Asian J Agric & Biol. 2023(2).
DOI: 10.35495/ajab.2022.071

AJAB

Original Article

Screening barley genotypes in terms of some quantitative and qualitative characteristics under normal and water deficit stress conditions

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Received:
May 06, 2022
Accepted:
July 27, 2022
Published Online:
September 19, 2022

Abstract

This study aimed to evaluate promising lines and commercial barley cultivars in terms of some quantitative and qualitative characteristics under normal and water deficit conditions. The experiment was conducted as a split-plot based on a randomized complete blocks design with three replications. According to the results, water deficiency significantly reduced the chlorophyll index (34.64 %), leaf relative water content (RWC) (15.11%), the leaf area index (42.90%), the number of seeds per spike (6.04%), 1000- grain weight (60.19%), biological yield (37.46%), grain yield (42.79%), starch (6.15%), and grain ash (20.16%) content while increasing superoxidase (33.89%) and catalase (CAT) (50.0%) enzyme activity and the grain protein content (19.58%) compared to normal conditions. In both environmental conditions, the highest grain yield was attributed to M-88-2 and M-86-5 lines and the Jonoob cultivar. However, the M-88-2 line had higher chlorophyll content, relative water content, antioxidant enzyme activity, and grain ash content in both environmental conditions compared to the Jonoob cultivar. Under normal conditions grain yield showed a positive and significant phenotypic correlation with 1000-grain weight. Furthermore, under water deficit conditions, we detected a positive phenotypic correlation between the grain yield and leaf RWC as well as the number of spikes per square meter and a positive phenotypic and genetic correlation with the biological yield. According to the results of the present study, the promising M-88-2 line can be used in future breeding programs for drought resistance as well as its quantitative and qualitative characteristics.

Keywords: Barley, Drought stress, Grain yield, Genotypes

How to cite this:

Fatemi R, Yarnia M, Mohammadi S, Vand EK and Mirashkari B. Screening barley genotypes in terms of some quantitative and qualitative characteristics under normal and water deficit stress conditions. Asian J. Agric. Biol. 2023(2): 2022071. DOI: https://doi.org/10.35495/ajab.2022.071

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Introduction

Barley (*Hordeum vulgare* L.) is one of the five major crop species in the world which is widely used for stock feed, human food, and malting (Ullrich, 2010). Barley grains in comparison to other cereals are a main source of P, Ca, K, Mg, Na, Cu, and Zn, especially selenium, which plays a crucial role in human health.

Barley's health benefits are provided by the β-glucan fiber fraction, which is associated with lowering blood cholesterol levels and glycemic index, as well as weight loss (Baik and Ullrich, 2008).
The barley final yield is dependent on water supply, and it is more adversely influenced when drought is imposed on the pollination and flowering phases (Ceccarelli et al., 2007). Therefore, post-anthesis drought conditions cause physiological changes and influence barley grain yield (Al-Ajlouni et al., 2016). Low precipitation causes water deficiency and results in drought stress in plants. The reaction to water deficit stress differs widely between related species and even between various varieties and genotypes within one specific variety and genotype (Saed-Moucheshi et al., 2022). Thus, selecting plants with suitable genes and higher adaptability to water shortage as well as screening tolerant genotypes is a critical step in any breeding program (Abdel-Ghani et al., 2015).
In the study by Saed-Moucheshi et al. (2022), Danesiah, Eram, and Yoosef genotypes were screened as suitable genotypes for normal irrigation conditions and water deficit stress based on tolerant indices. In the study by Saygili et al. (2021), 25 new barley lines were compared with "Tokak 157/37" cultivar in terms of quantitative and qualitative characteristics, they showed that 15 lines were superior to the standard "Tokak 157/37" cultivar in terms of yield and grain yield components. It has been reported that with the increase of antioxidant enzyme activity, the level of plant resistance to damage caused by reactive oxygen species is enhanced (Gapinska et al., 2008). The activity of antioxidant enzymes is one of the physiological mechanisms in cereals that plays a vital role in drought stress (Cossania et al., 2012). In the study by Hafez and Soleiman (2017), traits such as the grain yield and its components, chlorophyll index, leaf relative water content (RWC), grain starch, and ash content were decreased and the level of antioxidant enzyme activity and grain protein content was reduced by water deficit stress. Therefore, this study aimed to 1) Explore the effect of drought stress on some quantitative and qualitative characteristics, 2) Evaluate the genetic variation in terms of some quantitative and qualitative characteristics of barley genotypes in normal and water deficit conditions, and 3) Evaluate phenotypic and genetic relationships between the studied traits, 4) Screening genotypes and identifying drought-tolerant genotypes.

Material and Methods

To evaluate the genetic variation in barley genotypes, an experiment was conducted at the agricultural and natural resources research station of Miandoab (36° 58′ N, 46° 6′ E; 1314 m above sea level)), Iran in 2016 and 2018. This region is one of the semi-arid areas of Iran. In both years the experiment was conducted as a split-plot based on a randomized complete blocks design with three replications. Each plot consisted of two rows 2.5 m long and the inter-row and interplant spacing's were 20 and 5 cm, respectively. Planting was conducted in early April with a density of 350 plants per square meter. Soil characteristics are presented in Table 1. Plant materials included 12 varieties and advanced lines of barley (Table 2).
Irrigation under stress and non-stress treatments was carried out after 90 mm evaporation from class A pan until the heading stage. Under the water deficit treatment, irrigation was stopped at the heading stage, whereas under normal irrigation, it was continued until the maturity stage. All genotypes implemented standard agricultural plant protection practices such as fertilization, irrigation, and weed control throughout the growing period.
The application of chemical fertilizer was performed based on the soil analysis. N, P, and K were applied at the levels of 80 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1}, and 70 kg P_2O_5 ha^{-1}, respectively. Total P (triple superphosphate) was applied to the soil during land preparation (as the base fertilizer). One-third of N was applied during land preparation (as the basal fertilizer), and the rest was administered in the form of topdressing during the tilling and flowering stages.

Table-1. Characteristics of studied barley genotypes

1	Tajadin	
2	FAJRE30	
3	JONOOB	
4	ARASS	
5	RIHANE 03	
6	SINA	
7	Chaldoran	
8	M-84-14	Cr115/Por//Bc/3/Api/CM67/4/Giza120/5/H272/Bgs/3/ Mzq/Gva//Alanda-01
9	M-86-5	Bgs/Dajia//L.1242/4/L.B.IRAN/Una8271//Gloria'S'/3/ Alm/Una80
10	M-88-2	Kavir/Badia/3/Torsh/9cr.279-07/Bgs/4/Karoon/Kavir
11	MD-88-15	Rojo/3/LB.IRAN/Una8271//Gloria"S"/Com"S"
12	W-83-4	Zrn/Shiroodi/6/Zrn/5/Omid/4/Bb/Kal//Ald/3

Table- 2. Physical and chemical characteristics of soil testing

%Sp	EC (ds/m)	W.P	B.D	pH	%T. N. V	O.C%	N%	P(ppm)	K(ppm)	Sand %	Silt%	Clay%	Soil texture
43	1.3	12	1.4	8	4.7	1.3	0.13	14.2	444	16	56	28	loam silt clay

Measurements

Morphological and physiological measurements

To measure the leaf area index, 10 plants from each plot were randomly selected at the heading stage. Aria Leaf Meter (Model: Li-Cor 3100, USA) was employed to measure the leaf area, and the leaf area index was obtained by dividing the measured leaf area by the specific ground area for 10 plants (Meier, 2001).
The chlorophyll content was measured using a manual chlorophyll meter (SPAD-502, Minolta Sensing Ltd, Japan), and the highest developed leaf in the main stem during the heading stage was employed to record the SPAD (Meier, 2001). RWC was estimated at the heading stage B, (Meier, 2001) by utilizing completely developed leaves, weighted for the fresh weight (FW). Turgid weight (TW) was calculated by rehydrating the gathered leaves in pure water in a closed container at +10°C in the dark for 24 h and then weighing them again. Dry weight (DW) was measured for the same leaves after oven-drying for 72 h at +65°C. RWC was calculated as follows:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Antioxidant enzymes

Fresh plant leaves were used to measure the activity of antioxidant enzymes (At the 50% flowering stage and 10 days after the application of water stress).
About 0.5 g of leaves was homogenized at 0–4ºC in 3 mL of 50 mM TRIS buffer (pH 7.8), containing 1.0 mM ethylenediaminetetraacetic acid disodium (EDTA-Na_2) and 7.5% polyvinylpyrrolidone.
The homogenized samples were centrifuged at 12000 rpm for 20 min at 4.0ºC and then the activities of total soluble enzymes were measured spectrophotometrically in the supernatant (Hafez et al., 2012). All samples were poured into semi-micro-cuvettes and the absorbance was detected at +25ºC using a spectrophotometer at 430 nm (UV-160A, Shimadzu, Japan). Catalase (CAT) activity was determined spectrophotometrically according to the method described by Aebi (1983). Changes in the absorbance at 240 nm were detected at 30-sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min^{-1} g^{-1} FW. Guaiacol peroxidase activity was directly determined using the crude enzyme extract as described by Hammerschmidt et al. (1982). Changes in the absorbance at 470 nm were recorded at 30-sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min^{-1} g^{-1} FW.

Yield and its components:

Barley grain yield (14% moisture) (Ullrich, 2010) and biological yield were obtained by harvesting the whole two rows after removing the margin effects from the middle of each plot, while plant height, the number of fertile tillers per square meter, the number of seeds per spike, and 1000-grain weight were determined by collecting 10 plants from the two outer rows within each plot.

Quality traits

Nitrogen was measured using the standard micro cavitation digestion method with sulfuric acid.
The nitrogen content was then multiplied by 5.83 to estimate the grain protein content.
To measure the ash content of grains, 1.0 g of the ground grain sample was used. The samples were dried overnight at +105°C in an oven and then they were weighed (W1). In the next step, the samples were placed in a muffle furnace at 580°C for 8 h, then cooled in a desiccator before being weighed again (W2). The ash content was calculated using the formula W2/W1, and the net value was multiplied by 100 to acquire the ash percentage.
Approximately 100 mg of the milled sample (0.5 mm) was weighed to analyze the starch content using an assay kit (K-TSTA-50A/K-TSTA-100A, Megazyme, Wicklow, Ireland) and the protocol provided by the manufacturer with a UV spectrophotometer (Model UV-1800 240V IVDD, Shimadzu Inc., Kyoto, Japan).

Statistical analysis

The performed statistical analyses including the Shapiro-Wilk normality test and analysis of variance were carried out using SAS 9.2 and SPSS19 software. The Duncan test at the 0.05 probability level was used to compare the means of treatments
Phenotypic and genetic correlation coefficients were

calculated to evaluate the relationships between the traits using phenotypic and genetic variances and covariances via formulas proposed by Miller et al. (1957).

$r_g = \sigma_{g\ xy} / (\sigma_{gx} \times \sigma_{gx})$ $\qquad$ $r_p = \sigma_{p\ xy} / (\sigma_{gx} \times \sigma_{gx})$

$\sigma_{g\ xy}$ = Calculated genetic covariance between two traits

σ_{gx} and σ_{gx} = Genetic standard deviation of x and y traits

$\sigma_{g\ xy}$ = Calculated phenotypic covariance between two traits

σ_{gx} and σ_{gx} = phenotypic standard deviation of x and y traits

Results

The results of the combined analysis of variance revealed that there was a significant difference between the two years in terms of plant height ($P \leq 0.05$) and the number of spikes per square meter ($P \leq 0.01$). The effect of irrigation conditions on all the studied traits except for plant height and spike numbers ($P \leq 0.01$) was significant. The interaction effect of year and irrigation was significant only on plant height and grain ash ($P \leq 0.01$). There was a significant difference ($P \leq 0.01$) between the studied genotypes in terms of all the studied traits. The interaction effect of year and genotype on plant height, the leaf area index, 1000-grain weight, biological yield, and the grain ash percentage ($P \leq 0.01$) and on the grain number per spike ($P \leq 0.01$) was significant. The interaction effect of genotype and conditions was also significant on all the traits except for 1000-grain weight and seed ash ($P \leq 0.01$) (Table 3).

Table-3. Combined variance analysis of studied traits in barley lines and cultivars in two two-years and two conditions (in normal and water deficit conditions)

SOV	DF	MS								
		Plant Hieght	Chlorophyll content	Relative water content	Leaf area index	spike number / m^2	Grain number / spike	1000 grain weight	Grain yield	Biological yield
Year (Y)	1	393.36**	17.50^{ns}	556.08^{ns}	0.17^{ns}	1187219**	10.54^{ns}	26.26^{ns}	1.06^{ns}	0.07^{ns}
Y(R)	4	15.51	13.34	87.79	0.14	53964.5	3.17	12.05	0.84	0.01
Irrigation (I)	1	103.36^{ns}	2216.93**	2800.39**	232.11**	202^{ns}	92.12**	221.25**	205.31**	30.50**
Y× I	1	559.11**	52.36ns	28.39ns	3.88ns	42539^{ns}	6.07^{ns}	19.13^{ns}	0.34^{ns}	0.07ns
Error1	8	44.9	65.81	10.21	1.42	152755	4.52	5.81	11.74	1.32
Genotype (G)	11	1160.55**	81.12**	555.78**	80.83**	113409**	367.47**	407.95**	80.01**	0.92**
Y× G	11	242.44**	31.17^{ns}	85.26ns	4.76**	20469^{ns}	17.66*	90.12**	6.60**	0.13ns
G× I	11	108.09**	83.11**	150.04**	2.54**	53906**	63.20**	15.45^{ns}	9.95**	0.070**
G×Y× I	11	45.20ns	6.26^{ns}	40.78^{ns}	0.65^{ns}	14945^{ns}	9.19^{ns}	8.45^{ns}	7.43**	0.21ns
Error 2	88	38.53	22.37	16.35	0.45	11682	9.27	9.90	1.66	0.26
(%CV)	-	8.53	17.66	10.85	9.42	17.54	10.46	7.07	17.03	19.80
Ns, * and **: non-Significant, Significant at 0.05 and 0.01 probability levels, respectively										

Continuation of Table 3

SOV		MS				
		Peroxidase activity	Catalase activity	Grain protein	Grain starch	Grain Ash
Year (Y)	1	837.8^{ns}	0.010^{ns}	0.018^{ns}	1.07^{ns}	0.71^{ns}
Y×R	4	143.3	0.0013	1.96	5.93	0.013
Irrigation (I)	1	35470.8**	0.18**	262.99**	386.40**	2.24**
Y× I	1	280.1^{ns}	0.0008^{ns}	4.97^{ns}	27.05ns	1.66**
E1	8	1088.0	0.00008	3.29	7.61	0.006
Genotype (G)	11	1297.8**	0.004**	45.16**	55.62**	0.62**
Y× G	11	1329.7ns	0.0002^{ns}	1.57^{ns}	19.77ns	0.14**
G× I	11	900.2**	0.005**	21.57**	31.69**	0.03ns
G×Y× I	11	498.7^{ns}	0.0002^{ns}	17.55**	60.96**	0.04ns
E2	88	358.0	0.0006	5.60	12.40	0.04
(%CV)	-	17.66	14.24	15.82	6.39	14.77
Ns, * and **: no Significant, Significant at 0.05and 0.01 probability levels, respectively						

Table-4. Comparison of barley lines and variety in terms of agronomic traits under normal water deficit condition

	Plant height (cm)		Chlorophyll (SPAD)		RWC (%)		LAI	
Irrigation	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
	73.62a	66.93b	30.70a	22.85b	67.17a	58.35b	8.46a	5.92b
Genotypes								
Tajadin	73.0de	67.33bc	32.57a-d	20.11def	67.50bcd	61.43d	7.50de	4.12f
FAJRE30	59.58f	53.66d	26.26d	22.7cde	74.75b	67.58bc	7.00e	5.02de
JONOOB	93.91a	77.33a	35.94abc	23.46bcd	72.75bc	75.37a	8.46bcd	5.37d
ARASS	84.04b	72.91a	31.1bcd	19.2f	57.50ef	54.87ef	8.23bcd	5.00de
RIHANE	72.91cd	63.16c	30.45cd	27.01a	54.87f	51.18f	9.17ab	6.77bc
SINA	74.91cd	75.41a	27.16d	20.73def	61.37def	58.43de	8.55bc	8.05a
M-84-14	67.25de	63.26c	27.23d	24.55abc	64.87de	63.68cd	8.81abc	4.75e
M-86-5	67.33de	62.00c	26.7d	23.46bcd	64.75de	59.37de	8.54bc	6.67bc
M-88-2	74.00cd	64.66c	38.37a	26.30ab	85.25a	70.00ab	8.46bcd	6.60bc
MD-88-15	79.41bc	68.41bc	37.7ab	19.98ef	63.2de	60.2de	9.6a	7.1b
W-83-4	63.00ef	62.33c	27.4d	25.81 abc	66.3cd	69.0bc	7.9cd	5.1de
Chaldoran	75.16bc	71.75ab	27.4d	20.92def	72.8bc	62.0d	9.1ab	6.3c
Means in each column with the same letter are not significantly different at P<0.05								

Continuation of Table 4

	spike number / m^2		Grain number / spike		1000 grain weight (g)		Biological yield (t/ha)		Grain yield (t/ha)	
Irrigation	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
	616.60a	598.12a	29.12a	27.46b	45.72a	28.54b	8.77a	6.38b	3.07a	2.15b
Genotypes										
Tajadin	880.01a	620bc	18.36d	15.02f	46.75b-d	37.85b-f	8.89cd	6.63de	3.25b	2.42b
FAJRE30	772.50bc	535.83b-e	30.90b	28.10c	44.58cde	37.07c-f	8.06cde	7.02cd	2.62c	2.11bcd
JONOOB	815.83ab	726.67a	38.27a	35.94a	56.16a	41.35a	12.31a	9.80a	4.42a	3.44a
ARASS	708.33cd	502.50b-e	22.61c	21.95d	45.08cd	34.2f	8.89c	6.83cde	3.11bc	2.01bcd
RIHANE	720cd	637.50b	31.72b	27.72c	43.58de	40.89ab	7de	4.63g	3.04bc	1.86cd
SINA	580e	418.33e	31.37b	30.82b	46.58bcd	37.25cf	9.68bc	5.28fg	2.71bc	1.86d
M-84-14	741.37cd	576.67bcd	31.24b	29.87bc	40.83e	37.25cf	9.02c	6.58de	2.72bc	2.21bcd
M-86-5	741.50cd	490.83cde	35.81a	28.12c	46bcd	40.4abc	9.39bc	5.69efg	2.67bc	2.15bcd
M-88-2	880a	799.17a	37.47a	34.46a	47.91bc	38.08a-e	19.02ab	8.88ab	4.33a	3.75a
MD-88-15	877.50a	753.33a	35.90a	36.06a	49.25b	41.5a	12.31a	8.59b	4.93a	3.66a
W-83-4	670.83d	637.50	37.07a	27.27c	43.50de	36.71def	8.70cd	6.13def	2.74bc	2.39b
Chaldoran	545e	475de	21.17cd	18e	46.66bcd	40a-d	6.20e	7.83bc	2.74bc	2.33bc
Means in each column with the same letter are not significantly different at P<0.05										

Continuation of Table 4

	Peroxidase activity mM H_2O_2 g^{-1} FW min^{-1}		Catalase µmol tetra-guaiacol g^{-1} FW min^{-1}		Protein (%)		Starch (%)		Ash content (%)	
	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress	Normal
Irrigation	91.72a	122.81a	0.14a	0.21a	11.17b	13.89a	56.77a	53.48b	1.49a	1.24b
Genotypes										
Tajadin	80.45de	130.28a-d	0.109gh	0.168d	8.38d	8.65d	57.31a-d	53.08a-d	1.50bcd	0.70g
FAJRE30	95.82abc	115.07d	0.137defg	0.187cd	11.93b	14.05c	54.99cde	52.573bcd	1.21d	1.26e
JONOOB	93.85bcd	143.79abc	0.128efgh	0.220b	14.13a	14.12c	58.57abc	55.185abc	1.53bc	1.50a
ARASS	76.81e	124.41bcd	0.151bcde	0.217bc	10.17bcd	10.40d	55.79bcd	49.70d	1.59ab	1.03f
RIHANE	108.08a	121.83cd	0.164abc	0.231ab	10.11bcd	14.83bc	54.13de	56.397ab	1.51bcd	1.36b-e
SINA	82.93cde	108.67d	0.156abcd	0.216bc	11.12b	17.01ab	57.07abcd	52.759bcd	1.51bcd	1.45abc
M-84-14	98.20ab	108.92d	0.183a	0.214bc	14.36a	15.15bc	51.30e	51.095d	1.55abc	1.42a-d
M-86-5	93.84bcd	106.81d	0.165abc	0.212bc	10.89bc	14.99bc	59.73ab	55.266abc	1.44bcd	1.28de
M-88-2	105.20ab	153.49a	0.170ab	0.223ab	11.88b	15.53abc	60.86a	53.581a-d	1.84a	1.49ab
MD-88-15	79.93de	151.17ab	0.14cdef	0.25a	11.58b	13.41c	59.84ab	57.03a	1.23cd	1.35cde
W-83-4	103.26ab	109.72d	0.10h	0.21bc	10.40bc	17.81a	54.010de	52.212cd	1.71ab	1.068f
Chaldoran	83.72cde	109.62d	0.11fgh	0.21bc	9.07cd	10.70d	57.67a-d	52.915bcd	1.21d	0.993f

Morphological and physiological characteristics

The results of the present study indicated that water deficit stress reduced the chlorophyll index, leaf RWC, and the leaf area index by 34.64%, 15.11%, and 42.90%, respectively, compared to normal irrigation conditions (Table 4).

Among the studied genotypes under normal irrigation conditions, Jonoob and Fajr 30 cultivars had the highest and lowest plant heights with an average of 91.31 and 59.58 cm, respectively. Under water stress conditions, Jonoob, Aras, and Sina cultivars showed the highest plant heights with an average of 77.33, 72.29, and 75.41 cm, respectively, whereas the Reyhan 3 cultivar and M-84-14, M-86-5, M-88-2, and W- 83-4 lines had the lowest plant heights with an average of 63.16, 63.26, 62.00, and 64.66 cm, respectively. As can be observed, promising lines in both environmental conditions had lower plant height compared to commercial cultivars, and the Jonoob cultivar had the highest plant height in both conditions. The results demonstrated that under normal irrigation conditions, the M-88-2 line had the highest chlorophyll content, while the lowest value of this index was attributed to the W-83-4 line and the Chaldoran cultivar. Under water stress conditions, the Reyhan 3 cultivar had the highest leaf chlorophyll content and the Aras cultivar had the lowest content (Table 4). Under normal irrigation conditions, the phenotypic correlation of chlorophyll index with plant height (rp = 0.60*) was positive and remarkable (Table 5).

Under normal irrigation conditions, the M-88-2 line had the highest leaf RWC with an average of 85.25%, while the Reyhan 3 cultivar showed the lowest leaf RWC with an average of 54.87%. Under water stress conditions, although the Jonoob cultivar showed the highest leaf RWC with an average of 75.37%, there was no significant difference between this cultivar and the M-88-2 cultivar. Under these conditions, the lowest values of this trait were related to Seyed Tajuddin and Chaldoran cultivars with an average of 61.43% and 62.0%, respectively.

Based on the results of the correlation between traits table (Table 5), under normal irrigation conditions, RWC had a positive and significant phenotypic relationship with chlorophyll index (0.64*).

Comparison of mean genotypes in terms of the leaf area index revealed that the Fajr 30 cultivar had the highest value and the MD-88-15 line had the lowest value under normal conditions. Under water deficit conditions, the maximum and minimum indices were ascribed to Sina and Seyed Tajuddin cultivars, respectively. In this study, a positive and considerable phenotypic correlation was detected between the leaf area index and plant height (rp = 0.67*) as well as the chlorophyll index (rp = 0.69**) under normal irrigation conditions (Table 5).

Table 5. Phenotypic correlation (Numbers placed under the diameter) and genetic correlation (Numbers placed above the diameter) coefficients between the studied traits under normal irrigation conditions

	Plant Height	Chlorophyll	RWC	LAI	Spike number	Grain number	TKW	Biological yield	Grain yield	Peroxidase	Catalase	Protein	Starch	Ash
Plant Height	1	0.26	0.42	0.55	0.02	0.01	0.26	0.51	0.18	0.29	0.08	0.17	0.04	0.12
Chlorophyll	0.60*	1	0.42	0.28	0.29	0.18	0.29	0.66*	0.39	0.70**	0.62*	0.53	0.17	0.04
RWC	0.38	0.64*	1	.0.25	0.17	0.19	0.25	0.60*	0.29	0.27	0.36	0.19	0.39	0.19
LAI	0.67*	0.69*	0.45	1	0.24	0.02	0.08	0.51	0.19	0.41	0.31	0.29	0.18	0.17
spike number	-0.39	-0.11	0.03	0.03	1	0.17	0.28	0.12	0.50	0.08	0.15	0.11	0.19	0.41
Grain number	-0.04	0.02	-0.20	0.07	0.28	1	-0.09	0.31	0.26	0.18	0.39	0.24	0.36	0.09
TKW	0.16	0.16	0.46	0.13	0.33	0.48	1	0.51	0.55	0.12	0.41	-0.09	0.71**	0.18
Biological yield	0.698*	0.75**	0.75**	0.63*	-0.17	-0.02	.18	1	0.52	0.61*	0.50	0.09	0.09	0.16
Grain yield	0.08	0.16	-0.07	0.09	0.44	0.23	.83*	0.05	1	0.29	0.25	-0.17	0.16	0.08
Peroxidase	0.63*	0.97**	0.75**	0.71**	-0.08	0.01	.29	0.80*	0.09	1	0.52	0.39	0.19	0.25
Catalase	0.29	0.73**	0.64*	0.46	0.20	0.30	.39	0.62*	0.52	0.73**	1	0.15	0.32	0.13
Protein	0.65*	0.78**	0.74**	0.62*	-0.35	-0.21	.20	0.84**	-0.2	0.86**	0.44	1	0.23	0.32
Starch	-0.01	0.27	0.43	0.29	0.59*	.30	.21	0.30	0.29	0.39	0.53	0.31	01	0.20
Ash	0.05	0.40	0.59*	-0.16	0.14	-0.20	0.64*	0.27	0.01	0.42	0.41	0.32	0.05	1

* and **, Significant at 0.05 and 0.01 probability levels, respectively

Yield and yield components

In this study, water shortage led to a reduction of 3.08%, 6.04%, 60.19%, 37.46%, and 42.79% in the number of spikes per square meter, the number of grains per spike, 1000-grain weight, biological yield, and grain yield, respectively (Table 4).

According to the results of the present study, mean comparison of genotypes revealed that the Seyed Tajuddin cultivar and M-88-2 and MD-88-15 lines had the maximum spike numbers with an average of 880.01, 880, and 877.5 spike number/m^2, and the Chaldoran cultivar with an average of 545 spike number/m^2 showed the minimum value of this trait under normal conditions (Table 5). Under the water deficit condition, the Jonoob cultivar and M-88-2 and MD-88-15 lines exhibited the highest spike number/m^2 with an average of 726.67, 799.17, and 753.33, respectively, and the minimum number belonged to the Sina cultivar with an average of 418.33 (Table 5).

Under water deficit stress conditions, a positive and remarkable phenotypic correlation (rp = 0.95*) was detected between the number of spikes per square meter and the leaf RWC.

Based on the results, under normal conditions, the maximum value of the grain number per spike was recorded in the Jonoob cultivar and M88-2, W-83-4, MD-88-15, and M-86-5 lines with averages of 38.27, 37.47, 37.07, 35.90, and 35.81 grains, respectively. However, the minimum value was observed in the Seyed Tajuddin cultivar with an average of 18.63 grains (Table 4). Under water deficit conditions, the Jonoob cultivar and MD-88-15 and M-88-2 lines had the highest number of grains per spike with an average of 35.94, 36.06, and 34.46 grains, respectively, and the Chaldoran cultivar with an average of 18.00 grains had the lowest value (Table 4).

Under normal conditions, the highest 1000-grain weight was observed in the Jonoob cultivar with an average of 51.6 g while the lowest value was detected in the Fajr 30 cultivar and the M-88-2 line. Furthermore, the highest 1000-grain weight under water deficit conditions belonged to the M-88-2 line and the Jonoob cultivar with an average of 41.50 and 41.35 g, respectively, while the lowest 1000-grain weight was observed in the Aras cultivar with an average of 34.2 g (Table 4).

Based on the results, the highest biological yield under normal conditions belonged to the Jonoob cultivar and the MD-88-15 line with an average of 12.31 and 12.31 ton/ha, respectively, and the Chaldoran cultivar with an average of 6.20 ton/ha had the lowest biological yield. However, under the water deficit condition, Jonoob and Reyhan 3 cultivars had the highest and lowest biological yields with an average of 9.80 and 4.63 ton/ha, respectively.

Under normal irrigation conditions, the biological yield exhibited a positive and meaningful phenotypic and genetic correlation with the chlorophyll index (rg = 0.66 *, rp = 0.75**) as well as leaf RWC (rg = 0.60 *, rp = 0.75 **), and a positive and remarkable phenotypic relationship was also detected between the biological yield and plant height (rp = 0.69 **) as well as the leaf area index (rp = 0.63 *) (Table 4). Under drought stress conditions, a positive and significant phenotypic and genetic correlation was recorded between the biological yield and the relative leaf water content (rp = 0.80 **, rg = 0.60 **) as well as the number of spikes per square meter (rp = 0.60 *, rg = 0.61 *) (Table 5).

Results demonstrated the highest grain yield was obtained in M-88-2, Jonoob, and M-86-5 genotypes with an average of 4.93, 4.42, and 4.33 ton/ha under normal conditions, respectively, while the lowest yield was observed in the Fajr 30 genotype with an average of 2.62 ton/ha (Table 5). Furthermore, the highest grain yield under water deficit conditions belonged to M-86-5 and M-88-2 lines as well as the Jonoob cultivar with an average of 3.75, 3.66, and 3.44 ton/ha, respectively (Table 4), while the lowest yield was observed in the Sina cultivar with an average of 1.86 ton/ha.

A positive and significant phenotypic correlation was observed between grain yield and 100-grain weight (rp = 0.61*) under normal conditions. Moreover, under water deficit conditions, we observed a positive phenotypic correlation between the grain yield and leaf RWC (rp = 0.79**) as well as the number of spikes per square meter (rp = 0.85**) and a positive phenotypic and genetic correlation with the biological yield (rp = 0.86**, rg = 0.70**) (Table 5).

Antioxidant enzyme activity

The results of the present study revealed that water deficiency increased the activity of superoxidase and CAT enzymes by 33.89% and 50.00%, respectively, compared to normal irrigation conditions (Table 4).

Under normal irrigation conditions, although the highest peroxidase activity was ascribed to the Reyhan 3 cultivar, the difference between this cultivar and the Fajr 30 cultivar and M-88-2 and W-83-4 lines was not significant. The lowest peroxidase activity was assigned to the Aras cultivar. Under water deficit conditions, the M-88-2 line had the highest amount of antioxidant enzyme activity, but no significant difference was observed between this cultivar and the Jonoob cultivar and the MD-88-15 line. Among the studied genotypes, the lowest amount of peroxidase activity was related to Fajr 30, Sina, and Chaldoran cultivars as well as M-84-14, M-86-5, and W-83-4 lines. Under normal irrigation conditions, a considerable phenotypic and genetic relation was obtained between peroxidase activity and the chlorophyll index (rp = 0.97 **, rg = 0.70 **) as well as the biological yield (rp = 0.80 **, rg = 0.61 **) (Table 5). In addition, peroxidase activity had a positive and considerable phenotypic relation with plant height (rp = 0.63 *), relative leaf water content (rp = 0.75 **), and the leaf area index (rp = 0.71 **). Under water deficit stress, a positive and remarkable phenotypic and genetic correlation was relationship between peroxidase activity and biological yield (rp = 0.72 **, rg = 0.62 **) as well as the grain yield (rp = 0.87 **, rg = 0.65 *) and a positive and considerable phenotypic correlation was detected between peroxidase activity and leaf RWC (rp = 0.59 *) (Table 5). Under normal irrigation conditions, the M-84-14 line showed the highest amount of CAT activity. There was no significant difference between this line and M-86-5 and M-88-2 lines and Reyhan 3 and Sina cultivars. The lowest amount of CAT activity was observed in the W-83-4 line and Chaldoran and Seyed Tajuddin cultivars. Under water shortage, MD-88-15 and M-88-2 lines and the Reyhan 3 cultivar had the highest and Seyed Tajuddin cultivar had the lowest CAT activity. Under normal irrigation conditions, CAT activity had a positive and considerable phenotypic and genetic relation with the chlorophyll content (rp = 0.73 **, rg = 0.62**). Moreover, a positive and significant phenotypic correlation was detected between CAT activity and leaf RWC (rp = 0.64*), biological yield (rp = 0.62*), and peroxidase activity (rp = 0.73**). Under water deficit stress, CAT activity showed a positive and significant phenotypic correlation with leaf area index (rp = 0.67 *) and the number of grains per spike (rp = 0.64 *) (Table 5).

Grain quality

The results indicated that water stress compared to normal conditions decreased the starch and grain ash content by 6.15% and 20.16%, respectively, while increasing the grain protein content by 19.58% (Table 4). According to the results, under normal irrigation conditions, the Jonoob cultivar and M-84-14 line had the highest protein content with an average of 14.13% and 14.36%, respectively, and the Seyed Tajuddin cultivar with an average of 8.38% had the lowest grain protein content. There was no significant difference in terms of protein percentage between the Seyed Tajuddin cultivar and Aras, Reyhan 3, and Chaldoran cultivars. Under water deficit conditions, the highest grain protein content belonged to the W-83-4 line with an average of 17.81%, and no significant difference was observed between the mentioned line and the M-88-2 line and Sina cultivar. The lowest grain protein content was attributed to Seyed Tajuddin, Aras, and Chaldoran cultivars with an average of 8.65, 10.40, and 10.70%, respectively (Table 5).

Under normal irrigation conditions, grain protein content exhibited a positive and considerable phenotypic relation with plant height (rp = 0.65 *), the chlorophyll content (rp = 0.78 **), leaf RWC (rp = 0.74 **), leaf area index (rp = 0.62 *), the biological yield (rp = 0.84 **), and peroxidase activity (rp = 0.86 *). Under water stress conditions, grain protein content had a positive and considerable phenotypic relation with the chlorophyll index (rp = 0.64 *) and the number of grains per spike (rp = 0.69 *) (Table 5). In this study, the highest percentage of starch was attributed to the M-88-2 line with an average of 60.86%. It should be noted that no significant difference was observed between this line and MD-88-15 and M-86-5 lines. The M-84-14 line had the lowest percentage of starch under normal conditions with an average of 51.30%. Under water deficit conditions, the highest and lowest grain starch content were ascribed to the MD-88-15 line and Aras cultivar with an average of 57.03 and 49.70, respectively (Table 4). Under normal irrigation conditions, starch content had a positive and significant genetic correlation with 1000-grain weight (rg = 0.71 **) and showed a positive and significant phenotypic correlation with the number of spikes per square meter (rg = 0.59 *). Under water stress conditions, the genetic correlation of starch content with 1000-grain weight (rg = 0.71 **) was positive and meaningful. In addition, a positive and considerable phenotypic relation of starch content with chlorophyll content (rp = 0.64 *) and number of grains per spike (rp = 0.69 **) was detected in these circumstances (Table 5).

Among the studied genotypes, the highest ash content was related to the M-88-2 line with an average of 1.84%; however, the difference between the mentioned line and W-83-4 and M-84-14 lines as well as the Aras cultivar was not significant.

Table 6. Phenotypic correlation (Numbers placed under the diameter) and genetic correlation (Numbers placed above the diameter) coefficients between the studied traits under normal irrigation conditions

	Plant Height	Chlorophyll	RWC	LAI	Spike number	Grain number	TKW	Biological yield	Grain yield	Peroxidase	Catalase	Protein	Starch	Ash
Plant height	1	0.12	-0.01	0.41	0.31	0.12	-0.21	0.61	0.39	0.41	0.17	0.12	0.12	0.18
Chlorophyll	-0.46	1	0.12	0.17	0.21	0.52	0.17	-0.10	0.17	-0.09	0.43	0.35	0.18	0.51
Rwc	-0.03	0.08	1	-0.10	0.51	0.14	0.51	0.60*	0.62*	0.31	0.14	0.30	0.19	0.11
Lai	0.246	0.04	-0.18	1	-0.12	0.51	0.12	0.32	0.31	0.05	0.41	0.37	0.25	0.28
Spike number	-0.05	0.40	0.65*	-0.08	1	-0.18	-0.22	0.61*	0.71**	0.25	0.51	0.12	0.02	0.25
Grain number	0.02	0.38	0.37	0.45	0.51	1	0.15	0.18	0.33	0.15	0.34	-0.070	0.25	0.51
Tkw	0.11	0.19	0.30	0.42	0.37	0.35	1	0.16	0.22	0.49	0.40	0.13	0.71**	0.25
Biological yield	0.31	-0.14	0.80**	-0.13	0.60*	0.35	0.26	1	0.70**	0.62*	0.14	0.12	0.23	0.42
Grain yield	0.20	0.07	0.79**	0.09	0.85**	0.56	0.42	0.86**	1	0.65*	0.19	0.12	0.21	0.23
Peroxidase	0.24	-0.01	0.59*	0.06	0.55	0.44	0.31	0.72**	0.87**	1	0.15	-0.41	0.40	0.32
Catalase	0.27	0.18	0.04	0.64*	0.35	0.67*	0.42	0.19	0.39	0.35	1	0.20	0.52	0.31
Protein	-0.23	0.64*	0.04	0.43	0.10	0.69*	0.06	-0.22	0.02	-0.18	0.39	1	0.23	0.36
Starch	-0.01	0.22	0.29	0.51	0.46	0.44	0.91**	0.13	0.42	0.42	0.46	0.16	1	0.25
Ash	0.03	0.45	0.08	0.54	0.28	0.91**	0.33	0.18	0.32	0.23	0.64*	0.69*	0.35	1

* and **, Significant at 0.05 and 0.01 probability levels, respectively

The lowest ash content was attributed to Fajr and Chaldoran cultivars with an average of 1.21%. Under water stress conditions, although the Jonoob cultivar had the highest percentage of grain ash with an average of 1.50%, the difference between this cultivar and M-84-14 and M-88-2 lines as well as the Sina cultivar was not statistically significant. The lowest ash content belonged to the Seyed Tajuddin cultivar with an average of 0.70% (Table 4). Under normal irrigation conditions, the phenotypic correlation of the grain ash content with the relative leaf water content (rg = 0.59*) and 1000-grain weight (rg = 0.64*) was positive and meaningful. Under water deficit conditions, the grain ash content showed a positive and meaningful phenotypic relation with the number of grains per spike (rg = 0.91**), CAT content (rg = 0.59*), and grain protein content (rg = 0.69*) (Table 5).

Discussion

One of the important goals of plant breeding is to develop new genotypes with the ability to adapt to adverse environmental conditions, especially in areas where the plant is faced with intermittent periods of different drought intensities.

In this study, the effect of irrigation levels on all the traits was significant except for plant height and the number of spikes per square meter. Given that water deficit stress was applied at the end of the season, and at this stage, the mentioned traits were completely formed, such results were not unexpected. In this study, a significant difference was observed between the studied genotypes in terms of all the traits. This significant difference indicates the existence of appropriate genetic diversity among the studied population, which in turn implies the effectiveness of the selection process among the population. Other studies have also reported genetic variation in barley landraces (Hua et al., 2015; Shakhatreh et al., 2015)

In our study, water deficiency significantly reduced the chlorophyll index and leaf RWC. Moreover, it lowers turgor pressure, closing the pores and reducing the level of photosynthesis in the leaves (Wu et al., 2008; Hafez and Kobata, 2012). Decreased RWC in leaves can be due to the reduced water supply for leaves (Ghotbi-Ravandi et al., 2014). Some researchers have suggested that the negative effect of water stress on stomatal conductance in leaves and reduced photosynthesis can decrease the chlorophyll content and leaf area index (Ghotbi-Ravandi et al., 2014). In the study by Hafez and Soleiman (2017), water deficiency significantly diminished the leaf area index, chlorophyll content, and relative leaf water content compared to normal irrigation conditions. Comparison of the mean of genotypes in terms of morphological traits revealed that the Jonoob cultivar exhibited the highest plant height in both conditions. While the highest chlorophyll content and relative leaf water content in both conditions were attributed to the promising M-88-2 line, there was no significant difference between the Jonoob cultivar and the M-88-2 line in terms of the leaf area index under normal irrigation conditions; however, the leaf area index in the M-88-2 line was higher than that in the Jonoob cultivar under water shortage conditions. Various researchers have stated that physiological traits such as the chlorophyll content and relative leaf water content are intensely related with water deficit tolerance in barley (Rong-Hua et al., 2006; De Mezer et al., 2014). In the study by Zhao et al. (2010), a significant difference was observed between different barley genotypes in terms of leaf soluble sugar content and water use efficiency. In their study, the XZ5 genotype showed the highest amount of resistance to drought stress. The results revealed that water shortage stress significantly reduced the grain yield, yield components, and biological yield. A decrease in the supply of water required in the plant is one of the most important causes of the decline of the grain yield and its components (Samarah, 2005). Due to water shortage, the uptake of the required nutrients from the soil by the plant is diminished. As a result, cell division and differentiation, which are related to the grain yield and yield components, are reduced (Pecio and Wach, 2015). This decline might be associated with a decrease in plant growth which results in a reduction in the capacity of source and sink size in drought-stressed plants compared to well-watered plants. Similarly, it was reported that a drop in the grain yield occurred in wheat under drought stress during the pre-anthesis stage, which resulted from a decrease in the photosynthetic rate (Arisnabarreta and Miralles, 2008). In the study by Hafez and Soleiman (2017), the grain yield and yield components of barley, including the number of spikes per square meter, number of grains, and 1000-grain weight, in the water deficit treatment showed a significant decrease. In accordance with our results, it was reported that applying water deficit treatment during the pre-anthesis growth stage decreased the grain weight in barley (Al-Ajlouni et al., 2016).In the present study, the highest grain yield and grain yield components belonged to the Jonoob cultivar and the promising M-88-2 line (except for 1000-grain weight under drought stress conditions), It should be noted that the difference between the two genotypes in the M-88-15 line was not significant in terms of grain yield. Among the studied genotypes, the Chaldoran cultivar showed the highest yield reduction, and the Reyhan 3 cultivar showed the lowest yield reduction in response to water deficit conditions. The yield reduction of the two promising lines, M-88-2 and M-88-15, was less than the Jonoob commercial cultivar, indicating that the two lines were more resistant to drought conditions compared to the Jonoob cultivar. In the study by Saygili et al. (2021), 25 new barley lines were compared with "Tokak 157/37" cultivar in terms of quantitative and qualitative characteristics. They showed that 15 lines were superior to the standard "Tokak 157/37" cultivar in terms of yield and grain yield components. In a study on barley, Danesiah, Eram, and Yoosef cultivars were recognized as the most appropriate genotypes for both normal irrigation and water shortage conditions (Saed-Moucheshi et al., 2022). A positive and correlation was detected between the grain yield and 1000-grain weight under normal conditions. Furthermore, under water deficit conditions, we observed a positive correlation between the grain yield and leaf RWC, number of spikes per square meter, and biological yield. Therefore, the high grain yield in the Jonoob cultivar under normal irrigation conditions was attributed to the 1000-grain weight component. In addition, the high grain yield in the M-88-2 line under water deficit stress conditions can be related to the number of spikes per square meter, relative water content, and biological yield. In the study by Hebbache et al. (2021), drought stress significantly reduced the relative leaf water content and leaf chlorophyll content in barley. In their study, the Rahma cultivar had the highest relative leaf water content and the Jaidor cultivar had the highest leaf chlorophyll content. In a study on barley, Saed-Moucheshi et al. (2022) reported a positive and significant correlation between the grain yield and the biological yield, number of fertile spikes, number of grains per spike, and leaf area index. The results revealed that water deficiency increased the activity of antioxidant enzymes. Enhanced activity of antioxidant enzymes allows plant growth to continue under water stress conditions and is a vital indicator for resistance to stress conditions (Noctor et al., 2014). Increased activity of antioxidant enzymes under stress conditions neutralizes the harmful effects of reactive oxygen produced under osmotic stress (Hafez et al., 2012). Increased activity of barley leaf antioxidant enzymes has also been reported in the study by Hafez and Soleiman (2017). In the study by Martínez-Subirà et al. (2021), the activity of antioxidant enzymes in barley was enhanced due to temperature stress. Among the studied genotypes, the M-88-2 line in both conditions and the M-88-15 line under water deficit conditions had high levels of peroxidase and CAT activity. These two lines also showed the highest activity of the two enzymes compared to other genotypes in response to drought stress. High levels of antioxidant enzyme activity can be an indicator of a cultivar's resistance to adverse environmental conditions. In this study, under water deficit stress conditions, the phenotypic correlation between the grain yield and peroxidase activity was positive and significant. The high grain yield in M-88-2 and M-88-15 lines can also be attributed to high peroxidase activity. In our research, water stress decreased the starch and grain ash and increased the grain protein content compared to normal conditions. In the study by Hafez and Soleiman (2017), the highest percentage of grain protein was ascribed to drought stress treatments, and the highest percentage of starch and grain ash in barley was related to the normal irrigation treatment. Under water deficit conditions, the M-88-2 line had a high content of protein, starch, and grain starch. Under normal irrigation conditions, this line had a higher amount of starch and ash compared to other cultivars.

Conclusion

In the present study, the highest grain yield in both environmental conditions was attributed to the M-88-2 and MD-88-15 lines, and the Jonoob commercial cultivar. Furthermore, the grain yield reduction in these two lines was less than the Jonoob cultivar under water shortage stress compared to normal conditions. In addition, the values of the chlorophyll content, leaf RWC, antioxidant enzyme activity, and grain ash content in both environmental conditions in the M-88-2 line were higher than in the Jonoob cultivar. Therefore, the M-88-2 line has the genetic potential for future breeding programs aimed at producing high-yielding cultivars with suitable quality characteristics, which are also tolerant to dehydration stress.

Acknowledgements

We thank Dr. Kahrarian for his valuable input on data analysis.

Disclaimer: None
Conflict of Interest: None
Source of Funding: None.

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Contribution of Authors

Fatemi R: Designed and directed the project, performed the experiments, analysed spectra and wrote the article.
Yarnia M: Designed and directed the project and analysed spectra.
Mohammadi S, Vand EK & Mirashkari B: Performed the experiment and wrote the article.