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Seed germination and seedling growth of hexaploid wheat (*Triticum aestivum* L.) varieties as influenced by different levels of sodium chloride

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ABSTRACT

This experiment was conducted in the laboratory of plantation crops/Faculty of Agriculture/University Putra Malaysia in 2015 to evaluate salinity effects on seed germination percentage and some seedling growth traits of eight bread wheat cultivars and genotypes (*Triticum aestivum* L.). Salinity treatments were measured (0, 50, 100, 150, 200, 250 and 300 mM sodium chloride). Distilled water was added to control treatment for each cultivar for comparison. Data regarding germination percentage, water uptake (%), plumule and radicle length (cm), strong seed index and salt tolerance index were counted. Based on salt tolerance index, only one genotype (G8) fell in the tolerant class; three accessions (G1, G6 and G10) were moderately salt tolerant; one genotype (G2) was moderately salt susceptible and three accessions (Abo-Graib, Forat and Dijla) were salt susceptible. Results showed that increasing concentration of NaCl solution resulted in the gradual reduction in all studied parameters in all wheat varieties and genotypes. The G8 genotype showed significant superiority on the other genotypes and varieties in all traits above which can be used as an improved genotype against salt stress in breeding programmes, while the Abo-Graib gave the lowest rate under the probability level of significance of 0.05. These were positively and significantly ($P \leq 0.01$) correlated between the traits studied under six concentrations (mM) of NaCl stress except the relationship between germination percentage and each of water uptake, plumule length, radicle length, while strong seed index was positively but not significantly correleted. Whereas the correlation was positive and significant ($P \leq 0.05$) between water uptake and plumule length, radicle length and strong seed index.

Key words : Germination percentage**,** hexaploid wheat, salinity, salt tolerant index, seedling traits

INTRODUCTION

Wheat is a plant of grasses family (Poaceae) and Triticum genus that its scientific name is *Triticum aestivum.* Wheat is one of the most established developed plants for man to develop the broadest and the most elevated quality taken. It is simple for sowing to adjust to diverse climatic conditions as one of the main groups in grain yield production and daily utilization of bread is a dire need to produce a huge yield of this group. Saltiness is a standout amongst the most vital abiotic burdens restricting yield production in dry and semi-arid areas, where soil salt substance is regularly high and precipitation can be lacking for draining (Krasensky and Jonak, 2012). In recent years, it is often observed that the productivity of wheat declines significantly due to the influence of various factors, such as abiotic and biotic stress particularly high salinity (Kilic and Yagbasanlar, 2010; Ayed-Slama *et al.,* 2015).

Saltiness influences numerous

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physiological, morphological and biochemical techniques, including seed germination, plant development, water and supplement uptake (Bahrami *et al.,* 2012 ; Chapman, 2013). Wheat (*Triticum aestivum* L.) is the staple nourishment for more than 35% of world population (Datta *et al.,* 2009).

New wellsprings of saltiness resilience are required for harvests becoming on the salt-influenced area (Rengasamy, 2002). There is more than 900 million hectares of the area around the world, approx. 20% of the aggregate rural area (FAOSTAT, 2010) is influenced by salt, representing more than 6% of the world's aggregate area range. NaCl is the prevalent salt bringing about salinization, and it is obvious that plants have developed components to control its amassing (Munns and Tester, 2008). Salinity retards growth and the growth reduction is manifested by a number of discrete responses that are linked with the build-up of salt in the shoot, or are independent of salt accumulation in the shoot (Roy *et al.,* 2014). Seed germination is an essential and powerless stage in the life cycle of physical angiosperms and decides seedling foundation and plant development. In spite of the essentialness of seed germination under salt stress (Chapman, 2013), the mechanism(s) of salt tolerant in seeds is moderately ineffectively seen, particularly when contrasted and the measure of data as of now accessible about salt resilience physiology and organic chemistry in vegetative plants (Kanai *et al.,* 2007).

Saltiness influences seed germination through osmotic impacts (Khatun *et al.,* 2013), particle harmfulness (Hampson and Simpson, 1990) or a blend of the two (Huang and Redmann, 1995). In vegetative plants, salt anxiety reasons diminished cell turgor and discouraged rates of root and leaf elongation (Fricke *et al.,* 2006), proposing that ecological saltiness acts fundamentally on water uptake. Moreover, high intracellular concentrations of both Na^+ and Cl^- can hinder the metabolism system of isolating and extending cells (Abbas *et al.,* 2012), hindering germination and actually prompting seed passing. Ionic impacts may be recognized from osmotic impacts by contrasting the impact of salt arrangements and iso-osmotic arrangements of an inactive osmoticum, for example, polyethylene glycol (PEG; in fact basically a matricum) that can't enter the cell wall. Many researchers attempted to select the salt tolerant lines or cultivars of several species at the seedling growth stage (Khayatnezhad *et al.,* 2010). The aim of this study was to focus the impacts of saltiness on seed germination percentage, water uptake, plumule length, radicle length and strong seed index record of some bread wheat cultivars and genotypes at different concentrations of NaCl, and choice of saline tolerant cultivars for breeding programmes.

MATERIALS AND METHODS

Plant Material

This experiment was conducted in laboratory of plantation crops/Faculty of Agriculture/University Putra Malaysia in 2015 to find out the effect of salinity on germination, water uptake, plumule length, radicle length, strong seed index and salt tolerance index of seeds and seedling of some varieties of bread wheat. The latitude and longitude of it : 2°59' 57" N, 101°42'28" E. Seeds of eight wheat (*Triticum aestivum* L.) cultivars : Abo-Graib, Dijla, Forat, genotypes of G1, G2, G6, G8 and G10 were obtained from the State Board of Agricultural Research, Baghdad, Iraq. The seeds were surface sterilized by dipping the seeds in 70% ethanol solution for 1 min, 20% Clorox for 20 min and rinsed thoroughly with distilled water. In this experiment, 25 seeds were placed per Petri dish with seven sodium chloride salts (NaCl) concentrations (0, 50, 100, 150, 200, 250 and 300 mM). Each experimental unit included 1 Petri dish with 100 × 150 mm dimension. Each contained 25 healthy seeds. Below and top seeds filter paper was placed. In each Petri dish, 25 ml of salt was added. To the control treatment to Petri dish distilled water was added. The treatments were placed in culture room with temperature 25°C. Counting germinated seeds after eight days, germination percentage was determined by counting the number of germinated seeds and expressed as a percentage.

Determination of Water Uptake by Seeds

The weight of 25 seeds from each variety and genotype was counted and placed in Petri dishes and added a 25 ml of NaCl, control treatments and taken out after 24 h and removed the water from the surface of the seeds and re-weighed and then extracted the amount of water absorbed by the seed after 24 h. Water uptake per cent was calculated by the formula given below (Rahman *et al.,* 2008) :

$$\text{Water uptake (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

Where,

W_1=Initial weight of seed.

W_2=Weight of seed after absorbing water in a particular time.

Determination of Germination (%)

When the plumule was longer than half length of the seed and the radicle was equal to or greater than the seed length, a seed was deemed as germinated seed. The final germination percentage (GP) of seeds was determined using the equation given below (Rauf, 2005) :

GP=(Ni/N) × 100 (Ni=Number of germinated seeds till i days and N=Total number of seeds)

Determination of Radicle Plumule and Radicle Length (cm)

Plumule and radicle length was measured for 10 seedlings from each treatment after two weeks of sowing and the result was converted to the plant.

Strong Seed Index

This parameter was determined by the following equation (Abdul-Baki and Anderson, 1970).

Strong seed index={Germination percentage × Means of seedling length (Radicle+Plumule) (cm)}/100

Preparation of Salt Solution

Salinity levels using pure salts were prepared with the following equation (Chu, 1978) :

$$C_1 \times V_1 = C_2 \times V_2$$

Where,

C_1 is the initial conentration (mM) of stock solution.

V_1 is the initial volume.

C_2 is the final concentration.

V_2 is the final volume.

Salt Tolerance Trait Index

An estimation of the average of salt tolerance trait index (STTI) using the formula of Ali *et al.* (2007) will give the salt tolerance index as under :

$$\text{STTI} = \frac{\text{Value of trait under stress condition}}{\text{Value of trait under no stress}} \times 100$$

Statistical Analysis

All experiments were conducted using split plot design using randomized complete block design (RCBD) in three replications, where the whole plot represents salinity levels, the sub-plots represent the genotypes and blocks represent the replicates. All data were subjected to one-way ANOVA using the SPSS software package, version 22 as well as Least significant difference (LSD) was used at significance level of 0.05 to compare among the means. Kwon and Torrie (1964) formula was used to calculate the correlation coefficients among traits.

RESULTS AND DISCUSSION

Water Uptake

A direct relationship was observed between the water absorption of the seeds and the increase in the level of NaCl concentration (Fig. 1). The water uptake of the seeds decreased when there was a greater concentration of NaCl (300 mM) in the soil, compared to controlled soil conditions. There was 15% of water uptake when the level of NaCl was at 300 mM; the water uptake was 69% under controlled soil conditions with 0 mM of NaCl (Fig. 1) due to under saline conditions, the growth of seedlings was severely impaired (Tezara *et al.,* 2003) because of slow or reduced mobilization of reserve foods (Kayani *et al.,* 1990), which led to suspended cell division, hypocotyls injury (Assadian and Miyamoto, 1987) and cell enlargement (Meiri and Poljakoff-

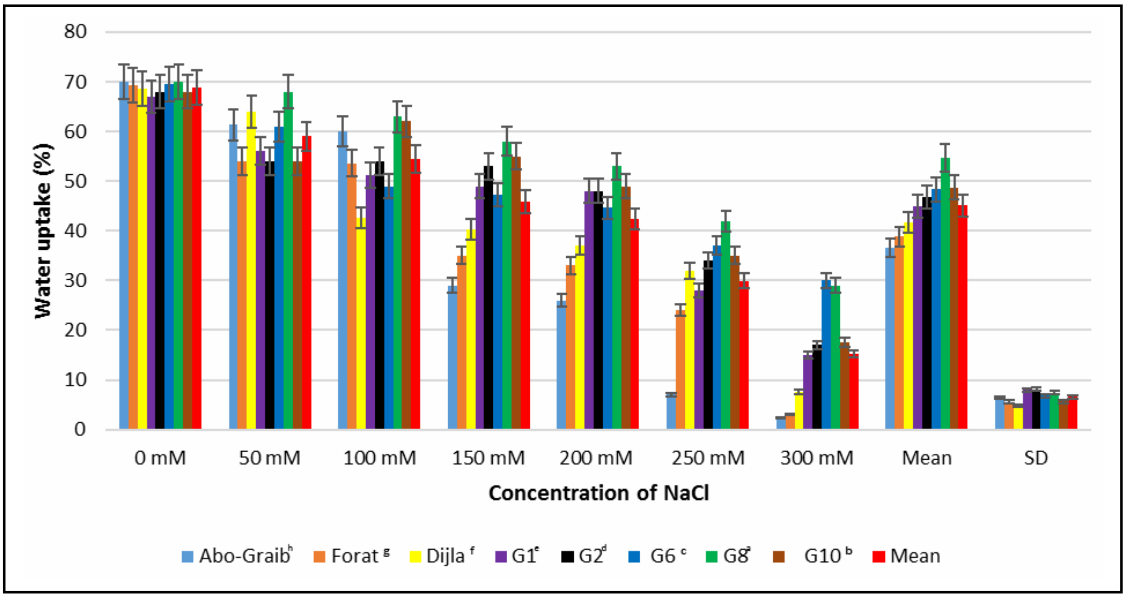

Fig. 1. Effect of NaCl concentration on water uptake (%).

Mayber, 1970). When probability level was at P≤0.05, substantial variances were observed among different varieties (Table 1). The genotype Abo-Graib exhibited about an average of 36.5% of water uptake, while the genotype G8 demonstrated the highest amount of water uptake at 55%. Based on the significant interaction effect (varieties x NaCl), it was observed that under controlled conditions, the percentage of water uptake by G8, G6 and Abo-Graib seeds was the highest (70%), while the percentage of water uptake of Abo-Graib in 300 mM NaCl was the lowest (2.3%) (Fig. 1). Akbari *et al.* (2007) reported similar findings where it was mentioned that water uptake by seeds declined remarkably with the increase in salt concentration in the soil. The water uptake based on the salt tolerance index average was recorded at 66% (Table 2). The range of water uptake, on an average, was from 78% (G8 genotype) to 52% (Abo-Graib and Forat varieties).

Seed Germination Percentage

A significant decrease in the germination percentage was noticed at statistical level 0.05, corresponding to the germination percentage synchronised with increasing stress levels. Furthermore, 99% was the highest rate of germination percentage observed under controlled conditions (0 mM of NaCl), while 32% was the lowest rate of germination percentage observed during reduced water update by seeds at 300 mM level of NaCl (Fig. 2). There are two ways in which

Table 1. Result of variance analysis on germination percentage and growth of seedling characteristics under various concentrations of NaCl

Source	d. f.	Mean squares				
		Water uptake (%)	Germination percentage	Plumule length (cm)	Radicle length (cm)	Strong seed index
Corrected model	55	2159.941	6554.039	39.999	40.867	228.517
Intercept	1	694330.583	1573857.190	9841.008	10340.762	27886.074
Varieties	7	1548.543*	13115.993*	40.142*	40.823*	232.359*
NaCl	6	16405.917*	32603.329*	283.932*	290.905*	1575.150*
Varieties × NaCl	42	226.701*	1739.053*	5.127*	5.155*	35.501*
Error	280	1.010	1.695	.004	0.008	0.082
C. V. (%)		14.64	15.8	16.7	10.9	16.1
Total	336					

*Significant at P=0.05 level.

Table 2. Salt tolerant categories of eight wheat cultivars according to salt tolerance index (STI) under six concentrations (mM) of NaCl

Salt tolerance category	Range of salt tolerance index (%)	No. of accessions	Accessions
Tolerant	80-100	1	G8
Moderately tolerant	67-80	3	G1, G6, G10
Moderately susceptible	54-66	1	G2
Susceptible	Below 54	3	Abo-Graib, Forat, Dijla

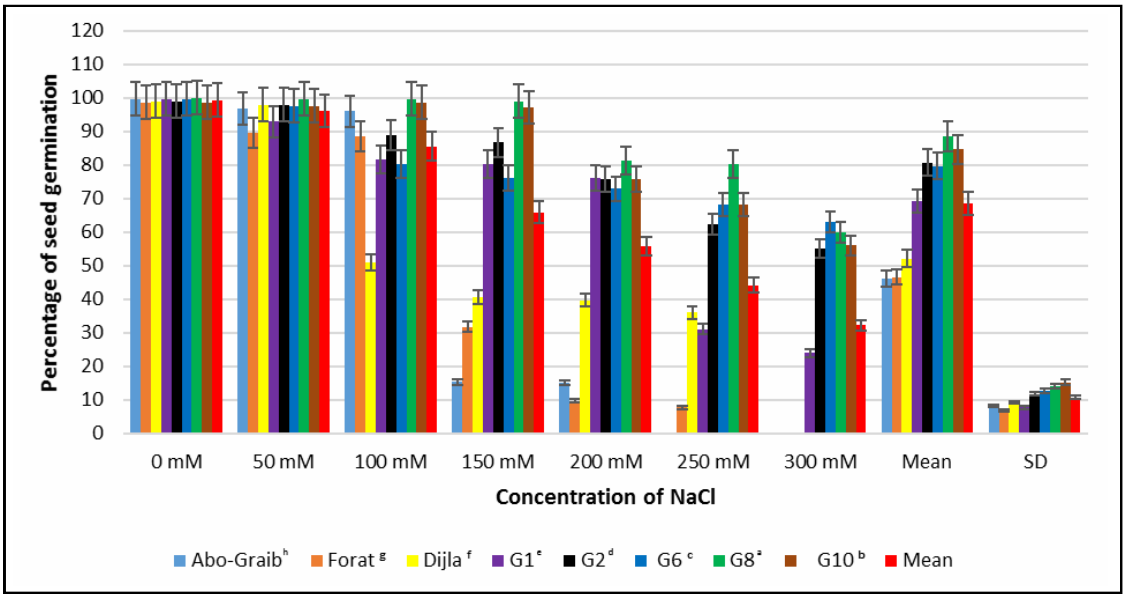

Fig. 2. Effect of NaCl concentration on wheat seed germination percentage.

salinity affects seed germination. In the first, the concentration of salt in the medium can become high enough to induce reduction in the osmotic potential of the cells to such an extent where the water uptake gets retarded or prevented, resulting in hindrance of nutrient mobilization necessary for germination. In the second way, the accumulation of salt or ions may become toxic to the embryo, damaging its growth potential. At probability level P≤0.05, considerable variances were seen among different varieties (Table 1). The G8 genotype exhibited the highest rate at 89% (Fig. 2), while the lowest rate was observed in the Abo-Graib variety at 46%. These findings are in agreement with the observations and data presented by Datta *et al.* (2009) and Rahdari and Hoseini (2015) on wheat. Based on the significant interaction effect (varieties x NaCl), it was seen that the germination percentage of the G8, G6, G1 genotypes and Abo-Graib variety under control conditions was the highest (100%), while the germination percentage was the lowest (0%) for the Forat, Dijla and Abo-Graib varieties in soil with 300 mM of NaCl. These results are similar to the observations made by Hester *et al.* (2001), who reported that crop performance and salt concentration of soil had a negative relationship between them. The germination percentage based on the average salt tolerance index was recorded at 68% (Table 2). The range of the germination percentage was from 89% (G8 genotype) to 46% (Abo-Graib and Forat varieties).

Plumule and Radicle Length

Figs. 3 and 4 depict the influence of salinity stress on the plumule and radicle length of the genotypes, respectively. Substantial variances in the radicle and plumule length were observed at 0.05% probable level (Table 1). A comparison of the plumule and radicle length of the genotypes at varying levels of salinity (0, 50, 100, 150, 200, 250 and 300 mM) revealed that when there was an increase in the salinity level, the plumule and radicle length of the seedlings decreased. Under controlled conditions, the highest values of the plumule and radicle length recorded were

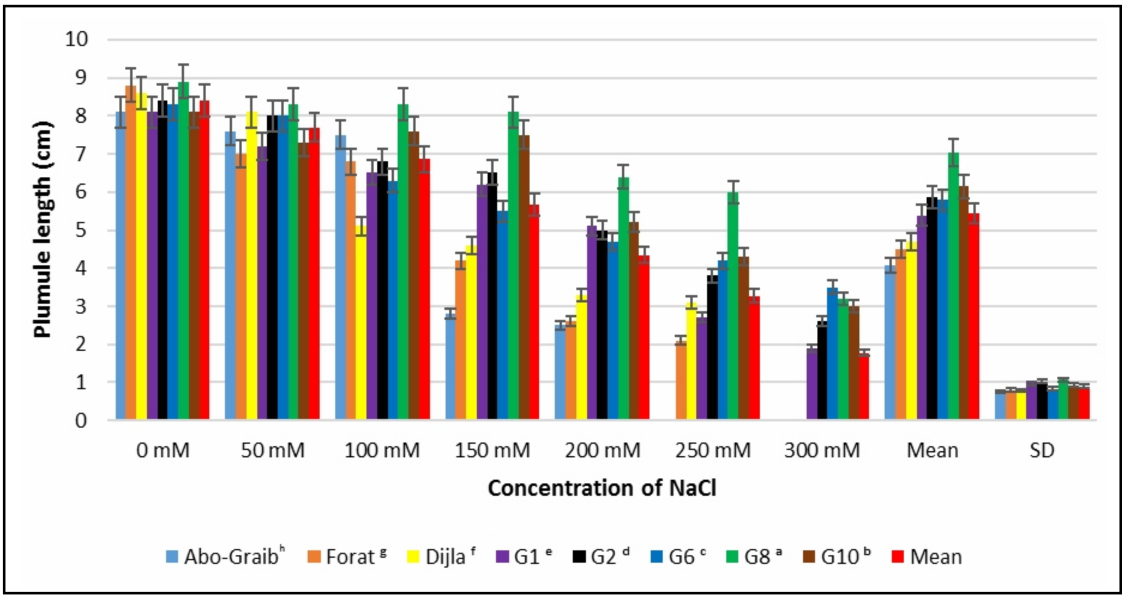

Fig. 3. Effect of NaCl concentration on plumule length.

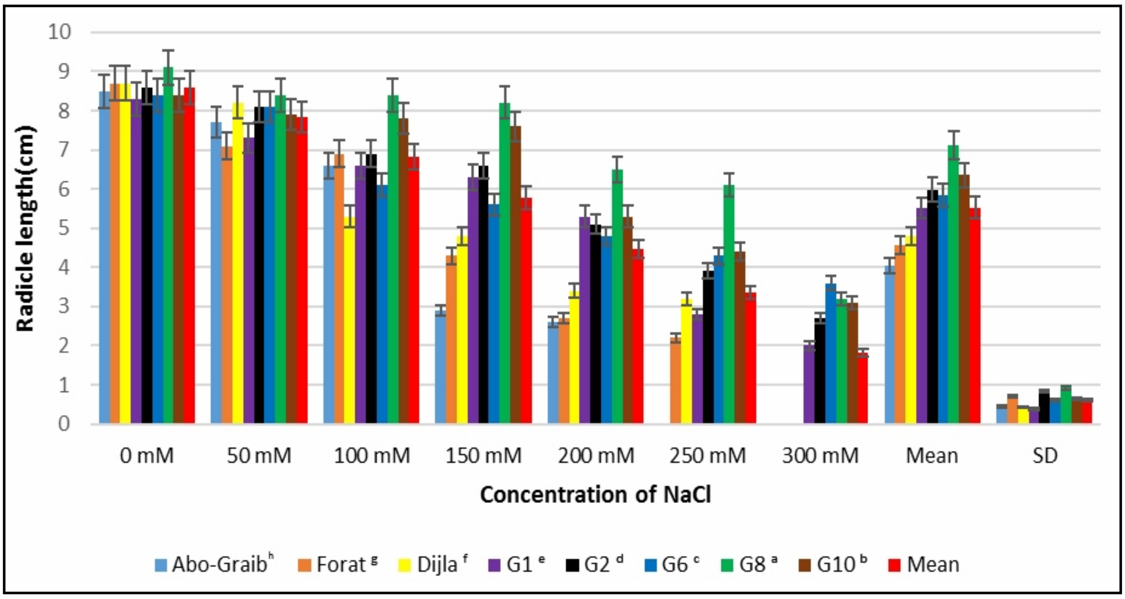

Fig. 4. Effect of concentration of NaCl on radicle length.

8.4 and 8.6 cm, respectively. On the other hand, at 300 mM of NaCl salinity of soil, the maximum plumule length (Fig. 3) and radicle length (Fig. 4) were found to be 1.8 and 1.8 cm, respectively. Abdelkader *et al.* (2015) proposed that the growth of the plumule and radicle length decreased as the salinity level increased. Since an increase in the salinity level results in a decrease in water absorption, consequently the cell division and differentiation in the seedlings also get reduced, which leads to a decrease in the growth of the plumule and radicle length. Besides, substantial variances in plumule and radicle length were observed among different varieties under varying concentration levels of NaCl present in the soil (Table 1).

The G8 genotype exhibited the highest growth in the plumule and radicle length at 7 and 7.1 cm, respectively, while the Abo-Graib cultivar showed the lowest growth in the plumule and radicle length at 4.1 and 4 cm, respectively (Figs. 3 and 4). In relation to these attributes, a significant interaction effect was observed between the different varieties and NaCl. Under controlled environment, the G8 genotype exhibited the highest growth in plumule and radicle length at 8.9 and 9.1 cm, respectively, while at a NaCl concentration level of 300 mM, the Dijla, Forat and Abo-Graib varieties produced no increase in the plumule and radicle length (0 cm). These results are in agreement with the observations made by Gupta and Srivastava (1989). The findings implied that the salt tolerant genotypes showed greater growth in the radical length than the plumule length. This is in line with earlier reports on wheat (Akbarimoghaddam *et al.,* 2011). The plumule length based on the average salt tolerance index was 65%, while for radicle length, it was 64% (Table 2). The range of plumule length growth was from 79% (G8 genotype) to 50% (Abo-Graib and Forat varieties), while the range for radicle length growth was from 78% (G8 genotype) to 49% (Abo-Graib variety) (Table 3).

Strong Seed Index

Depending on the probability level (P≤0.05), the strong seed index showed a substantial variance in the different varieties in relation to the varying salinity levels (Table 1). With an increase in the NaCl concentration, the strong seed index decreased (Fig. 5). The greatest strong seed index was 16.8 under controlled conditions, whereas the least was 2 at 300 mM of NaCl concentration (Fig. 5). In the presence of salinity, in general, there is a direct relationship between the seed vigour index and reduced water potential. The findings are similar to the observations made by (Ozhan and Hajibabaei, 2015), who reported that if salinity increased, the seed characteristics would decrease. Moreover, considerable differences in this trait were observed among the different varieties (Table 1). The G8 genotype seed index was the highest at 13%, while the Forat variety exhibited the least value of seed index at 6.5%. There was a big interaction effect (varieties x NaCl) influencing the strong seed index.

The highest seed index at 17.7% was of the G8 genotype under a controlled environment, whereas the least value was 0% shown by the Forat, Dijla and Abo-Graib varieties at 300 mM of NaCl stress. The results are in line with the findings reported by Siti-Aishah *et al.* (2010) on sorghum. The strong seed index based on the average salt tolerance index was 55% (Table 3). The range of the strong seed index values was from 74% (G8 genotype) to 37% (Abo-Graib variety).

Eight wheat genotypes were categorized into four groups depending on the salt tolerance index (Table 2) : salt susceptible (STI, below 54%), moderately salt susceptible (STI, 54-66%), salt tolerant (STI, 80-100%) and moderately salt tolerant (STI, 67.0-80%). There was one genotype (G2) that was moderately salt susceptible, and only one genotype (G8) that was salt tolerant. The three varieties (Forat, Dijla and Abo-Graib) were salt susceptible, and the three genotypes (G1, G6 and G10) were

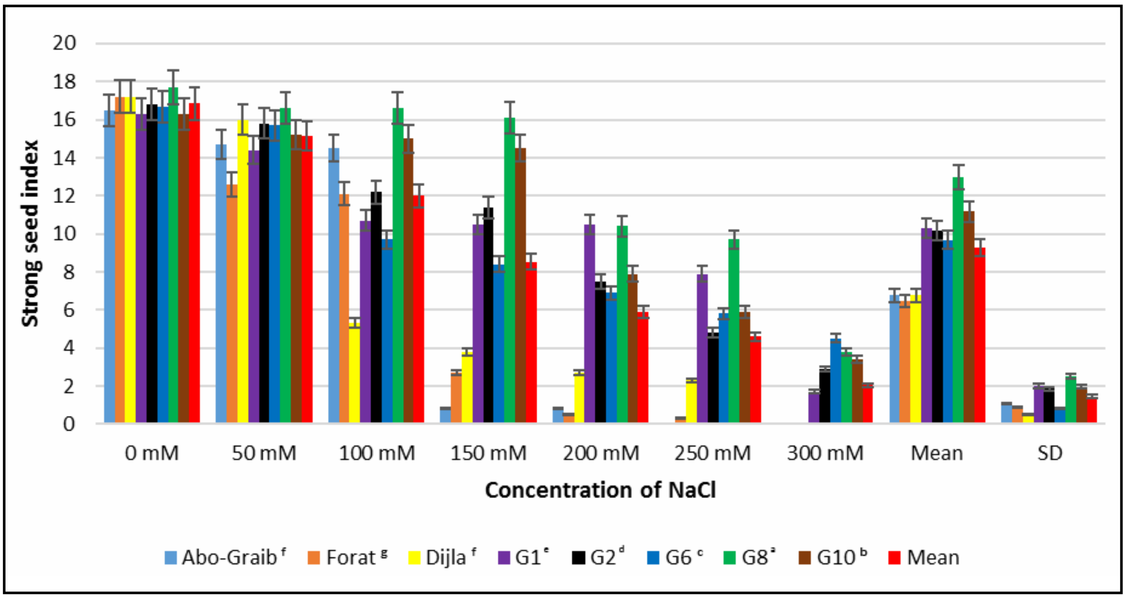

Fig. 5. Effect of NaCl concentration on strong seed index.

Table 3. Salt tolerance trait indices (STTIs) of five traits studied in eight wheat cultivars under six concentrations (mM) of NaCl

Variety	Water uptake	Germination percentage	Plumule length	Radicle length	Strong seed index	Mean
Abo-Graib	52	46	50	49	37	46.8[h]
Forat	56	46	51	52	38	48.6[g]
Dijla	61	47	54	55	45	52.4[f]
G1	68	71	66	66	56	65.4[e]
G2	71	82	70	68	60	70.2[c]
G6	69	80	69	69	58	69[d]
G8	78	89	79	78	74	79.6[a]
G10	74	85	76	76	68	75.8[b]
SE_{diff}[x]	0.74	0.48	0.20	0.20	0.242	

[x]Standard error of the difference between means of salt tolerance trait index/salt tolerance index.

Table 4. Correlation between six traits in eight varieties of wheat under various concentrations of NaCl

Trait	Water uptake (%)	Plumule length (cm)	Radicle length (cm)	Strong seed index	Salt tolerance index
Germination percentage	0.26^{ns}	0.03^{ns}	0.002^{ns}	0.24^{ns}	90**
Water uptake (%)		0.41*	0.50*	0.48*	99**
Plumule length (cm)			0.68**	0.89**	94**
Radicle length (cm)				0.85**	93**
Strong seed index					93**

*,**Significant at P<0.05 and P<0.01 levels, respectively. NS : Not Significant.

moderately salt tolerant.

Under the six varying concentrations (mM) of NaCl, the five traits studied were positively and significantly (P≤0.01) correlated with each other. However, there was an exception with the relationship between germination percentage and each of water uptake, plumule length, radicle length and strong seed index, which was positive but not significant (Table 4). In spite of the exception, the relationship between water uptake and each of plumule length, radicle length and strong seed index was still positive and significant (P≤0.05).

CONCLUSION

The results of this study suggest that increase in salinity reduces seed germination percentage, plumule and radicle length and strong seed index in all wheat varieties Abo-Graib, Forat, Dijla, genotypes G1,G2,G6,G8 and G10. The G8 genotype under 0, 50, 100, 150, 200, 250 and 300 mM had superiority over other varieties and genotypes in all studied traits because it had more tolerance for salinity. On the other hand, Abo-Graib variety showed less tolerance for salinity. Therefore, G8 genotype can be used in breeding programmes to improve ability of wheat varieties for salt tolerance.

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