Oxidant related biochemical traits are significant indices in triticale grain yield under drought stress condition

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Abstract

Two separate experiments, one as drought stress condition and the other one as the normal irrigation condition based on randomized complete block design (RCBD) with three replications were implemented to examine 20 triticale (Triticoseclae wittamack X) genotypes of which 19 genotypes have recently been generated by crossing different parent lines and the other one was Javaniloo, common cultivar. Based on the results of different statistical techniques and comparing relationships among traits for normal irrigation and stress condition, enzymatic antioxidant could be used as criteria for screening tolerant genotypes of triticale. On the other hand, it is pointed out that superoxide dismutase (SOD) are the most important criteria to achieving higher tolerant genotypes through indirect selection. Furthermore, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content with having highly relationship with rain yield are also different possible criteria for screening triticale genotypes for water

stress conditions in which genotypes with lower content of these traits could be screened.

Keywords: Wheat, yield, drought stress, superoxide dismutase, malondialdehyde

Introduction

In the recent decades, efforts have brought about great progresses in breeding for higher yield under different environmental conditions for several crops. Numerous experiments have been carried out providing essential ideas and models as well as understanding the molecular and biochemical responses of crops to drought and water shortage conditions coupled with detection of the relationships and biochemical changes regarding this condition, still a large gap exists between the optimal yields and productivity under water shortage conditions (Araus et al., 2002). Furthermore, direct selection toward crop productivity and grain yield for environmental stresses and especially water stress conditions has encountered some difficulties which are rooted form some genetically issues to name a few epistasis, polygenic control, and some sort of significant genotype × environment impacts (Van Eeuwijk et al., 2005). Hence, evaluations of the new methods and traits which are connected with grain yields and have significant impact on these traits as indirect selection have been of the greatest concern of the scientists.

Since lacking water resources and occurring water stress is increasingly becoming one of the influential problems in agriculture (Tilman et al., 2002; Pointillart et al., 1987), evaluating and developing drought tolerant genotypes is of the substantial and essential efforts of the scientific researchers now a days. In regards to providing new genetic resources and species, triticale (*Triticosecale wittmack* X) could be mentioned as one of the most prosperous cereals which have been provided and developed by humankind. This crop has synthetically been developed to attain exclusive grain quality of wheat (*Triticum ssp.*) and

tolerance of rye (*Secale spp.*) to biotic and abiotic stresses as parental species (Zalewski et al., 2001; Aniol and Gustafson, 1984). Lonbani and Arzani (2011) and also Chen and Bushuk (1970) found that triticale is showing a high tolerance to drought and other environmental stresses such as nutrient shortage, soil acidity, and element toxicities of the soil. Accordingly, serious breeding efforts have been sustained to develop modern cultivars of triticale which their production can be on par with the superior common wheat cultivars in terms of grain yield potential under favorable conditions (Akbarian et al., 2011; Kuleung et al., 2004; Fox et al., 1990; Müntzing, 1979; Clarke et al., 2016).

For many years, agronomic traits such as grain yield and its components have been applied for evaluating drought tolerance and screening genotypes under drought stress (Sinclair, 2011). However, agronomic traits are not able to completely reflect the different levels of stress tolerance or susceptibility between different genotypes and/or species. Indeed, considering drought tolerance at the biochemical level can provide more accurate information about the characteristics fundamentally to drought tolerance in plants (Passioura, 2012). At the biochemical level, one of the most important effects of drought stress on plants is generation of the reactive oxygen species (ROS) containing O_1^-, O_2^-, $OH^\cdot$ and H_2O_2 that are extremely reactive in nature and they can interact with a number of other molecules and metabolites such as DNA, pigments, proteins, lipids, and other essential cellular molecules which lead to a series of destructive processes (Gill and Tuteja, 2010; Hossain et al., 2015). Plants' response at this level is to produce ROS scavengers (Ashraf, 2009). Antioxidant enzymes, e.g., superoxide dismutase (SOD), catalase (CAT), ascorbic peroxidase (APX), peroxidase (POD), and glutathione reductase (GR) are known to considerably reduce the levels of ROS in plants (Zduńczyk et al., 2006; Viuda-Martos et al., 2013). There are several reports available on the interrelationship and association between elevating the level of antioxidants' activities and tolerance to abiotic stresses in various plants, e.g., rapeseed

(Mirzaee et al., 2013), soybean Krüger, 2002), rice (Guo et al., 2006), barely (Guo et al., 2006), wheat (Khanna-Chopra and Selote, 2007; Guo et al., 2006), and triticale (Żur et al., 2014; Zduńczyk et al., 2006; Gorji et al., 2011); however, these results have been often based on the limited numbers of genotypes.

This research aims to consider the relationship between and among grain yield and biochemical characteristics in regards to reactive oxygen species (ROS) under water stress condition and to providing possible traits for indirect selection in this condition along with comparing these relationships with normal condition. Another key aim of the current work is to introduce some high potent genotypes to be screened for the future cultivars.

Materials and methods

Methodology

Two distinct and separate experiments, one as drought stress condition and the other one as the normal irrigation condition were implemented to examine 20 triticale (*Triticoseclae wittamack* X) genotypes of which 19 genotypes have recently been generated by crossing different parent lines in the Agriculture and Natural Resources Research Institute of Eghlid, Fars, Iran. The one other cultivated cultivar named Javaniloo is a common cultivar. The genotypes' name and the number for each one of them that is used in place of their complete name in current paper is presented in Table 1. The experiments were carried out for two consecutive years from 2016 to 2017. Experimental Station was placed in Eghlid, Fars province (52° 46' E, 29° 50' N, altitude 1,810 m above sea level), Shiraz, Iran. The experimental design of both conditions for both years were randomized complete block design (RCBD) with 20 genotypes as treatments and three replications having overall 60 plot in each condition per year. Each plot had 2 by 1 m area with four cultivated row having 2 m long. The only difference between the two conditions was the number of irrigations. The irrigation for water stress condition had been suspended in the flowering stage to the end of

the experiment; whereas, the normal condition had been irrigated regularly to the final stage of the growing season. In order to determine the proper time for irrigation, the soil samples of the experimental station were randomly collected in about 5 days after the last irrigation as a daily based from 0-40 cm depth and the field capacity (FC) was measured based on weighing method. When the samples' FC were reached 65 to 60 percent, the irrigations were applied.

Measuring traits

Except for the grain yield that was harvested in the final stage of the plants' life, all other traits were sampled during the experiment in about 25 days after starting the water stress treatment. Total protein content was measured based on the method of Bradford (1976), using bovine serum albumin (BSA) as a standard and absorbance was read at 595 nm. Total proline content was measured using Bates et al. (1973) method. Briefly, 2 ml acid ninhydrin and 2 ml glacial acetic acid were mixed and incubated with sample tissue in a water bath at 100°C for 1 h. The reaction was terminated by placing the mixture in an ice bath. Free proline content of the solution was finally extracted with 4 ml toluene. The absorbance was recorded at 520 nm. The pigments' content comprising of total chlorophyll (Chl T), chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid (Car) contents were measured based on the method of Lichtenthaler and Buschmann (2001) using the following formula:

$$\text{Total chlorophyll (mg/ml)} = 20.2\ (A645) + 8.02\ (A663)$$

$$\text{Chlorophyll } a \text{ (mg/ml)} = 12.7\ (A663) - 2.69\ (A645)$$

$$\text{Chlorophyll } b \text{ (mg/ml)} = 22.9\ (A645) - 4.68\ (A663)$$

$$\text{Carotenoid (mg/ml)} = (1000A470 - 3.27[\text{Chl a}] - 104[\text{Chl b}])/227$$

Where, A is recorded number in spectrophotometer and Chl *a* and Chl *b* denoted for chlorophyll *a* and chlorophyll *b* content.

Antioxidant enzyme activities namely superoxide dismutase (SOD), peroxidase (POD), ascorbic peroxidase (APX), and catalase (CAT) were measured by the methods of Beauchamp and Fridovich (1971), Chance and Maehly (1955), Nakano and Asada (1981), and Dhindsa et al. (1981), respectively. The activities of the antioxidants were described according to their abilities to decompose related oxidants in protein unit using spectrophotometric tools. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) as the cell injuries representation traits were measured using the methods described by Alexieva et al. (2001) and Heath and Packer (1968), respectively. For hydrogen peroxide, potassium phosphate was applied as buffer (pH 7.0), and after adding potassium iodide (KI) to the extract, the absorbance of supernatant was read at 390 nm and adjusted according to the standard curve. For measuring MDA, Leaf samples were homogenized in 4 ml of 10% trichloroacetic acid (TCA) and the supernatant was mixed with 0.6% thiobarbituric acid, and after 30 min heating at 95°C and then cooling down on ice, the absorbance was measured at 450, 532, and 600 nm wavelengths.

Statistical analysis

Combination of separate experiments in this study containing two different water stress conditions as well as two consecutive years representing environmental influence were subjected to combine analysis of variance (ANOVA), where year, stress, genotypes, and the combination of their interactions were taken as sources of variations for ANOVA table using SAS v. 9.4. Stepwise regression was also executed by SAS software to pull out substantial traits through the final grain yield. Minitab software was used for multivariate statistical methods containing principal component analysis and cluster analysis. Genetic parameters consist of heritability, genotypic coefficient of variation and phenotypic coefficient of variation were calculated by means of the SAS code written by the authors using interactive matrix language (IML) of the software.

Results

Analysis of variance

Taking year as random effect and stress along with genotype as fixed effects, the combined analysis of variance was implemented, where each main and interaction effects were tested by their own defined error terms specified by SAS software. Table 2 represents combined analysis of variance for all measured traits consist of hydrogen peroxide ($H_2 O_2$), malondialdehyde (MDA), pigment content (chlorophyll a and b, total chlorophyll, and carotenoid), total protein content, superoxide dismutase activity (SOD), catalase activity (CAT), ascorbic peroxide activity (APX), proline content, and grain yield of the triticale genotypes for two consecutive years under both normal irrigation and water stress conditions. Except for the total chlorophyll (Chl T) and chlorophyll b (Chl b), the effect of year was significant for the rest of the traits. The impact of the water stress was significant regarding all measured traits, while the interaction effect of year by stress was not significant for protein content, SOD, POD, and APX. Chl a content showed no significant difference among the genotypes based upon combined ANOVA table (Table 2), but all other traits showed highly significant differences among the genotypes. In regards to two-way interaction effect between year and genotype, Chl b and proline content showed no significant effect; and related to two-way interaction effect of stress and genotype, Chl a in line with Car content as well as H_2O_2 were not significant. The three-way interaction effect of year by stress by genotype was significant for Chl a, Chl T, SOD, POD, proline, and grain yield. The blocks within year and stress conditions showed no differences for both MDA and APX.

Genetic parameters

In order to determine the variability among triticale genotypes; genetic parameter namely genotyping coefficient of variation (GCV) and phenotyping coefficient of variation

(PCV) as well as heritability of the traits were reckoned (Table 3). The lowest calculated values of heritability were obtained for proline content (4.41%) along with POD (2.65%), while the highest values were recorded for both SOD (58.88%) and MDA (57.55%) in comparison to all other measured traits. Grain yield showed a relatively modest heritability (39.69%). The highest PCV and GCV were calculated for Chl b (94.58%) and CAT (46.2%), respectively, whereas, the lowest PCV was obtained in protein (34.89%) and the lowest GCV was shared between proline content (14.04) and POD (14.98%). In line with the grain yield, MDA and POD also showed high PCV and GCV.

Genotypic and phenotypic correlation coefficient were determined along with other genetic parameters (Table 4). Since the numbers of the data used for reckoning the correlation coefficients were high (240 numbers for two consecutive years under both water stress and normal condition) and the degree of freedom for correlation coefficients is highly dependent on the number of the observations for calculating the probability value or the significant level, all the high or low coefficients are significant in Table 4. However, high phenotypic correlations with grain yield were observed in H_2O_2 (-0.72), MDA (-0.66) protein content (-0.76), SOD (-0.72), POD (-0.73) with inverse relationships (negative correlation) and CAT (0.86) with positive association. The highest genotypic correlation with grain yield was recorded for H_2O_2 (-0.85), SOD (-0.89), POD (-0.86), CAT (-0.87), and proline content (-0.60), all of which showing negative relationships.

Principal component analysis (PCA)

In order to get an outstanding view regarding the structural relationship feasibly presented among the measured variables, principal component analysis (PCA) as a multivariate statistical method was implemented. PCA was separately executed for both normal irrigation and water stress condition. Overall, the first two components under both conditions showed higher eigenvalues than the criteria (1), which make them to be influential

toward the explaining of the variability (Table 5). The first two components of the normal irrigation condition explained about 79%, while the first two components of the water stress condition have accounted for about 75% of overall variability. Taking into consideration that just two components can be portrayed in a 2-dimential surface, coupled with the fact that the third component has relatively much lower eigenvalue, so that to explain variability in comparison to the two first ones, the biplot depiction was portrayed using the first two components under both separate conditions (Figure 1 and Figure 2). As the biplot for normal irrigation condition is represented in Figure 1, the relationship among the variables based on these two components is considerable. The pigments showed close relationship to one another and also with total protein content, while they showed negative associations with POD, CAT, APX, and proline content. Protein content, carotenoid, MDA, and H_2O_2 had the closest relation to the grain yield. Under water stress condition, the structure of the relationship has reformed as compared to the normal condition (Figure 2). Grain yield showed close associations with pigments' content and total protein, while it showed relatively no relationship with proline content. All enzymes' activities along with MDA and H_2O_2 showed negative correlations with the grain yield, where the strongest negative relationship with the grain yield was obtained for SOD.

Regression analysis

Implementing stepwise regression analysis for screening the most influential variables towards the grain yield pointed out that the proline content and MDA under normal irrigation condition and H_2O_2, SOD, and MDA under water stress condition are the most effective traits on the grain yield of triticale (Table 6). The model R-Square (coefficient of determination) for normal irrigation condition was relatively low (0.33), whereas its value under irrigation condition was high and acceptable (0.79). Final model of regression analysis implied that proline content under the normal irrigation condition has a negative relationship with grain

yield, while MDA has a positive one. The two of the variables screened in water stress condition consisted of H_2O_2 and MDA showed a negative association with grain yield, while the third one, SOD, showed a positive correlation. All the variables under both conditions had significant probability levels at <0.01. In order to better understand the structural model relationship between the grain yield and the screened variables under both normal irrigation and water stress conditions, path coefficient analysis was carried out. The highest direct effect under stress condition was belonged to the H_2O_2 content having a negative sign. The indirect effects of H_2O_2 through MDA and SOD were negative and positive, respectively. The direct effect of MDA was relatively high and negative, and its indirect effects were negative for H_2O_2, while positive for SOD. Proline and MDA that screened out as the most significant variables for grain yield modeling under normal condition showed a positive and a negative direct effect through grain yield (Table 7).

Cluster analysis

Dendrogram of cluster analysis in order to considering the overall relationship among traits under both separate conditions was carried out by which the results of the principal component analysis (biplot) along with regression analysis were approved. Under normal irrigation condition, yield showed much more relationship with SOD, APX, MDA, and H_2O_2 (Figure 3). Also, under water stress condition, the pigments, apart from carotenoid, showed much more close association with grain yield along with proline and protein contents, while all other variables were grouped in one separate cluster (Figure 4).

Discussion

Photosynthetic plant cells are especially at risk for oxidative damage due to their oxygenic condition and to the abundance of photosensitizers and polyunsaturated fatty acids in the chloroplast envelope and thylakoids (Sankhla et al., 1992). Thus, it has been estimated

that 1% of the oxygen consumed by plants is diverted to produce reactivated oxygen in various subcellular loci (Foyer et al., 1994). Different environmental conditions may also enhance the production of reactive oxygen species (ROS), including superoxide radical (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen (O_1^-) (Saed-Moucheshi, Pakniyat, et al., 2014). Excessive ROS generation damages macromolecules, including proteins, nucleic acids, and lipids, and plants have developed a variety of antioxidant enzymes and scavenging molecules to prevent these damages (Saed-Moucheshi, Shekoofa, et al., 2014).

Altering environmental conditions caused by weather and other natural factors is capable to differ the ROS and accordingly antioxidant state of the plants. Water stress induces a higher generation of free radicals, as a result of lipid peroxidation, causes oxidative damages to plants' cells. Superoxide dismutase catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, a product which is relatively stable and detoxified by CAT and POD (Tewari et al., 2007). Therefore, higher activity of antioxidant enzymes is related to higher stress tolerance in plants. On the other hand, these antioxidant enzymes are of known indicators to evaluate the status of oxidation-reduction in plants (Ashraf, 2009). However, environmental condition and its changes could affect reactive oxygen metabolism and antioxidant production, genetics content and expression of important genes are still highly involved (Sinha et al., 2012). Therefore, genetic background and self-regulation of different involved genes which are usually different in divergent genotypes has an important role in this situation (Ashraf, 2009).

Generation of H_2O_2 is increased in response to a wide variety of abiotic and biotic stresses, and some authors have suggested that H_2O_2 plays a dual role in plants: at low concentrations, it acts as a messenger molecule involved in stress signaling, triggering tolerance against various abiotic and biotic stresses, and at high concentrations, it orchestrates

programmed cell death (Gechev et al., 2002; Lin and Kao, 2000). Thus, it appears likely that H_2O_2 accumulation in specific tissues, and in the appropriate quantities, may benefit plants by mediating plant acclimation and cross-tolerance to both biotic and abiotic stresses (Bailly et al., 1996). On the contrary, if the content of H_2O_2 gains higher than a steady state in the cellular constituents, it could be dangerous and destructive for tissues (Ali et al., 2005). On the other hand, the higher generation of MDA is usually related to higher negative effect of stresses. Also, proline is a kind of efficient osmotic regulation substance with affinity in plants. Although accumulation of proline is generally considered to be positively correlated with osmotic stress tolerance, the dispute about proline exists all the time (Zhang et al., 2015; Filek et al., 2015).

One of the most detrimental effects of water stress in the membrane exposed to water stress is lipid peroxidation, measured in terms of MDA content. 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 (Savvides et al., 2015). MDA reacts with thiobarbituric acid to form a dark-colored chromogen (TBARS) and, hence, it is a convenient biomarker for the determination of lipid peroxidation. Environmental stresses result in overproduction of ROS, which may cause lipid peroxidation by initiating a variety of auto-oxidative chain reactions on membrane-unsaturated fatty acids, thus yielding lipid hydro peroxides and consequent membrane injuries since peroxidation of membrane lipids would enhance membrane fluidity and electrolyte leakage (DaCosta and Huang, 2007). In the study of (Pérez-López et al., 2009), MDA was much higher in sensitive genotype than tolerant ones.

Drought stress or water shortage is seriously restricting crop yield potential. Therefore, it is imperative to identify genetic resources tolerant to drought stress in an effort for sustainable agricultural production and make the best of the lands and the natural

resources. The results of the current study show that the activity of the enzymatic antioxidant in addition to cell injuries and pigment's contents are highly susceptible to environmental changes which could be observed by the effect of the years in ANOVA table and the mean comparison between years. Comparing water shortage condition to the normal condition implies that water stress condition significantly increases the amount of H_2O_2 and MDA in triticale genotypes which results in higher activities of antioxidant enzymes. With the aim to detoxifying the generated ROS under stressful condition, plant shares more energy in production of antioxidant lead to lowering the production of the pigments, so that lower final yield. Considering phenotypic and genotypic correlation coefficient for overall stressful and normal condition have approved this fact. On the contrary, stepwise regression picked up SOD with a positive coefficient under stressful condition related to grain yield. This result in addition to indicating the importance of the SOD under this condition through production and yield of the triticale genotypes, is implying that under stressful condition higher activity of SOD which leads to lowering cell injury has a positive effect on the stable grain yield. Also, both H_2O_2 and MDA have been extracted as influential traits toward the grain yield under stressful condition asserting the importance and substantial impact of the ROS and cell injury on the plant product. With regards to the impact of these traits under two normal and stressful conditions, it is pointing out that genotypes having higher activity of antioxidant, especially SOD, and lower content of H_2O_2, and MDA are more suitable genotypes to be screened in this situation. Under normal condition, with taking the results of biplot and PC analysis to the consideration, providing that the genotypes with higher pigment contents to be screened in this condition are genotypes that might result in a higher yield in the next generations. Arough et al. (2016) reported that even under nutritional stress, the activity of enzymatic antioxidant in triticale is risen in comparison to normal nutritional condition. Effects of soil drought on crop yield of 4 strains and 7 cultivars of spring triticale was investigated under

field condition by Grzesiak et al. (2003). In their study, the yield and physio-biochemical traits were investigated, and they pointed out that in the triticale genotypes using indirect selection by means of physio-biochemical traits is proper and valuable method for screening the new varieties of this plant.

Among antioxidant enzymes, SOD activity showed a higher variation among barley varieties, this points out that this enzyme is an important trait to be exerted in the breeding programs. Studying different genotypes of triticale, Gorji et al. (2011) reported a high heritability for enzymatic antioxidant in which additive effect in POD and CAT was significant, but in SOD was not. Vosough et al. (2015) found highly genotypic and phenotypic correlation of the enzymatic antioxidant with grain yield of wheat genotypes. They also stated that because of low heritability of grain yield in wheat, while higher heritability of the antioxidant, these traits are proper criteria for indirect selection in wheat genotypes under stressful condition. Seckin et al. (2010) observed the similar patterns in the activities of SOD, CAT, POD, APX, and GR enzymes in response to sodium chloride (NaCl) stress in *Hordeum vulgare*. Significant increase in the activities of SOD, POD, APX, and glutathione reductase (GR) in the NaCl stressed leaves was highly correlated with the regulation of the constitutive related isoenzymes. Many researchers have reported intensifying of these enzymatic antioxidants under drought and deficit irrigation conditions (Ahmed, Dai, et al., 2013; Ahmed, Cao, et al., 2013; Jiang and Huang, 2001; Acar et al., 2001; Habibi, 2013; Krüger, 2002; Guo et al., 2009).

Conclusions

Based on the results from different statistical techniques and comparing relationship among traits for normal irrigation and stress condition, enzymatic antioxidant could be used as criteria for screening tolerant genotypes of barley. On the other hand, it was found SOD

are the most important trait to achieving higher tolerant genotypes through indirect selection. Furthermore, H_2O_2 and MDA contents with having highly relationship with grain yield are also different possible traits for screening triticale genotypes for water stress conditions in which genotypes with lower content of these traits could be screened.

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Table 1. The number and origin of the genotypes used in the experiments

Number	Name	Origin
1	ELTTCL1	LIRON_2.5.DIS B5. 3. SPHD. PVN. YOGUI_ 6.4. KER_3. 6. BULL_10. MANATI_1. 7. ARDI_1. TOPO 1419. ERIZO_9.3.2 *KETTU_1
2	ELTTCL2	AR.SNP6.TARASCA 87_3. C, S10.3. URON_ 5. TATU_ 1.4. BULL_ 10. MANATI_ 1.3. ELK54. BUF_ 2. NIMIR _3.5. DAHBI_ 6.3. ARDI_1. TOPO 1419.ERIZO_9
3	ELTTCL4	BW32-1.CENT.SARDEV.7.LIRON_2.5.DIS B5. 3. SPHD. PVN. YOGUI_ 6.4. KER_ 3.6. BULL_ 10. MANATI _ 1.8. MERINO. JLO. REH. 3. HARE _267. 4. ARDI_ 4.5. PTR. CSTO. GLT.3. RHINO_4-1.4.HARE_7265.YOGUI _3.6.BULL_10.MANATI_1
4	ELTTCL7	DRIRA.2*CMH77A.1165.8.NIMIR_3.ERIZO_12.5.GC.3.733.EB.MPE.3.LA MB_3.4.BUF_2.6.POLLMER_2.7.FAHAD_8-2.9. ARDI_ 1. TOPO 1419. ERIZO_9 .3. LIRON_1-1.4. FAHAD_4. FARAS_1
5	ELTTCL8	CMH80.1212.CMH81A.1239.3.YOGUI_3.ERIZO_11.ONA_2.POSS_1-2.7.LIRON_2.5.DIS B5. 3. SPHD. PVN. YOGUI_ 6.4. KER_ 3.6. BULL_ 10. MANATI_1
6	ELTTCL9	CMH82.1082.ZEBRA 31.7.LIRON_2.5.DIS B5. 3. SPHD. PVN. YOGUI_6.4.KER_3.6.BULL_10.MANATI_1.8.LIRON_2.5.DIS B5.3.SPHD.PVN.YOGUI_6.4.KER_3.6.BULL_10.MANATI_1
7	ELTTCL10	FD-693. 2* FAHAD_ 4. POLLMER_ 4.3. POLLMER_ 2.1.4. FARAS. CMH84. 4414.6. RHINO_ 3. BULL_ 1-1.5. CMH77. 1135. CMH77A. 1165.2 *YOGUI_1.3.IBEX.4.JLO 97.CIVET
8	ELTTCL12	LIRON_2.5.DIS B5. 3. SPHD. PVN. YOGUI_ 6.4. KER_ 3.6. BULL_ 10. MANATI_1.7. DAHBI_6.3. ARDI_1. TOPO 1419. ERIZO_9
9	ELTTCL15	ARDI_1.TOPO 1419. ERIZO_ 9.3. LIRON _1 -1.4. FAHAD_4. FARAS_1.5. DAHBI.3. FAHAD-2-8*2. PTR.PND-T
10	ELTTCL18	HX87-244.HX87-255. 3. T1502_ WG. MOLOC_ 4. RHINO_ 3. BULL_1-1
11	ELTTCL19	HX87-244.HX87-255.5.PRESTO.2*TESMO_1.MUSX 603.4.ARDI_1.TOPO 1419.ERIZO_9.3.SUSI_2
12	ELTTCL20	POPP1_2.TX93-57-7.7.LIRON_2.5.DIS B5.3.SPHD.PVN.YOGUI_6.4.KER_3.6.BULL_10.MANATI_1
13	ELTTCL21	TAHARA.TREAT.7.LIRON_2.5.DIS B5.3.SPHD.PVN.YOGUI_6.4.KER_3.6.BULL_10.MANATI_1
14	ELTTCL22	POLLMER_2.2.1*2.FARAS.CMH84.4414.4.DAHBI_6.3.ARDI_1.TOPO 1419.ERIZO_9
15	ELTTCL24	LIRON_2.5.DIS B5. 3. SPHD. PVN. YOGUI_ 6. 4. KER_ 3. 6. BULL_1 0. MANATI_ 1. 7. RHINO_ 3. BULL_ 1-1.8. BAT* 2. BCN. CAAL. 3. ERIZO_7. BAGAL_ 2. FARAS_1
16	ELTTCL25	PRESTO. 2* TESMO_ 1. MUSX 603. 4. ARDI_ 1. TOPO 1419. ERIZO_ 9.3. SUSI_2.5. POPP1_ 1.6. BULL_ 10. MANATI_1*2. FARAS. CMH84. 4414
17	ELTTCL28	LIRON_2.5.DIS B5. 3. PHD. PVN. YOGUI_ 6.4. KER_ 3.6. BULL_ 10. MANATI_1* 2.7. TUKURU
18	ELTTCL29	LIRON_ 2. 5. DIS B5. 3. SPHD. PVN. YOGUI_ 6. 4. KER_ 3. 6. BULL_ 10. MANATI_1* 2.7. TUKURU
19	ELTTCL30	LIRON_ 2. 5. DIS B5. 3. SPHD. PVN. YOGUI_ 6. 4. KER_ 3.6. BULL_ 10. MANATI_1* 2. 7. TUKURU
20	Javaniloo	Common triticale cultivars in Iran.

Table 2. Combined analysis of variance for genotype × year × stress for two consecutive years under both normal and stress conditions in measured traits

Source	DF	H_2O_2	MDA	Chl a	Chl b	Chl T	Car	Protein	SOD	POD	CAT	APX	Proline	Yield
Y	1	10319.8**	33319.8**	5124.79*	70907ns	19967ns	173898*	847.67*	50439.59*	26364*	2236.15*	4484282*	8198.29*	880561*
E1[&]	2	310.53	137	81.98	513.34	10737.07	1579	86.23	1094.93	6186.23	548.63	1705102	3748	62740
C	1	3470.78*	936556**	1918.66*	9192.07*	2998.62**	232770**	78849**	176418*	182712*	3781.28*	9503826**	11226.62*	3016986**
E2	2	409.71	17840	73.7	792.67	521.84	2548	57.59	1100.2	6172.33	547.46	99961	3734	95326
Y*C	1	409.85**	17839**	372*	1793.02*	521.32**	40549**	751ns	1103.85ns	6164.01ns	547.22*	1999943ns	23735**	62484**
E3	8	8.29	5.91	32.71	235.15	22.45	438.84	719.09	3405.23	1371.34	98.64	196.16	140.61	2500.87
G	19	21.86**	808.03**	20.37ns	129.11*	226.23**	445**	557.62*	330.57**	9214.56**	56.26*	11178.73*	56.91**	75013**
E4	38	1.3	8.8	17.37	26.91	22.58	41.53	54.09	31.97	208.11	3.19	5548.76	59.18	33228
Y*G	19	1.43**	177.79**	15.68**	6.59ns	22.05**	42.87**	47.5**	335.63**	1199.79**	2.95**	5530.67**	60.38ns	385.26**
C*G	19	0.61ns	210.79**	7.4ns	27.26**	56.82*	11.68ns	18.86*	440.89**	1185.9**	4.78**	1189.05*	445.61*	32972**
E5	76	0.75	9.78	5.7	6.94	6.29	13.01	12.27	44.55	177.58	1.54	370.96	46.8	129
Y*C*G	19	0.75ns	39.78ns	5.7**	6.94ns	6.29**	13.01ns	12.27ns	44.55**	177.58**	1.54ns	3370.96ns	46.8**	7200.83**
Er	34	0.59	36.98	2.05	7.88	1.49	11.47	9.15	13.95	68.97	1.2	1250.59	5.8	129

**, * and ns are respectively indicating significant at 1%, 5% level of probability and not significant.

Y: year; E: error; C: condition; G: genotype; H_2O_2: hydrogen peroxide; MDA: malondialdehyde; Ch: chlorophyll; Car: carotenoid; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; APX: ascorbic peroxidase; Er: Residual error.

[&]: Errors are based on calculated values from SAS's output taking year and all its interaction as random effect.

Table 3. Genotypic parameters related to measured traits for both normal and stress condition together and for all two years

Parameter	H_2O_2 (umol g^{-1} FW)	MDA (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})	Yield (g m^{-2})
Heritability	17.52	57.55	23.72	10.84	23.03	19.44	19.15	58.88	2.65	34.49	19	4.41	39.69
PCV	77.5	65.25	45.7	94.58	38.78	35.51	34.89	49.99	70.61	60.31	63.76	51.27	52.72
GCV	42.32	64.57	29.03	40.61	24.27	20.42	19.92	50.03	14.98	46.2	36.25	14.04	43.32
Mean	5.88	11.39	9.79	4.74	15.07	6.01	24.37	29.49	29.92	0.98	153.05	15.09	506.94
Std Dev	3.75	7.95	3.93	2.21	4.54	2.63	11.56	18.26	20.43	0.5	52.53	8.3	251.9
Minimum	1.98	1.49	2.84	1.05	4.68	2.17	7.56	2	4.22	0.16	47.1	2.31	78
Maximum	27.92	29.28	21.42	14.3	27.36	14.56	50.51	65.63	88	2.52	261.24	40.8	1260

PCV: phenotypic coefficient of variation; GCV: genotypic coefficient of variation; Std Dev: standard deviation.

Table 4. Genotypic (below the main diagonal) and phenotypic (above the main diagonal) correlation coefficient based on Pearson method

	H_2O_2	MDA	Chlorophyll a	Chlorophyll b	Total Chl	carotenoid	Protein	SOD	POD	CAT	APX	Proline	Grain yield
H_2O_2	1	0.612**	-0.491**	-0.318**	-0.506**	0.607**	0.590**	0.647**	0.655**	-0.674**	0.384**	0.076*	-0.717**
MDA	-0.037*	1	-0.366**	-0.349**	-0.403**	0.485**	0.838**	0.983**	0.509**	-0.608**	0.506**	0.142**	-0.662**
Chlorophyll a	0.204**	-0.126**	1	0.372**	0.900**	-0.286**	-0.267**	-0.439**	-0.466**	0.452**	-0.464**	-0.359**	0.486**
Chlorophyll b	0.001ns	-0.363**	0.53**	1	0.501**	-0.095*	-0.271**	-0.377**	-0.359**	0.276**	-0.433**	-0.062	0.315**
Total chl	-0.025ns	-0.234**	0.922**	0.866**	1	-0.203**	-0.330**	-0.492**	-0.523**	0.499**	-0.465**	-0.330**	0.545**
carotenoid	-0.019ns	0.1**	0.991**	0.97**	0.97**	1	0.402**	0.499**	0.501**	-0.354**	0.229**	-0.207**	-0.380**
Protein	0.532**	0.122**	0.97**	0.487**	0.839**	0.924**	1	0.850**	0.610**	-0.718**	0.342**	0.239**	-0.761**
SOD	-0.034*	0.97**	-0.158**	-0.38**	-0.259**	0.094**	0.16**	1	0.572**	-0.656**	0.530**	0.203**	-0.715**
POD	0.428**	-0.96**	0.347**	0.557**	0.382**	-0.547**	0.466**	-0.96**	1	-0.672**	0.456**	0.383**	-0.727**
CAT	-0.651**	0.741**	-0.246**	-0.421**	-0.321**	-0.304**	0ns	0.759**	-0.96**	1	-0.340**	-0.352**	0.859**
APX	-0.29**	0.725**	-0.474**	-0.666**	-0.408**	-0.572**	-0.744**	0.674**	-0.96**	0.54**	1	0.272**	-0.409**
Proline	-0.815**	-0.832**	-0.711**	0.936**	0.358**	0.97**	-0.96**	-0.864**	-0.96**	-0.96**	0.066**	1	-0.409**
Grain yield	-0.85**	0.484**	-0.142**	-0.296**	-0.167**	-0.18**	-0.153**	-0.893**	-0.86**	-0.87**	0.42**	-0.596**	1

**, * and ns are respectively indicating significant at 1%, 5% level of probability and not significant.

Y: year; E: error; C: condition; G: genotype; H_2O_2: hydrogen peroxide; MDA: malondiadehyde; Ch: chlorophyll; Car: carotenoid; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; APX: ascorbic peroxidase.

Table 5. Eigenvalues and percent of variance explained by each component under both normal and stressful conditions.

Components	Eigenvalues	Percent of Variance	Cumulative %
Normal			
1	6.338	48.7538	48.7538
2	3.983	30.6385	79.3923
Stress			
1	6.926	53.2769	53.2769
2	2.852	21.9385	75.2154

Table 6. Summary of Stepwise Selection

Step	Variable Entered	Variable Removed	Label	Partial R-Square	Model R-Square	F Value	Pr > F
Normal							
1	proline		proline	0.2106	0.2106	32.01	<.0001
2	MDA		MDA	0.1201	0.3307	21.35	<.0001
Stress							
1	H_2O_2		H_2O_2	0.7252	0.7252	316.64	<.0001
2	SOD		SOD	0.0502	0.7754	26.63	<.0001
3	MDA		MDA	0.0137	0.7891	7.65	0.0066

Table 7. Estimating the parameters of the final regression model for grain yield

	DF	Parameter	SE	t Value	Pr > |t|	Standardized Estimate
normal						
Intercept	1	714.8634	28.18401	25.36	<.0001	0
MDA	1	19.22048	4.15929	4.62	<.0001	0.34747
Proline	1	-7.11375	1.23292	-5.77	<.0001	-0.43385
Stress						
Intercept	1	348.1497	17.95248	19.39	<.0001	0
H_2O_2	1	-21.9741	1.18717	-18.51	<.0001	-1.0371
MDA	1	-13.1165	4.74183	-2.77	0.0066	-0.6907
SOD	1	8.07094	2.20602	3.66	0.0004	0.95545

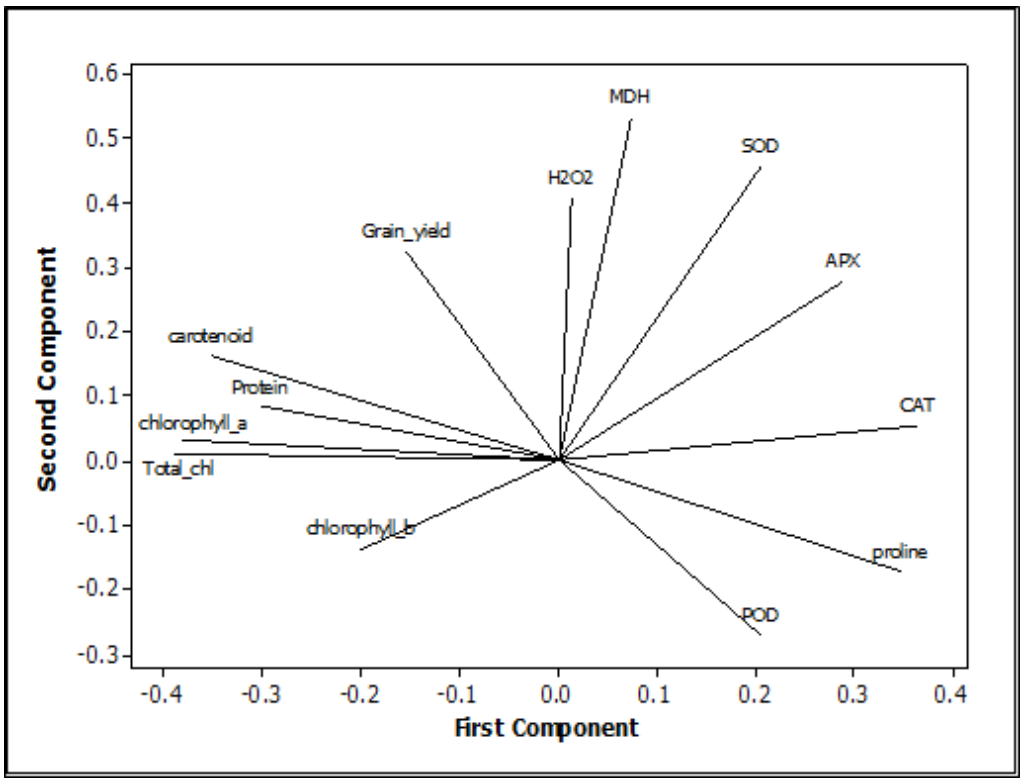

Figure 1. First two components and the measured traits scattered in two dimensions' area under normal condition.

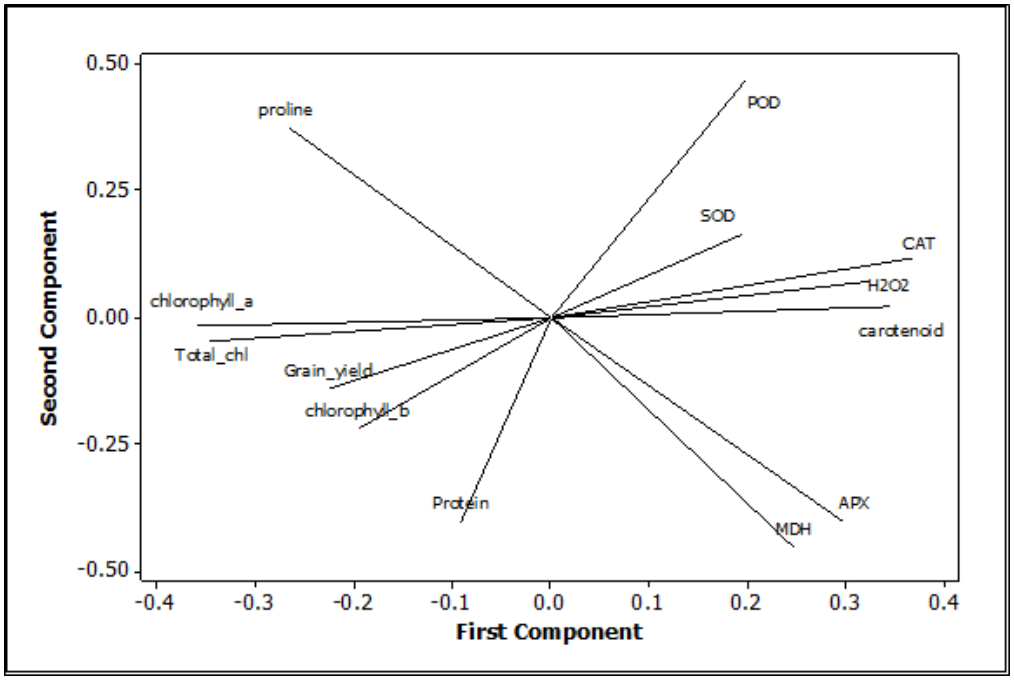

Figure 2. First two components and the measured traits scattered in two dimensions' area under Stress condition.

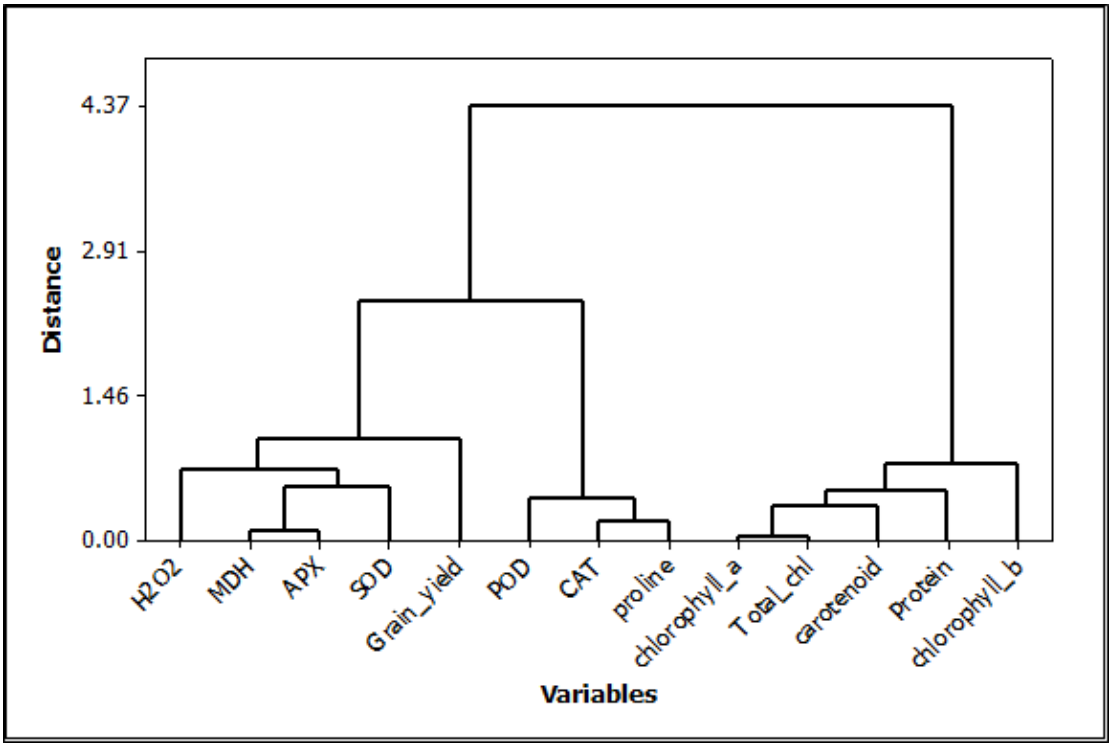

Figure 3. Cluster analysis of all measured traits under normal condition.

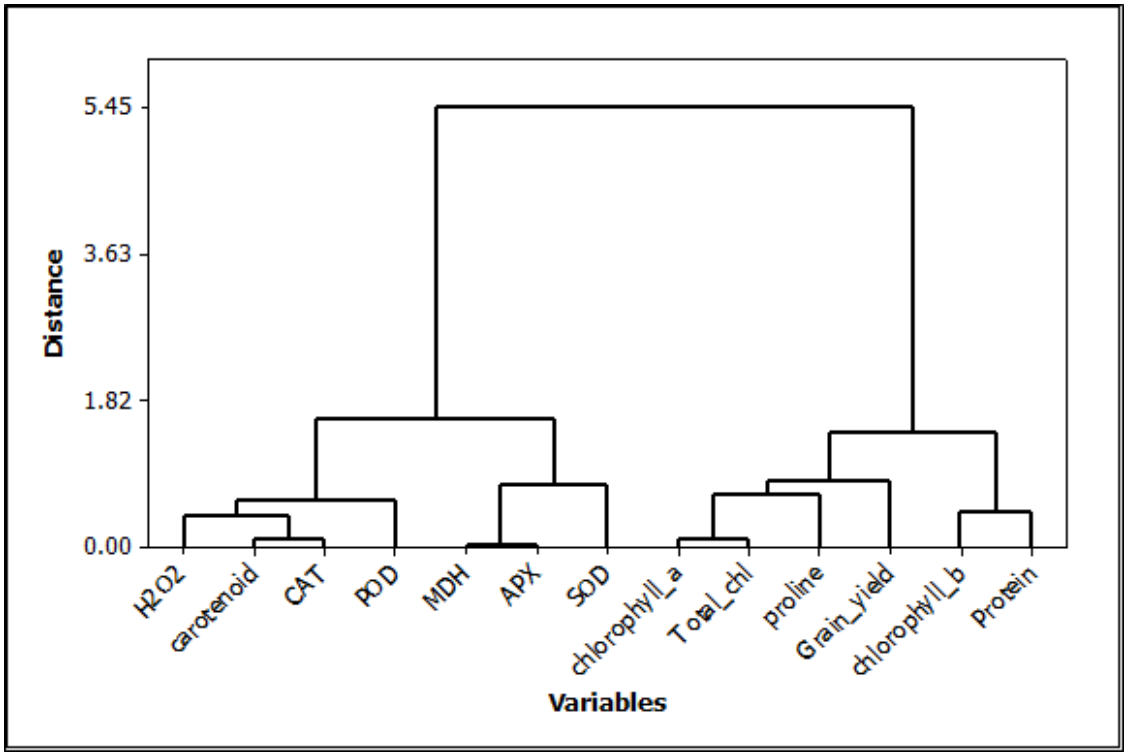

Figure 4. Cluster analysis of all measured traits under water stress condition.