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Association of biochemical traits with grain yield in triticale genotypes under normal irrigation and drought stress conditions

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Abstract

Effectiveness of selection of high-yield genotypes under drought stress environments can be improved by using biochemical traits as indirect selection indicators. The objective of this study was to determine the extent to which biochemical traits are associated with triticale grain yield under normal irrigation and drought stress conditions. This field study was conducted to evaluate grain yield and various biochemical traits of 58 triticale genotypes during three consecutive growing seasons (2013-2016). Normal irrigation was applied when 40% of available soil water capacity was depleted. Drought stress was imposed by withholding irrigation for about 40 days from early heading stage to harvest time. In addition to grain yield, proline, malondialdehyde (MDH), hydrogen peroxide (H_2O_2), protein content, carotenoid, chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll along with antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), ascorbic peroxidase (APX), and catalase (CAT) were measured. The contents of proline, H_2O_2, MDH and protein as well as activities of the antioxidant enzymes significantly increased in response to drought stress. Statistical analyses including correlation coefficients, principal component analysis (PCA), stepwise regression and path coefficients revealed that selection of triticale genotypes with low MDH, proline contents and SOD activity as well as high amounts of Chl a and total chlorophyll may improve grain yield under normal irrigation condition. PCA showed negative association between grain yield and antioxidant enzymes under drought stress condition. The results also suggested that low H_2O_2 and malondialdehyde contents along with high activity of SOD were the selection indicators with significant contributions to triticale grain yield under drought stress condition.

Keywords: Indirect selection; Antioxidant enzymes; Superoxide dismutase; MDH; Proline

Abbreviations: ANOVA_ Analysis of variance; APX_ Ascorbic peroxidase; Car_Carotenoid; CAT_Catalase; Chl_Chlorophyll; GCV_Genotypic coefficient of variation; H_2O_2_Hydrogen peroxide; LSD_Least significant difference; MDH_Malondialdehyde; PCV_Phenotypic coefficient of variation; POD_Peroxidase; SOD_Superoxide dismutase.

Introduction

In arid and semi-arid regions such as south of Iran, water shortage is the most significant constraint for crop production. Drought stress predominantly coincides with flowering and grain filling stages of winter cereals in these regions. Thus, development of drought tolerant cultivars is a key target in cereal breeding programs. Triticale (X. *Triticosecale Wittmack*) is a synthetic amphiploid cereal resulting from crossing between wheat and rye. Triticale was developed to combine high grain quality of wheat (*Triticum ssp.*) with tolerance to abiotic stresses of rye (*Secale spp.*) (Zalewski et al., 2001). Triticale shows a number of agronomic advantages including tolerance to water deficit, soil toxic elements, and low nutrient availability (Lonbani and Arzani, 2011). Hence, triticale is considered as an alternative crop to other small grain cereals to grow in unfavorable environments. The area under triticale cultivation has been increasing in Iran and triticale has become a crop of interest for drought prone areas. The existence of ample genetic variation for triticale grain yield (Blum, 2014) has made possible development of triticale cultivars with grain yield potential comparable with superior wheat cultivars (Akbarian et al., 2011).

Drought tolerance is a complex trait governed by numerous genes involved in various adaptive responses. Screening drought tolerant genotypes solely based on grain yield may not be efficient enough because grain yield is a polygenic trait, largely affected by environment and genotype-by-environment interaction (Van Eeuwijk et al., 2005). Accordingly, effectiveness of selection of high-yield genotypes under drought stress environment may be improved by using biochemical traits, as indirect selection indicators. Therefore, it is essential to know the extent to which biochemical traits are associated with grain yield.

Elevated generation of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide and hydroxyl radicals, is one of the major effects of drought stress on plants (Saed-

Moucheshi et al., 2014). ROS can interact with cellular macromolecules including proteins, lipids and nucleic acids, leading to damage cellular components (Saed-Moucheshi et al., 2014). Excessive ROS results in lipid peroxidation which eventually leads to cell membrane damage. The level of lipid peroxidation can be estimated by measuring malondialdehyde (MDH) content (Guo et al., 2006). Hence, MDH can be used as an indicator of oxidative damage and sensitivity to stress in plants. Plants possess enzymatic and non-enzymatic antioxidants, as ROS scavengers, to alleviate oxidative stress (Saed-Moucheshi et al., 2014). The activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) generally increases in response to abiotic stresses to substantially reduce ROS level in plants (Acar et al., 2001; Guo et al., 2006; Ahmed et al., 2013). Furthermore, proline accumulation occurs under drought conditions to facilitate osmotic adjustment in plants. Proline may function as ROS scavenger to protect cells against oxidative stress (Ashraf, 2009). There are several reports implying relationship between increased activity of antioxidants and tolerance to abiotic stresses in various plant species such as rice (Guo et al., 2006), barely (Zahedi et al., 2016) and wheat (Khanna-Chopra and Selote, 2007). Such relationship has also been reported in triticale (Zduńczyk et al., 2006; Gorji et al., 2011; Żur et al., 2014), however the results were generally based on the evaluation of limited number of genotypes.

This study was performed to measure the effect of drought stress on biochemical traits and grain yield of the 58 triticale genotypes and to determine relative significance of biochemical traits as selection indicators for improvement of triticale grain yield under normal irrigation and drought stress conditions.

Results

Effect of drought stress on traits of the triticale genotypes

Combined analysis of variance (Supplementary Table 1) was performed for biochemical traits and grain yield of the 58 triticale genotypes measured during three growing seasons under normal irrigation and drought stress conditions. The growing season had a significant effect on the traits, except chlorophyll b (Chl b), protein content, and SOD. The ANOVA also showed that drought stress significantly affected the biochemical traits as well as grain yield. Moreover, highly significant differences were observed among the genotypes for all the traits. There was a significant interaction effect between genotype and growing season for the biochemical traits and grain yield. The ANOVA also revealed a significant interaction between genotype and irrigation regime for the traits except Chl b and APX.

Comparisons of the means (Table 1) showed a significant reduction in grain yield in response to drought stress. Activities of the antioxidant enzymes (SOD, POD, CAT, APX) as well as the contents of proline and protein of the triticale genotypes were significantly higher under stressful condition than those of under normal irrigation condition. Conversely, chlorophyll content significantly decreased under drought stress condition. Oxidative damage, measured by MDH and H_2O_2, significantly increased in the genotypes under drought stress condition. The average grain yield of the genotypes under both conditions is shown in Table 2. The extent of yield reduction varied among the triticale genotypes. Under normal irrigation condition, the genotypes 48, 36, 39 and 38 showed the highest grain yield, whilst under drought stress condition; maximum grain yield was obtained in the genotypes 46, 37 and 32, respectively. As a result, this study identified the triticale lines with higher grain yield than the main commercial triticale cultivars (the genotypes 56, 57 and 58) in Iran.

Coefficients of variation and heritability estimates

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) of the traits are shown in Table 3. In general, substantial variation was observed for the traits among the triticale genotypes. Under normal irrigation condition, the maximum PCV was obtained for SOD (52.52%), followed by MDH (50.31%), whereas the minimum PCV were recorded for protein content (16.07%) and carotenoid (16.58%). GCV ranged from 41.84% for SOD to 5.77% for protein content. Under drought stress condition, Chl b (40.78%) and SOD (15.69%) showed the highest and the lowest PCVs, respectively. In addition, H_2O_2 (18.04%) and carotenoid (7.84%) showed the maximum and minimum GCV under drought stress condition, respectively. A wide range of heritability estimates were observed for the traits. SOD and MDH exhibited the highest heritability under both conditions, whereas the lowest values of heritability were estimated for Chl b. Grain yield showed heritability estimates of 32.14% and 29.62% under normal irrigation and drought stress conditions, respectively, implying the significance of environmental effects.

Correlation analysis

The correlation coefficients among the traits were obtained for normal irrigation and drought stress conditions (Table 4). The results showed that the interrelationships among the traits were influenced, to some extent, by irrigation condition. Under normal irrigation condition, grain yield was positively and significantly correlated with total chlorophyll and Chl a. On the contrary, grain yield showed significant negative correlations with H_2O_2, proline and MDH contents as well as activities of SOD, CAT and APX. In addition, significant positive correlations were generally observed between the cell injury related traits (H_2O_2 and MDH) and activities of the antioxidant enzymes. Under drought stress condition, grain yield had a strong negative correlation with H_2O_2 content. Grain yield also showed negative and significant, but not strong, correlations with Chl b, MDH, protein and carotenoid contents as well as POD activity. On the other hand, grain yield showed significant positive correlations with APX and SOD activities. No correlation was found between grain yield and proline content under drought stress condition.

Principal component analysis

Principal component analysis (PCA) was applied to analyze relationships among the variables using reduced set of uncorrelated principal components (PCs). PCA was separately performed for normal and drought stress conditions. The results of PCA for each irrigation regime (Table 5) revealed that the first three PCs had eigenvalues greater than the criteria (1.0) making them significant to explain total variability among the triticale genotypes. The first three PCs explained about 75% of the total variation among the data of normal irrigation condition. The first PC accounted for 41.06% of the variation, whereas the second and third PCs explained 22.94% and 10.82% of the total variation, respectively. The contents of Chl a, Chl b and Chl T followed by protein content showed strong positive contribution to the first PC. They were also positively associated with grain yield. On the other hand, APX, SOD, proline, POD and MDH were the major negative contributors to the first PC. The second PC was related to carotenoid, MDH, SOD and protein content which had the highest positive weights. The loading plot for the first two PCs under normal irrigation condition is presented in Fig 1. Finally, strong positive contributions were observed for POD and CAT in the third PC, while they were in negative association with Chl b.

Under drought stress condition, the results of PCA showed that the first three PCs contributed to about 78% of the total variation. The first PC explained 45.58% of the variation, whilst the second and the third PCs accounted for 17.32% and 15.04% of the variation, respectively. The structure of relationships among the traits altered in comparison with the normal irrigation condition. Based on the first PC, grain yield showed positive association with proline, carotenoid, and protein contents. In addition, POD, SOD, and MDH exhibited strong negative contributions to the first PC and eventually negative associations with grain yield. The second PC was basically loaded by chlorophyll and protein contents with positive weights; as well as SOD, MDH and APX with negative weights. Fig. 2 shows the loading plot for the first two PCs under drought stress conditions. Grain yield showed the highest positive contribution to the third PC. Furthermore, H_2O_2 and CAT had strong negative associations with grain yield under stressful condition according to the third PC.

Stepwise regression and path analysis

Stepwise regression (Table 6) was separately carried out for normal irrigation and drought stress conditions in order to identify biochemical indicators which had significant contributions to triticale grain yield. Comparison of the final regression models revealed that the significance of biochemical traits influencing grain yield altered regarding the irrigation regimes. Under normal irrigation condition, MDH and proline contents remained in the final regression model, and cumulatively explained 90% of grain yield variation. They showed significant negative associations with grain yield. Under drought stress condition, the final regression model consisted of H_2O_2, MDH and SOD which cumulatively explained 92% of triticale grain yield variation. While H_2O_2 and MDH significantly and negatively contributed to grain yield, SOD had a significant positive relationship with grain yield under drought stress condition.
Path coefficient analysis (Table 7) was further performed in order to elucidate direct and indirect effects of the biochemical traits, selected as the significant indicators by final regression models, on grain yield. Under normal irrigation condition, MDH and proline contents exhibited relatively the same negative direct effects on grain yield. Under drought stress condition, H_2O_2 showed high negative direct effect and negligible indirect effects via MDH and SOD on grain yield. In spite of negative correlation between MDH and grain yield, MDH had a negligible positive direct effect on grain yield. In fact, MDH was related to grain yield by a negative indirect effect through SOD. In contrast to H_2O_2 and MDH, SOD had a positive direct effect on grain yield, while its indirect effects through H_2O_2 and MDH on grain yield were negligible.

Discussion

Drought triggers a series of plant biochemical reactions which eventually affect plant productivity. Thus, those biochemical traits which show high variability and heritability as well as association with grain yield can be exploited as selection indicators to enhance selection efficiency of breeding programs. Grzesiak et al. (2003) identified traits such as leaf water potential, as proper indicators to select high-yield triticale genotypes under drought stress condition. Our study measured grain yield and biochemical traits of the 58 triticale genotypes during three growing seasons to robustly evaluate interrelationships between biochemical traits and grain yield and to ultimately identify reliable biochemical indicators for improvement of triticale grain yield under normal irrigation and drought stress conditions.

The results of statistical analyses revealed that H_2O_2 content was a key negative determinant for triticale grain yield under drought stress. H_2O_2 content showed high variability among the triticale genotypes, although its heritability was relatively similar to grain yield. This finding suggested that selection against H_2O_2 content may lead to identify triticale genotypes with high yield under drought stress. H_2O_2 content was not recognized as a major indicator for grain yield under normal irrigation condition, however the association between H_2O_2 and grain yield was detected by correlation analysis. Drought stress generally enhances generation of ROS such as superoxide radical and H_2O_2 (Saed- Moucheshi et al., 2014). This suggests that H_2O_2 plays a dual role in plant cells: at low concentrations, it functions as a secondary messenger involved in signal transduction triggering abiotic stress tolerance, and at high concentrations it mediates oxidative stress and programmed cell death (Lin and Kao, 2000).

In agreement with the previous studies (Dacosta and Huang, 2007; Zahedi et al., 2016), this study showed that MDH content significantly increased in response to drought stress. MDH is known as a biomarker for cell membrane lipid peroxidation induced by overproduction of ROS (Savvides et al., 2016). Environmental stresses cause ROS toxicity leading to peroxidation of cell membrane-unsaturated fatty acids (Dacosta and Huang, 2007). In the current study, MDH was accordingly taken into account by all the analyses as a significant negative biochemical trait influencing triticale grain yield under both normal and drought stress conditions. This finding indicated that high-yield triticale genotypes had lower levels of lipid peroxidation because of having higher ROS-scavenging capacities. Interestingly, MDH showed high variability and heritability, compared to the other traits, making it a reliable indicator for triticale grain yield. MDH was known as a critical biochemical trait associated with grain yield in barley (Zahedi et al. 2016). Moreover, a salt-sensitive barley genotype showed a significantly higher level of MDH compared to a salt-tolerant barley genotype (Pérez-López et al., 2009).

The present study revealed that activities of the antioxidant enzymes such as SOD, APX and POD significantly increased in the triticale genotypes under drought stress condition. Similar results were obtained in different crop plants (Acar et al., 2001; Guo et al., 2006; Ahmed et al., 2013).

Table 1. Means of the biochemical traits and grain yield averaged over **(a)** the triticale genotypes and the irrigation conditions, **(b)** the triticale genotypes and the growing seasons, and **(c)** the triticale genotypes only.

(a) Year		H_2O_2 (umol g^{-1} FW)	MDH (umol g^{-1})	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Chl T (mg g^{-1})	Car (mg g^{-1})	Protein (mg g^{-1})	SOD (U g^{-1})	POD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	Proline (µM g^{-1})	Grain Yield (g m^{-2})
2013-2014		4.78b	10.82a	12.49a	5.05a	17.57a	4.86b	25.28a	26.72b	24.27b	3.01b	139.88b	12.82b	557.4a
2014-2015		6.94a	11.87a	7.09b	4.42b	12.59b	7.1a	23.45a	32.07a	35.75a	6.81a	167.24a	17.43a	460.99b
2015-2016		4.87b	4.57b	12.86a	5.02a	16.76a	4.94b	25.65a	24.53b	24.53b	2.74b	128.89b	11.76b	552.48a
(b) Condition														
Normal		3.34b	4.09b	12.6a	5.73a	18.14a	5.21b	14.51b	12.14b	13.26b	2.09b	122.53b	8.94b	741.99b
Stress		7.71a	14.08a	9.03b	3.92b	13.14b	6.06a	35.07a	43.41a	43.11a	6.28a	168.14a	19.07a	305.25a
(c) Year	Condition													
2013-2014	Normal	3.4c	5.14b	14.47a	5.86a	20.32a	5.59b	15.12b	12.52b	11.11	1.49c	112.89c	4.11c	788.47a
2013-2014	Stress	6.15b	16.5a	10.52b	4.23b	14.82b	4.13c	35.44a	40.92a	37.43	4.52b	166.86ab	21.53a	326.33c
2014-2015	Normal	3.19c	4.68b	8.38c	5.38a	14.48b	4.29c	12.84b	15.3b	17.18	3.39bc	145.01b	14.67b	662.21b
2014-2015	Stress	10.68a	19.06a	5.8d	3.45b	10.7c	9.91a	34.06a	48.83a	54.33	10.22a	189.47a	20.18a	259.76d
2015-2016	Normal	3.43c	2.45b	14.96a	5.96a	19.62a	5.74b	15.59b	8.58b	11.48	1.39c	109.67c	2.52c	775.3a
2015-2016	Stress	6.31b	6.69b	10.75b	4.08b	13.9b	4.14c	35.7a	40.47a	37.58	4.08b	148.1b	21a	329.67c

H_2O_2: Hydrogen peroxide; MDH: Malondialdehyde; Ch: Chlorophyll; Car: Carotenoid; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; APX: Ascorbic peroxidase.
Means within a column followed by the same letter are not significantly different according to LSD test at P≤0.05.

Table 2. Means of grain yield (g m^{-2}) measured in the 58 triticale genotypes grown under normal irrigation and drought stress conditions in three growing seasons.

Genotype	Normal	Stress	Genotype	Normal	Stress	Genotype	Normal	Stress	Genotype	Normal	Stress
1	600.98	292.48	16	644.11	299.40	31	728.66	292.95	46	795.37	400.37
2	720.48	338.68	17	737.55	326.15	32	871.57	379.07	47	845.38	287.20
3	696.22	304.75	18	762.45	309.08	33	742.10	282.63	48	991.02	339.90
4	707.46	331.97	19	664.64	225.13	34	676.59	297.50	49	725.43	309.22
5	673.04	247.84	20	676.99	235.28	35	739.99	273.12	50	846.27	314.24
6	746.43	358.69	21	707.92	318.79	36	964.94	367.15	51	757.03	377.42
7	680.46	216.56	22	630.88	272.68	37	765.68	399.10	52	790.29	306.29
8	594.66	303.32	23	607.74	258.77	38	892.50	307.17	53	800.03	349.65
9	686.95	269.40	24	755.28	312.69	39	925.88	288.31	54	703.67	308.29
10	789.60	322.49	25	737.16	320.00	40	641.36	317.39	55	807.88	253.86
11	698.98	322.06	26	676.88	252.74	41	815.12	353.36	56	730.94	343.55
12	764.23	245.69	27	664.81	263.97	42	732.35	298.12	57	679.48	276.03
13	703.92	356.77	28	576.82	201.44	43	738.59	336.91	58	806.11	313.52
14	785.36	287.46	29	826.62	257.95	44	673.37	320.47			
15	819.98	302.42	30	696.50	360.83	45	812.84	296.45			

LSD (5%) = 15.37

Table 3. Mean, maximum, minimum, phenotypic and genotypic coefficient of variation, and heritability of the traits measured in the fifty-eight triticale genotypes grown under normal irrigation and drought stress conditions during three growing seasons.

Parameter	H_2O_2 (umol g^{-1} FW)	MDH (umol g^{-1})	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Chl T (mg g^{-1})	Car (mg g^{-1})	Protein (mg g^{-1})	SOD (U g^{-1})	POD (U g^{-1})	CAT (U g^{-1})	APX (U g^{-1})	Proline (µM g^{-1})	Grain Yield (g m^{-2})
Normal irrigation													
Heritability (%)	31.67	56.04	30.06	12.44	24.14	21.78	12.91	63.48	17.08	32	14.33	20.47	32.14
PCV (%)	16.78	50.31	21.91	35.96	17.87	16.58	16.07	52.52	41.94	39.18	40.5	29.17	21.13
GCV (%)	9.44	37.66	12.01	12.68	8.78	7.74	5.77	41.84	17.33	22.16	15.33	13.2	11.98
Mean	3.34	4.08	12.63	5.74	18.17	5.22	14.52	12.13	13.31	2.09	122.1	8.93	737.91
Minimum	1.98	1.09	4.51	1.21	8.68	2.35	7.56	1.29	4.22	0.19	43.33	0.23	286.8
Maximum	5.23	11.06	22.31	14.3	27.91	7.88	21.9	28.38	36.1	6.54	261.24	29.99	1260
Drought stress													
Heritability (%)	31.38	41.87	21.95	9.54	18.11	16.61	27.17	64.16	28.46	16.05	28.26	32.5	29.62
PCV (%)	32.2	22.08	22.34	40.78	19.41	19.24	15.73	15.69	31.01	30.26	17.29	23.66	26.6
GCV (%)	18.04	14.29	10.47	12.6	8.26	7.84	8.2	12.57	16.54	12.12	9.19	13.49	14.48
Mean	7.77	14.1	9.04	3.92	13.14	6.08	35.11	43.44	43.11	1.75	167.86	19.08	303.52
Minimum	2.39	2.4	2.84	1.05	4.68	2.17	18.99	21.53	13.79	0.66	86.91	2.89	78
Maximum	27.92	29.28	15.13	9.85	24.48	14.56	50.51	65.63	88	5.35	235.81	40.8	637.31

PCV: Phenotypic coefficient of variation; GCV: Genotypic coefficient of variation; H_2O_2: Hydrogen peroxide; MDH: Malondialdehyde; Ch: Chlorophyll; Car: Carotenoid; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; APX: Ascorbic peroxidase.

Table 4. Pearson's correlation coefficients between the traits under normal irrigation (above main diagonal) and drought stress (below main diagonal) conditions.

	H_2O_2	MDH	Chl a	Chl b	Chl T	Car	Protein	SOD	POD	CAT	APX	Proline	Grain Yield
H_2O_2	1	0.57**	-0.83**	-0.12	-0.64**	0.01	0.23*	0.80**	0.21*	0.64**	0.52**	0.58**	-0.80**
MDH	0.11	1	-0.62**	0.13	-0.39**	0.35**	0.21*	0.72**	-0.16	0.54**	0.52**	0.46**	-0.57**
Chl a	-0.02	.63**	1	0.13	0.63**	-0.04	-0.18	-0.84**	-0.18	-0.69**	-0.67**	-0.59**	0.98**
Chl b	0.46**	0.31**	0.21*	1	0.23*	0.50**	-0.03	-0.01	-0.60**	0.06	0.22*	-0.07	0.179
ChlT	0.06	0.55**	0.90**	0.43**	1	0.35**	-0.43**	-0.59**	-0.29**	-0.40**	-0.22*	-0.34**	0.63**
Car	0.29**	0.10	0.46**	0.49**	0.59**	1	-0.19	0.32**	-0.71**	0.20	.435**	-0.04	0.03
Protein	0.40**	0.08	0.29**	0.59**	0.34**	0.56**	1	0.28**	0.07	0.24*	-0.17	0.12	-0.14
SOD	-0.06	-0.88**	-0.59**	-0.28*	-0.54**	-0.05	0.14	1	-0.10	0.71**	0.65**	0.50**	-0.78**
POD	0.04	0.84**	0.67**	0.01	0.50**	0.02	-0.05	-0.86**	1	-0.12	-0.26*	0.38**	-0.22*
CAT	-0.25*	0.22*	0.06	-0.18	-0.01	-0.20	-0.43**	-0.42**	0.28**	1	0.51**	0.27**	-0.65**
APX	-0.44**	-0.69**	-0.38**	-0.65**	-0.40**	-0.37**	-0.53**	0.51**	-0.42**	0.01	1	0.28**	-0.64**
Proline	-0.29**	0.24*	0.07	-0.33**	0.09	-0.28**	-0.72**	-0.47**	0.28**	0.68**	0.24*	1	-0.59**
Grain Yield	-0.86**	-0.29**	-0.09	-0.48**	-0.16	-0.26*	-0.33**	0.30**	-0.26*	0.04	0.47**	0.12	1

* and**: Significant at 5% and 1%, respectively.

H_2O_2: Hydrogen peroxide; MDH: Malondialdehyde; Ch: Chlorophyll; Car: Carotenoid; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; APX: Ascorbic peroxidase.

Table 5. Eigenvectors and eigenvalues of the first three components of principal components analysis for grain yield and various biochemical traits measured in the 58 triticale genotypes under **(a)** normal irrigation and **(b)** drought stress conditions.

Trait	**(a)**		**Principal Component**			**(b)**
	1	2	3	1	2	3
H_2O_2	-0.532	0.497	0.34	-0.425	-0.113	-0.806
MDH	-0.669	0.682	-0.061	-0.682	-0.597	0.313
Chl a	0.652	0.271	0.6	-0.19	0.576	0.229
Chl b	0.622	0.473	-0.522	0.434	0.757	-0.048
Chl T	0.802	0.419	0.304	0.098	0.879	0.148
Car	0.335	0.807	-0.125	0.773	0.485	0.044
Protein	0.574	0.633	-0.134	0.665	0.648	0.189
SOD	-0.721	0.64	-0.091	-0.683	-0.636	0.241
POD	-0.61	-0.093	0.73	-0.823	0.238	-0.187
CAT	-0.467	0.428	0.621	-0.071	-0.426	-0.615
APX	-0.769	0.315	-0.026	-0.335	-0.526	0.345
Proline	-0.717	0.456	-0.354	0.797	0.264	-0.008
Grain yield	0.475	0.312	0.146	0.591	-0.199	0.771
Eigenvalue	5.338	2.938	1.407	5.926	2.252	1.955
Proportion (%)	41.063	22.943	10.825	45.581	17.322	15.038
Cumulative (%)	41.063	64.006	74.832	45.581	62.903	77.942

H_2O_2: Hydrogen peroxide; MDH: Malondialdehyde; Ch: Chlorophyll; Car: Carotenoid; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; APX: Ascorbic peroxidase.

Table 6. Stepwise regression models to identify significant biochemical indicators for triticale grain yield in normal irrigation and drought stress conditions.

Model	Parameter Estimate	Standard Error	Standardized Parameter Estimate	Partial R^2	Model R^2
Normal					
Intercept	614.863	22.1840	0		
MDH	-19.221	4.1593	-0.986	0.5406	0.511
Proline	-8.114	1.5329	-0.434	0.3601	0.902
Stress					
Intercept	148.150	14.9525	0		
H_2O_2	-21.974	1.1872	-1.0371	0.525	0.72
MDH	-12.117	4.2418	-0.6907	0.230	0.83
SOD	9.0890	2.2060	0.95545	0.098	0.92

Table 7. Path coefficients showing direct (main diagonal, italics and bold) and indirect effects of the biochemical traits, selected by the stepwise regression models, on triticale grain yield under normal irrigation and drought stress conditions.

Stress				
	H_2O_2	MDH	SOD	Correlation with Grain yield
H_2O_2	*-0.847*	0.008969	-0.0195	-0.858
MDH	-0.09071	*0.083746*	-0.286	-0.293
SOD	0.051132	-0.07399	*0.32373*	0.30087
Residual	0.200775			

Normal			
	MDH	Proline	Correlation with Grain yield
MDH	*-0.37703*	-0.1892	-0.566
proline	-0.17154	*-0.41585*	-0.587
Residual	0.542249		

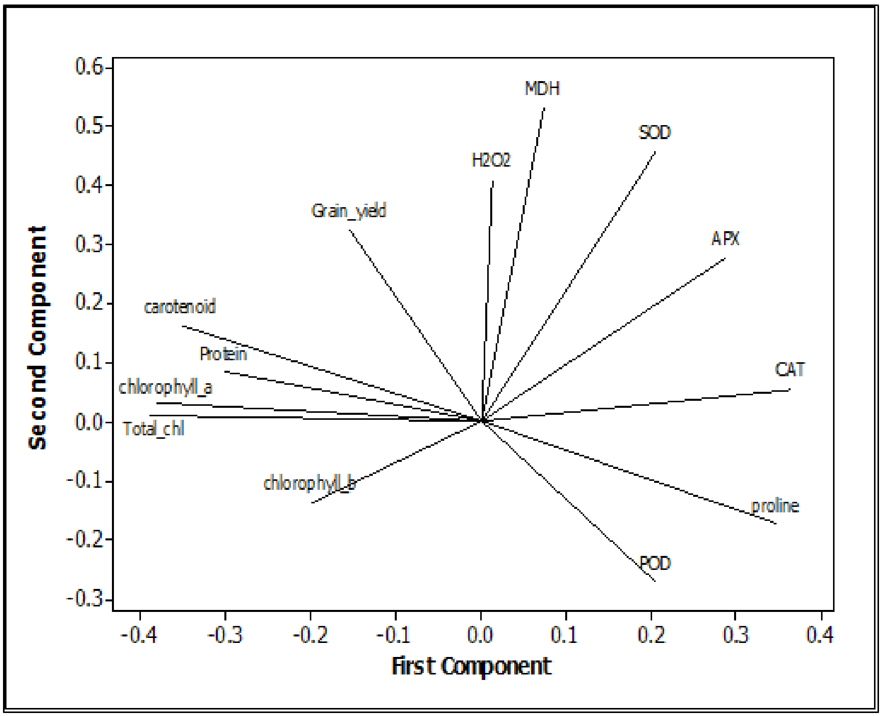

Fig 1. The loading plot of the first two principal components for grain yield and various biochemical traits measured in the 58 triticale genotypes under normal irrigation condition.

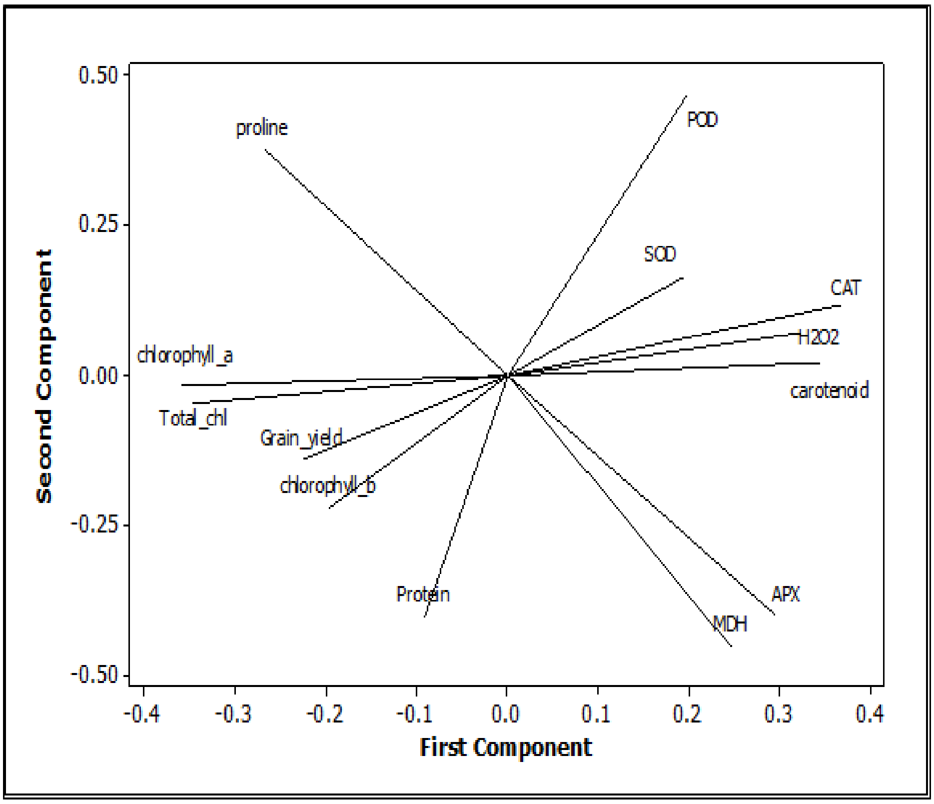

Fig 2. The loading plot of the first two principal components for grain yield and various biochemical traits measured in the 58 triticale genotypes under drought stress condition.

The results indicated that irrigation regime affected the relationships between grain yield and activities of the antioxidant enzymes in triticale. Under normal irrigation condition, the correlation coefficients and PCA showed negative association between grain yield and the antioxidant enzymes particularly SOD and APX. SOD exhibited higher genetic variability among the triticale genotypes and correspondingly higher heritability than APX, making it a more reliable indicator for grain yield under normal condition. Antioxidant enzymes are known as indicators to assess the status of redox in plants (Ashraf, 2009). Under normal irrigation condition, plant cells generate antioxidant enzymes as much as required to maintain ROS at appropriate level for signal transduction and metabolic homeostasis. On the contrary, SOD and APX were positively, but not strongly associated with triticale grain yield under drought stress condition according to the correlation coefficients and the third principal component of PCA. Furthermore, SOD was picked up by stepwise regression and path analysis as a major positive contributor to grain yield under drought stress. Therefore, the triticale genotypes with high SOD activity can be selected for drought stress environments because they are presumably able to more efficiently alleviate damaging effects of oxidative stress and consequently show less grain yield reduction. Previous reports have also pointed out the association of high antioxidant enzymes activities with tolerance to abiotic stresses in crop plants (Ahmed et al., 2013; Zahedi et al., 2016). It is noteworthy that, while SOD and grain yield had relatively similar genetic variation under drought stress condition, SOD showed higher heritability. This evidence further indicated that SOD can be utilized as a proper indirect selection indicator in triticale breeding programs towards drought tolerance improvement. The previous studies also reported high heritability for antioxidant enzymes in triticale (Gorji et al., 2011).

The statistical approaches almost consistently showed that proline content had a significant negative contribution to triticale grain yield under normal irrigation condition. As a result, low proline content may be used as a biochemical indicator for selection of high yield triticale genotypes under normal irrigation condition, although it should be noted that proline exhibited low heritability. On the other hand, proline content showed positive relationship with grain yield under drought stress condition according to PCA, whilst no association was found between proline and grain yield by the other statistical approaches. Under stressful condition, proline may act as an osmoregulatory agent to scavenge free radical molecules (Ashraf, 2009). It yet remains uncertain whether proline accumulation is positively associated with drought tolerance or is simply a common adaptive response to drought stress (Zhang et al., 2015). In accordance with the previous report on triticale (Lonbani and Arzani, 2011), chlorophyll content showed a significant reduction under drought stress condition. Total chlorophyll, Chl a and Chl b were not known as good indicators for triticale grain yield under drought stress condition because no strong association was observed between grain yield and chlorophyll contents. On the other hand, triticale grain yield was positively associated with total chlorophyll and Chl a under normal irrigation condition according to correlation coefficients and PCA.

Materials and Methods

Plant materials and experimental procedure

The study was conducted on a silty loam soil at the research station of School of Agriculture, Shiraz University, Shiraz, Iran (52^{o} 46' E, 29^{o} 50' N; 1,810 m altitude) during three consecutive growing seasons (2013-2014, 2014-2015, and 2015-2016). The 58 triticale genotypes, originated from the International Maize and Wheat Improvement Center (CIMMYT), were sown in early November of each year in two separate experiments under normal irrigation and drought stress conditions. The triticale genotypes consisted of three commercial cultivars (Sanabad, Juanillo, and Paj) and 55 advanced lines obtained from the Agricultural and Natural Resources Research and Education Centers of Khorasan-Razavi and Fars provinces, Iran. The pedigree information of the genotypes designated by numbers 1 to 58, is shown in Supplementary Table 2. Each experiment was laid out in a randomized complete block design with three replicates. Each experimental plot consisted of four two-meter-long rows spaced 20cm apart. Normal irrigation was applied when 40% of available soil water capacity was depleted. Soil water content at field capacity and wilting point was determined in the root zone to estimate plant available water capacity. In order to implement the irrigation schedule, soil water content was regularly determined at depth of 0-40cm by weighing soil samples before and after oven-drying at 105°C for 24h and working out their original water content. Drought stress was imposed by withholding irrigation for about 40 days from early heading stage to harvest time. A total precipitation of 187mm, 143mm and 240mm occurred during the experiments in the three consecutive growing seasons, while no rainfall was recorded after heading when drought stress was applied.

Traits measured

Two central rows per plot were harvested to measure grain yield. In order to measure biochemical traits, leaf samples were collected from each plot after imposing drought stress when around 80% of available soil water capacity was depleted. The samples were immediately frozen in liquid nitrogen and stored at -80°C. Total protein content was assayed by the method of Bradford (1976). Proline content was estimated using the protocol of Bates et al. (1973). Contents of pigments including chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl T) and carotenoid (Car) were determined according to the method of Lichtenthaler and Buschmann (2001). Activity of antioxidant enzymes including SOD, POD, APX, and CAT was measured spectrophotometrically according to the methods of Beauchamp and Fridovich (1971), Chance and Maehly (1955), Nakano and Asada (1981), and Dhindsa et al. (1981), respectively. Contents of H_2O_2 and MDH as indicators of oxidative damage were estimated following the protocols of Alexieva et al. (2001) and Heath and Packer (1968), respectively.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) to test the effects of drought stress, genotype, growing season and their interactions on grain yield and biochemical traits of triticale. Mean comparisons were made using LSD test at $P \leq 0.05$. Genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and heritability were calculated for each trait by the SAS codes written by the authors using interactive matrix language (IML) of SAS based on expected mean squares for sources of variation in the ANOVA. Various statistical approaches including correlation coefficients, principal component analysis (PCA), stepwise regression and path coefficients were employed to evaluate relationships among the traits and to find out relative significance of the biochemical traits on triticale grain yield under normal irrigation and drought stress conditions. Statistical analyses were done by SAS version 9.4 and Minitab. The path analysis was performed using agricolae package in R program version 3.4.2.

Conclusion

This study represented solid information about the significance of biochemical traits as selection indicators for triticale grain yield using the data obtained from a large number of triticale genotypes tested in a multi-year experiment. Significant variation was observed for grain yield and the biochemical traits among the triticale genotypes. Under normal irrigation condition, selection of triticale genotypes which had low MDH, proline and SOD contents as well as high amounts of Chl a and total chlorophyll may lead to improve grain yield. Under drought stress condition, screening based on low H_2O_2 and MDH contents along with high activity of SOD may result in identification of high yield triticale genotypes.

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