorophyll, protein and ions homeostasis (Munns *et al.*, 2006; Flowers *et al.*, 2015; Munns *et al.*, 2016), The effect of salinity on dry weight is obvious (Table 2) , dry weight reduced at 150 and 200 mM NaCl. This finding support that results obtained previously by other authors (El-Hendawy *et al.*, 2011; Hussein *et al.*, 2011) who reported that increasing water salinity depressed plants dry weight. However, increased at 100 mM NaCl concentration than control. There was observed a large variation in dry weight of genotypes under saline stress, among the wheat genotypes,

Buhooth10, Saberbeg and Buhooth158 were ranked as the most salt-tolerant genotypes compared with the others. Iba99 and AbuGraib were the most salt sensitive genotypes (Figure 2). The result indicates that these genotypes have a mechanism to adjust osmotic pressure at high salinity by increasing organic solute in cytoplasm. Plant area including shoot and leaves has influence significantly by salinity. Cell expansion under salt stress reduces in shoot and leave due to decreasing energy necessary because it uses for salinity defense (Munns and Gilliham, 2015). There was a large variation in plant area of 11 genotypes of wheat. Nevertheless, Buhooth10 and Furat have high mean of plant area, whereas the genotypes Iba99, Uruk and Axad 9 have a minimum plant area (Table 1). This phenomenon of decreasing plant area shows also in salinity concentration (Table 2). Wheat plant is a moderate tolerance to salinity stress (Munns and Tester, 2008). Therefore, tolerant genotypes at high level of salinity (200 mM NaCl) are a typical attribute for some wheat genotypes depending on specific mechanism and environmental conditions. However, the mechanism of salt tolerance in several genotypes is still poor understanding due to the genetic and physiological complexity of salinity trait (Zhu *et al.*, 2015). It is presumed that high levels of Na⁺ in leaf would enhance premature senescence of old leaves and inhibit photosynthetic performance of younger leaves (Benderradji *et al.*, 2011). Results of this study revealed that wheat genotypes responded differently to salinity stress. On the basis of plant area the genotypes Saberbeg, Furat and Fath were ranked as the most salt-tolerant genotypes compared with the others. Axad9, AbuGraib, Iraq and Iba99 were the salt sensitive genotypes. The Uruk and Buhooth10 genotypes maintained area close to the area of control plants at the level of salinity 200 mM NaCl (Figure 3). Sodium chloride applied in small amount is known to enhance the growth and dry matter production. However, when its concentration in the nutrient solution, irrigation water or soil increases above threshold level the reduction in growth and growth characteristic starts (Jennings, 1976). El-Hendawy *et al.* (2011) suggested that seedling parameters are the most important criterion for the screening of genotypes for salt tolerance at the early growth stages.

Plant height was significantly different between genotypes. Saberbeg showed significantly higher plant height than others especially Uruk which gave shorter height (Table 1). Salts present in the soil solution reduce the ability of plant to absorb water which slow down plant growth, on the basis of mean comparison, plant height was more affected at 200 mM NaCl (Table 2). This is in agreement with the finding of Hussein *et al.* (2011) who stated when increasing water salinity from 3000 to 6000 ppm depressed plants height, also with Sadeghi and Emam (2011) who reported that plant height was decreased upon increasing salinity level. Furthermore, Buhooth10 and Iraq were sensitive at moderate and high salinity levels and, to become more tolerant at low salinity levels, depending on the results of this study, maintaining the salinity at low levels is an important strategy for improving the growth of these two genotypes. The inhibition of plant height is a typical occurs at high salinity due to spending more organic solutes and energy for osmotic adjustment and decreasing the net photosynthesis because of stomatal closure (Flowers *et al.*, 2015). The 11 genotypes responded differently to salinity. The Saberbeg genotype exhibited good plant height, Hashimia and Buhooth10 genotypes maintained area close to the height of control plants at the level of salinity 200 mM NaCl. Whereas other genotypes height reduced especially Uruk which gave shorter height (Figure 4). El-Hendawy *et al.* (2011) obtained that salinity affected plant height and concluded that the measurements of shoot growth may be effective criteria for screening wheat genotypes for salt tolerance at early growth stages. Wheat genotypes could be able to continue grow in high salinity stress or at least survive owing to the tissue tolerance for salinity stress (Munns *et al.*, 2016). Results of El-Hendawy *et al.* (2011) study have shown that height and dry weight of shoot, and the shoot/root ratio demonstrated the greatest variation in the salt tolerance among genotypes. In this study, whole Na⁺ and K⁺ ions tested together in shoot and leaves to determine which genotype is able to exclude Na⁺ under salinity stress. The genotypes shows significant differences for accumulation of K⁺ and Na⁺ in their tissues, K⁺ decreased and Na⁺ concentrations increased in the genotypes with increased salinity to a varying degree (Table 1). The genotype Furat has the maximum content of K⁺. In addition, Fath and Buhooth10 accumulated higher Na⁺. On other hand Uruk genotype has the minimum mean content of K⁺ and Na⁺. The results indicates that Buhooth10 has low ability to exclude Na⁺ ion from root to prevent accumulation of Na⁺ in shoot or leaves in spite of it has maximum plant growth such as fresh, dry weight and plant area. Adequate maintenance of K⁺ in tissues is dependent upon the selective uptake of K⁺, cellular compartmentalization and distribution of K⁺ and Na⁺ ions in shoots (Siringam *et al.*, 2011). However, maintenance of high K⁺/Na⁺ ratio under saline environment is considered as an important salt tolerance selection criterion (Chinnusamy *et al.*, 2005; Siringam *et al.*, 2011), Other authors (Dashti *et al.*, 2009; Ahmadi *et al.*, 2009; Sadeghi and Emam, 2011) found that accumulation of Na⁺ in the shoots of salt tolerant cultivars was lower than the salt sensitive cultivars. Wheat genotypes were different with regard to tissues K⁺/Na⁺

ratio (Table 1). The least Na⁺/K⁺ ratio was observed in genotypes Buhooth158 and Saberbeg. The highest ratio of K⁺/Na⁺ was found in Uruk and Furat (Table 1). The sensitivity of some crops to salinity has been attributed to the inability for maintenance of Na⁺ and Cl⁻ ions out of the transpiration stream (Munns *et al.*, 2002). Asgari *et al.* (2012) observed highest leaf K⁺ concentration and K⁺/Na⁺ ratio and lower leaf Na⁺ and Cl⁻ concentrations in tolerant genotypes. K⁺ accumulation showed a decreasing tendency with the increasing of NaCl levels (Table 2) salinity had positive and significant effect on Na⁺ and Cl⁻ concentrations and ratio of Na⁺/K⁺ (Keshavarz *et al.*, 2013). Exclusion of Na⁺ and Cl⁻ and maintenance of high K⁺/Na⁺ ratios in the tissues are among the important aspects of mechanisms of salt tolerance in most plants (Shahbaz *et al.*, 2011). In this study salt stress caused a marked increase in Na⁺ concentration in the tissues of all wheat genotypes under salt stress. The relationship between K⁺ and Na⁺ ion relatively related with increasing salinity (Table 2). The earth crust contains potassium about 2.4% by weight. However, soil environment shows available potassium to plant at just 1-2% as exchangeable K⁺, non-exchangeable K⁺ and dissolve K⁺ and almost 98% of potassium is unavailable (Jaiswal *et al.*, 2016).

The genotypes responded differently under salt concentrations for the accumulation of Na⁺, K⁺ and K⁺/Na⁺ ratio in tissues. The concentration of Na⁺ was increases while the K⁺ accumulation decreased in all the genotypes with increased Salinity (Figures 5, 6). However, in the non tolerant, Buhooth158 and Saberbeg the Na⁺ concentration was greater than the tolerant genotypes under stress. The tolerant genotypes Uruk, AbuGraib, Iraq and Furat accumulated low Na⁺ and maintained higher K⁺ and K⁺/Na⁺ ratio in their leaves. Akbari ghogdi *et al.* (2012) stated that the salinity tolerance in tolerant cultivars as manifested by lower decrease in grain yield is associated with the lower sodium accumulation and higher K⁺/Na⁺ compared to the sensitive cultivars. Also Hussein *et al.* (2011) indicate that the uptake of N²⁺, P³⁺, K⁺, Ca²⁺ and Mg²⁺ in shoot of wheat plants decreased by increasing irrigation water salinity from 3000 to 6000 ppm compared to tap water (control), but increasing irrigation water salinity increased the Na⁺ and Cl⁻ uptake of wheat shoot, Na⁺ concentration increased in all wheat cultivars under saline regime.

The high K⁺ concentration at higher salinity level is good criteria for selecting salt tolerant genotypes (Flowers *et al.*, 1977). The sensitive genotypes had less than 1 ratio of K⁺/Na⁺ at higher salinity levels and the tolerant had more than 1 showing their tolerance consistently (AbuGraib at 150 and Uruk at 200 mM NaCl). It was evident from the results that some tolerant genotypes also showed the ratio below 1 (Iraq and Furat), which is not in agreement with the findings of Wyn Jones *et al.* (1979) who suggested a minimum ratio of 1 for K⁺/Na⁺ for normal growth of plants subjected to saline conditions. Tolerant plants compartmentalize the toxic concentrations of salts in their tissues (older leaves) and cells (vacuoles), and osmotic adjustments are accomplished by the synthesis of sugars in the cytoplasm (Gorham and Wyn Jones, 1993). The ability of plants to maintain a high level of cytosolic K⁺/Na⁺ ratio is an indicator for enhancing salt-tolerance in plants

(Maathuis and Amtmann, 1999; Anschütz *et al.*, 2014). Zhang (2010) indicated that the K^+/Na^+ ratio in root is not connection with salinity tolerance in the plasma membrane due to the independently of Na^+ exclusion and tissue tolerance mechanisms.

However, some reports have pointed out there is no relationship between salt resistance and K^+/Na^+ selectivity. It was proposed that the K^+/Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. However, some reports have pointed out there is no relationship between salt resistance and K^+/Na^+ selectivity. It was proposed that the K^+/Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. However, some reports have pointed out there is no relationship between salt resistance and K^+/Na^+ selectivity. It was proposed that the K^+/Na^+ selectivity of a cation channel in the plasma membrane of root cells does not differ in salt-tolerant and salt-sensitive wheat species. Interaction of genotypes with salinity demonstrates the ability of some genotypes to increase the K^+/Na^+ ratio at 150 mM NaCl (Figure 7), such as Axad9, Uruk, Buhooth158 and AbuGraib. High salinity stress at 200 mM NaCl decreases the K^+/Na^+ ratio in the majority of genotypes with the exception of the genotypes Uruk, Abu Ghraib, Fath and Acad9 (Figure 7). The variation in K^+/Na^+ ratio depends on the capability of plants to retain K^+ and efflux Na^+ from the roots. Increasing salinity tolerance in bread wheat correlates positively with increasing K^+ ion in plant tissues (Cuin *et al.*, 2008). Therefore, providing optimum amounts of K^+ and Nitrogen in external solution close to roots enhance the ability of K^+ translocation from root to shoot. This phenomenon presents in some wheat genotypes that have high level of K^+/Na^+ ratio in shoot and leaves at 150 mM NaCl (Figure 7). Conversely, the K^+/Na^+ ratio declines at high NaCl concentration in around the roots leading K^+ efflux from shoot to root (Abbasi *et al.*, 2016).

The variations of the salt tolerance indices among genotypes were less at low salinity concentration than at high salinity concentration (Figure 7). This result suggests that the selection criteria can be considered appropriate for screening wheat genotypes only when they were measured under high salinity concentrations. The net Na^+ uptake and its ratio to K^+ have a strong correlation with leaf area and crop yield (Zeng *et al.*, 2003; Asgari *et al.*, 2012). In this study, K/Na ratio was correlated with plant area for the genotypes Uruk and Furat, but not for AbuGraib and Iraq (Figure 7). In salt-sensitive genotypes of wheat, Na^+ was less effectively excluded from the transpiration stream as it entered the leaf blade, so resulting in a higher Na^+ accumulation (Benderradji *et al.*, 2011).

Furat genotype maintained a same ratio under all salinity levels which shows that it has a mechanisms of tolerance make it maintains the balanced proportions of potassium and sodium in the tissues. Furthermore, the genotype Uruk is semi-dwarf plant so it gave less results for vegetative

traits, but it gave the highest ratio of potassium to sodium, which is one of the mechanisms of salt tolerance making it one of Iraq's most promising plants to salt tolerance.

CONCLUSION

From the above mentioned data it could be concluded that salinity adversely affected of growth and nutrients uptake, however, salt tolerance in the 11 wheat genotypes was found to be linked to K^+/Na^+ ratio in plant tissues. So, the genotypes such as Uruk and Furat were more salt tolerant than the other genotypes examined in this study. On the basis of these plant growth parameters, K^+ and Na^+ contents and K^+/Na^+ ratio measured at the seedling stage can be considered for screening wheat genotypes at high salinity concentrations.

REFERENCES

- Abbasi, H., Jamil, M., Haq, A., Ali, S., Ahmad, R., and Malik, Z. (2016) Salt stress manifestation on plants, mechanism of salt tolerance and potassium role in alleviating it: a review. *Žemdirbyst (Agriculture)*, **103**: 229-238.
- Ahmadi, A., Emam, Y., and Pessaraki, M. (2009) Response of various cultivars of wheat and maize to salinity stress. *J. Food Agri. Environ.* **7**: 1 2 3 - 1 2 8.
- Akbari ghogdi, E., Izadi-Darbandi, A., and Borzouei, A. (2012) Effects of salinity on some physiological traits in wheat (*Triticum aestivum* L.) cultivars. *Indian Journal of Science and Technology*, **5** (1): 1901 -1906.
- Anschütz, U., Becker, D. & Shabala, S. (2014) Going beyond nutrition: Regulation of potassium homeostasis as a common denominator of plant adaptive responses to environment. *Journal of plant physiology*, **171**: 670-687.
- Asgari, H.R., Cornelis, W., and Van Damme, P. (2012) Salt stress effect on wheat (*Triticum aestivum* L.) growth and leaf ion concentrations. *Int. J. Plant Prod.* , **6**: 195-208.
- Bado, S., Forster, B.P., Ghanim, A.M.A., Jankowicz-Cieslak, J., Berthold, G., and Luxiang, L. (2016a). Introduction. In " Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley". Springer International Publishing, Cham, pp. 1-7.
- Bado, S., Forster, B.P., Ghanim, A.M.A., Jankowicz-Cieslak, J., Berthold, G., and Luxiang, L., (2016b) Protocol for Screening for Salt Tolerance in Barley and Wheat. In" Protocols for Pre-Field Screening of Mutants for Salt Tolerance in Rice, Wheat and Barley". Springer International Publishing, Cham, pp. 33-37.
- Benderradji, L., Brini, F., Amar, S.B., Kellou, K., Azaza, J., Masmoudi, K., *et al.* (2011). Sodium transport in the seedlings of two-bread wheat (*Triticum aestivum* L.) genotypes showing contrasting salt stress tolerance. *Aust. J. Crop Sci.*, **5**:233-241.
- Berger, B., de Regt, B., and Tester, M. (2012) Trait dissection of salinity tolerance with plant phenomics. *Plant Salt Tolerance: Methods and Protocols*, 399-413.

- Chinnusamy, V., Jagendorf, A., and Zhu, J. (2005). Understanding and improving salt tolerance in plants. *Crop Sci.*, 45:437–448.
- Christen, E.W., Saliem, K.A. (2012) Iraq Salinity Assessment Current State causes and impact, Current State, Causes, and Impacts. In, The Iraq Salinity Assessment - Report 1: Situation Analysis. pp. 1-48.
- Cuin, T.A., Betts, S.A., Chalmandrier, R., and Shabala, S. (2008) A root's ability to retain K^+ correlates with salt tolerance in wheat. *Journal of Experimental Botany*, 59 : 2697-2706.
- Dashti, A., Khan, A.A., and Collins, J. C. (2009) Effect of salinity on growth, ionic relations and solute content of *Sorghum bicolor* (L.) Moench. *J. Plant Nutr.*, 23:1219-1236.
- El-Hendawy, S.E., Hu, Y., Sakagami, J.I., and Schmidhalter, U. (2011). Screening Egyptian wheat genotypes for salt tolerance at early growth stages. *International Journal of Plant Production*, 5 (3): 283-298.
- FAO, (2011). Crops.FAOSTAT: [p://faostat.fao.org/site/567/default.aspx#ancor](http://faostat.fao.org/site/567/default.aspx#ancor).
- Ferdose, J., Kawasaki, M., Taniguchi, M., and Miyake, H. (2009). Differential sensitivity of rice cultivars to salinity and its relation to ion accumulation and root tip structure. *Plant Prod. Sci.*, 12 (4): 453-461.
- Flowers, T.J., Munns, R., and Colmer, T.D., (2015). Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Annals of Botany*, 115 : 419-431.
- Flowers, T.J., Troke and P.F., and Yeo, A. R. (1977). The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.*, 28: 89–121.
- Gorham, J. and Wyn Jones, R.G. (1993). Utilization of *Triticeae* for improving salt tolerance in wheat. In "Towards the Rational use of High Salinity Tolerant Plants H. Leith & A.A. Massoum (Eds.), Kluwer Acad. Pub. :The Netherlands. pp.27-33.
- Houmani, H. and Corpas, F.J. (2016). Differential responses to salt-induced oxidative stress in three phylogenetically related plant species: *Arabidopsis thaliana* (glycophyte), *Thellungiella salsuginea* and *Cakile maritima* (halophytes). Involvement of ROS and NO in the control of K^+/Na^+ homeostasis. *AIMS Biophysics*, 3(3): 380-397.
- Hussein, M.M., Abd El-Rheem, Kh. M., Khaled, S. M., and Youssef, R. A. (2011) Growth and nutrients status of wheat as affected by ascorbic acid and water salinity. *Nature and Science*, 9(10):64-69.
- Jaiswal, D.K., Verma, J.P., Prakash, S., Meena, V.S., and Meena, R.S. (2016) Potassium as an Important Plant Nutrient in Sustainable Agriculture: A State of the Art. In "Potassium Solubilizing Microorganisms for Sustainable Agriculture". V.S. Meena, B.R. Maurya, J.P. Verma, R.S. Meena (Eds.), Springer, New Delhi : India, pp. 21-29.
- Jennings, D.H. (1976) Effects of sodium chloride on higher plants. *Biological Reviews of the Cambridge Philosophical Society*, 51 : 453 – 486 .
- Kanwal, H., Ashraf, M. and Hameed, M. (2013) Water relations and ionic composition in the seedlings of some newly developed and candidate cultivars of wheat (*Triticum aestivum* L.) under saline conditions. *Pak. J. Bot.*, 45(4): 1221-1227.
- Keshavarz, L., Saffari, M., and Golkar, P. (2013) Effect of salinity stress on agro-physiological characters of wheat genotypes (*Triticum aestivum* L.). *International Journal of Agriculture: Research and Review*, 3 (3): 584-589.
- Kingsbury, R.W. and Epstein, E. (1984) Selection for salt resistant spring wheat. *Crop Sci.*, 24: 310–315.
- Lucini, L., Roupael, Y., Cardarelli, M., Canaguier, R., Kumar, P., and Colla, G. (2015) The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. *Scientia Horticulturae*, 182:124-133.
- Maathuis, F.J. and Amtmann, A. (1999). K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. *Annals of Botany*, 84 :123-133.
- Maas, E.V. (1986) Salt tolerance of plants. *Appl Agric Res.*, 1:12–26.
- Mass, E.V. and Grieve, C.M. (1994) Tiller development in salt-stressed wheat. *Crop Sci.*, 34: 1594–1603.
- Memory, H.R. and Mrinal, B. (2011) Cereals and Abiotic Stresses: Roles of Aquaporins and Potential in Wheat Improvement. In Almeida, M. T. (Eds.), *Wheat : Genetics, Crops and Food Production*. NOVA Science Publishers, Inc, New York, pp. 121-151.
- Munns, R. (2005) Genes and salt tolerance: bringing them together. *New Phytologist*, 167 : 645-663.
- Mohammadi-Nejad, G., Singh, P.K., Arzani, A., Rezaie, A.M., Sabouri, H., and Gregorio, G.B. (2010) Evaluation of salinity tolerance in rice genotypes. *Int. J. Plant Prod.*, 4 (3): 199-208.
- Munns, R. and Gilliam, M. (2015) Salinity tolerance of crops – what is the cost? *New Phytologist*, 208 :668-673.
- Munns, R., Hare, R.A., James, R.A., and Rebetzke, G.J., (2000) Genetic variation for improving the salt tolerance of durum wheat. *Australian Journal of Agricultural Research*, 51 :69-74.
- Munns, R., Husain, S., Rivelli, A.R., James, R.A., Condon, A.T., Lindsay, M.P. (2002) Avenues for increasing salt tolerance of crops, and the role Nutrition:

Plenary Lectures of the XIV International Plant Nutrition Colloquium. Springer, pp. 93-105.

Munns, R., James, R.A., Gilliam, M., Flowers, T.J., and Colmer, T.D. (2016) Tissue tolerance: an essential but elusive trait for salt-tolerant crops. *Functional Plant Biology*, 43: 1103-1113.

Munns, R., James, R.A. and Läuchli, A. (2006) Approaches to increase the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57:1025- 1043.

Munns, R. and Tester, M., (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59, : 651-681.

Munns, R., Wallace, P.A., Teakle, N.L., and Colmer, T.D., (2010) Measuring soluble ion concentrations (Na⁺, K⁺, Cl⁻) in salt-treated plants. *Plant Stress Tolerance: Methods and Protocols*, 371-382.

Negrão, S., Schmöckel, S.M., and Tester, M., (2016) Evaluating physiological responses of plants to salinity stress. *Annals of Botany*.

Ortiz, R. (2011) Advance in Wheat Genetic Enhancement for Global Food Production. *In* Almeida (Ed.), WHEAT: Genetics, Crops and Food Production. Nova Science Publishers, Inc., New York, pp. 153-178.

Richards, R.A. (1983) Should selection for yield in saline regions be made on saline or non-saline soils? *Euphytica*, 32 : 431-438.

Sadeghi, H. and Emam, Y. (2011) Chemical composition, yield and yield components of two wheat cultivars in response to salt stress. *Journal of Plant Physiology and Breeding*, 1(1): 39-47.

Sauer, D. and Burroughs, R. (1986) Disinfection of seed surfaces with sodium hypochlorite. *Phytopathology*, 76 :745-749.

Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat methods*, 9:671-675.

Setia, R., Smith, P., Marschner, P., Gottschalk, P., Baldock, J., Verma, V., Setia, D., and Smith, J. (2012) Simulation of salinity effects on past, present, and future soil organic carbon stocks. *Environmental science and technology*, 46 :1624-1631.

Shahbaz, M., Ashraf, M., Akram, N.A., Hanif, A., Hameed, S., Joham, S. (2011) Salt-induced modulation in growth, photosynthetic capacity, proline content and ion accumulation in sunflower (*Helianthus annuus* L.). *Acta Physiol. Plant.*, 10: 639- 649.

Sharbatkhari, M., Galeshi, S., Shobbar, Z.S., Nakhoda, B. and Shahbazi, M. (2013) Assessment of agro-physiological traits for salt tolerance in drought-tolerant wheat genotypes. *International Journal of Plant Production*, 7 (3): 437-454.

Sheikh, B. (2006) Hydroponics: key to sustain agriculture in water stressed and urban environment. *Pak. J. Agric., Agril. Eng., Vet. Sci.*, 22 :53-57.

Siringam, K., Juntawong, N., Cha-um S., and Kirdmanee, C. (2011) Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt sensitive rice (*Oryza sativa* L. spp. *indica*) roots under isoosmotic conditions. *Afr. J. Biotechnol.*, 10(8): 1340- 1346.

Steel, R.G. and Dickey, J.H. (1997) Principles and procedures of statistics a biometrical approach.

Tsegay, B.A. and Gebreslassie, B. (2014) The effect of salinity (NaCl) on germination and early seedling growth of *Lathyrus sativus* and *Pisum sativum* var. *abyssinicum*. *African Journal of Plant Science*, 8: 225-231.

Wyn Jones, R.G., Brady, C.J. and Speirs, J. (1979) Ionic and Osmotic Regulation in Plants. *In*: "Recent advances in the Biochemistry of Cereals". D.L. Laidman, R.G. Wyn Jones (Eds.), Academic Press: London, P. 63-103.

Zeng, L., Poss, J.A., Wilson, C., Draz, A.D.E., Gregorio, G.B., and Grieve, C.M. (2003) Evaluation of salt tolerance in rice genotypes by physiological characters. *Euphytica*, 129: 281-292.

Zhang, J.L., Flowers, T.J., and Wang, S. M. (2010) Mechanisms of sodium uptake by roots of higher plants. *Plant and Soil*, 326: 45-60.

Zhu, J.K. (2003) Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.*, 6: 441-445.

Zhu, M., Shabala, S., Shabala, L., Fan, Y., and Zhou, M. (2015) Evaluating predictive values of various physiological indices for salinity stress tolerance in wheat. *J. Agron. Crop Sci.*, 10 :1111.