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Evaluation of Antioxidant Enzymes, Lipid Peroxidation and Proline Content as Selection Criteria for Grain Yield under Water Deficit Stress in Barley

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Abstract

Screening plant genetic resources under water deficit environment using reliable selection criteria is an essential step in breeding programs towards drought tolerance improvement. In this greenhouse experiment, the twenty-five barley (*Hordeum vulgare* L.) cultivars were subjected to well-watered and water deficit stress conditions in order to measure changes of antioxidant enzymes activities, malondialdehyde (MDA), proline and protein contents in response to water deficit stress and to evaluate the significance of the biochemical traits as selection criteria for improvement of grain yield. Significant variations were observed among the barley cultivars for grain yield as well as the biochemical traits including the antioxidant enzymes, MDA, protein and proline contents. Water deficit stress caused a significant increase in MDA, proline and protein contents as well as the antioxidant enzymes activities including catalase, peroxidase, superoxide dismutase and ascorbic peroxidase. The results of correlation coefficients and regression modeling showed that the relationships between grain yield and biochemical traits were somewhat influenced by water regimes. The results suggested that high proline content along with low protein content can be used as the criteria for selection of high-yield barley cultivars under well-watered conditions. Catalase and superoxide dismutase activities as well as proline content were known as the selection criteria with significant contributions to grain yield of barley under water deficit conditions. Catalase and superoxide dismutase activities were significantly positively related to grain yield, while proline content had a negative relationship with grain yield under water deficit conditions.

Keywords: *Hordeum vulgare*, Superoxide dismutase, Catalase, Protein content, MDA

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the world's fourth most important crop after rice, wheat and maize in terms of cultivated area [1]. Barley is known as a versatile crop due to various beneficial properties. In arid and semi-arid regions, growth and production of barley are adversely affected by water deficit stress during either a particular growth period or throughout the whole growth season. Thus, development of drought tolerant barley cultivars is of great priority to ensure more consistent production. Screening genetic resources under water deficit environment using reliable selection criteria is an essential step in breeding programs towards drought tolerance improvement [2]. For many years, agronomic traits such as grain yield and its components have been applied for evaluation of drought tolerance [3], however agronomic traits may not properly reflect different levels of drought tolerance between genotypes. Indeed, evaluation of drought tolerance at the level of biochemical responses may provide more accurate information about attributes intrinsically related to drought tolerance [4].

Water deficit stress increases reactive oxygen species (ROS) such as superoxide (O-2.), hydrogen peroxide (H2O2), hydroxyl radical (OH.) and singlet oxygen (1O2) [5]. ROS, at low concentrations, may act as messenger molecules involved in stress signal transduction, triggering tolerance against various abiotic and biotic stresses [5, 6, 7, 8]. On the other hand, higher amounts of these reactive agents cause oxidative stress which eventually has damaging effects on cellular constituents [9, 10, 11]. In fact, the degree of damage by ROS depends on rates of generation and removal of ROS.

Plants have the ability to scavenge/detoxify ROS by producing different types of antioxidants. High activities of antioxidants in response to abiotic stresses have been widely observed in various plant species [12, 13, 14, 15, 16]. Antioxidants can be generally categorized into two types comprising of enzymatic such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), and non-enzymatic antioxidants such as α-tocopherol, ascorbic acid and β-carotene [11]. Enzymatic antioxidants are in charge of removing oxidative reactive agents. Antioxidant enzymes are known to substantially decrease the levels of superoxide and hydrogen peroxide in plants [17]. Superoxide dismutase catalyzes the dismutation of O2− to molecular oxygen and H2O2. It is one of the most important enzymes in the plant defense system against oxidative stress, and it occurs ubiquitously in every cell of all types of plants [11]. In addition, Catalase and peroxidase catalyze a redox reaction in which dismutation of hydrogen peroxide (H2O2) gives rise to water and oxygen. Ascorbate peroxidases, alternatively, are the key enzymes for scavenging hydrogen peroxide in chloroplast and cytosol of plant cells [18]. Accumulation of ROS, as a consequence of water deficit stress, causes lipid peroxidation which in turn, results in the breakdown of cell membrane functions [19]. The extent of lipid peroxidation can be assessed by measurement of malondialdehyde (MDA) content which is presumed as a sign of membrane injury [20]. The amount of MDA is an indirect marker of oxidative damage and accordingly sensitivity to stress in plant species. Furthermore, accumulation of proline occurs under water deficit stress in plants to facilitate osmoregulation [21]. Proline may act as a scavenger of ROS to alleviate oxidative damage [22].

There are several reports indicating the relationship between the increased antioxidant enzymes activities and tolerance to abiotic stresses in various plant species such as wheat [14, 23], rice [13], rapeseed [15, 24] and soybean [25]. The association between the enhanced activities of antioxidant enzymes and tolerance to environmental stresses

has been also reported in barley [12, 26, 27, 28, 29], however the results were often obtained based on the assessment of limited number of genotypes.

The present study aimed to measure changes of antioxidant enzymes activities, MDA and proline contents in response to water deficit stress in twenty-five barley cultivars and to evaluate the relative significance of these biochemical characteristics as selection criteria for improvement of grain yield.

MATERIALS and METHODS

The experiment was carried out at the greenhouse of Department of Crop Production and Plant Breeding, College of Agriculture, Shiraz University, Shiraz, Iran. A factorial experiment based on a completely randomized design with three replications was used to evaluate the effects of two water regimes (well-watered and water deficit stress) on different biochemical characteristics and grain yield of twenty-five barley cultivars including Valfajr, Jonoob, Shirin, Nimrouz, Kavir, Reyhan, Karoon, Goharjo, Bahman, Torkaman, Gorgan4, Fajr30, Zarjo, Yousef, Nosrat, Torsh, Aras, Sahra, Afzal, Alger-Ceres, Sina, Danesiah, Makooei, Eram, and Dasht. The physical and chemical properties of the soil samples are shown in Table 1. Five plants were grown in each pot filled with 5 Kg 4mm-sieved air-dried soil under greenhouse conditions with 16 hours daylight and 28°C/15°C day/night temperature. 150 mg N kg-1 soil was used as urea 46% N fertilizer in all the pots. The seeds were treated with ethanol 98% for about 20 s and were then washed three times with distilled water and kept at 20 °C. The well-watered regime was performed by regularly irrigating the plants to keep soil moisture around field capacity (FC) during the experiment. The water deficit regime was imposed on the plants at the flowering to the end of maturity stage by withholding water to reduce soil moisture to 45% FC. For each water regime, the pots were daily weighed and watered until they reached the desired FC level.

Leaf samples (0.5 g) were homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and the solution was filtered using Whatman No. 2 filter paper. Total protein content was estimated using Bradford's protocol [30], in which bovine serum albumin (BSA) was used as a standard. The activity of the superoxide dismutase (SOD) was determined based on its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) [31]. One unit (U) of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT at 560 nm. Peroxidase (POD) activity was assayed [32] at 436 nm based on its ability to convert guaiacol to tetraguaiacol (ε = 26.6 mM cm−1). The activity of catalase (CAT) was determined by monitoring the disappearance of H2O2 at 240 nm (ε = 40 mM cm−1) [33]. Ascorbic peroxidase (APX) activity was measured based on method described by [34]. At the end of the growth and maturing period, grain yield was measured to considering its relationship with antioxidant enzymes' activities. Malondialdehyde content was also measured following the protocol of Heath and Packer [35]. The proline content was determined using the method of Bates [36].

Analysis of variance (ANOVA) was performed to test the effects of cultivars and water regimes and their interaction. Least Significant Difference (LSD α= 0.05) test was used for mean comparisons. The data were analyzed in SAS software (V. 9.3). Stepwise selection procedure and correlation coefficient technique were also employed to detect the most important variables affecting grain yield using PROC REG and PROC CORR of SAS software. Genotypic, environmental, and phenotypic variance and also heritability, phenotypic, and genotypic coefficient of variation were calculated based expected values of two-way factorial ANOVA using PROC VARCOMP of SAS software.

Table 1: Physical and chemical properties of the soil used in this experiment.

PWP (%)	FC (%)	SP (%)	pH		EC (dS m-1)	Clay (%)	Silt (%)	Sand (%)
9.1	25.3	54.7	7.9		0.5	33	49	18
Cu (mg kg-1)	Mn (mg kg-1)	Zn (mg kg-1)	Fe (mg kg-1)	K (mg kg-1)	P (mg kg-1)	TKN (%)	CaCO3 (%)	OC (%)
2	11.3	1.7	5	240	15	0.06	11	1.3

EC: electrical conductivity of saturated paste; **OC:** organic carbon; **SP, FC, and PWP:** soil moisture at saturation, field capacity, and permanent wilting point, **TKN:** total Kjeldahl N; **K:** NH4OAc-extractable potassium, and DTPA-Extractable Fe, Cu, Mn, and Zn.

RESULTS

The results of analysis of variance (Table 2) showed that water deficit stress significantly affected all the traits. In addition, significant variation among the barley cultivars as well as the interaction effect between the water regimes and the cultivars were observed for all the traits. The biochemical traits as well as grain yield showed considerable genotypic and phenotypic variations (Table 3). MDA, proline and SOD showed the maximum variation. The highest heritability was obtained for APX and CAT activities (Table 3), respectively, whilst grain yield had the minimum heritability indicating the magnitude of environmental effects on it.

The results of mean comparison (Table 4) clearly showed that the cultivars under water deficit stress had higher CAT activity (31%) compared to normal condition. Under normal condition, the cultivars Alger-Ceres and Makooei showed the highest and the lowest CAT activity, respectively; while under stress condition the cultivars Torsh and Shirin had maximum and minimum CAT activities. Moreover, APX and POD activities under stressful conditions were significantly higher than those of under normal condition with increase of 43% and 38%, respectively. Under well-watered conditions, the highest activities of APX and POD were respectively obtained for Kavir and Danesiah,while the cultivars Afzal and Yousef showed the lowest APX and POD activities. In the water deficit conditions, cultivar ranking differed in respect to APX and POD activities. The highest and the lowest activities of APX were recorded for the cultivars Jonoob and Eram. On the other hand, Eram had the maximum POD activity under water stress environment.

Water deficit stress caused a significant increase (129%) in SOD activity. The cultivar Bahman showed the highest SOD activity under both irrigation regimes.. The cultivars Yousef and Dasht had the minimum SOD activity under both normal and water stress conditions, respectively. Lipid peroxidation, measured by MDA content, significantly increased in all the cultivars due to water deficit stress (Table 5). The cultivar Nosrat exhibited the highest amount of MDA under water deficit stress indicating the maximum rate of damaged cell membranes. The cultivars Valfajr and Danesiah had the lowest level of MDA under water deficit stress. Proline content significantly accumulated in response to water deficit stress. The maximum proline content in both conditions was observed for the cultivar Valfajr, although the cultivar Alger-Ceres showed the highest level of increase in proline during water stress (Table 5). The amount of total protein also significantly increased (20%) in response to water deficit stress. Sina and Jonoob gained the maximum protein content under normal and water stress conditions, respectively. A significant reduction (39%) was observed for grain yield due to water deficit stress. The cultivars Nosrat and Danesiah showed the maximum grain yield under normal and stress environments, respectively (Table 5). The cultivar Valfajr had the lowest reduction for grain yield due to water deficit stress. Under normal and water stress conditions, the minimum grain yield was recorded for Gorgan4 and Goharjo, respectively (Table 5).

Correlation coefficients among the traits for normal and water deficit irrigation regimes are shown in Table 6. The results revealed that the interrelationships between the traits were somewhat influenced by water regimes. In the well-watered conditions, POD activity showed significant negative correlations with CAT and APX activities. On the other hand, POD activity had significant negative correlations with APX and SOD activities under water deficit stress. In normal conditions, grain yield had no significant correlations with antioxidant enzymes. Proline and protein contents were the only attributes that showed significant, but not strong, correlations with grain yield under normal conditions. In the water deficit stress conditions, grain yield had significant positive correlations with CAT and SOD activities. Also significant negative correlations were found between grain yield and some biochemical traits including MDA, protein and proline contents. Proline content showed significant positive correlations with CAT, APX, POD and MDA under water deficit stress, whilst no significant correlations were found between proline content and other biochemical traits under well-watered conditions.

Stepwise regression was separately performed for normal and stress conditions in order to determine the most important biochemical criteria which had significant contributions to grain yield (Tables 7 and 8). In accordance with correlation coefficients, the regression models indicated that the significance of biochemical traits differed in the two water regimes. Under well-watered conditions, proline and protein contents were the characteristics which significantly affected grain yield. Similar to correlation results, the regression model showed that proline and protein had positive and negative relationships with grain yield. Under water deficit conditions, stepwise regression found CAT, SOD and proline as the indicators with significant contributions to grain yield. CAT and SOD activities were significantly positively related to grain yield, while proline content had a negative relationship with grain yield under water deficit conditions.

DISCUSSION

It has been estimated that 1% of the oxygen consumed by plants is diverted to produce reactive oxygen species in various subcellular compartments [37]. Different environmental cues may enhance the production of ROS which damages macromolecules including proteins, nucleic acids, and lipids [5]. Plants have developed a variety of antioxidant enzymes and scavenging molecules, as a part of plant defense systems, to reduce such damaging effects [11].

Drought seriously limits crop yield potential. Therefore, it is essential to identify genetic resources tolerant to drought stress in an effort for more consistent agricultural production. Water deficit stress increases generation of ROS which may cause oxidative damages to plants cells. Superoxide dismutases catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, a product which is relatively stable and detoxified by CAT and POD [38]. Our results showed significantly higher activities of enzymatic antioxidants such as POD, CAT and SOD under water deficit conditions. This is in consistent with the previous findings [12, 14, 16, 26, 28, 29]. In addition, a substantial genetic variation for the antioxidants activities was observed among the barley cultivars. As previously mentioned [39, 40], this finding highlighted the key role of genetic control on the metabolic pathways which eventually create different capacities for production of antioxidant enzymes. The Results of correlation coefficients and regression modeling showed that the relationships between grain yield and enzymatic antioxidants are affected by environmental conditions. Under well-watered irrigation, there was no relationship between grain yield and antioxidant enzymes. In this condition, plant cells do not require to produce high amounts of antioxidant enzymes as the essential role of antioxidative systems is to maintain a balance between production and removal of ROS to keep them at appropriate levels for signaling and control of metabolic homeostasis. In addition, these antioxidant enzymes are known as indicators to evaluate the status of oxidation-reduction in plants [39]. On the contrary, the results revealed the importance of antioxidant enzymes as selection criteria for grain yield improvement under water deficit stress conditions. In particular, CAT and SOD activities significantly contributed to grain yield in water stress condition. When oxidative stress occurs, higher activity of antioxidant enzymes is required to scavenge oxidative compounds and to prevent plants from losing much energy and decreasing yield. The cultivars with higher performance of CAT and SOD can be selected for drought stress conditions. The previous studies have also reported that higher activity of antioxidant enzymes is related to tolerance to environmental stresses in plants [14, 15, 24, 28, 41, 42]. Among the antioxidant enzymes, SOD activity showed a higher variation among the barley cultivars implying that this enzyme can be exploited as an indirect selection indicator in barley breeding programs towards drought tolerance improvement.

Water deficit stress caused an increase in proline content; however there were different levels of proline concentrations among the barley cultivars. Proline content was recognized to be one of the significant contributors to grain yield under both normal and water deficit conditions. The results suggested that high proline content can be used as a criterion for selection of high-yield cultivars under well-watered conditions. Conversely, the negative relationship between proline content and grain yield under water defecit stress

indicated that the cultivars with high grain yield generally had lower proline contents. It seems that the level of proline content reflects the level of damaging effects of water deficit stress on the plants. Proline is an osmoregulatory agent which can scavenge free radical molecules in order to prevent oxidative damage caused by reactive oxygen species [22]. It still remains uncertain whether proline accumulation is simply a common adaptive biochemical response to stress or it can be considered as an indicator associated with the level of drought tolerance in a given genotype [43, 44].

In accordance with the previous reports [14, 16, 24, 45], the results displayed that MDA content significantly increased in the barley cultivars due to water deficit stress. Environmental stresses result in overproduction of ROS, which may cause lipid peroxidation measured in terms of MDA content as a convenient biomarker. MDA (small hydrocarbon fragments) is the final product of plant cell membrane lipid peroxidation induced by free radicals and its accumulation reflects the level of ROS toxicity [20, 46]. In the well-watered conditions, the high-yield cultivars generally had lower levels of lipid peroxidation indicating a higher free radical-scavenging capacity of these cultivars .On the other hand, MDA was not known as a critical indicator for grain yield under water deficit stress.

In conclusion, the barley cultivars significantly varied for grain yield as well as biochemical traits including antioxidant enzymes, MDA and proline contents under both well-watered and water deficit conditions. Water deficit stress increased the activity of the antioxidant enzymes in order to alleviate deleterious effects of ROS. Our results also implied that CAT and SOD activities as well as proline content are the reliable selection criteria for improvement grain yield of barley under water deficit conditions. Further progress can be achieved for drought tolerance improvement in crop plants by modulating the amounts of antioxidant enzymes.

Table 2. Analysis of variance for the traits measured in the twenty-five barley cultivars under normal and water deficit stress conditions.

Source	DF	CAT	APX	POD	SOD	MDA	Proline	Protein	Grain yield
Stress (S)	1	1005.296**	342720.41**	36454.75**	2138.62**	0.332**	654.56**	2.22**	16.26**
Variety (V)	24	847.39**	76024.57**	4458.62**	6205.12**	0.235**	467.81**	0.42**	0.39**
S*V	24	187.24**	3226.93**	249.86**	2896.07**	0.123**	154.76	0.12**	0.21**
Error	100	10.93	534.65	154.54	363.55	0.014	53.67	0.02	0.08
Coefficient of Variation (%)		5.42	4.68	12.91	16	8.56	21.78	10.19	20.88

*** and ****: Significant at 5 and 1% of probability respectively.
CAT: catalase; **APX:** ascorbic peroxidase; **POD:** peroxidase; **SOD:** superoxide dismutase; **MDA:** malondialdehyde.

Table 3. Mean, maximum, minimum, phenotypic and genotypic coefficient of variation, and heritability of the traits measured in the twenty –five barley cultivars under normal and water deficit stress conditions.

Trait	Mean	Minimum	Maximum	Heritability(%)	PCV (%)	GCV (%)
CAT	60.97	30.53	101.63	92.73	20.11	19.37
APX	837.76	365.71	1415.71	94.99	20.89	20.36
POD	96.27	22.71	149.74	82.27	30.67	27.82
SOD	303.81	46.01	946.76	80.8	36.52	32.83
MDA	0.22	0.15	0.3	83.23	47.98	42.34
Proline	25.98	5.77	43.42	62.52	46.03	36.76
Protein	1.32	0.5	2.32	78.92	22.19	19.71
Grain yield	1.35	0.45	3.02	30.55	26.86	16.89

PCV: phenotypic coefficient of variation; **GCV:** genotypic coefficient of variation; **CAT:** catalase; **APX:** ascorbic peroxidase; **POD:** peroxidase; **SOD:** superoxide dismutase; **MDA:** malondialdehyde.

Table 4. Mean comparison for the antioxidant enzymes measured in the twenty –five barley cultivars under normal and water deficit stress conditions.

Cultivar	CAT (u/mg dw)		APX(u/mg dw)		POD (u/mg dw)		SOD(u/mg dw)	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Valfajr	39.98	53.52	911.19	1251.67	97.64	138.7	155.33	248
Jonoob	67.76	75.05	843.1	1409.52	30.58	75.49	279.33	517.33
Shirin	44.86	41.43	558.57	744.76	94.96	137.72	184	309.33
Nimrouz	50.43	51.85	730.24	1247.38	70.33	84.11	202	786.67
Kavir	52.72	87.45	1108.33	1211.19	89.7	120	245.33	528.67
Reyhan	45.68	82.25	710	1023.57	113.18	129.75	191.33	323.33
Karoon	49.05	66.05	535.95	802.14	112.01	136.52	158	325.33
Goharjo	61.22	76.53	593.57	1118.1	94.14	124.31	122.67	470
Bahman	47.43	65.44	606.43	839.52	47.92	59.52	340	820
Torkaman	39.17	59.22	504.52	799.52	102.58	139.67	257.33	584
Gorgan4	52.72	77.89	665.48	1056.9	62.36	86.34	150.67	270.67
Fajr30	56.12	78.76	892.14	969.76	31.68	71.7	130	311.33
Zarjo	49.05	54.9	606.43	979.76	99.07	118.3	118.67	364
Yousef	54.42	69.73	810.24	1388.57	26.42	57.22	73.33	493.33
Nosrat	76.53	90.14	524.05	739.76	46.09	82.08	149.33	318
Torsh	49.63	99.8	733.33	1248.57	91.15	124.94	250	522
Aras	57.82	68.03	770.95	903.81	63.48	96.17	305.33	487.33
Sahra	54.42	68.03	595.71	854.76	83.03	109.15	148.67	280.67
Afzal	48.54	65.75	452.38	708.57	98.87	128.05	170.67	344
Alger-Ceres	78.23	96.94	691.43	760.24	53.66	130	151.33	453.33
Sina	51.02	67.64	620.24	951.9	89.92	119.92	279.33	516
Danesiah	38.78	52.72	679.29	891.19	121.43	139.32	140	330.67
Makooei	33.23	49.13	722.14	989.29	115.11	140.78	200.67	390
Eram	49.54	59.03	496.19	684.05	109.77	144.61	76.67	342.67
Dasht	66.67	76.19	850	1101.43	71.85	102.06	130	244
LSD (5%)	5.36		63.46		20.14		78.75	

CAT: catalase; **APX:** ascorbic peroxidase; **POD:** peroxidase; **SOD:** superoxide dismutase.

Table 5. Mean comparison for grain yield, MDA, proline and protein contents measured in the twenty –five barley cultivars under normal and water deficit stress conditions.

Cultivar	Proline (µmol/gr dw)		MDA(µmol/L fw)		Protein(mL/gr dw)		Grain Yield(g/m2)	
	Normal	Stress	Normal	Stress	Normal	Stress	Normal	Stress
Valfajr	30.89	42.98	0.23	0.23	1.58	1.73	1.43	1.22
Jonoob	14.73	21.19	0.26	0.28	1.41	1.85	1.68	1.01
Shirin	14.39	26.97	0.23	0.32	1.27	1.6	1.38	1.02
Nimrouz	11.57	13.52	0.24	0.29	1.05	1.61	2.03	1.08
Kavir	12.69	16.23	0.18	0.27	0.81	1.14	1.8	1.12
Reyhan	8.4	15.86	0.25	0.27	1.13	1.4	1.7	1.17
Karoon	12.41	17.39	0.19	0.26	1.5	1.78	1.8	1.1
Goharjo	14.89	30.73	0.23	0.25	1.26	1.61	1.13	0.7
Bahman	10.81	12.47	0.18	0.3	1.4	1.82	1.47	0.9
Torkaman	6.26	7.49	0.21	0.31	1.19	1.47	2.07	0.8
Gorgan4	16.65	32.56	0.24	0.25	1.25	1.54	0.92	0.74
Fajr30	15.79	27.08	0.2	0.26	1.19	1.43	1.37	0.74
Zarjo	13.07	21.88	0.25	0.28	1.23	1.72	1.8	0.96
Yousef	10.51	15.63	0.21	0.27	1.53	1.76	1.81	1.24
Nosrat	14.73	18.04	0.3	0.36	0.86	1.13	2.63	0.9
Torsh	10.66	15.84	0.19	0.26	1.22	1.63	1.1	0.81
Aras	11.79	17.53	0.3	0.34	0.77	1	2.43	1.12
Sahra	13	20.42	0.27	0.32	0.85	1.07	1.63	1.05
Afzal	12.02	19.55	0.29	0.31	0.74	1.13	1.7	0.98
Alger-Ceres	13.28	29.94	0.27	0.28	1.01	1.57	1.4	1.1
Sina	14.11	27.72	0.28	0.31	1.97	1.4	1.23	0.98
Danesiah	20.8	27.36	0.22	0.23	0.97	0.65	1.97	1.53
Makooei	11.93	13.94	0.23	0.32	1.39	0.92	2.02	1
Eram	13.99	22.35	0.25	0.29	1.25	1.51	1.97	1.35
Dasht	12.83	21.63	0.25	0.28	1.05	1.48	1.6	0.98
LSD (5%)	3.45		0.023		0.22		0.46	
MDA: malondialdehyde								

Table 6. Correlation coefficient among the traits under stress (above main diagonal) and normal (below main diagonal) conditions.

	CAT	APX	POD	SOD	MDA	Proline	Protein	Grain yield
CAT	1	0.171	-0.219	0.21*	0.31**	0.3**	0.088	0.379**
APX	0.078	1	-.349**	0.213	0.23	0.21*	.337**	-0.028
POD	-.593**	-.301**	1	-.350**	0.16	0.34**	-0.238*	0.176
SOD	-0.113	0.107	-0.058	1	0.27*	0.15	0.260*	0.268*
MDA	0.31**	0.13	0.06	0.27*	1	0.29**	0.31**	-0.21*
Proline	0.13	0.14	0.14	0.15	0.19	1	0.26*	-0.29**
Protein	-.241*	-0.013	0.032	0.072	0.34**	0.11	1	-.246*
Grain yield	0.012	-0.065	-0.01	0.02	0.11	0.24*	-.293*	1

*** and **:** Significant at 5 and 1% of probability
CAT: catalase; **APX:** ascorbic peroxidase; **POD:** peroxidase; **SOD:** superoxide dismutase; **MDA:** malondialdehyde.

Table 7. Summary of stepwise selection of the biochemical traits as independent variables significantly influencing grain yield under normal irrigation condition.

Variable Entered	Partial R-Square	Model R-Square	F Value	Pr > F
Protein	0.4858	0.4858	8.85	0.0107
Proline	0.3256	0.8012	7.45	0.0241

Variable	Estimate	Standard Error	SS	F Value	Pr > F
Intercept	1.24069	0.23413	20.0869	103.73	<.0001
Protein	-0.56709	0.27841	2.52563	6.85	0.0107
Proline	0.45672	0.12348	2.34571	5.78	0.0189

Table 8. Summary of stepwise selection of the biochemical traits as independent variables significantly influencing grain yield under water deficit conditions.

Variable Entered	Partial R-Square	Model R-Square	F Value	Pr > F
CAT	0.4609	0.4779	11.67	0.001
SOD	0.2432	0.7123	8.08	0.001
Proline	0.1954	0.9011	7.07	0.002

Variable	Estimate	Standard Error	SS	F Value	Pr > F
Intercept	1.8655	0.27014	5.7401	84.9	<.0001
CAT	0.1153	0.0403	0.8743	10.51	0.0017
SOD	0.2794	0.0675	0.2785	7.88	0.0072
Proline	-0.2011	0.0974	0.3540	5.64	0.0192

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