Evaluation of some Libyan Barley *Hordeum vulgare* L Genotypes for Salinity Tolerance at Booting Stage

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Abstract

Sustainable development of agriculture in the North Africa region, especially in Libya, is severely restricted by soil salinity and shortage of fresh water. In the coastal regions of the country saline water is used for crops irrigation. Therefore, to determine the genotypes of salinity-tolerant barley, a field experiment was conducted in the fall of 2018 to evaluate some genotypes of Libyan barley for salinity-tolerance. In this study, 7 Libyan barley (*Hordeum vulgare* L.) genotypes were irrigated with saline or non-saline water 0, 150 and 200 mM NaCl, (EC value of < 1.5, 15.9 and 21.7 dSm^{-1}) at booting stage. A total of three salinity treatments of saline water and 7 barley genotypes were arranged in a randomized complete design with four replicates of each treatment. Growth and yield measurements were taken at maturity. The result indicated that plant growth traits were reduced when irrigated with saline water. Considerable variation for shoot and above ground biomass was found within genotypes, with some genotypes being able to maintain significant growth and yield under salinity stress, whereas others genotypes showed significant decrease in plant growth and yield traits. Genotypes such as Rayhab3, California, and Neboula1 had the best yield under saline condition, while genotypes include Wadi Mymon, Wadi Zart, and Beecher were salt sensitive genotypes. This study shows that the most salt-tolerant barley genotypes were those that contain both higher harvest index and higher gain yield. These differences may distinguish the extent of the salinity tolerance between genotypes. These findings should be investigated in more detail to be used in the breeding programs aimed to the selection of barley salinity tolerance genotypes.

Key words: *Hordeum vulgare* L, salinity, grain yield, booting stage,

Introduction

Salinity is one of the most severe stresses, endangering crop yields worldwide (Shahbaz and Ashraf, 2013; Gupta and Huang, .2014). The global climate change is further increasing the area of salt effected soils, particularly in dry areas of the world. Worldwide, more than 20% of the world's agricultural land and 33% of irrigated land are affected by salinity; this number is increasing every day (Flowers, 2004; Ismail and Horie, 2017; Machado and Serralheiro, 2017). And more than 40% of soils in the Mediterranean basin are salt affect soils (Nedjimi, 2014). Salinity is one of the major abiotic stresses, which affect productivity of agricultural lands. Generally, high salinity has a negative effect on plant growth, causing decreases in plant productivity (Munns, 2005; Rengasamy, 2006; Munns and Tester, 2008; Machado and Serralheiro, 2017; Ehtaiwesh and Rashed, 2020). High levels of salts in the soil reduce the ability of plant root cells to absorb water, and high levels of salts inside a plant lead to toxicity (Munns and Termaat, 1986; de Oliveira et al, 2013; Yadav et al., 2019; Ehtaiwesh and Abuiflayjah, 2020).

Barley (*Hordeum vulgare* L.) is ranked fourth among the worldwide production of cereals after rice, wheat and maize (Singh et al, 2014; Taddese et al., 2019). Worldwide barley used as animal feed, human food and industrial use (Druka et al., 2011). The world population is increasing rapidly and is projected to reach about 8 billion by 2024 (United Nations, 2019). Barley is adapted to different conditions and tolerant to several abiotic stresses, such as temperature, salinity, and drought (Maas and Hoffman, 1997; Goyal and Ahmed, 2012; Ferreira et al,. 2016). However; barley is considered to be a moderately salinity tolerant crop (Ullrich, 2002; Munns et al., 2006, Hassan et al., 2021). Though it shows significant variation over genotypes in salinity tolerance (Slavich et al., 1990; Jaradat et al., 2004). Some studies show that higher salt tolerance of some barley genotypes is attributed to less Na^+ accumulation, stronger anti-oxidative capacity and strong enzyme activity (Jabeen et al.., 2020, Thabet et al., 2021, Ouertani et al., 2022).

In Libya barley is used as food resources for both human and animal and is one of the most commonly cultivated cereals after wheat (FAO, 2019). It represents one of the most important cereals cultivated in many regions of the country. However; due to the arid conditions in the country, irrigation is becoming a common practice to supply water to crops (Abagandura and Park, 2016). In many cases salinity stress may occurs during booting and may extend to flowering and up to grain filling, when there is not enough rain fall, which affects the spike umber, number of seeds per spike and grain weight (Sallam et al, 2019). Evaluation of genetic variety levels and salinity stress tolerance of crops is important for developing effective breeding programs to enhance crop productivity (El-Esawi et al, 2018, Oubaidou, et al., 2021). Screening large numbers of genotypes for salinity tolerance in the field is difficult due to different physiological mechanisms of salinity tolerance, different phenotypic responses of plants at different growth stages and due to spatial heterogeneity of soil chemical and physical properties (Arzani, 2008). Crops salinity tolerance vary with their growth stage (Maas and Poss, 1989; Mass and Grieve, 1994; Ehtaiwesh, 2022b). And also; plant species differ in their salt tolerance (Kingsbury and Epstein, 1984). Therefore, there is an urgent need to develop an appropriate technique for the screening of barley genotypes for salt tolerance. The evaluation of salinity tolerance of barley genotypes will also improve the understanding of mechanisms of salt tolerance in this crop and allow the development of selection criteria of direct value to the breeders. Breeders often use indirect selection and use well-correlated traits with the yield to improve grain yield in under abiotic stress (Sallam et al, 2014; 2021 ،راشد و احتيوش). These yield traits include plant height, days to heading, days to maturity, spike length, number of spikelet spike^{-1}, and number of grains spike^{-1}, thousand grain weight, grain yield spike^{-1}, grain yield, dry biomass, and harvest index (Sallam et al, 2019). In addition there are two common ways for evaluating stress tolerance by sowing the selected genotypes under common and stressed condition; (1) estimate of the reduction in a trait due to stress for each genotype, and (2) Drought susceptibility index (Sallam et al, 2019). The identification of selection criteria will be a step towards the urgent goal of developing barley varieties with a better ability to grow and produce grain in places where barley is grown inefficiently. Improvement of grain yield in barley is an important objective in breeding program. Therefore, the assessment of final grain yield and other yield related components determining grain yield is an important feature of breeding programs. Therefore; the present study was conducted to screen different genotypes of barley for their growth and grain yield performance and tolerance to salinity under different saline environment.

Materials and Methods

To evalute Libyan barley genotypes for salinity tolerance, a field experiment was conducted at Jodaam farm in Zawia, Libya, during the fall season 2018, and lab work was done at plant science department, University of Zawia.

Plant Material

Seeds of 7 Libyan barley genotypes were used in this experiment include: (Wadi Mymon, Rayhan3, Wadi Zart, California, Neboula1, Beecher, and Wadi Alhay), these genotypes obtained from Misurata agricultural research station and from Libyan national gen-bank.

Experimental and treatment conditions

The soil at the experiment location is a sandy loam soil and experimental field had not been cultivated for the last two years. The plot size was 2 m × 1.5 m, containing 6 rows, each 15 cm apart. Approximately 20 seeds (at 5 cm spacing and 2 cm depth) were hand-sowing per row. Experimental plots arranged in a randomized complete design (RCD) with three replications. Control treatment 0mM NaCl and two other treatments 150, and 200mM NaCl (EC value of < 1.5, 15.9 and 21.7 dSm^{-1}) respectively were applied. The electrical conductivity of its saturated extract (ECe) is 1.5 $dS.m^{-1}$. 7 days before sowing, fertilization with phosphorus (P) was applied at a rate of 6.5 g P2O5 per plot. Granular urea nitrogen (N) fertilizer was applied once at a rate of 7.5 g urea per plot at 46% N), three weeks after planting. An application of NPK fertilizer (20-19-19) at a rate of 6g per plot was also made at four, six and ten weeks after planting. The plots were irrigated twice per week. Control plots were irrigated with water of 1.5 dS.m−1 from sown till harvesting. Saline plots also were irrigated with 1.5 dS.m−1 water until booting stage. Around booting stage, treated plants were irrigation with saline water of 150 and 200mM NaCl (15.9 $dS.m^{-1}$ and 21.7 $dS.m^{-1}$ respectively. The irrigation with saline water las for 3 weeks, and then all plants (the control and two saline plots) were irrigated with water of 1.5 $dS.m^{-1}$ until plants get to physiological maturity. At and after harvesting, the growth and yield traits were measured under control and saline conditions.

Data collection

At maturity, four plants from each replicate were hand-harvested by cutting them at the soil level. Data on plant height, number of tillers $plant^{-1}$, number of spike $plant^{-1}$ were recorded at the day of harvesting. Plant height was determined as the distance between bases of the plant to the tip of the main stem spike including awns. Tiller number $plant^{-1}$ contained both fertile (with spikes) and non-fertile tillers (without spike). After drying for 3 d, the main spikes were hand threshed to separate grains, and grain number per spike was counted manually. Grain yield for main spike and per plant were calculated and 1000 grain weight was calculated. Harvest index was calculated as the ratio of grain yield to the total vegetative dry weight for each plant.

Experiment design and data analysis:

The experimental design was a randomized complete design (RCD) with four replications. Salinity was the main plot factor (three levels 0, 150, 200 mM NaCl), genotype was assigned to sub plots. Data were analyzed using GLM procedure in statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for mean and standard error estimation. Separation of means was carried out using the least significant differences (LSD; $P < 0.05$).

Results

The P-values for growth and yield traits obtained with SAS GLM procedure are presented in Table 1. The independent effects of salinity, was significant (P < .0001) for all studied traits. Likewise, the independent effect of genotype was significant (P < .0001) for tiller number, spike number, grain number plant, grain yield plant, dry weight and harvest index. The independent effect of genotype was significant (P < 0.001) for plant height, grain number spike, grain yield spike and 1000 grain weight. The interaction effects of salinity x genotypes were significant (P < 0.05) for all traits included in the study (Table 1).

Table 1. Probability values of the effects of salinity (S), genotype (G), and S x G interaction on various growth and yield traits of barley.

Traits	Salinity (S)	Genotype (G)	S x G
Plant height (cm)	<.0001	0.0020	0.0492
Tiller number plant^{-1}	<.0001	<.0001	0.0481
Spike number plant^{-1}	<.0001	<.0001	0.0473
Grain number spike^{-1}	<.0001	0.0012	0.0445
Grain number plant^{-1}	<.0001	<.0001	0.0384
Grain yield spike^{-1} (g)	<.0001	0.0011	0.0496
Grain yield plant^{-1} (g)	<.0001	<.0001	0.0325
1000 grain weight (g)	<.0001	0.0022	0.0342
Dry weight plant^{-1} (g)	<.0001	<.0001	0.0498
Harvest index (%)	<.0001	<.0001	0.0335

Results of the effect of salinity on growth and yield traits are presented in (Table 2). All growth and yield parameters decreased under saline condition (150 and 200mM NaCl). The data on table 2 clearly showed that sodium chloride solution has a clear inhibitory effect on the growth of some barley genotypes. Low concentrations of sodium chloride solution (150mmM NaCl) have low osmotic potential slightly effected growth and traits of some barley genotypes, while with an increase in the concentration of sodium chloride (200mmM NaCl), the osmotic potential of the solution increased and therefore most growth and yield traits of some barley genotypes significantly decreased.

Table 2. The effect of salinity on various growth and yield traits of 7 barley genotypes. Data are averaged across 7 genotypes, 4 replications of each genotype and 3 salinity levels. Means was estimated using the GLM procedure in SAS.

Traits	0mM NaCl	150mM NaCl	200mM NaCl
Plant height (cm)	71.3^{a}	63.1^{b}	52.5^{c}
Tiller number $plant^{-1}$	6.9^{a}	5.3^{b}	4.5^{c}
Spike number $plant^{-1}$	6^{a}	4.8^{b}	3.6^{c}
Grain number $spike^{-1}$	40.4^{a}	32.8^{b}	27.5^{c}
Grain number $plant^{-1}$	246^{a}	158^{b}	102^{c}
Grain yield $spike^{-1}$ (g)	1.7^{a}	1.3^{b}	1.1^{c}
Grain yield $plant^{-1}$ (g)	10.3^{a}	6.3^{b}	4^{c}
1000 grain weight (g)	43.2^{a}	34.5^{b}	22.2^{c}
Dry weight $plant^{-1}$ (g)	19.6^{a}	15.5^{b}	13.5^{c}
Harvest index (%)	0.52^{a*}	$0..40^{c}$	0.29^{d}

[*] Individual value is the mean of 7 genotypes under different salinity levels. Values followed by different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

Also, the result showed that the genotypes of the same species differ greatly in their growth under salinity stress conditions, so some barley genotypes grow rather well in saline conditions, while others have poor growth, which affecting final yield. It was noted from Figure (1A) that plant height was negatively and significantly affected with the increase in salinity concentration in most barley genotypes. However, the cultivar (Rayhan3) achieved the highest rate of plant height, as it was associated with the lowest percentage decrease (19%), while the cultivar (Wadi Zart) recorded the highest decrease (34%) at the concentration (200mM NaCl).

Also, table (2) showed that both salinity levels (150 and 200mM NaCl) have led to a decrease in plant dry weight compared with the control (0mM NaCl). The results also showed that there was a significant difference between the cultivars in the extent to which the plant dry weight was affected by the salinity of the irrigation water. As shown in figure (1B) the genotypes showed a difference in the extent of their response to salinity, while the cultivar Rayhan3 recorded the least decrease (28%), the cultivar (Wadi Zart) recorded the highest decrease (37%) at the concentration of 200mM NaCl compared to the control 0mM NaCl.

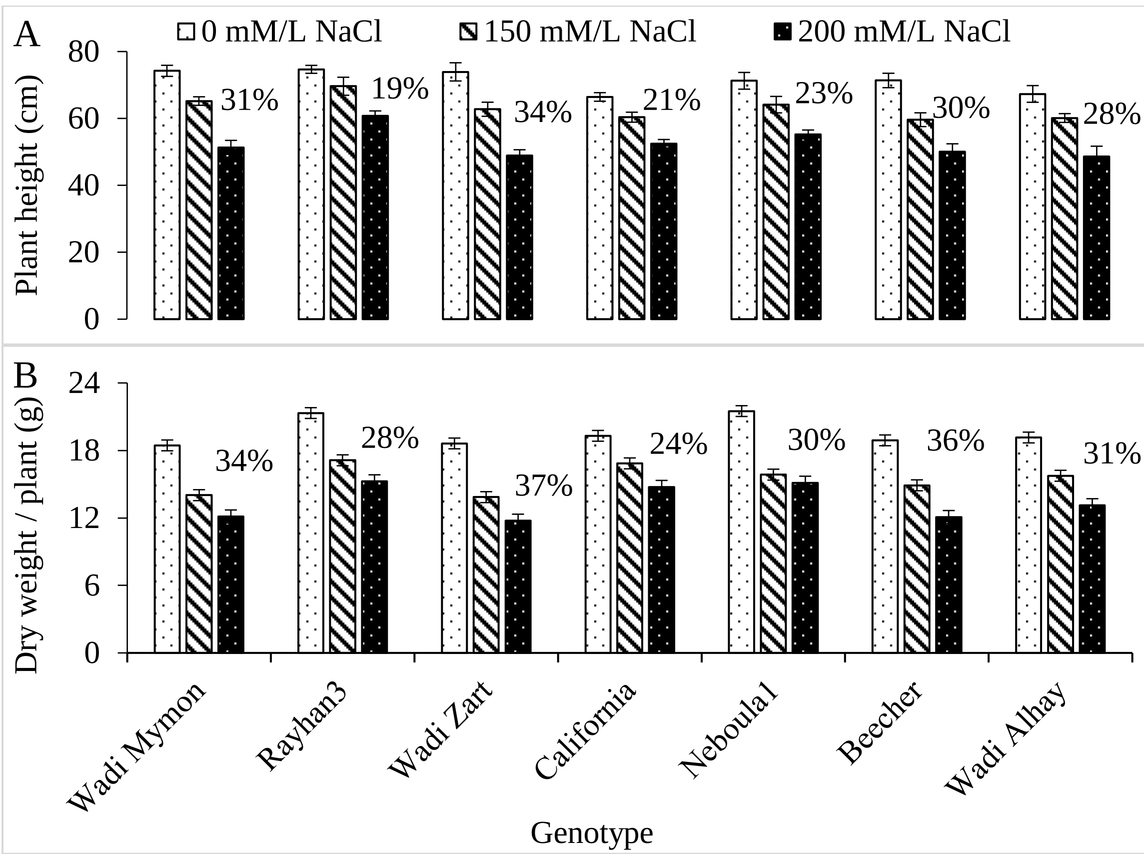

Fig. 1: Effect of salinity on (A) plant hight (cm) and (B) total dry weight plant^{-1} (g) of 7 barley genotypes.Values shown are mean ± SE. Values in parenthesis indicates the percent differences from 0mM NaCl to 200mM NaCl salinity treatment.

Correspondingly, salinity stress led to a significant decrease in tillers number of some barley genotypes. The number of tillers for barley plants were reduced when the salinity stress was increased (150 and 200mM NaCl). Yet the results showed that there was a difference in the response of different cultivars of barley to salinity stress, as the most affected genotype by salinity was the cultivar (Wadi Mymon), which recorded the most decrease in tillers number plant^{-1}, which recorded with 44% decline, while the most tolerant cultivar was Rayhan3 cultivar where the percentage of decline was recorded about 26% (Fig. 2A).

With regard to the number of spikes plant^{-1}, treating barley genotypes with saline solution led to a significant decrease in the number of spikes plant^{-1}, as the shown in table2 the number of spikes plant-1 were 3.6 spikes plant^{-1} under the high concentration of salinity (200mM aCl) compared to the control treatment (0mM NaCl). mol) which were 7 spikes plant^{-1} (Table 2). The results also indicted that barley genotypes responded differently to salinity stress, so the results pointed out that the most affected genotype by salinity stress was the cultivar (Wadi Zart), which recorded the most decrease in the number of spikes plant^{-1} with 55% decrease, while the most tolerant cultivar was Neboula1 cultivar where the percentage of decrease was recorded about 30% (Fig. 2A).

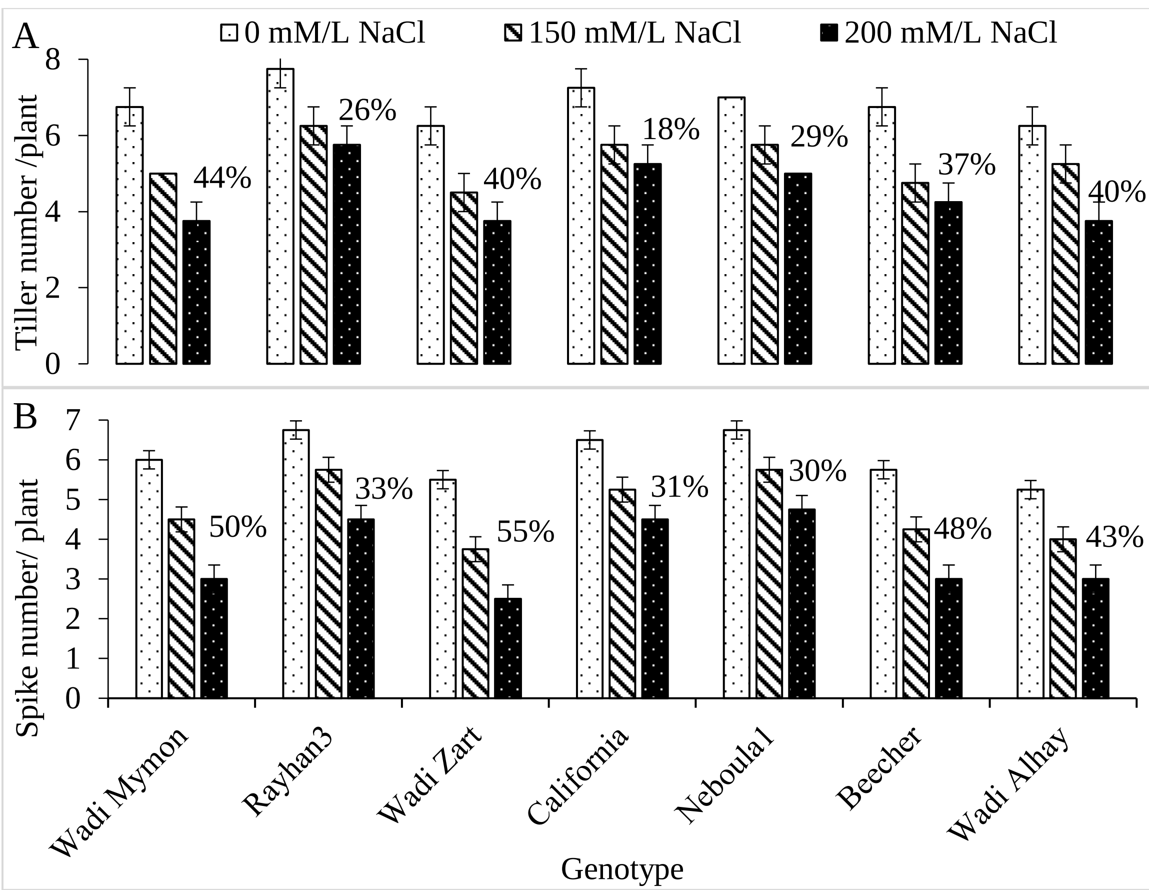

Figure 2: Effect of salinity on (A) tiller number plant $^{-1}$ and (B) spike number plant $^{-1}$ of 7 barley genotypes. Values shown are mean ± SE. Values in parenthesis indicates the percent differences from 0mM NaCl to 200mM NaCl salinity treatment.

In addition, the results in table 2 showed that the treatment of plants with saline solution resulted in a significant decrease in grain number, and this decrease is directly proportional to the level of salinity stress. The grain number spike^{-1} of barley genotypes that treated with both level of salinity (150 and 200mM NaCl) were 32.8 and 27.5 grains spike^{-1} respectively, while the number of grains spike^{-1} was 40.4 grains in the control treatment (0mM NaCl). Nevertheless, barley genotypes showed different response to salinity stress. The result in figure 3A showed that some barley genotypes was more tolerant to salinity stress at high level of salinity 200m NaCl, these genotypes include Rayhan3, Neboula1 and California showed %24, %25, %26, reduction over the control respectively.

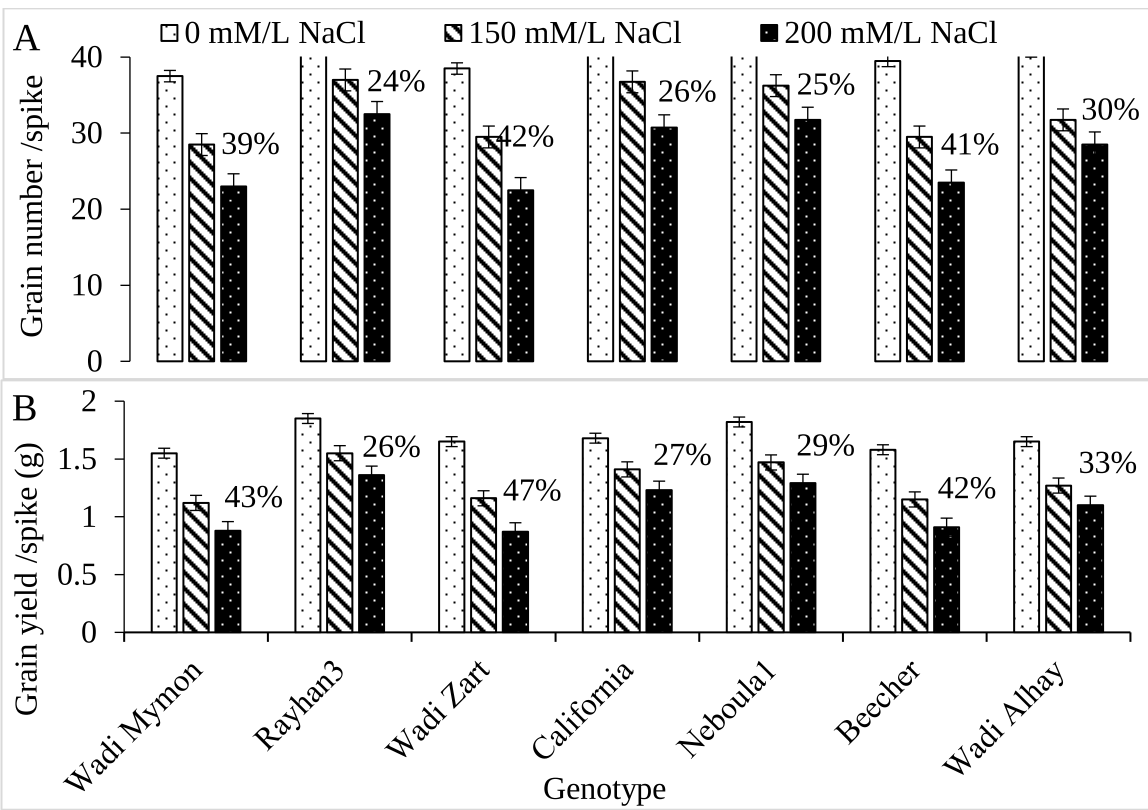

Figure3: Effect of salinity on (A) grain number spike^{1} and (B) grain yield spike^{-1} (g) of 7 barley genotypes.Values shown are mean ± SE. Values in parenthesis indicates the percent differences from 0mM NaCl to 200mM NaCl salinity treatment.

Moreover, the effect of salinity stress was similar on grain yield spike^{-1}. The average of grain yield spike^{-1} under salinity conditions 150 and 200mM NaCl were 1.1 and 1.3 grams respectively, while the average of grain yield spike^{-1} in the control was 1.7 grams table 2. Besides barley genotypes showed diverse response to salinity stress, Figure 3B showed a percent reduction over the control in all barley genotypes due to salinity stress. The results showed that genotypes Rayhan3, California and Neboula1 had the lowest reduction of grain yield spike^{-1} which were about 26%, 27% and 29% respectively, whereas other genotypes such as Wadi Zart, Wadi Mymon, and Beecher had reduction of 47%, 43% and 42% respectively.

Additionally 1000 grain weight of barley was significantly ($P < 0.001$) affected by salinity stress table 1. The mean value of 1000 grain weight at non-saline condition was 43.2g, however, this value was significantly decreased by both salinity levels 150 and 200mM NaCl to 34.5g and 22.2g respectively table 2 which indicated decreased 1000 grain weight due to salinity stress. Further, genotypes were significantly different for 1000 grain weight. The values of 1000 grain weight at high salinity level 200mM NaCl ranged between 17-28g. The result showed that genotypes Rayhan3, Neboula1 and California had the lowest reduction over the control for 1000 grain weight (Fig. 4A).

In regarding to harvest index trait, table 2 showed that as the level of salinity stress increased, harvest index of barley plants decreased as a result of the decrease in both plant dry weight and grain yield. The results showed that the salt stress had a stronger effect on the harvest index. Figure 4B showed that the genotypes recorded a variance in their response to salinity, as the cultivar Wadi Zart recorded the highest percentage of decline 62% compared to the control treatment. The lowest percentage of decline was recorded by the cultivar Neboula1, which was (29%) compared to the control.

Discussion

Barley (*Hordeum vulgare* L.) represents one of the most important crop cultured worldwide. The aims of the study were to evaluate the effects of salinity stress on growth and yield of some Libyan barley genotypes, and to screen those genotypes for salinity tolerance. Salinity is well-known to reduce the growth and yield of most crop plants. The extent of this effect is interrelated to the level of salinity, the plant species sensitivity, and the environment (Tsonev et al., 1998). The results of the present study displayed the adverse effect of salinity and found that salinity stress decreased growth and yield traits of barley as shown in Table 2. This finding agreed with some early studies on different crops such as wheat (Ehtaiwesh, 2016; EL Sabagh et al., 2021), Pea (*Pisum sativum*

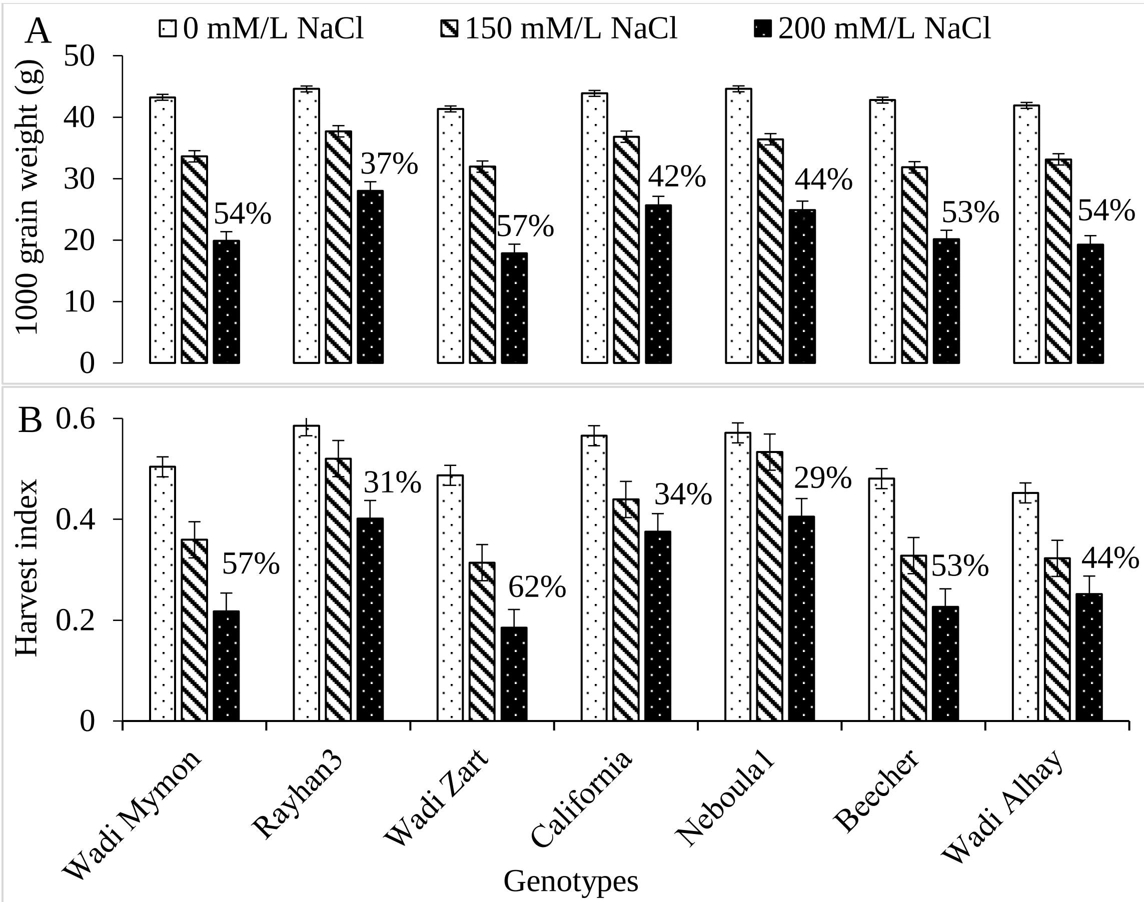

Figure 4: Effect of salinity on (A) 1000 grains weight (g) and (B)) harvest index (%) of 7 barley genotypes.Values shown are mean ± SE. Values in parenthesis indicates the percent differences from 0mM NaCl to 200mM NaCl salinity treatment.

L) (Ehtaiwesh and Emsahel, 2020; Yousef et al., 2020), faba beans (Bimurzayev et al., 2021; Ehtaiwesh, 2022), beans (*Phaseolus vulgaris*) (Ehtaiwesh and Abuiflayjah, 2020; Al-huraby and Bafeel, 2022), and barley (Sabagh et al., 2019). However the study suggest that some barley genotypes exhibited better performance than others. Several investigators in many researchers reported that many plant genotypes within the same species responded differently to abiotic stress such as: the response of winter wheat to high temperature and salinity stress (Ehtaiwesh, 2016; Ehtaiwesh and Rashed, 2020), the response of barley to drought (Harb et al., 2020; Hebbache et al., 2021), and response of winter and spring wheat to salinity (Ehtaiwesh, 2019). Salinity stress effecting plant productivity because it causes osmotic stress and ion toxicity, through increasing the assimilation of Na+ ion and so decreasing the Na+/K+ ratio due to lower osmotic potential within the plant roots, (EL Sabagh et al., 2021). Also, plants growing in saline condition may exposed to suffer from drought stress which may lead to reduced growth and productivity. Plants employ a several physiological and biochemical mechanisms as adaptation strategies to mitigate the adverse effects of stress (Sabagh et al., 2019). The results herein showed that the high concentrations of salinity had a strong negative effect on growth and yield of some barley genotypes, and the treatment with high salinity (200mM NaCl) led to a significant decrease in the studied trait compared to the control (0mM NaCl), The negative effect of salinity on plant growth could be due to a decrease in the cell division process, as well as a decrease in cell size due to a decrease in the turgor pressure of plant. In barley, yield is determined by yield components which include number of spikes, grain number and grains weight. This study found that grain yield was decreased as salinity level increased. Several studies agreed with this founding and they have indicated that an increase in the concentration of salts in the growth medium led to a decreased spikes number plant$^{-1,}$ grains number spike^{-1}, and 1000 grains weight, which resulted in decrease in grain yield (Pakar et al., 2016; Alkharabsheh et al., 2021). Furthermore; harvest index which is an important traits of plant yield and had very significant relationship with grain yield and plant dry biomass. In this study, a significant decrease of grain yield and harvest index of barley genotypes was noted with increased salinity level. These results analogous with the results reported from other studies, which reported that grain yield and harvest index were reduced under salinity stress (Harris et al., 2010; Ehtaiwesh and Rashed, 2020). This study also found that some genotypes were able to perform well under salinity stress by having high grain yield as well as high harvest index. This outcome agreed with prior studies in wheat (Goudarzi and Pakniyat, 2008; Ehtaiwesh, 2016), barley (Tavakkoli et al., 2011; Ahmed et al., 2013), and triticale (Mohammadi Kale Sarlou et al., 2022).

Conclusion

Field experiments were conducted to investigate genotypic differences in response to salinity between barley genotypes. In nature plants are exposed to a variety of abiotic stresses and reveal deferent responses to these stresses. Tolerance to abiotic stresses such as salt stress is an important breeding objective. In this study the responses of 7 barley genotypes was evaluated under field non-saline and saline conditions using salty irrigation water. All barley genotypes exposed to 150 and 200mM NaCl. The study found that salinity stress highly influenced growth and yield traits of some barley genotypes. Interestingly, the study revealed that barley genotypes exhibited different response under saline condition, where some barley genotypes performed well under saline treatments. In conclusion, investigating genetic variability of barley is important for enhancing the crop productivity. We therefore recommend that in the case of limited possibility of irrigation by non-saline water some barley genotypes maybe irrigated with low to moderate salinity level without a significant decrease in product of the grain yield.

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