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Screening Barley Hordeum vulgare L Genotypes for Drought Tolerance

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Abstract
Barley (*Hordeum vulgare* L.) is one of the oldest domesticated crops that is cultivated globally. However, drought is one of the major limitations to barley production in arid and semi- arid regions, so the development of drought tolerant genotypes is an issue of global concern to ensure food security. The present study was conducted to investigate barley genotypes in response to drought stress to find out the tolerant genotypes. Therefore, eight barley genotypes include (Acsad 176, Wadi Mymon, Rayhan3, Wadi Zart, California, Neboula1, Beecher, and Wadi Alhay) were subjected to drought stress at heading stage. A filed experiment was conducted in randomized complete design (RCD) with three replications. Three different treatments were applied which were: control (normal condition), moderate drought stress and severe drought stress). The drought stress was initiated by withholding of water for 2 and 3 weeks for moderate and severe drought stress respectively. The result showed that all growth and yield trait were significantly (P<.001) altered in all genotypes under severe drought stress conditions. However, spike number plant-1, grain number spike-1 grain yield and harvest index, were significantly higher in California, Acsad and Rayhan3 genotypes than those in the rest of barley genotypes included in this study, particularly under severe drought stress conditions. Overall, the interaction between drought stress and genotypes was significant (p<0.05), as some genotypes were achieved higher grain yield under severe drought stress. These genotypes may labeled as drought tolerant genotypes and may suggested for cultivation in drought stress regions.
Keywords: Barley *Hordeum vulgare* L, drought stress, drought tolerance, growth, yield.

1. Introduction

Climate changes may cause gradually severe and frequent drought and may result in intensifying the abiotic stress on agricultural productivity worldwide. In North Africa region farming is the main income for many families However; many limitations are facing farming in dry and warm regions such that in North Africa. Farming needs adequate water and good soil conditions, these are the most two factors effecting crop production in this region (soil salinity and water shortage). Dehydration stress caused by drought is the most widespread abiotic stress that limits plant growth and productivity. In cases where plants do not obtain adequate rainfall or irrigation for a period, drought stress causes growth and yield reduction more than all other environmental stresses combined (Cattivelli et al. 2008). Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration and evaporation. Drought stress effected plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, and nutrient metabolism (Hellal et al., 2018; Ferioun et al., 2023). In addition, drought strongly affects plant phenology by shortening the plant growth life cycle. At early growth stage, water stress inhibits cell elongation; therefore, it results in reduced leaf area and short stems, which in turn affect dry matter production (Mitchell et al., 1996; González et al., 2007; Liu et al., 2020; Fernández-Calleja et al., 2021). It also inhibits cell expansion and cell growth because of the low turgor pressure (Yasseen and Al-Maamari, 1995; Hellal et al., 2018; Abd El-Samad et al., 2019). Due to drought, soil water potential decrease and water movement from the soil to plant root is reduced. This causes a reduction in cell pressure potential and increase in cell osmotic potential, and disorders in the cell membrane structure and composition. Many studies showed that the most affected process in plant metabolism is photosynthesis (Ashraf et al., 2006; Ghotbi-Ravandi et al., 2014; Daszkowska-Golec et al., 2019). Plant photosynthesis decreases with the reduction in the relative water content and leaf water potential (Chaves 1991; Horobets et al., 2021). The reduction in photosynthesis rate under drought stress is mostly due to stomata closure and reduced chlorophyll content of leaves (Hasanuzzaman et al., 2022; Lv et al., 2023). Also some studies reported that photosynthesis reduction under drought stress is due oxidative damage of the chloroplast and decreases activity of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Zhou et al. 2007; Jedmowski et al., 2014; Skowron and Trojak, 2021). In addition, drought stress induces synthesis of some harmful compounds such as reactive oxygen species (ROS) like super oxides and peroxides, which damage membranes and causing cellular damage (Carvalho 2008; Zhanassova at al., 2021; Islam et al., 2023).

Plants have developed mechanisms to cope with and adapt to different types of abiotic stress. They have different strategies to deal with drought stress that commonly involve a combination of morpho-physiological and biochemical traits of stress tolerance (Fang and Xiong, 2015Fatemi et al., 2022). The response of plants to drought stress differs with various plant species and plant growth stages and depending upon intensity and duration of stress (Blum, 1996; Laxa et al., 2019; Seleiman et al., 2021). Drought tolerance is defined as a plant's ability to extract water from the environ, maintain that water, and use the water in processes of producing chemical energy from light energy to sythesize plant tissues, in developing reproductive organs (Elakhdar et al., 2022). In general, under drought stress plants undergo some physiological, biochemical, anatomical, and morphological changes to overcome the stress. Major tolerance mechanisms that plant employ: changes in membrane lipid composition, ion transporters, proteins, and antioxidants (Srivastava el al. 2012).

Barley (*Hordeum vulgare* L.) is the second most cultivated cereal in Libya and used for consumption and for animal feeding. However, its production is limited due to some environment factors such as heat, salinity and drought. Thus, the selection of drought tolerant barley genotypes is the best strategy to ensure the security of barley production. In the field, crops may encounter drought stress at any growth stage. Therefore, genotypes are better be tested for their drought tolerance at appropriate and different growth stages because some genotypes could be tolerate drought at germination, but these may be very sensitive to drought at the other stages (Sallam et al., 2019). In barley, heading and grain filling stages of plant growth and development are susceptible to drought stress (Appiah

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et al., 2023). Heading stage which defined as the head emergence from flag leaf, is considered a key growing stage because it associated with adaptation to the environment and an element of grain yield (Alqudah and Schnurbusch, 2017; Cammarano et al., 2021). Reducing the exposure to drought stress throughout susceptible crop stages requires selection of plant drought tolerant genotypes (Appiah et al., 2023). There for this work aimed to evaluate ad screen eight Libya barley genotypes for drought tolerant.

2. Materials and Methods

Field experiment was conducted at Jodaam farm in Zawia, Libya, during the fall-winter season 2018 to evaluate 8 Libyan barley genotypes for drought toleran

2. 1.Plant Material

Seeds of eight Libyan barley genotypes were used in this experiment include (Acsad 176, Wadi Mymon, Rayhan3, Wadi Zart, California, Neboula1, Beecher, and Wadi Alhay), genotypes obtained from Misurata agricultural research station and from Libyan national gen-bank in Tripoli.

2. 2.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 × 2 m, containing 8 rows, each 20 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 (irrigated twice a week) and two other drought treatments, which were moderate drought stress and severe drought stress that were initiated by withholding water for 2 and 3 weeks respectively.

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 for 15 minutes each time. Control plots were irrigated with water of 1.5 dS.m−1 from sown until harvesting. Drought stress plots also were irrigated with 1.5 dS.m−1 water until heading stage. When barley plants reached the heading stage, treated plots were subjected to a drought stress treatments and the control plot was maintained at optimal water conditions. Moderate and severe drought stress were applied by withholding water for 2 and 3 weeks respectively. After that all plants (the control and the two drought stress plots) were maintained at optimal water conditions until plants get to physiological maturity.

2. 3.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}, spike length 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

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plant^{-1} contained both fertile (with spikes) and non-fertile tillers (without spike). Vegetative dry weight was determined as the weight of leaves, stems, and spikes per plant. 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.

2. 4. **Experiment design and data analysis:**

The experimental design was a randomized complete block design (RCBD) with three replications. Drought stress was the main plot factor (control, moderate and severe drought stress), genotype was assigned to sub plots. Data were analyzed using GLM procedure in SPSS program for mean and standard error estimation. Separation of means was carried out using the least significant differences (LSD; $P < 0.05$).

3. Results

Table 1 showed the p- value of drought (D), genotypes (G) and the interaction of D and G. Highly significant (P<.001) differences were found among the 8 barley genotypes under both optimum and drought stress conditions for all traits. The interaction between drought and genotypes was significant (P<.05) for all traits inclouded in this study.

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

Traits	Drought (D)	Genotype (G)	D x G
Plant height (cm)	<.001	<.001	0.044
Tiller number plant^{-1}	<.001	<.001	0.049
Spike number plant^{-1}	<.001	<.001	0.035
Spike length (cm)	<.001	<.001	0.047
Grain number spike^{-1}	<.001	<.001	0.041
Grain number plant^{-1}	<.001	<.001	0.024
Grain yield spike^{-1} (g)	<.001	<.001	0.046
Grain yield plant^{-1} (g)	<.001	<.001	0.029
1000 grain weight (g)	<.001	<.001	0.038
Dry weight plant^{-1} (g)	<.001	<.001	0.040
Harvest index (%)	<.001	<.001	0.049

The result in table 2 represented the effect of drought on various growth and yield traits of eight barley genotypes. The results shown in table2 confirm the results of table1. Significant effect of drought stress on all traits included in this study. The results indicated that drought stress reduced all growth and yield traits of barley genotypes compared to the control.

Table 2. The effect of drought on various growth and yield traits of eight barley genotypes. Data are averaged across eight genotypes ad 4 replications each. Means was estimated using the GLM procedure in SPSS.

Traits	Control	Moderate drought	Severe drought
Plant height (cm)	68.6[a]	65.1[b]	50.5[c]
Tiller number plant^{-1}	7.4[a]	6.6[b]	5[c]
Spike number plant^{-1}	6.1[a]	5.8[b]	3.8[c]
Spike length (cm)	8.4[a]	7.8[b]	5.8[c]
Grain number spike^{-1}	47[a]	39[b]	32[c]
Grain number plant^{-1}	287[a]	229[b]	124[c]
Grain yield spike^{-1} (g)	2.3[a]	1.7[b]	1.0[c]
Grain yield plant^{-1} (g)	14[a]	10[b]	4[c]
1000 grain weight (g)	9.3[a]	43.5[a]	32.7[b]
Dry weight plant^{-1} (g)	24.5[a]	19.7[b]	14.0[c]
Harvest index (%)	0.58[a*]	0..50[c]	0.28[d]

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

Table 3 showed the performance for each barley genotype under optimum and drought stress. The result indicted that amongst all the eight genotypes included in this study, the genotypes California had the highest values for all traits excluding plant height trait. Also the genotype Acsad, recorded as a second barley genotype that achieved the highest values for all traits excluding plant height, spike length (cm), and dry weight plant^{-1} (g) traits, followed by Rayhan3 which had the heights values for plant height, grain number spike^{-1}, Grain yield spike^{-1} (g) and 1000 grain weight (g). At the same, the genotypes such as Wadi Mymon and Wadi Zart recorded the lowest in term of Spike number plant^{-1}, Grain number plant^{-1}, Grain yield plant^{-1} (g), and harvest index whereas the genotype Wadi Allhy had the lowest value for Grain yield spike^{-1} (g) and 1000-grain weight (g). The result therefore consider California, Acsad and Rayhan3 genotypes as drought stress tolerant genotypes whereas Wadi Zart, Wadi Mymon, and Wadi Allhy genotypes as drought stress sensitive genotypes. The result also consider that the other two barley genotypes (Neboula1 and Beecher) as moderately drought tolerant genotypes.

Table 3. The effect of genotypes on various growth and yield traits of eight barley genotypes. Data are averaged across three drought levels and four replications each. Means were estimated using the GLM procedure in SPSS.

Traits	Wadi Mymo	Rayhan 3	Wadi Zart	California	Neboula1	Beecher	Wadi Alhay	Acsad
Plant height (cm)	58.9[c]	**72.6[a]**	59.4[c]	59.5[c]	60.8[bc]	58.4[c]	59.1[c]	62.3[b]
Tiller number plant^{-1}	5.3[c]	7.0[b]	5.3[c]	**8.8[a]**	5.1[c]	5.3[c]	5.1[c]	**8.8[a]**

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Spike number plant^{-1}	4.4^{d}	6.0^{b}	4.4^{d}	**6.4^{a}**	4.5^{cd}	4.8^{c}	4.9^{c}	**6.3^{ab}**
Spike length (cm)	5.9^{c}	9.4^{b}	6.0^{c}	**10.3^{a}**	5.9^{c}	6.0^{c}	5.8^{c}	9.2^{b}
Grain number spike^{-1}	37.5^{b}	**41.4^{a}**	38.0^{b}	**42.6^{a}**	38.3^{b}	37.8^{b}	37.3^{b}	**42.3^{a}**
Grain number plant^{-1}	172^{d}	251^{b}	176^{cd}	**276^{a}**	182^{cd}	190^{c}	191^{c}	**269^{a}**
Grain yield spike^{-1} (g)	1.56^{bc}	**1.85^{a}**	1.63^{b}	**1.92^{a}**	1.56^{bc}	1.59^{bc}	1.49^{c}	**1.90^{a}**
Grain yield plant^{-1} (g)	7.5^{d}	11.5^{b}	7.8^{cd}	**12.7^{a}**	7.7^{cd}	8.3^{cc}	7.9^{cd}	**12.4^{a}**
1000 grain weight (g)	40.2^{bcd}	**44.3^{a}**	41.8^{b}	**44.7^{a}**	39.6^{cd}	41.1^{bc}	38.9^{d}	**44.1^{a}**
Dry weight plant^{-1} (g)	17.5^{c}	21.9^{b}	18.0^{c}	**23.1^{a}**	17.6^{c}	17.5^{c}	17.6^{c}	21.9^{b}
Harvest index (%)	0.39^{d}	0.50^{b}	0.39^{d}	**0.53^{ab}**	0.40^{cd}	0.43^{c}	0.41^{cd}	**0.54^{a}**

* Individual value is the mean of each genotype under three drought levels. Values followed by different letters are significantly different according to Duncan's multiple range test (*$P < 0.05$*).

In addition, the results shown in Fig 1 approves the results of Table1. Significant differences were found between genotypes in optimum and drought stressed conditions. Further, drought stress influenced most of growth and yield traits of some barley genotypes. The result shows that drought stress reduced plant height trait of barley compared to the control for all cultivars studied. However, the genotypes responded differently to drought stress. It appears that 'Rayhan3 genotype was tolerant genotype and less influenced by drought as the plant height was decreased by 18%, yet Wadi Zart genotype was the most influenced genotype where plant height was decreased by about 34% in stressed plants compared to the control Fig 1a. also, the result indicate that tiller number plant-1 was effect under drought stress conditions and genotypes showed different response to the stress, tiller number plant-1 was reduced by 16% in California genotype and by 50% in Wadi Alhay genotype Fig 1b. The same trend was seen in whole plant dry weight, as genotypes illustrated different responses to drought stress, the result showed that dry weight trait decreased by 33% in Rayhan3genotype ad by 47% in Wadi Mymon genotype Fig 1c.

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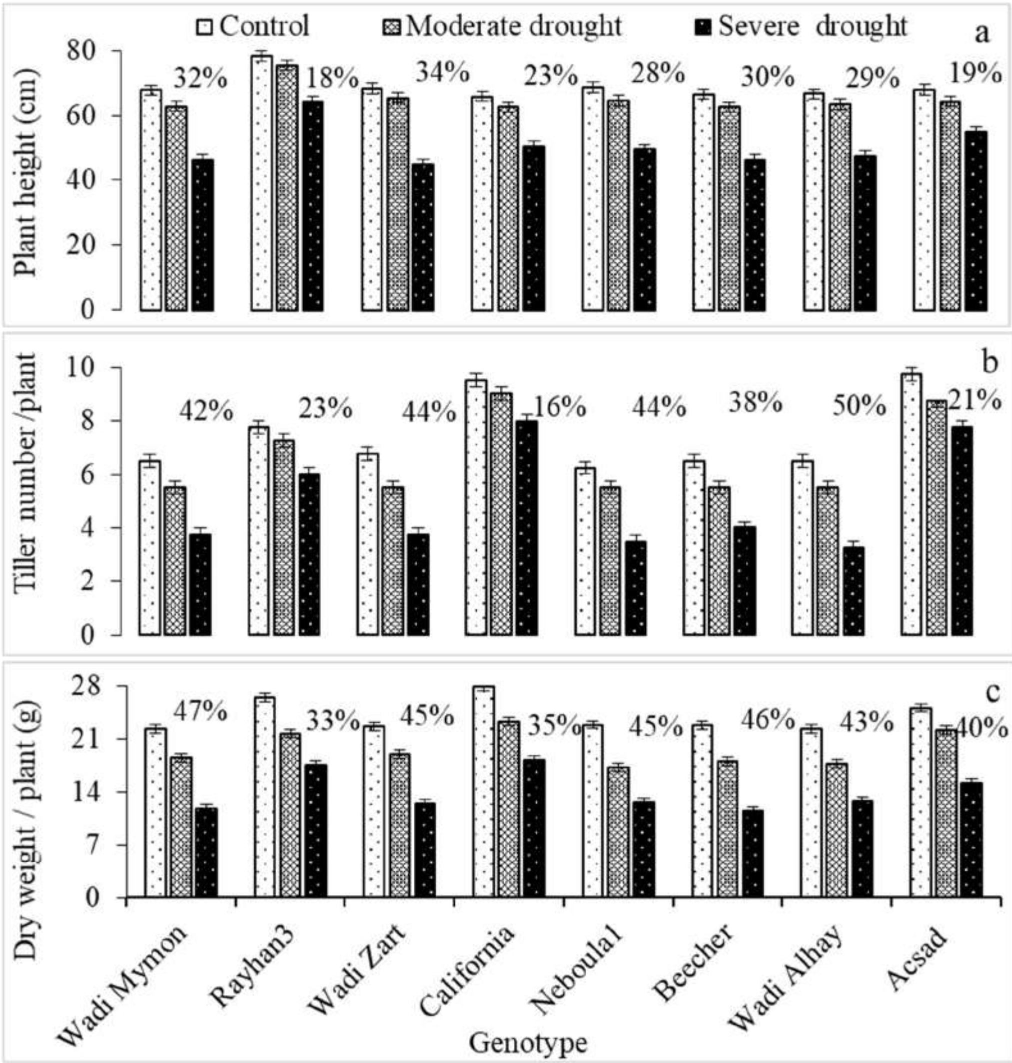

Figure 1: Effect of drought on (A) plant height (cm),) tiller number (plant $^{-1}$) and (C) total dry weight (g plant^{-1}) of 8 barley genotypes. Values shown are mean ± SE. Values in parenthesis indicates the percent differences from control to severe drought treatment.

Moreover, the results indicated that drought stress reduced all yield traits of barley genotypes compared to the control. Although, all genotypes showed a significant decrease of spike length (cm), spike number plant^{-1}, and grain number spike^{-1}, but some genotypes performed better than others (Fig.2). For example, spike length (cm) was decreased by 18% in California genotype and by 45% in Wadi Alhay genotype Fig 2a. Also spike number plant^{-1} was reduced by 26% in Rayhan3 genotype and by 48% in Wadi Zart genotype Fig 2b. Likewise, grain number spike^{-1} was reduced by 22% in California genotype and by 41% in Wadi Zart genotype Fig 2c

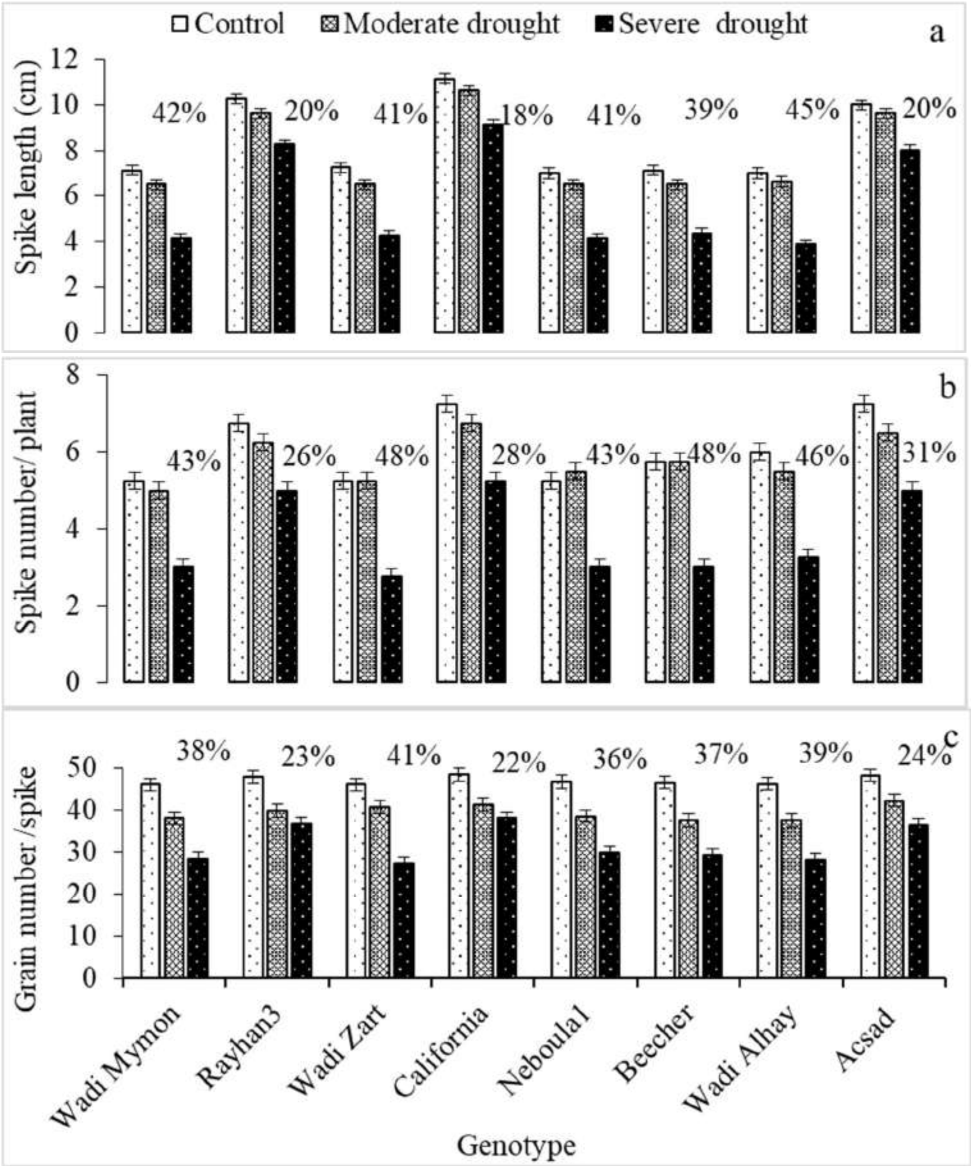

Figure 2: Effect of drought on (A) spike length (cm), (B) spike number (plant $^{-1}$) and (C) grain number (spike $^{-1}$) of 8 barley genotypes. Values shown are mean ± SE. Values in parenthesis indicates the percent differences from control to severe drought treatment.

Regarding other yield traits such as grain yield spike^{-1}, 1000 grains weight (g) and harvest index (%), severe drought stress decreased significantly those traits in all barley genotypes, still the genotypes responded differently to the stress as some genotypes had better performance than others Fig3. Fig3a showed that grain yield spike^{-1} trait was reduced for all barley genotypes, however when compared to control the reduction was higher in some genotypes than others. To illustrate, in Wadi Mymon genotype the reduction reached to 63%, but in California genotype the reduction was 44%. The same result found with 1000 grains weight (g), where genotypes showed different response under severe drought stress as compared to

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control. As shown in Fig. 3b 1000 grain weight trait was reduced by 41% in Wadi Mymon genotype and by 26% in California genotype. Further, the result in Fig. 3c revealed that harvest index (%) trait decreased by 42%, 39% and 39% in Rayhan3, California and Acsad genotypes respectively due to drought stress. The same traits decreased by 63%, 61% and 60 % in Wadi Zart, Wadi Alhay and Wadi Mymone genotypes respectively.

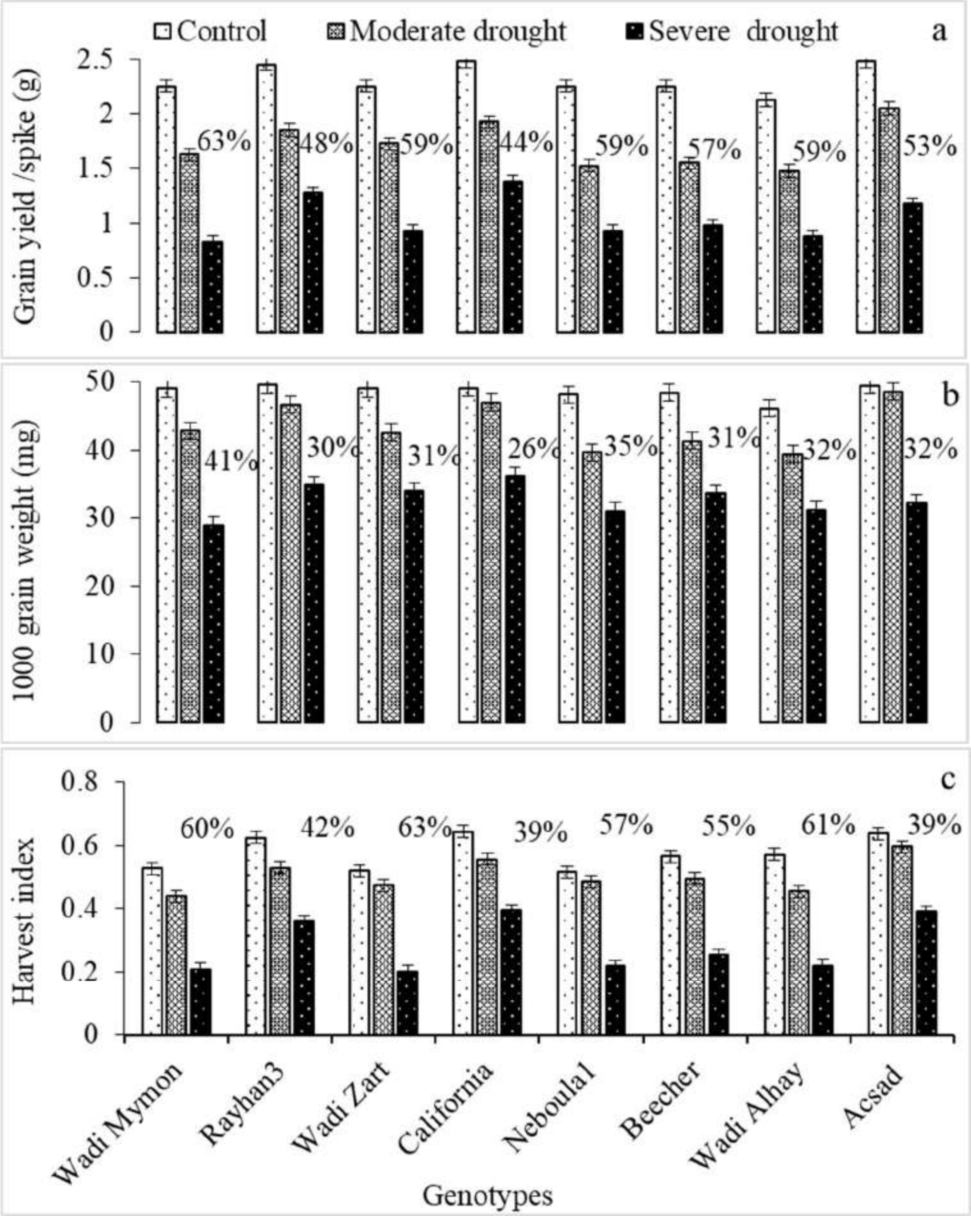

Figure3: Effect of drought on (A) grain yield (g/spike[1]) (B) 1000 grains weight (g) and (C) harvest index (%) of 8 barley genotypes. Values shown are mean ± SE. Values in parenthesis indicates the percent differences from control to severe drought treatment.

4. Discussion

In the field, plants are normally exposed to different abiotic stresses, such as drought, heat, chilling, and freezing. These conditions usually result in dehydration of plants. This study aimed to evaluate the response of eight barley genotypes grown under optimum and drought stress treatments. To develop genotypes with enhanced tolerance to drought and ensure the well-being of the crop under adverse conditions. Drought stress affects plant growth and crops production by reducing water potential, creating osmotic stress and inducing reductions in uptake and translocation of macronutrients, which resulted in reducing growth and yield traits (Sallam et al., 2019). This work indicated significant effects of drought on the measured traits. Some early researches found similar result of the effect of drought in different crops such as barley (Harb et al., 2020; Cai et al., 2020), wheat (Guo et al., 2020; Mohi-Ud-Din et al., 2021), maize (Badr et al., 2020; Saad-Allah et al., 2021), and rice (Auler et al., 2021). The outcome if this study pointed out that drought stress caused a decrease in all growth and yield traits and indicated variability among the eight barley genotypes in response to drought (Table 2 and Figures 1-3). Other studies report similar respective (). Results of the present study highlight that growth traits such as plant height, tiller number and spike length and number plant $^{-1}$ were reduced under severe drought stress. This result is consistent with our previous results (Ghorbanpour et al., 2020). Additionally, the present study indicated that drought stress reduced grain yield, which could be due to decreasing in number of spike plant^{-1}, number of grain spike^{-1} and/or 1000-grain weight. This study confirmed the known impact of drought stress on crop growth and development (Samarah, 2005; Alghabari and Ihsan, 2018; Al-Tawaha et al., 2020). Many researchers reported that plant species and genotypes within the same species exhibit different responses under optimum and stressed condition (Ehtaiwesh, 2016; Wasaya et al., 2021). Such findings recommended that screening of drought-tolerant plant genotypes significantly enhance crop productivity as well as food security. The study herein found that barley genotypes California, Rayhan3 and Acsad achieved the greatest values for grain yield and produced maximum values regarding harvest index under optimum and drought stress Similar result was indicated when these genotypes were grown under salinity stress (Ehtaiwesh, 2022). These findings require more investigations to clarify whether early mentioned genotypes would perform better under drought stress at different growth stages to be used in breeding programs. Then these genotypes could be recommended and selected for cultivation where drought stress is expected to occur.

Conclusion

The primary objective of this study was to identify genotypes that would be most suitable for growth under drought stress condition. Genetic development for drought tolerance in many crop plants is one of the most cost effective and sustainable solutions to intensify crop productivity and yield constancy. The present study demonstrated that barley genotypes California, Rayhan3 and Acsad achieved the greatest values for grain yield and produced maximum values regarding harvest index under optimum and drought stress. Therefore, these genotypes are suitable materials for developing drought-breeding programs.

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غربلة بعض الاصناف الوراثية للشعير *Hordeum vulgare* L لتحمل الجفاف

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الملخص

يعد الشعير (*Hordeum vulgare* L.) أحد أقدم المحاصيل التي يتم زراعتها عالميا. ومع ذلك، يعد الجفاف أحد العوائق الرئيسية أمام إنتاج الشعير في المناطق الجافة وشبه الجافة، لذا فإن تطوير اصناف وراثية تتحمل الجفاف يعد مسألة ذات اهتمام عالمي لضمان الأمن الغذائي. أجريت الدراسة الحالية لتقييم استجابة بعض الاصناف الوراثية للشعير للإجهاد المائي (الجفاف) وذلك لتحديد الاصناف الوراثية المتحملة للجفاف. شملت الدراسة تقييم ثمانية اصناف من الشعير وهي (أكساد 176، وادي ميمون، ريحان 3، وادي زرت، كاليفورنيا، نبولة 1، بيتشر، ووادي الحي) حيث تم تعريض هذه الاصناف للإجهاد المائي في مرحلة تكون وتطور السنابل. أجريت تجربة حقلية وفق التصميم العشوائي الكامل بثلاثة مكررات. تم معاملة أصناف الشعير بثلاث معاملات مختلفة وهي: (الشاهد، وإجهاد مائي متوسط، وإجهاد مائي شديد). تتم معاملة النباتات بالإجهاد المائي من خلال إيقاف الري لمدة 2 و 3 أسابيع للإجهاد المتوسط والشديد على التوالي. أظهرت النتائج أن جميع صفات النمو والانتاجية قد تأثرت معنويا ($P<.001$) في جميع الاصناف الوراثية تحت ظروف الإجهاد المائي الشديد. ومع ذلك، كانت هناك بعض الصفات مثل عدد السنابل لكل نبات، وعدد الحبوب لكل سنبلة وإنتاجية الحبوب ودليل الحصاد، التي أعطت قيم عالية تحت الإجهاد المائي لبعض الأصناف مثل كاليفورنيا وأكساد وريحان3 مقارنة بتلك التي اعطتها بقية الاصناف الوراثية للشعير المشمولة في هذه الدراسة، وخاصة في ظل ظروف الإجهاد المائي الشديد. بشكل عام، كان التداخل بين إجهاد الجفاف والتراكيب الوراثية معنويا ($P<0.05$)، حيث حققت بعض التراكيب الوراثية إنتاجية حبوب أعلى تحت إجهاد الجفاف الشديد. يمكن تصنيف هذه الأصناف على أنها اصناف وراثية تتحمل الجفاف ويمكن ان يتم اختيارها لزراعتها في المناطق التي تعاني من الجفاف.

الكلمات المفتاحية: الشعير, *Hordeum vulgare* L, إجهاد الجفاف, تحمل الجفاف, النمو, الانتاجية.