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Integrative assessment of agronomic and enzymatic characteristics for identifying drought-tolerant genotypes of barley (*Hordeum vulgare* L.)

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Abstract

Objective: One of the most important factors limiting the yield of a crop, including barley, is water-deficit stress during critical stages of growth. Water-deficit stress reduces barley yield and associated traits, compared to optimal conditions. Previous studies have reported that drought stress significantly increases the activity of antioxidant enzymes. This study aimed to identify water-deficit-stress-tolerant barley genotypes by assessing some morphological and enzymatic traits.

Methods: In this study, the tolerance of 18 barley genotypes to water-deficit stress was evaluated based on some morphological and enzymatic traits. Two field experiments were conducted in a randomized complete block design with three replications: one under optimal irrigation and the other under limited irrigation, where water was withheld at the 50% flowering stage (Zadok's 10.5.2). Subsequently, some enzymatic and agronomic characteristics were measured, including catalase (CAT) and peroxidase (POD) activity, 1000-grain weight, number of tillers, spike length, number of grains per spike, plant height, biomass, and grain yield. For the measured traits, genetic coefficient of variation, phenotypic coefficient of variation, and heritability were estimated. Also, cluster analysis was conducted to group genotypes under both water-deficit stress and normal conditions. In addition, the stress tolerance index (STI) was calculated to identify water-deficit-stress tolerant genotypes.

Results: There were significant differences among the barley genotypes for all traits, except for POD and CAT. Also, water-deficit stress significantly affected plant height, biomass, grain yield, peroxidase, and catalase. However, the interaction between genotypes and irrigation conditions was significant only for grain yield and biomass. According to the analysis of variance, the estimates of genetic coefficients of variation, and cluster analyses, there was appreciable variation among the barley genotypes for most of the traits measured in this experiment. Genotypes 12, 17, and 10 exhibited a higher grain yield in both normal and water-deficit stress conditions, and also the highest STI values among the genotypes. Enzyme activity analysis suggested that CAT is a more reliable indicator than POD for alleviating the adverse effects of water-deficit stress. Also, CAT exhibited a higher genetic coefficient of variation and higher heritability than POD under both conditions. The number of grains per spike showed noticeably higher heritability values than grain yield under both normal and water-deficit stress conditions. Also, a high heritability was observed for1000-grain weight under stress conditions. Thus, indirect selection for grain yield through the number of grains per spike and 1000-grain weight may be useful in segregating generations under water-deficit stress conditions, and through the number of grains per spike under normal conditions.

Conclusion: Genotypes 12, 17, and 10, with higher grain yield under normal and water-deficit stress conditions, and the highest STI values, can be recommended for future evaluation in breeding programs for drought tolerance.

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Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop ranking fourth globally in production, after maize, wheat, and rice (FAO 2020). It exhibits greater drought-tolerance than wheat, making it a suitable alternative in arid regions where precipitation is inadequate for wheat cultivation (Briggs 1978).

The severity of drought stress is increasing worldwide, and is projected to reduce crop yields by up to 30% by 2025, compared to 2014 (Rosenzweig *et al*. 2014). Drought negatively impacts the photosynthetic process, which leads to a decrease in grain yield. This reduction occurs due to decreased stomatal conductance, restricted synthesis, and accelerated degradation of Rubisco, a loss in chlorophyll content, and the promotion of premature leaf senescence (Farooq *et al*. 2017). The flowering stage of barley is the most sensitive phase of development to drought. During this time, water deficit reduces fertile florets, leading to a lower number of grains per spike (Emam 2011). Ahmadzadeh *et al*. (2018) reported significant differences among barley genotypes under both water-deficit stress and non-stress conditions. Similarly, Hafez and Seleiman (2017) found that water-deficit stress negatively impacted barley, affecting both grain and straw production. They also noted a decrease in yield-related traits, including 1000-grain weight, number of grains per spike, and the number of spikes per square meter.

Farshadfar *et al*. (2008) highlighted that drought tolerance is a complex trait influenced by physiological, morphological, and molecular factors, making it challenging to measure directly. Drought conditions during the grain-filling stage have a particularly strong impact on grain development and overall yield. Consequently, plant breeders focus on selecting cultivars that have improved nitrogen and carbon remobilization capabilities and on developing genotypes with high photosynthetic efficiency (Hajibarat *et al*. 2024). The responses of plants to drought vary depending on the intensity of the environmental stress, resulting in changes to morphophysiological characteristics, biochemical composition, and mineral nutrients (Brahmi *et al*. 2023).

Plants subjected to drought stress often undergo an oxidative burst, characterized by excessive production of reactive oxygen species (ROS) (Farooq *et al*. 2009; Hussain *et al*. 2018). To protect cellular components from oxidative damage and keep ROS at safe levels, plants engage in various enzymatic and non-enzymatic antioxidant defense mechanisms. The main function of the enzymatic antioxidant system is to decompose hydrogen peroxide (H_2O_2), using enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Tale Ahmad and Haddad 2011).

In barley, antioxidant enzyme activity fluctuates significantly under stress. Salt stress increased the activity of SOD and CAT in barley, whereas drought stress primarily impacted SOD (Alsamadany *et al*. 2024). Another study on barley indicated that drought stress significantly enhanced enzyme activity, particularly CAT, in tolerant genotypes, especially at field capacities of 25-30% (Shirvani *et al*. 2024). Furthermore, Hafez and Seleiman (2017) reported a significant increase in CAT and POD activity in barley subjected to water-deficit stress when treated with chemical inducers.

This study aimed to identify water-deficit-stress-tolerant barley genotypes by evaluating 18 different genotypes. We assessed their morphological and biochemical characteristics, and the stress tolerance index (STI) to categorize these genotypes and identify suitable new genotypes for cultivation in areas experiencing water scarcity.

Materials and Methods

In this study, two Iranian barley cultivars and 16 advanced promising genotypes from the Gachsaran Rainfed Agricultural Research Center, Iran (Table 1) were evaluated in a field experiment. The 'Khorram' cultivar is well adapted to hot and dry conditions in tropical and semi-tropical rainfed regions. It matures early, has a high 1000-grain weight, exhibits strong resistance to lodging, and performs well in high-potential environments. The 'Mahour' cultivar is also early maturing and shows good tolerance to late-season drought stress (https://zoomkesht.com (In Persian); Vaezi *et al.* 2016). However, it has not gained wide acceptance among farmers due to the presence of the lemma on the grain after threshing.

Prior to sowing, all seeds were treated with Vitavax or Thiram fungicide to protect against fungal diseases such as hidden black spot. Two separate field experiments were conducted using a randomized complete block design with three replications. The first experiment was carried out under optimal irrigation conditions, while the second involved limited irrigation, with water withheld starting at the 50% flowering stage (Zadok's scale 10.5.2). To determine appropriate irrigation timing, the available soil moisture at a depth of 30-40 cm, corresponding to the typical root depth of barley, was measured in the control (fully irrigated) plots using the gravimetric method. Agronomic traits were assessed at two key growth stages: the tillering stage (first sampling) and after the final irrigation at the tillering stage (second sampling). Biochemical trait measurements were synchronized with sampling for agronomic traits. For this, entire plants were harvested at the soil surface, wrapped in aluminum foil, immediately placed on ice, and transported to the laboratory, where they were stored at –40 °C.

The central row of each plot was used for sampling and measuring various traits throughout the study. The morphological and agronomic traits measured included 1000-grain weight, number of tillers, spike length, plant height, number of grains per spike, biomass, and grain yield per square meter. The POD activity was determined following the method of Chance and Maehly (1955), and CAT activity was measured according to Aebi (1984).

Statistical analysis

Analysis of variance (ANOVA) was conducted to test the significance of differences among barley genotypes for the measured characteristics, and consequently, means were compared according to Duncan's multiple range test, using SAS 9.1 software. Genetic variance, phenotypic variance, and heritability were estimated using expected mean squares. Also, cluster analysis was conducted, using the UPGMA method under both water-deficit stress and non-stress conditions. The number of clusters was determined through the Hotelling's T-test and the Cubic Clustering Criterion. SPSS Statistics 22 was used to group the genotypes. STI was calculated for all genotypes using the following formula proposed by Fernandez (1992), where Ys is the yield under water-deficit stress, Yp is the yield under non-stress conditions, and $\bar{Y}p$ is the mean yield of the genotypes in the non-stress conditions.

$$STI = (Yp \times Ys)/(\bar{Y}p)^2$$

Table 1. Characteristics of the studied barley genotypes.

Code	Pedigree or name
1	Mahour (Tolerant to moisture stress at the end of the season)
2	Khorram (Adapted to dry and hot conditions of tropical and subtropical regions)
3	Arbayan-01//As46/Aths/3/Barjouj, ICB02-0406-0AP-8AP-0AP
4	Avt/Attiki//MAtt733371/3/Aths/Lignee686/4/Kabaa, ICB98-0796-0AP-15AP-0AP-14AP-0AP-8AP-0AP
5	Lignee527/NK1272/4/Avt/Attiki//Aths/3/Giza121/Pue, ICB95-0279-0AP-8AP-0AP-14AP-0AP
6	Rhn03/3/Mr2584/Att//Mari/Aths*302/4/Rhn03/Lignee527, ICB05-0272-3AP-0AP
7	Rhn03/3/Mr2584/Att//Mari/Aths*302/4/Ssn/Badia//Arar/3/Gloria'S'/Copal'S', ICB05-0292-7AP-0AP
8	Aths/Lignee686//Mari/Aths*2/3/Lignee527/NK1272//Alanda/6/JLB7001/5/DeirAlla106//DL70/ Pyo/3/RM1508/4/Arizona5908/Aths//Avt/Attiki/3/Ager, ICB05-0238-0AP-5AP-0AP
9	AwBlack/Aths//Arar/3/9Cr27907/Roho/4/CompCr229//As46/Pro/3/DeirAlla106//DL71/Strain205, ICB97-0605-0AP-10AP-0AP-5AP-0AP-1AP-0AP
10	E.ACACIA/DEFRA//PENCO/CHEVRON-BAR, CBSS02Y00319S-0M-0M-5Y-1M-0Y
11	SHENMAI NO.3/MSEL//CANELA, CBSS04Y00367T-I-2Y-2M-0Y-0M-0Y
12	SHENMAI NO.3/MSEL//CANELA, CBSS04Y00367T-D-3Y-1M-0Y-0M-0Y
13	ATAH92/2*M81//TOCTE/3/PENCO/CHEVRON-BAR, CBSS01M00733T-0TOPY-7M-2M-2Y-1M-0Y
14	DEFRA/CL128//PFC 88209, CBSS02Y00326S-0M-0M-4Y-1M-0Y
15	FRESA/LEGACY, CBSS05Y00125S-7Y-0M-0Y-0M-1AP
16	MoB1337/Wi2291//Mooroco9-75/3/Hml
17	TRADITION/6/P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA1/7/LEGACY// PENCO/CHEVRON-BAR, CBSS04M00295T-2M-0Y-0M-0Y-1M-0AP
18	LIMON/BICHY2000//NE167/CLE176, CBSS05Y00064S-29Y-0M-0Y-0M-3AP

Results

The results of ANOVA (Table 2) indicated that the effect of genotype was significant for all traits, except for POD and CAT. Additionally, the effect of water-deficit stress was significant for plant height, biomass, grain yield, peroxidase, and catalase. The interaction between genotype and environment was significant only for biomass and grain yield, while no significant interaction was observed for other traits.

Drought stress, caused by irrigation interruption, resulted in reductions in plant height, grain yield, biomass, and POD activity. Conversely, CAT enzyme activity increased under water-deficit stress conditions (Table 3). A comparison of the two conditions revealed that all genotypes experienced a decrease in biomass and grain yield under drought stress (Figures 1 and 2).

Table 2. Combined analysis of variance for agronomic traits and enzymes of barley genotypes under water-deficit stress and non-stress conditions.

SOV	df	1000-grain weight	Tiller number	Spike length	Plant height	No. of grains per spike	Biomass	Grain yield	Peroxidase	Catalase
Irrigation (S)	1	22.32	0.733	0.996	159.67^{*}	12.68	29.56^{**}	7.73^{**}	0.059^{**}	0.026^{**}
Replication/S	4	114.52^{**}	4.17^{**}	1.009^{*}	215.57^{**}	106.8^{**}	2.07^{**}	0.54^{**}	0.018^{**}	0.0008
Genotype (G)	17	69.49^{**}	1.14^{**}	1.596^{**}	155.16^{**}	686.6^{**}	0.39^{*}	0.163^{**}	0.004	0.00041
G × S	17	9.45	0.26	0.547	50.38	30.26	0.37^{*}	0.118^{*}	0.002	0.00051
Error	68	8.85	0.412	0.351	35.60	23.38	0.182	0.052	0.0037	0.00065
CV (%)	-	7.65	15.96	8.78	6.90	16.86	17.96	19.73	5.39	2.45

*,**: Significant at the 5 and 1 percent probability levels, respectively.

Table 3. Effect of water-deficit stress on some studied traits in barley.

Condition	Plant height (cm)	Grain yield (t/ha)	Biomass (t/ha)	Peroxidase (μmol/gr)	Catalase (μmol/gr)
Normal	89.48 a	1.63 a	3.26 a	0.19 a	0.04 b
Stress	77.38 b	1.01 b	2.11 b	0.17 b	0.10 a

In each trait, the means that have different letters are significantly different at 5% probability level based on Duncan's multiple range test.

Table 4 revealed that Genotype 9 had the highest 1000-grain weight, followed by Mahour, Genotypes 10, 13, 16, 18, and 6, and Khorram. The Mahour cultivar demonstrated the greatest number of tillers. Genotype 4 showed the longest spike length, followed by Genotypes 3 and 9. Both Mahour and Khorram cultivars, along with Genotypes 3, 11, and 13, achieved the tallest plant heights. Genotypes 3 and 4 also recorded the highest number of grains per spike, followed by Genotype 12.

As shown in Figure 1, Genotype 12 recorded the highest biomass under both water-deficit stress and non-stress conditions, followed by Genotype 17. Genotypes 12, 10, and 17 had the highest grain yield in non-stress conditions, while Genotypes 17, 12, 4, and 10 had the highest grain yield in water-deficit stress conditions. Overall, Genotypes 12, 17, and 10 were promising in both environments (Figure 2). The STI values were also highest for Genotypes 12, 17, and 10 (0.99, 0.96, and 0.86, respectively (Table 4).

Mahour is recognized for its ability to withstand late-season drought stress, which explains its minimal yield reduction. However, Genotype 6 showed an even smaller decrease in grain yield, although both genotypes showed low yields in both conditions.

Descriptive statistics and estimates of genetic parameters for agronomic traits, and POD and CAT activity under normal and water-deficit stress conditions are shown in Tables 5 and 6, respectively. CAT had a higher genotypic coefficient of variation than POD at both conditions. Also, the genotypic

Table 4. Comparing the barley genotypes in terms of some studied characteristics, averaged over normal and water-deficit stress conditions.

Genotype	1000-grain weight (gr)	Tiller number	Spike length (cm)	Plant height (cm)	Number of grains per spike	STI
Mahour	42 ab	5.3 a	7.0 a-d	91.3 a	21.2 d	0.40
Khorram	41 ab	4.2 bc	6.4 d-f	90.1 a	22.3 d	0.52
3	35 ef	3.8 b-d	7.5 ab	91.8 a	49.5 a	0.46
4	36 c-f	3.5 cd	7.7 a	88.3 ab	46.2 a	0.72
5	40 b-d	3.8 b-d	6.1 ef	88.1 abc	21.5 d	0.55
6	41 ab	4.0 bc	5.8 f	81.2 c	18.2 d	0.35
7	39 b-e	4.2 bc	6.2 ef	86.4 abc	22.8 d	0.50
8	34 f	3.2 d	7.0 a-d	82.8 bc	36.2 c	0.53
9	44 a	4.3 b	7.5 ab	82.1 bc	23.3 d	0.68
10	42 ab	4.0 bc	6.4 d-f	82.1 bc	22.5 d	0.86
11	36 d-f	4.0 bc	6.4 d-f	90.0 a	39.2 bc	0.64
12	33 f	4.2 bc	6.7 c-e	82.4 bc	44.0 ab	0.99
13	42 ab	3.8 b-d	6.8 b-e	90.7 a	23.0 d	0.61
14	36 c-f	4.3 b	7.2 a-c	87.4 abc	39.3 bc	0.63
15	40 b-d	4.3 b	6.6 cd	88.7 ab	21.3 d	0.66
16	42 ab	4.0 bc	6.4 d-f	81.8 bc	20.2 d	0.53
17	36 c-f	4.0 bc	7.0 a-d	73.6 d	20.7 d	0.96
18	42 ab	3.8 b-d	6.9 b-d	86.7 abc	21.2 d	0.48

In each trait, the means that have different letters are significantly different at 5% probability level based on Duncan's multiple range test.

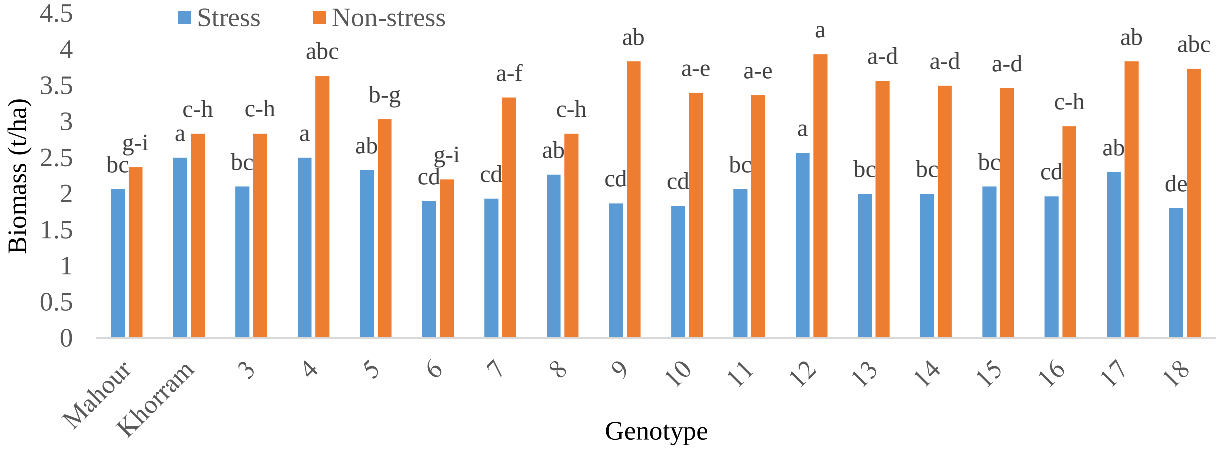

Figure 1. Biomass of barley genotypes under normal (non-stress) and water-deficit stress conditions; In each environment, the means that have different letters are significantly different at 5% probability level, based on

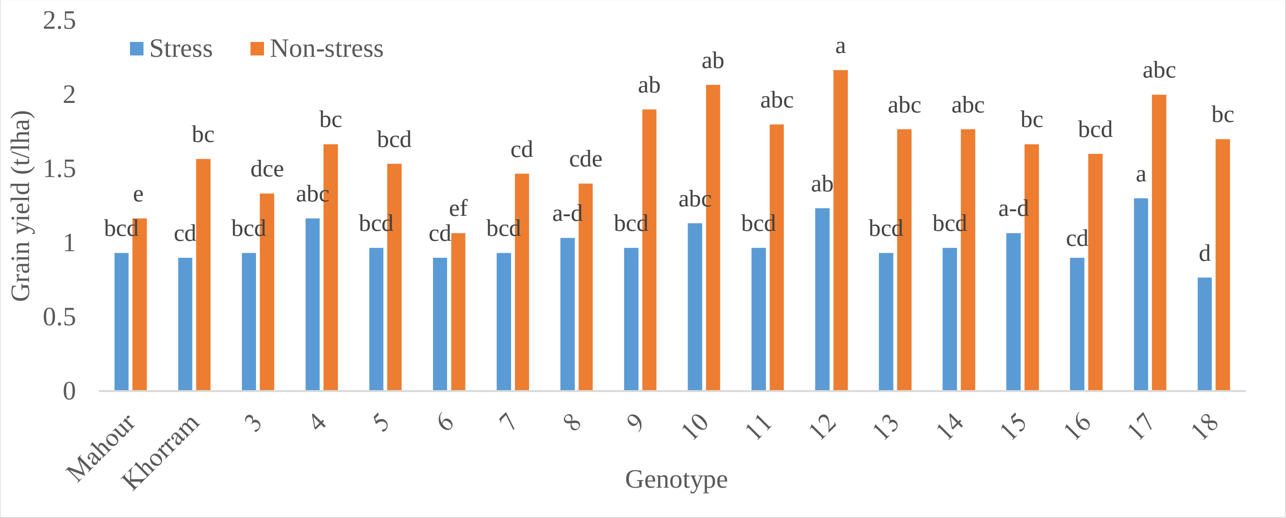

Figure 2. Grain yield of barley genotypes under normal (non-stress) and water-deficit stress conditions; In each environment, the means that have different letters are significantly different at 5% probability level, based on Duncan's multiple range test.

coefficient of variation of CAT increased substantially under water-deficit stress conditions as compared to normal conditions; however, POD activity decreased slightly in the water-deficit stress conditions.

Among the agronomic characteristics, the highest genotypic coefficient of variation belonged to the grain yield, followed by the number of grains per spike, biomass, and tiller number under normal conditions. However, under the water-deficit stress conditions, the number of grains per spike showed a much higher genotypic coefficient of variation than other characteristics.

The heritability value for CAT was much higher (89.99 and 91.66%) than that of POD (33.33 and 50%) under normal and water-deficit stress conditions, respectively. Among the agronomic characteristics, the number of grains per spike showed the highest heritability (94.76 and 90.90%) under normal and water-deficit stress conditions, respectively. The heritability for grain yield was high (68.35%) in the non-stress environment but moderate (32.56%) under water-deficit stress conditions.

The cluster analysis classified the genotypes under non-stress conditions into five groups (Figure 3). The first group consisted of two subgroups of A (Genotypes 6, 7, 9, 10, 16, and 18) and B (Mahour, Khorram, and Genotypes 5, 13, and 15). The second group consisted of only Genotype 17. The third group also had two subgroups of A (Genotypes 3, 11, and 14) and B (Genotype 4). The fourth group included Genotype 12, and the fifth group comprised Genotype 8.

Under water-deficit stress conditions, cluster analysis divided the genotypes into four groups. The first group included Mahour, Khorram, and Genotypes 5, 7, 12, 15, and 18. The second group contained Genotypes 6, 9, 10, 16, and 17. The third group consisted of genotypes 4, 8, 11, 12, and 14, and the fourth group was solely made up of Genotype 3 (Figure 4).

Table 5. Descriptive statistics and estimates of phenotypic variance, genetic variance, phenotypic and genetic coefficient of variation, and heritability for the studied enzymes and agronomic traits under normal irrigation (non-stress) conditions.

Trait	Mean	Min	Max	PV	GV	PCV (%)	GCV (%)	h^2 (%)
Peroxidase (µmol/gr)	0.19	0.034	0.339	0.006	0.002	40.77	23.54	33.33
Catalase (µmol/gr)	0.04	0.003	0.079	0.00067	0.0006	64.71	61.24	89.55
1000-grain weight (gr)	38.41	27.20	47.40	38.63	25.92	16.18	13.25	67.09
Tiller number	4.11	4.11	6.00	0.901	0.529	23.10	17.70	58.71
Spike length (cm)	6.84	5.22	8.29	0.900	0.486	13.87	10.19	53.94
Plant height (cm)	84.8	69.3	10.28	110.73	69.88	12.41	9.86	63.11
Number of grains per spike	26.9	17.00	60.00	37.82	35.84	22.86	22.26	94.76
Biomass (t/ha)	3.26	1.30	5.20	0.764	0.395	26.81	19.28	51.70
Grain yield (t/ha)	1.63	0.50	2.60	0.297	0.203	33.43	27.64	68.35

Min: Minimum; Max: Maximum; PV: Phenotypic variance; GV: Genetic variance; PCV: Phenotypic coefficient of variation; GCV: Genetic coefficient of variation; h^2: Heritability.

Table 6. Descriptive statistics and estimates of phenotypic variance, genetic variance, phenotypic and genetic coefficient of variation, and heritability for the studied enzymes and agronomic traits under water-deficit stress conditions.

Trait	Mean	Min	Max	PV	GV	PCV (%)	GCV (%)	h^2 (%)
Peroxidase (µmol/gr)	0.17	0.06	0.298	0.002	0.001	26.31	18.60	50.00
Catalase (µmol/gr)	0.10	0.02	0.182	0.012	0.011	109.54	104.88	91.66
1000-grain weight (gr)	39.15	31.90	48.70	37.55	32.23	15.65	14.50	85.81
Tiller number	3.98	3.00	7.00	0.458	0.073	17.00	6.79	15.94
Spike length (cm)	6.64	5.00	8.40	1.33	1.04	17.37	15.36	78.57
Plant height (cm)	77.38	70.00	96.40	71.51	50.36	10.93	9.17	70.42
Number of grains per spike	28.26	17.00	62.00	330.66	300.23	64.35	61.31	90.80
Biomass (t/ha)	2.11	1.40	3.20	0.163	0.061	19.13	11.71	37.42
Grain yield (t/ha)	1.01	0.60	1.50	0.043	0.014	20.53	11.72	32.56

Min: Minimum; Max: Maximum; PV: Phenotypic variance; GV: Genetic variance; PCV: Phenotypic coefficient of variation; GCV: Genetic coefficient of variation; h^2: Heritability.

Discussion

The observed differences in the responses of genotypes under non-stress and water-deficit stress conditions indicate a significant genotype × environment interaction for biomass and grain yield, but not for the other traits. This lack of significant genotype × environment interaction for other traits may be due to the timing of the stress application, which occurred late in the season, after spike emergence.

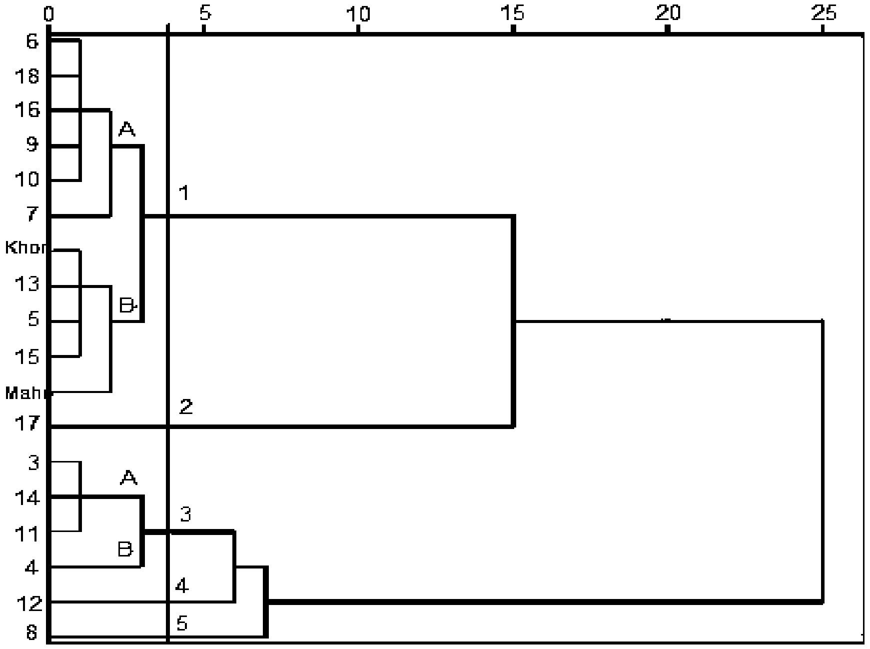

Figure 3. Dendrogram resulting from the cluster analysis of barley genotypes based on the studied traits under normal irrigation conditions.

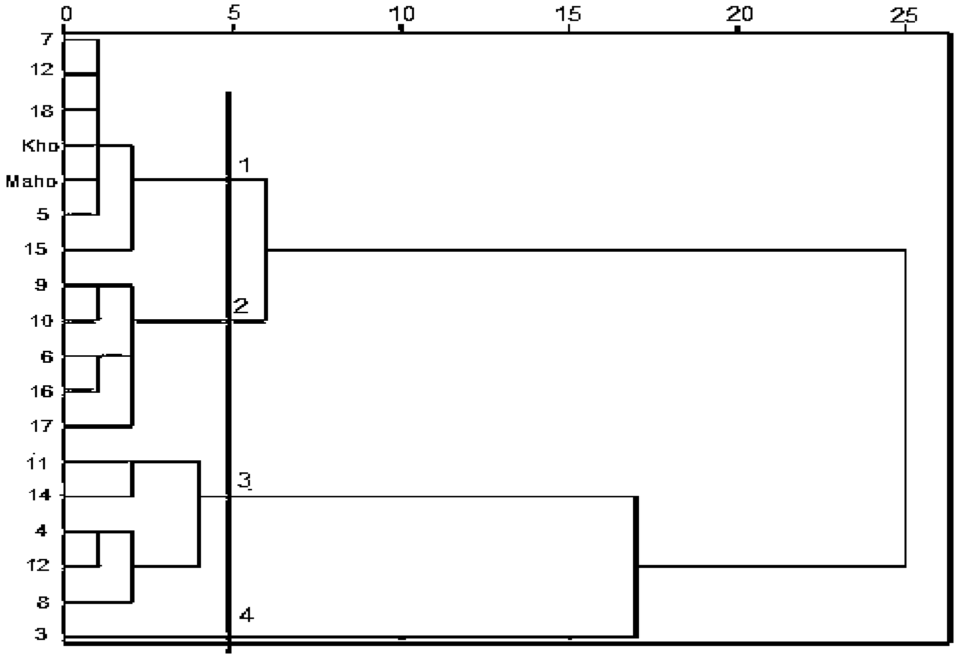

Figure 4. Dendrogram resulting from the cluster analysis of barley genotypes based on the studied traits under water-deficit stress conditions.

The results indicate that all genotypes experienced a reduction in grain yield under water-deficit stress conditions; however, the extent of the reduction varied among them. For instance, Genotype 18 experienced a significant decrease, with its grain yield dropping to less than half of its non-stress yield. In contrast, other genotypes, such as Genotype 6 and the Mahour variety, exhibited only slight reductions with a decrease of 233 kg and 160 kg, respectively; however, their grain yield was low. Soleimani *et al*. (2017) observed a 36% reduction in barley grain yield due to drought. Aghaali *et al*. (2016) emphasized that drought stress is a significant limiting factor in crop production, noting that terminal drought stress in chickpea results in a 50% yield loss. Alderfasi (2009) reported that drought stress during the flowering stage has a more pronounced effect on grain yield compared to drought stress during the vegetative stage.

Water deficit at any growth stage can reduce yield and yield components (including the number of fertile tillers, grains per spike, and grain weight) to varying degrees, depending on factors such as timing, duration, and intensity of the stress. Emam (2011) found that drought during the flowering stage in barley leads to a decline in the number of fertile florets and the number of grains per spike. Hafez and Seleiman (2017) also found that water-deficit stress significantly reduced both grain yield and straw yield, along with related traits such as 1000-grain weight, number of grains per spike, and number of spikes per square meter, in comparison to non-stress conditions. Although, in our study, water-deficit stress after flowering did not affect the yield components individually despite expectations for some components, the reduction in grain yield can be attributed to the cumulative effects of these components, including 1000-grain weight and number of grains per spike.

Ahmadikhah *et al*. (2016) reported that limited irrigation had a significant negative impact on several traits, including yield, plant height, tiller number, panicle length, and biomass. Brahmi *et al*. (2023) observed that drought stress in barley seedlings leads to a reduction in plant height. In our study, drought stress significantly affected plant height, biomass, and grain yield. However, the reduction in plant height was unexpected because the water-deficit stress was imposed at the 50% flowering stage when the plants had reached their maximum height. Therefore, the reduction in plant height may be attributed to sampling error and/or soil heterogeneity, not to the effect of the late-season water-deficit stress in the experiment.

The barley genotypes examined showed significant genetic variation for all characteristics, including 1000-grain weight, tiller number, spike length, plant height, number of grains per spike, grain yield, and biomass. Also, most of the genetic coefficients of variation were near or above 10% (ranging from 9.17 to 61.31%) across both conditions. These results indicated the existence of appreciable genetic variability among the studied barley genotypes, which allows for the selection of better genotypes for future studies. Ahmadzadeh *et al*. (2018) reported significant differences in spike length among barley genotypes under both non-stress and drought-stress conditions. In our study, 1000-grain weight was the most stable trait across normal and water-deficit stress conditions, followed by spike length, tiller number, and number of grains per spike. Shepherd *et al*. (2002) found that the number of spikes per square meter was more stable than the number of grains per spike. Although in our study, tiller number was also more stable than the number of grains per spike across normal and water-deficit stress conditions, the difference was not substantial (3.15% versus 5.06% change). Grain yield and biomass were the least stable traits with changes of 38.04% and 35.28%, much higher than those of the yield components.

This study found that drought stress resulted in a decrease in POD enzyme activity. Conversely, the activity of the CAT enzyme increased under water-deficit stress conditions. Thus, our results indicate that POD may not play a significant role in the antioxidant defense response to water-deficit stress. Instead, the increased activity of CAT suggests that it may have a more important role. In contrast to our study, Maghsoudi *et al*. (2016) noted that POD activity rose in the roots and stems of alfalfa cultivars as drought stress intensified. Similar findings to those of Maghsoudi *et al.* (2016) were reported for alfalfa (Wang *et al*. 2009), wheat (Esfandiari *et al*. 2009), rice (Lum *et al*. 2014), and sesame (Kadkhodaie *et al*. 2014).

Under both non-stress and water-deficit stress conditions, CAT displayed a much higher heritability (89.55% and 91.66%, respectively) and genetic coefficient of variation (61.24% and 104.88%, respectively) than POD with heritability of 33.33% and 50% and genetic coefficients of variation of 23.54% and 18.60%, respectively). According to Stansfield (1991), traits with heritability above 50 percent are considered to have high heritability, those between 20 percent and 50 percent have moderate heritability, and traits below 20 percent are considered to have low heritability. For traits with high heritability, effective selection can be conducted in early generations. In this study, POD and especially CAT showed good heritability under water-deficit stress conditions, indicating that selection based on these traits would be beneficial under such conditions.

Among agronomic characteristics, heritability values for the number of grains per spike (94.76 and 90.80%) were much higher than those for grain yield (68.35 and 32.56%) under normal and water-deficit stress conditions, respectively. Also, 1000-grain weight had a very high heritability under water-deficit stress conditions (85.81%). 1000-grain weight is the most important characteristic that determines grain yield under late-season drought. Indirect selection for grain yield would be efficient when the trait is correlated with grain yield and has higher heritability than the grain yield.

Since 1000-grain weight and the number of grains per spike are the two important components of grain yield, it therefore seems that, under conditions of this experiment, indirect selection for grain yield through these components can be useful in early segregating generations under water-deficit stress conditions, and through the number of grains per spike under normal conditions.

Cluster analysis resulted in five groups under normal conditions and four groups under water-deficit stress conditions. Similar to the estimates of the genetic coefficients of variation, these results indicate the existence of appreciable genetic variability among the barley genotypes under both normal and water-deficit stress conditions, which makes it possible to select superior genotypes for further evaluation. Genotypes 8 and 12, which were initially in separate groups under non-stress conditions, clustered together under water-deficit stress. In contrast, genotypes 3, 4, 11, and 14, which had been grouped together under normal conditions, became more dispersed under water-deficit stress conditions, with genotypes 3 and 4 moving to different groups. Some genotypes, specifically Mahour, Khorram, 5, and 18, remained within the same cluster under both water-deficit stress and non-stress conditions, indicating a degree of stability in their performance across different environments.

Table 4 indicated that Genotypes 12, 17, and 10 had the highest STI values (0.99, 0.96, and 0.86, respectively), while Mahour and Khorram cultivars recorded lower values (0.40 and 0.52, respectively). Therefore, Genotypes 12, 17, and 10 are regarded as more water-deficit tolerant and higher-yielding genotypes than the other genotypes.

Although the Mahour variety is known for its tolerance to late-season drought stress, it has not been widely adopted by farmers due to issues, such as the retention of the lemma on the grain after threshing. Furthermore, in our study, it demonstrated a much lower STI and grain yield compared to those of Genotypes 12, 17, and 10. Therefore, Genotypes 12, 17, and 10, with higher grain yield in both conditions and higher STI, can be candidates for future evaluation in breeding programs for drought tolerance.

Conclusion

The results of this study highlight the significant effects of genotype and water-deficit stress on most of the traits under study. However, the genotype by water-deficit stress interaction was only significant for grain yield and biomass, confirming the expectations about the differential influence of environmental conditions on these traits.

There was noticeable variation among genotypes for most of the traits under study, as evidenced by the analysis of variance table, the genetic coefficients of variation, and cluster analysis. Genotypes

12, 17, and 10 showed higher grain yield under both normal and water-deficit stress conditions and also the highest STI values, demonstrating greater drought tolerance than the other genotypes. Therefore, Genotypes 12, 17, and 10 can be recommended for further evaluation in breeding programs aimed at improving drought tolerance.

Data on enzymatic activity indicated that drought affected CAT and POD enzymes differently. POD showed a significant reduction, but CAT showed a significant increase under water deficit stress conditions, suggesting the importance of CAT in alleviating the harmful effects of water-deficit stress. Also, CAT showed a higher genetic coefficient of variation than POD in both normal and water-deficit stress conditions.

Heritability values for the number of grains per spike were appreciably higher than those for grain yield in both normal and water-deficit stress conditions. Also, 1000-grain weight showed a high heritability in the water-deficit stress environment. Therefore, it seems that indirect selection for grain yield through the number of grains per spike and 1000-grain weight can be practiced in segregating generations in water-deficit stress conditions, and through the number of grains per spike in non-stress conditions.

These findings provide valuable insights for selecting and developing barley genotypes with improved resilience and productivity under water-limited conditions.

Conflict of Interest

The authors declare no conflicts of interest with any individual or organization concerning the subject of this paper.

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