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Effect of Drought Stress on Agronomic Traits and Protein Electrophoretic Pattern of Barley

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Abstract: Drought stress is one of the most important abiotic factors that adversely affect plants' growth, metabolism and yield. The proteins are involved in plant stress response, so studying the changes in proteins under various stress conditions is important. This study aimed to investigate the effect of water deficit on ten Egyptian cultivars of barley under different levels of drought stress and detect the drought-tolerant cultivars of barley. The plant growth of barley cultivars was reduced significantly after exposure to drought stress. The cultivated barley displayed considerable genotypic variability under drought conditions. The drought-sensitive genotypes showed more reduction in agronomic traits than drought-tolerant ones. The electrophoretic patterns showed 29 bands, some of them were presented in the patterns of non-stressed plants, while others disappeared in the drought-stressed plants and vice versa. The three cultivars G123, G132 and G133 gave high values for most of the agronomic traits under normal and drought conditions. They showed a high number of bands in their protein patterns of control and treatments, with little values of reduction in shoot fresh weight and high tissue water content. The cultivars G132 and G133 also manifested specific protein bands after stress treatments indicating their tolerance to drought stress. Meanwhile, three bands disappeared in the protein pattern of the cultivar G130 after treatment with 20% Polyethylene Glycol, this cultivar showed also a reduction in the agronomic traits and low tissue water content under normal and drought conditions, indicating its sensitivity to drought stress. The other cultivars showed moderate values of agronomic traits and tissue water content under control and drought stress conditions besides the absence of some bands after treatments which are considered moderately resistant to drought stress. Developing drought-tolerant barley cultivars is the best option for barley production, yield improvement and stability under water deficit conditions.

Key words: Barley · Drought stress · Agronomic traits · Water relations · Protein electrophoresis

INTRODUCTION

Abiotic stresses such as drought induce a dramatic decline in photosynthesis, cellular water deficit (WD), cell membrane injury, loss of enzyme activities and severe crop yield reductions. Therefore, breeding stress-tolerant crops is the most efficient strategy to maintain productivity under environmental stress conditions. It is thus necessary to screen the genetic resources of different populations with high tolerance to abiotic stresses. It is very important to evaluate drought tolerance (DT) at the seedling stage because it affects all the subsequent stages and ultimately grain yield [1-6].

Barley (*Hordeum vulgare* L.) is characterized by having a relatively high DT, providing the potential to expand its production to areas affected by climate

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change. Investigation of the DT mechanisms in barley could facilitate an understanding of the genetic bases of DT and so enable the effective use of genetic approaches to improve its DT [7-9]. The morphophysiological traits in barley are adversely affected by continued drought stress (DS), while total soluble proteins are improved. DS could reduce the grain yield of barley by 49-87%. In barley, breeding drought-tolerant cultivars seems the most effective and economical approach to minimize the effects of DS on yield production [10-13].

Samarah [14] studied the effect of DS on grain growth and yield of the barley (*Hordeum vulgare*) cultivar "Rum" in a greenhouse experiment. He found that DS treatments reduced grain yield by reducing the number of tillers, spikes and grain weight. Akladious and Abbas [15] studied the DT of five barley genotypes that were grown in a pot experiment with DS levels of field capacity of 50% and 30%. They found that 30% field capacity led to reducing yield parameters in the G130 and G134 genotypes, while the G126 genotype displayed the highest and most stable yield under normal and drought conditions. The electrophoretic analysis showed that plants grown under drought showed induction or suppression of some polypeptide bands. Giza126 exhibited the best performance regarding the appearance of new bands in the protein profile.

A positive correlation was observed between relative water content (RWC) and grain yield in barley [16-18]. Nayyar and Gupta [19] showed that RWC and water potential were reduced significantly when leaves of barley were subjected to drought. Assessment of RWC could provide drought resistance screening parameters for developing drought-resistant barley cultivars. Siddique *et al*. [20] subjected four wheat (*Triticum aestivum* L.) cultivars grown in pots to water stress (WS) at vegetative and anthesis stages. They found noticeable decreases in leaf water potential and RWC. Wang *et al*. [21] exposed two accessions of hexaploid wheat to moderate and severe WS, the results showed that yields decreased by 29% and 61% under moderate and severe WS respectively in the two accessions. Mariey and Khedr [22] found that the Egyptian barley cultivars Giza 131, Giza 2000 and Giza 126 had the highest performance under normal and DS conditions for most of the studied traits. Pour-Aboughadareh *et al*. [23] performed an experiment to determine the effect of WS on morphological and physiological traits in 17 durum wheat genotypes under control and drought conditions. The results indicated that DS significantly reduced the plant height, grain yield, biomass and harvest index in all genotypes compared to the control.

Kausar *et al*. [24]. In a pot experiment, found that the growth of 20% Polyethylene Glycol (PEG)-treated plants of barley was adversely affected as compared to the control. The shoot length, root length, fresh weight and photosynthetic rate parameters decreased in the 20% PEG treatment plants as compared to the control. El-Denary and El-Shawy [25] studied the effect of WS induced by PEG on germination percentage, shoot length, root length and total dry matter in three barley genotypes. They reported that Giza126 and California Marriott varieties were tolerant and stable under different stress levels, while the sensitive variety Giza129 showed a sharp decrease in the studied traits. Cai *et al*. [26] screened 237 cultivated and 190 wild barley genotypes for DT at the seedling stage under WD and PEG-simulated drought. They found that water relation, photosynthetic activity and osmotic adjustment differed greatly between the contrasting genotypes under WD stress. Both WD stress significantly reduced shoot fresh weight (SFW), shoot dry weight (SDW) and tissue water content (TWC). This indicated that RWC in the youngest leaf is the suitable selection criteria for screening DT in barley at the seedling stage.

Under the condition of DS, the physiological status of barley had undergone a series of changes, in which the soluble protein concentration increased as an osmotic adjustment substance in cells. Stress protein synthesis is a common response to stressful conditions such as drought by hydrating cellular structures. The DS-induced proteins are required to maintain membrane stability and osmotic equilibrium in a stressful environment which allows plants to make biochemical and structural adjustments that enable plants to survive under stress [27-29]. Chmielewska *et al*. [30] studied the agronomic traits, RWC and protein changes in leaves of two genotypes of barley with contrasting drought tolerance Maresi (sensitive) and Cam/B1/CI (tolerant) subjected to WD. The results revealed a significant drought-related reduction for the agronomic traits and RWC for Maresi. The RWC indicated that Cam/B1/CI is less prone to water loss. Many of the proteins identified during this study are known as general indicators of abiotic stress and they gave higher constitutive accumulation levels in Cam/B1/CI than in Maresi. El-Mouhamady *et al*. [31] evaluated twenty-three rice genotypes for WS tolerance during two experiments. They showed that five rice genotypes were highly tolerant to WS compared to normal conditions and the presence of some protein bands is considered a genetic marker for WD tolerance in some rice genotypes.

Faw and Jung [32] reported that desiccation-hardened plants manifested an increase in soluble proteins and changes in their electrophoretic mobility. Cloutier [33] detected quantitative changes in the electrophoretic patterns of soluble proteins of different cultivars of winter wheat and rye grown in different environments. Vítámvás *et al*. [34] found that barley cv. Amulet reduced its growth and developmental rates and displayed increased levels of several protective proteins under drought conditions. Hellal *et al*. [35] investigated the effect of DS on the protein profile in ten Egyptian barley cultivars. They found that cultivars Giza127 and Giza134 showed the highest tolerance response under DS and the protein bands of 27 and 78 kDa showed high intensity after stress in almost all studied cultivars.

The objectives of this study were 1) to identify the best drought-tolerant cultivars among ten Egyptian genotypes of barley (*Hordeum vulgare* L.) under different drought conditions; 2) to identify barley leaf proteins that are regulated in response to DS and detect their relationships with WD as biochemical markers for DT.

MATERIALS AND METHODS

Plant Material: Ten Egyptian cultivars of barley (*Hordeum vulgare* L.) from different genetic origins were used in this study named; Giza123 (G123), Giza124 (G124), Giza126 (G126), Giza129 (G129), Giza130 (G130), Giza131 (G131), Giza132 (G132), Giza133 (G133), Giza134 (G134) and Giza2000 (G2000) as shown in Table 1. The cultivars were evaluated for DT at the greenhouse and laboratory levels using PEG at three concentrations: 0, 10 and 20%.

Response to Drought Stress Based on the Agronomic Traits and Grain Yield: The experiment was conducted on the ten cultivars of barley in a randomized complete block design in a greenhouse with three replications; each block consisted of 30 pots in which every three pots were planted by one cultivar for control and two WD conditions. Grains were sown in a greenhouse in 30 cm pots filled with a mixture of soil and sand (2:1) and watered with tap water (1000 mL/pot). The plants were subjected to DS; the control plants were irrigated every two weeks while the moderate and severe-stressed plants were irrigated every three and four weeks respectively. The Data were taken on individual plants for the following traits: plant height (PH), (cm); spike length (SL), (cm); the number of spikes/plant (NS/P); grain yield per plant (GY/P), (g); biological yield (BY), (g) and harvest index (HI), (%). The data were processed to obtain the parameters of yield performance for the ten cultivars of barley and estimate the effect of drought treatments on them.

Table 1: Pedigree of the ten cultivars of barley (*Hordeum vulgare* L.).

No.	Cultivar	Origin
1	G123	Giza117/FAO86
2	G124	Giza117/Bahtim52//Giza118/FAO86
3	G126	Baladi16/Bahtim52//SD729-Por12762-BC
4	G129	Deir Alla106/Cel//AS46/Aths*2
5	G130	Comp.cross229//BcoMr/ DZ02391/3/Deir Alla106
6	G131	CM67-B/CENTENO//CAM-B/3/ROW906.73/4/GLORIA-BAR/COME-B/5/FALCON-161 LINO
7	G132	Rihane-05//As46/Aths*2Aths/Lignee686
8	G133	CarboxGusto
9	G134	Alanda-01/4/W12291/3Api/CM67//L2966-69
10	G2000	Giza117/Bahtim52//Giza118/FAO86/3/Baladi16/Gem

Response to Drought Stress Based on Seedling Stage: The experiment was conducted in Petri dishes with a diameter of 15 cm at room temperature in a randomized complete block design with three replications, each plot consisted of 30 dishes in which every three dishes were planted by one cultivar as control and two WD (0.0, 10% and 20% PEG). The grains were immersed in sodium hypochlorite 1% for 5 min and washed with distilled water. Petri dishes and the barley grains were sterilized in an autoclave. Fifteen grains of each cultivar were transferred into each Petri dish in which the filter papers were placed. Five ml of distilled water was added to each Petri dish, then after five days 10 ml of PEG solution related to each treatment was added. The shoot length (SL), SFW, SDW and TWC were measured after 10 days of drought. SDW was obtained after drying at 70°C for 48 h. TWC is calculated as SFW minus SDW divided by SFW percentage.

Statistical Analysis: The data were subjected to the proper statistical analysis using a general data analysis software "Genstat 17th Edition" [36]. Treatment means were compared using the least significant difference LSD 0.05

Protein Electrophoresis: Ten seedlings were selected within each genotype from the control and drought treatments were used for protein analysis.

Samples of one gram from leaves exposed to DS besides control were used for protein analysis. Total soluble proteins were extracted from leaves of 15-day-old barley seedlings by salt buffer according to Stegemann [37] as follows; 50 m MTris-HCl buffer pH 8, 200 mM NaCl, 1% SDS and 2% β-mercaptoethanol. The solution was centrifuged for 10 min at 12,000 rpm and 4°C and the supernatants containing total soluble proteins were transferred to new Eppendorf tubes and reserved in a deep freezer until used for analysis. Extracted proteins were analyzed by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [38]. The gel was stained with Coomassie Brilliant Blue R 250. De-staining solution containing 300 ml methanol, 50 ml acetic acid and 650 ml distilled water was utilized to visualize the protein bands. The gel was photographed and scanned by Gel Doc Bio-Rad System (Gel-Pro analyzer V. 3).

RESULTS

Effect of Drought on Agronomic Traits and Yield Components: The mean performance values of six studied traits for the ten barley cultivars under control and dry treatments in a greenhouse are illustrated in Table 2. Under control: the mean values manifested that the cultivar G123 gave the highest values for three traits; SL (6 cm), GY/P (10.5 g) and BY (34 g). The cultivar G126 showed the highest values for PH (52.25 cm) and HI (30.97%). The cultivar G132 manifested the highest values for SL (6 cm) and NS/P (26). Meanwhile, cultivar G129 gave the lowest values for three traits; SL (4 cm), GY/P (6 g) and BY (23.5 g). The cultivar G130 gave the lowest value for NS/P (15.50). Under medium drought: the cultivar G123 gave the highest values for the three traits; SL (4 cm), GY/P (5 g) and HI (23.39 %). The cultivar G126 showed the highest values for PH (39 cm), while the cultivar G132 manifested the highest value for SL (4 cm). The cultivar G133 gave the highest value for three traits; SL (4 cm), NS/P (17.50) and GY/P (5.50 g). The two cultivars G134 and G2000 showed the highest value for BY (25 g). Meanwhile; G129 gave the lowest values for four traits; PH (20 cm), SL (2.50 cm), GY/P(2.50 g) and BY (15 g). The cultivar G131 manifested the lowest values for GY/P (2.50 g) and HI (15.62 %), while the cultivar G130 gave the lowest value for NS/P (9.50). Under severe drought: the cultivar G126 gave the highest values for

Table 2: Mean performance for six agronomic traits of ten barley cultivars grown in the two seasons 2020/2021 and 2021/2022 under different drought conditions in the greenhouse.

Irrigation	Genotypes	Plant height (cm)	Spike length (cm)	Number of spikes/plants	Grain yield/ plant (g)	Biological yield (g)	Harvest index (%)
Control	G.123	45.00	6.00	19.50	10.50	34.00	30.65
(every 2 weeks)	G.124	35.00	5.00	17.50	8.50	28.50	29.73
	G.126	52.25	5.50	21.50	9.00	29.00	30.97
	G.129	40.00	4.00	18.50	6.00	23.50	25.50
	G.130	37.50	5.00	15.50	7.50	28.00	26.30
	G.131	37.50	5.00	17.50	8.00	32.50	24. 56
	G.132	42.50	6.00	26.00	10.00	33.50	29.96
	G.133	47.50	5.50	24.00	9.50	32.00	29.75
	G.134	47.50	5.00	18.00	9.50	34.00	28.04
	G.2000	41.00	5.50	16.50	9.50	32.50	29.11
Medium Dry	G.123	22.50	4.00	11.00	5.00	21.00	23.39
(every 3 weeks)	G.124	23.50	3.00	12.00	4.00	19.50	20.42
	G.126	39.00	4.00	13.00	4.50	21.00	21.78
	G.129	20.00	2.50	10.00	2.50	15.00	16.39
	G.130	27.50	3.00	9.50	3.50	20.50	16.75
	G.131	21.00	3.00	12.50	2.50	15.50	15.62
	G.132	30.00	4.00	17.00	5.00	23.00	22.07
	G.133	31.00	4.00	17.50	5.50	21.50	20.10
	G.134	30.00	3.50	12.50	5.00	25.00	20.00
	G.2000	26.00	3.50	12.50	5.00	25.00	20.00
Severe	G.123	13.50	2.00	4.50	1.67	13.50	10.96
(every 4weeks)	G.124	10.50	2.00	7.00	1.133	13.50	9.17
	G.126	22.50	2.50	8.50	2.00	14.00	14.23
	G.129	14.50	1.00	7.00	0.90	8.50	10.68
	G.130	15.00	1.00	6.00	0.93	10.00	9.33
	G.131	12.50	1.00	6.50	0.93	10.00	9.33
	G.132	20.00	2.00	11.50	2.00	15.00	12.78
	G.133	19.50	2.00	9.50	1.50	12.50	13.08
	G.134	17.50	2.00	7.50	2.00	16.50	11.91
	G.2000	15.50	2.00	9.50	1.33	13.50	10.16
C.V		8.90	7.60	8.60	8.60	9.30	10.7
L.S.D	0.05	3.67	0.70	2.75	2.75	2.43	2.47

Table 3: Effect of PEG on shoot length, weight and tissue water content in ten barley cultivars

Barley cultivar	PEG levels (%)	Shoot length (cm)	Reduction in shoot length (%)	Fresh weight (mg/plant)	Reduction in fresh weight (%)	Dry weight (mg/plant)	Tissue water content (%)
G123	0	19.5		163		11	93.25
	10	13.6	43.38	142	14.79	8	94.37
	20	9.5	105.26	105	55.24	6	94.29
G124	0	17.4		155		10	93.55
	10	16.7	4.19	104	49.04	9	91.35
	20	11.2	55.36	65	138.46	6	90.77
G126	0	17.6		117		9	92.31
	10	14.2	23.94	72	62.50	8	88.89
	20	9.6	83.33	40	192.50	5	87.50
G129	0	15.8		143		9	93.71
	10	12.8	23.44	92	55.43	9	90.22
	20	8.2	92.68	36	297.22	6	83.33
G130	0	12.0		80		5	93.75
	10	9.1	31.87	70	14.29	5	92.86
	20	6.6	81.82	20	300.00	5	75.00
G131	0	16.0		110		9	91.82
	10	13.0	23.08	90	22.22	8	89.13
	20	11.0	45.45	70	57.14	8	88.57
G132	0	21.0		256		16	93.75
	10	15.5	35.48	199	28.64	11	94.47
	20	11.8	77.97	187	36.90	9	95.19
G133	0	18.5		229		11	95.20
	10	13.4	38.06	192	55.73	8	95.83
	20	8.4	120.24	141	62.41	7	95.04
G134	0	19.2		153		12	92.16
	10	16.4	17.07	106	44.34	10	90.57
	20	18.8	2.13	054	183.33	7	87.o4
G2000	0	22.7		290		14	95.17
	10	17.2	31.98	166	74.70	11	93.37
	20	8.9	155.03	100	190.00	6	94.00
C.V.:		10.80		8.18		10.20	
LSD (0.05):		4.73		12.90		1.37	

four traits; PH (22.50 cm), SL (2.5 cm), GY/P (2g) and HI (14.23 %). the cultivar G132 manifested the highest values for NS/P (11.50) and GY/P (2 g).The cultivar G124 gave the lowest values for PH (10.50 cm) and HI (9.17 %), while cultivar G129 gave the lowest value for the three traits; SL (1 cm), GY/P (0.90 g) and BY (8.50 g).

The reduction in all agronomic studied traits, except HI percentage, was significant after medium and severe drought treatments compared to the controls for the ten cultivars (Table 2). In conclusion; The cultivars G123, G132 and G126 gave high values for most of the studied traits under control and drought; while the cultivars G129 and G130 showed a reduction in most studied traits.

Effect of Drought Stress by PEG on Barley Seedlings: The mean values of three studied traits for the ten barley cultivars under normal and drought conditions using PEG are illustrated in Table 3. The cultivar G2000 gave the highest value of SL with 22.7 cm for control and 17.2 cm after treatment by 10% PEG with a reduction of 31.98%, while the cultivar G134 gave19.2 cm for control, 16.4 cm and 18.8 cm after treatments by 10% and 20% PEG with reduction of 17.07% and 2.13%, respectively comparing to the control. The cultivar G132 showed a high value of SL of 21.0 cm for the control and 15.5 cm and 11.8 cm after treatments by 10% and 20% PEG with a reduction of 35.48% and 77.97% compared to the control, respectively. The cultivar G124 manifested the best performance for SL (16.7cm) after treatment by 10% PEG which was reduced only by 4.19%, while the cultivar G134 showed the best performance for this trait (18.8cm) after treatment by 20% PEG which reduced by 2.13% compared to the control. Meanwhile, the cultivar G130 gave the lowest values for SL with 9.1cm and 6.6cm after treatments by 10% and 20% PEG, respectively. The reduction in SL for the cultivars G123, G132, G133 and G2000 was significant after treatments by 10% and 20% PEG compared to the controls.

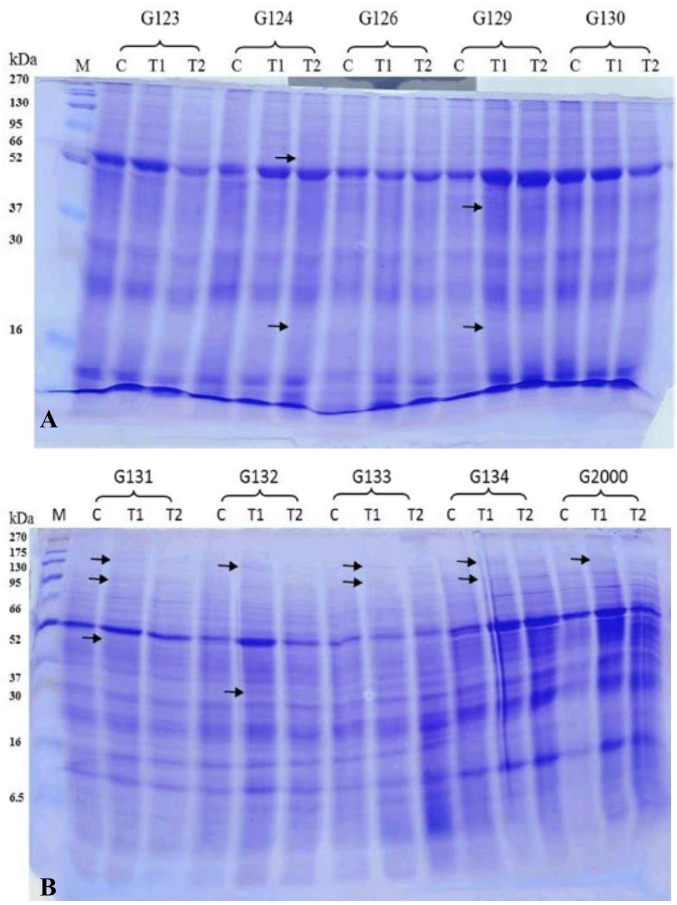

Fig. 1: Electrophoretic patterns of the ten barley cultivars (A: cultivars 1-5 and B: cultivars 6-10) for leaf water-soluble proteins exposed to drought stress. C: control, T1: treatment by PEG 10% and T2: treatment by PEG 20%. M: standard proteins.

Table 3 showed that the cultivars G133, G123 and G132 had the highest TWC in the control and DS after treatments by PEG with 10% and 20%. These cultivars reserved TWC of 95.20, 95.83, 95.04%; 93.25, 94.37, 94.29% and 93.75, 94.47, 95.19%, respectively. On the other hand, the cultivars G130 and G129 gave the least TWC of 75.00% and 83.33% after treatment by 20% PEG, respectively. The reduction in SFW was low in the cultivar G123 (14.79% and 55.24%) and the cultivar G132 (28.64% and 36.90%) after treatment by 10% and 20% PEG respectively compared to the control. The reduction in SFW percentage was very high in the cultivar G130 (300%) after treatment by 20% PEG compared to the control. Therefore, the results manifested that the cultivars G132, G123 and G133 were the most tolerant to DS which the TWC did not reduce significantly after treatments by PEG, while G130 was the most sensitive to DS which TWC was reduced significantly after treatments by 20% PEG. The reduction in SFW for all cultivars was significant after drought treatments compared to the controls. The reduction in SDW for the cultivars G123, G132, G133, G134 and G2000 was significant after treatments by 10% and 20% PEG compared to the control.

Protein Analysis: Water soluble proteins were extracted from leaves of the ten barley cultivars that were exposed to DS after ten days of treatments by 10% and 20% PEG besides control for use in protein analysis by electrophoretic technique. Protein performance for the ten cultivars manifested differences in the molecular weight and intensity of protein bands between the cultivars and treatments as illustrated in Figure 1 (A and B) and Table 4. The electrophoretic patterns showed 29 bands, some of them were presented in the patterns of non-stressed plants, while others disappeared in the drought-stressed plants and vice versa. The cultivar G123 gave the highest number of bands (29 bands) in its

Table 4: Electrophoretic patterns of leaf water-soluble proteins for the ten barley cultivars after exposure to drought treatments. C: control, T1: treatment by PEG 10% and T2: treatment by PEG 20%. (+) means presence and (-) means the absence of the band

B.N.	M W (kDa)	Barley varieties																													
		G123			G124			G126			G129			G130			G131			G132			G133			G134			G2000		
		C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2	C	T1	T2
1	188.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	181.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	156.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
4	98.9	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	+	+	+	+
5	93.3	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	76.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	70.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	65.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	58.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	55.8	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	50.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	47.9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
13	45.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+
14	41.0	+	+	+	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	38.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	35.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	33.0	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
18	28.5	+	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	24.6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	23.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
21	21.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22	18.5	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
23	15.7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
24	12.7	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	+	+	+	-	-	-
25	10.1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
27	6.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	4.8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	2.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Total bands		29	29	29	26	26	27	29	27	28	26	28	28	28	28	26	26	29	28	27	29	28	26	27	28	27	28	27	25	26	26

protein patterns of control and treatments by 10% and 20% PEG. Some of the other cultivars showed new bands compared to the control in their protein patterns after DS such as; G124 (58.8 and 18.5 kDa), G129 (41 and 18.5 kDa), G131 (156, 98.9 and 47.9 kDa), G132 (156 and 33 kDa), G133 (156 and 98.9 kDa), G134 (156 and 98.9 kDa) and G2000 (156 kDa).

These results are in agreement with those obtained in the greenhouse and laboratory experiments, especially for the cultivar G123, which also gave high values in the agronomic traits, SL and TWC under DS (Tables 2 and 3), indicating its tolerance to DS. On the other hand, three bands of 41, 35.3 and 12.7 kDa disappeared in the protein pattern of the cultivar G130 after treatment with 20% PEG. This cultivar gave low values in the agronomic traits and a high reduction in SFW and low TWC after treatment by 20% PEG, indicating its sensitivity to DS. Other bands also were absent in the DS patterns of some cultivars after treatments by PEG such as; 28.5 kDa (G124), 12.7 kDa (G131), 98.9 kDa (G132), 12.7 kDa (G133), 45 and 18.5 kDa (G134), which are considered moderately resistant to DS because they showed moderate values of agronomic traits and TWC under control and DS conditions.

DISCUSSION

Ten Egyptian cultivars of barley (*Hordeum vulgare* L.) were evaluated for DT under greenhouse and laboratory conditions using PEG at three concentrations; 0, 10 and 20% in this research. DS significantly reduced the plant growth of all barley genotypes. The cultivated barley displayed considerable genotypic variability in DT. The drought-tolerant genotypes showed less reduction in shoot biomass than drought-sensitive ones. Likewise, the trait of water relation adjustment differed between the genotypes under WD stress, suggesting the potential of this trait to be the selection criteria for DT. The three cultivars G123, G132 and G133 gave high values for the agronomic traits of SL, GY/P, BY and HI under normal and drought conditions. They showed a high number of bands in their protein patterns of control and treatments, with little values of reduction in fresh weight and high TWC. The cultivars G132 and G133 also manifested specific bands after stress treatments indicating their tolerance to DS. Meanwhile, three bands disappeared in the protein pattern of the cultivar G130 after treatment with 20% PEG. This cultivar showed also a reduction in the agronomic traits and low TWC under normal and drought conditions, indicating its sensitivity to DS.

Several studies on breeding for DT have proposed the importance of varied natural populations in the identification of useful parents for improving crop tolerance [39-40]. It has been previously reported that DS induced by PEG had similar effects on the growth parameters as did by water withholding and it generated reproducible effects at seedling stages on the growth of plants. Thus, PEG solutions have been widely applied in studies on evaluating DT [10, 41-42]. Fresh matter, dry matter and RWC at the seedling stage are the suitable selection criteria for screening DT in barley [26, 40].

Li *et al*. [43] measured several physiological and morphological traits under both drought and control conditions to estimate the DT of some genotypes of barley. They found that the yield losses due to DS were lower for the drought-tolerant varieties than for the drought-susceptible varieties. Our results coincided with that of Noaman *et al*. [44] who reported that the barley cultivar G132 is drought-tolerant which gave the highest value of the seedling fresh weight and exhibited wide adaptability under different levels of drought stress in rain-fed stress areas and in the newly reclaimed areas. Azzam *et al*. [45] reported also that cultivar G123 gave high values for plant height, grain yield and seedling dry weight while cultivar G132 gave the highest value of the seedling fresh weight, in an experimental field compared to nine other barley varieties. The results also are in agreement with that of Akladious and Abbas [15] who found that 30% field capacity led to reducing yield parameters in the G130 and G134 genotypes, while the G126 genotype showed the highest and most stable yield under normal and drought conditions.

The desiccation-hardened plants manifested an increase in the amount of soluble proteins and changes in their electrophoretic mobility [32]. Cloutier [33] detected quantitative changes in the electrophoretic patterns of soluble proteins of different cultivars of winter wheat and rye grown in different environments. Abou-Deif [46] found that the two wheat varieties Giza168 and Sonalika manifested four new bands in their protein electrophoretic patterns under drought conditions compared to the control. Vítámvás *et al*. [34] found that barley cv. Amulet decreased its growth and developmental rates and exhibited increased levels of several protective proteins after exposure to drought treatments. Hellal *et al*. [35] studied the effect of DS on the protein profile in ten Egyptian barley cultivars. They found that the protein bands of 27 and 78 kDa showed high intensity after stress in almost all studied cultivars.

CONCLUSION

Ten Egyptian cultivars of barley (*Hordeum vulgare* L.) were evaluated for DT under greenhouse and laboratory conditions using PEG at three concentrations; 0, 10 and 20% in this research. The three cultivars G123, G132 and G133 gave high values for the agronomic traits of SL, GY/P, BY and HI under normal and drought conditions. They showed a high number of bands in their protein patterns of control and treatments, with little values of reduction in fresh weight and high TWC indicating their tolerance to DS. The cultivar G130 showed a reduction in the agronomic traits and low TWC under normal and drought conditions, indicating its sensitivity to DS. The study manifested the importance of diverse natural populations in the identification of useful genotypes for improving DT in barley.

Abbreviations:

WD : Water deficit
DT : Drought tolerance
DS : Drought stress
WS : Water stress
RWC : Relative water content
TWC : Tissue water content
PEG : Polyethylene Glycol
SFW : Shoot fresh weight
SDW : Shoot dry weight
PH : Plant height
SL : Spike length
NS/P : Number of spikes/plant
GY/P : Grain yield per plant
BY : Biological yield
HI : Harvest index
SL : Shoot length
SDS-PAGE : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

REFERENCES

1. Yamaguchi-Shinozaki, K.and K. Shinozaki, 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annual Review of Plant Biology, 57: 781-803.
2. Cattivelli, L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia, C. Marè, A. Tondelli and A.M. Stanca, 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crops Research, 105: 1-14.
3. Tester, M. and P. Langridge, 2010. Breeding technologies to increase crop production in a changing world. Science, 327: 818-822.
4. Hameed, A., M. Goher and N. Iqbal, 2010. Evaluation of seedling survivability and growth response as selection criteria for breeding drought tolerance in wheat. Cereal Research Communication, 38: 193-202.
5. Lobell, D.B., W. Schlenker and J. Costa-Roberts, 2011. Climate trends and global crop production since 1980. Science, 333: 616-620.

6. Kadam, N.N., G. Xiao, R.J. Melgar, R.N. Bahuguna, C. Quinones, A. Tamilselvan, P.V. Prasad and K.S.V. Jagadish, 2014. Chapter Three - Agronomic and physiological responses to high temperature, drought and elevated CO2 interactions in cereals. Advances in Agronomy, 127, 111-156. DOI: 10.1016/B978-0-12-800131-8.00003-0
7. Talame`, V., N.Z. Ozturk, H.J. Bohnert and R. Tuberosa, 2007. Barley transcript profiles under dehydration shock and drought stress treatments: a comparative analysis. Journal of Experimental Botany, 58(2): 229-240.
8. Blake, T., V. Blake, J. Bowman and H. Abdel-Haleem, 2011. In Barley: Production, Improvement and Uses (ed. S. E. Ullrich) 522-531 (Wiley-Blackwell).
9. Wiegmann, M., A. Maurer, A. Pham, T.J. March, A. Al-Abdallat, W.T.B. Thomas, H.J. Bull, M. Shahid, J. Eglinton, M. Baum, A.J. Flavell, M. Tester and K. Pillen, 2019. Barley yield formation under abiotic stress depends on the interplay between flowering time genes and environmental cues. Scientific Reports, 9: 6397.
10. Szira, F., A.F. Ba´lint, A. Börner and G. Galiba, 2008. Evaluation of drought-related traits and screening methods at different developmental stages in spring barley. Journal of Agronomy and Crop Sciences, 19: 334-342.
11. Samarah, N.H., A.M. Alqudah, J.A. Amayreh and G.M. McAndrews, 2009. The effect of late-terminal drought stress on yield components of four barley cultivars. Journal of Agronomy and Crop Sciences, 195: 427-441.
12. Mora, F., Y.A. Quitral, I. Matus, J. Russell, R. Waugh and A. Del Pozo, 2016. SNP-based QTL mapping of 15 complex traits in barley under rain-fed and well-watered conditions by a mixed modeling approach. Frontiers in Plant Sciences, 7: 909.
13. Tahira, T., A. Riaz, F. Muhammad and M.A.B. Shahzad, 2018. Improving the drought tolerance in barley by osmo-priming and biopriming. International Journal of Agricultural Biology, 20: 1597-1606.
14. Samarah, N.H., 2005. Effects of drought stress on growth and yield of barley. Agronomy for Sustainable Development, 25(1): 145-149.
15. Akladious, S.A. and S.M. Abbas, 2014. Inter simple sequence repeat (ISSR) markers and some physiological attributes of barley (*Hordeum vulgare* L.) genotypes to drought and potassium nutrition. Journal of Animal and Plant Sciences, 24(2): 620-633.
16. Matin, M.A., J.H. Brown and H. Ferguson, 1989. Leaf water potential, relative water content and diffusive resistance as screening techniques for drought resistance in barley. Agronomy Journal, 81: 100-105.
17. Hu, H. and L. Xiong, 2014. Genetic engineering and breeding of drought-resistant crops. Annual Review of Plant Biology, 65: 715-741.
18. Yang, A., S.S. Akhtar, M. Amjad, S. Iqbal and S.E. Jacobsen, 2016. Growth and physiological responses of quinoa to drought and temperature stress. Journal of Agronomy and Crop Sciences, 202: 445-53.
19. Nayyar, H. and D. Gupta, 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: association with oxidative stress and antioxidants. Environmental and Experimental Botany, 58: 106-113.
20. Siddique, M.R.B., A. Hamid and M.S. Islam, 2001. Drought stress effects on water relations of wheat. Botanical Bulletin of Academia Sinica, 41: 35-39.
21. Wang, J.Y., N.C. Turner, Y.X. Liu, K.H.M. Siddique and Y.C. Xiong, 2017. Effects of drought stress on morphological, physiological and biochemical characteristics of wheat species differing in ploidy level. Functional Plant Biology, 44: 219-234.
22. Mariey, S.A. and R.A. Khedr, 2017. Evaluation of some Egyptian barley cultivars under water stress conditions using drought tolerance indices and multivariate analysis. Journal of Sustainable Agricultural Sciences, 43(2): 105-114.
23. Pour-Aboughadareh, A., R. Mohammadi, A. Etminan, L. Shooshtari, N. Maleki-Tabrizi and P. Poczai, 2020. Effects of drought stress on some agronomic and morpho-physiological traits in durum wheat genotypes. Sustainability, 12(14): 5610.
24. Kausar, R., M. Arshad, A. Shahzad and S. Komatsu, 2013. Proteomics analysis of sensitive and tolerant barley genotypes under drought stress. Amino Acids, 44: 345-359.
25. El-Denary, M.E. and E.E. El-Shawy, 2014. Molecular and field analysis of some barley genotypes for water stress tolerance. Egyptian Journal of Genetics and Cytology, 43: 187-98.
26. Cai, K., X. Chen, Z. Han, X. Wu, S. Zhang, Q. Li, M.M. Nazir, G. Zhang and F. Zeng, 2020. Screening of worldwide barley collection for drought tolerance: the assessment of various physiological measures as the selection criteria. Frontiers in Plant Science, 11: 1159.
27. Al-Jebory, E.I., 2012. Effect of water stress on carbohydrate metabolism during *Pisum sativum* seedlings growth. Euphrates Journal of Agricultural Sciences, 2012(4): 1-12.

28. Qaseem, M.F., R. Qureshi and H. Shaheen, 2019. Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. Scientific Reports, 9: 1-12.
29. Liu, H., G. Bao, Z. Dou, H. Liu, J. Bai, Y. Chen, Y. Yuan, X. Zhang and J. Xi, 2022. Response characteristics of highland barley under freeze-thaw, drought and artemisinin stresses. BMC Plant Biology, 22: 126.
30. Chmielewska, K., P. Rodziewicz, B. Swarcewicz, A. Sawikowska, P. Krajewski, L. Marczak, D. Ciesiolka, A. Kuczy´nska, K. Mikolajczak, P. Ogrodowicz, K. Krystkowiak, M. Surma, T. Adamski, P. Adamski and M. Stobiecki, 2016. Analysis of drought-induced proteomic and metabolomic changes in barley (*Hordeum vulgare* L.) leaves and roots unravel some aspects of biochemical mechanisms involved in drought tolerance. Frontiers in Plant Science, 7: 1108.
31. El-Mouhamady, A.B.A., A.M. Gad and G.S.A. Abdel-Karim, 2022. Improvement of drought tolerance in rice using line X tester mating design and biochemical molecular markers. Bulletin of the National Research Centre, 46: 1-20.
32. Faw, W.F. and G.A. Jung, 1972. Electrophoretic protein patterns in relation to low-temperature tolerance and growth regulation of alfalfa. Cryobiology, 9(6): 548-555.
33. Cloutier, Y., 1983. Changes in the electrophoretic patterns of the soluble proteins of winter wheat and rye following cold acclimation and desiccation stress. Plant Physiology, 71(2): 400-403.
34. Vítámvás, P., M.O. Urban, Z. Škodácekˇ, K. Kosová, I. Pitelková, J. Vítámvás, J. Renaut and I.T. Prášil, 2015. Quantitative analysis of proteome extracted from barley crowns grown under different drought conditions. Frontiers in Plant Science, 6: 479.
35. Hellal, F.A., H.M. El-Shabrawi, M. Abd El-Hady, I.A. Khatab, S.A.A. El-Sayed and C. Abdelly, 2018. Influence of PEG-induced drought stress on molecular and biochemical constituents and seedling growth of Egyptian barley cultivars. Journal of Genetic Engineering and Biotechnology, 16: 203-212.
36. Goedhart, P.W., 2014. Procedure VSEARCH. In: Biometris GenStat Procedure Library Manual 17th Edition (Editors: Goedhart, P.W. and J.T.N. MThissen). Biometris report 26.12.14, Biometris, Wageningen. http:// www.wageningenur.nl/ en/ show/ GenStat-Procedures.htm.
37. Stegmann, H., 1979. Electrophoresis and focusing in slabs using the Pantaphor apparatus for analytical and preparative separations in gel (Polyacrylamide, Agarose, Starch, Sephadex). Messeweg 11, D-3300, Braunschweig Institute of Biochemistry, West-Germany, pp: 1-29.
38. Laemmli, U.K., 1970. Cleavage of structural protein during the assembly of bacteriophage T4. Nature, 227: 680-685.
39. Jabbari, M., B.A. Fakheri, R. Aghnoum, N.M. Nezhad and R. Ataei, 2018. GWAS analysis in spring barley (*Hordeum vulgare* L.) for morphological traits exposed to drought. PloS One, 13: e0204952.
40. Sallam, A., A.M. Alqudah, M.F.A. Dawood, P.S. Baenziger and A. Börner, 2019. Drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. International Journal of Molecular Sciences, 20: 3137.
41. Molna´r, I., L. Ga´spa´r, E´. Sa´rva´ri, S. Dulai, B. Hoffmann, M.M. Láng and G. Galiba, 2004. Physiological and morphological responses to water stress in *Aegilops biuncialis* and *Triticum aestivum* genotypes with differing tolerance to drought. Functional Plant Biology, 31: 1149-1159.
42. Luo, J., S. Tang, X. Peng, X. Yan, X. Zeng, J. Li, X. Li and G. Wu, 2015. Elucidation of cross-talk and specificity of early response mechanisms to salt and PEG-simulated drought stresses in Brassica napus using comparative proteomic analysis. PloS One, 10: e0138974.
43. Li, R., P. Guo, M. Baum, S. Grando and S. Ceccarelli, 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agricultural Sciences in China, 5: 751-757.
44. Noaman, M.M., A.A. El-Sayed, R.A. Abo El-Enein, I.A. Ahmed, A.S. El-Gamal, A.M. El-Sherbiny, Kh. A. Amer, M.F. Saad, H.A. Ashmawy, R.A. Rizk and Y.M. Abdel-Tawab, 2006. Giza132, a new drought-tolerant six-rowed barley cultivar. Egypt Journal of Applied Science, 21: 46-58.
45. Azzam, C.R., M.F.S. Abd El-Rahman and E.A.I. Mohamed, 2011. Genetic relationships of some barley cultivars based on phenotypic, seed quality and molecular analyses. Egypt Journal of Plant Breeding, 15(4): 11-37.
46. Abou-Deif, M.H., 2006. Effect of water stress on protein electrophoretic patterns in hexaploid wheat. Bulletin of the National Research Centre, 31: 135-145.