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In vitro-examination of genetic parameters and estimation of seedling physiological traits under drought and normal conditions in durum wheat

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Abstract

An investigation was carried out to evaluate genetic parameters of *in-vitro* traits including callus size (CS), fresh weight (FW), dry weight (DW) and relative water content (RWC) under normal and drought conditions for seventeen durum wheat genotypes in a factorial experiment. Analysis of variance revealed significant differences between the genotypes for all traits. The results indicated that the phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all traits. High heritability and genetic advance of traits showed relatively low environmental effects on the traits and thus the chance of effective selection. Genotypic response to drought stress under *in-vitro* condition in terms of mean comparison demonstrated that the genotypes, 19E-M84859 (G6) and 19E-M141994 (G9) achieved higher levels of CS and RWC under both normal and stress conditions. The genotypes, 19E-TOPDY (G4) and 19E-142069 (G16) had minimum difference between fresh and dry weights in normal and stress conditions. These genotypes had higher genetic potential for response to drought stress. The germplasm evaluation based on physiological traits showed variation for some antioxidant enzyme activities and biomass of genotypes under both normal and stress conditions. The results of present study on genetic diversity among the genotypes may be utilized in wheat breeding programs.

Key words: Durum wheat, genetic parameters, drought stress, *In-vitro* culture, physiological traits

Introduction

Durum wheat (*Triticum durum* Desf.) is considered to be an important cereal crop for planting in most of the rainfed regions in Iran. Iranian durum wheat generally exhibit a great genetic variation for quantitative and qualitative traits even under abiotic stresses such as drought (Heidari et al. 2017; Ehdaie and Waines 1989). A successful breeding program mainly depends on the availability of genetic variation. However, utilization of genetic resources as a source of variability requires their proper systematic evaluation (Belay et al. 1993). One of approaches has been used to study the magnitude of genetic variation in wheat is to assess the variability of different traits among the genotypes (Ceccarelli and Grando 2000; Ehdaie and Waines 1989) under diverse ecological conditions but *in-vitro*-examination of genetic variability and evaluation of genetic advance have been rarely done in durum wheat. Existence of diversity in the culture medium of genotypes under *in-vitro* condition will not only accelerate the selection process but also enhance the selection efficiency of desirable traits at cellular level and over the later stages in plant tolerance level. In this procedure, the callus is exposed to stress such as Nacl, Mannitol and PEG leading to the possibility that the resulted plants would achieve high tolerance against stress. Some researchers have adopted *in-vitro* techniques to study drought tolerance in wheat and reported genetic parameters such as heritability, variance components and genetic advance for *in-vitro* traits. The screening has facilitated the selection of superior genotypes suitable for different environmental conditions (Chaghakaboodi et al. 2012; Ghafari et al. 2014; Hsissou and Bouharmont 1994; Farshadfar et al. 2012; Razmjoo et al. 2015). The significance of these studies in terms of breeding will help in gain more by partitioning of the observed variability into its heritable and non-heritable components and by estimating the expected genetic advance for *in-vitro*

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traits. Heritability plays a predictive role in plant breeding and has a direct link between heritability and response to selection called genetic advance providing more effective conditions for selection (Tazeen et al. 2009). Drought stress is the most common adverse environmental condition that can seriously reduce crop productivity. Selection of drought resistant genotypes would be the most economical approach to improve productivity. To survive against the stress, plants have evolved a number of morphological, physiological, biochemical, and metabolic responses. These mechanisms enabling plants to cope with drought stress and maintain their growth under those conditions (Gao et al. 2008). Therefore, a large part of breeding studies have been dedicated to plant response to water deficiency and drought stress at physiological level (Demangnet et al. 1991). Previous studies focused on the antioxidant enzyme activity and structural capacity of the plant to deal with oxidative activities under stress conditions (Sreenivasulu et al. 1999). Recent research shows that drought stress can lead to formation kind of reactive oxygen and finally cause of intensify the antioxidant enzyme activity. By producing a variety of antioxidant enzyme compounds, plants are able to eliminate the oxygen free radicals and their toxic effects (Meloni et al. 2003). There is a wide variety of antioxidant enzymes including superoxide dismutase, catalase, ascorbate peroxidase and peroxidase, which protect cells against hydrogen peroxide by functioning as H_2O_2 sweepers (Dixit et al. 2001) thus playing a crucial role in gaining resistance to oxidative stress in cellular adaptive responses (Jose et al. 1999). AL-Ghamdi (2009) with the examination of drought stress in two wheat varieties (drought resistant and susceptible) indicated that the weak antioxidant enzymes response of drought susceptible varieties leading to enhanced membrane damage during severe drought stress, indicated by the accumulation of malondialdehyde (MDA). The drought acclimated varieties exhibited increase in the activity of H2O2 scavenging enzymes particularly APX and CAT and maintenance of ascorbate redox pool by efficient function of APX enzyme. Additional reports have been provided by Yong et al. 2006; Lascano et al. 2005; Demiral et al. 2004; Gregersen and Holm 2007; Unyayar and Cekic 2005; Nayyar and Gupta 2006 on physiological traits. Therefore, the aim of current study was to evaluate the callus induction and its growth in durum genotypes under normal and stress conditions and estimating the genetic parameters associated with these traits. Further, this study was also intended to examine the genetic diversity of 17 durum wheat genotypes in terms of physiological traits measured in seedling stage under normal and stress conditions.

Materials and methods

Seventeen wheat genotypes including three control genotypes *viz*., Zardak, Saji and Sardari were used in this investigation. Names of the tested durum wheat genotypes are given in Table 1. The experiment was performed at the Dryland Agricultural Research Institute (DARI), Sararood, Kermanshah, Iran. Sampling was done from plants and seeds which were collected from DARI.

Table 1. The list of wheat genotypes along with their pedigree studied

Geno-types	Name/ pedigree	Genotypes	Name/ pedigree
G1(C)	Saji*	G10	19E-M141995
G2(C)	Zardak	G11	18E-M142005
G3(C)	Sardari	G12	19E-M142017
G4	19E-TOPDY	G13	19E-M142025
G5	19E-RASCON	G14	19E-M142038
G6	19E-M84859	G15	19E-M142045
G7	19E-M141979	G16	19E-M142069
G8	19E-M141982	G17	19E-M142070
G9	19E-M141994		

C = Control

In callus culture, the seed samples were disinfected with 1.5% of sodium hypochlorite for 10 minutes. They were rinsed with distilled water. The MS medium was prepared with 15 gr sucrose, 7 gr agar in pH=5.8 (Murashige and Skoog 1962). The sub-samples were maintained in a MS medium supplemented with 2,4-D and a medium under osmotic stress with mannitol 2% until callus induction. Traits of callus size (CS) in mm, callus fresh (FW) and dry weights (DW) in grams, callus relative water content (RWC) were measured in the experiment conducted in a completely randomized design with three replications. *In-vitro* traits under normal and stress conditions were subjected to analysis of variances according to the formula suggested by Steel and Torrie (1960). The genotypic and phenotypic variances and expected genetic advance (GA) were estimated as per the procedure suggested by Johnson et al. (1955). Genotypic (GCV) and phenotypic (PCV) coefficients

of variation were computed according to Burton and Devane (1998). Heritability in broad sense (h^2b) was estimated using the formula adopted by Allard (1960). Genetic advance over mean was estimated by using the formula proposed by Comstock and Robinson (1952). The Duncan's method was employed to compare the mean values of durum wheat genotypes in relation to the tissue culture traits. The physiological traits were measured at the seedling stage in durum wheat genotypes. Physiological traits, their abbreviated names along with units of measurement and measurement methods including, Malondialdehayde (MDA), uM/g FW (Stewart and Bewley 1980), Superoxide Dismutase (SOD), unit/mg protein (Moon and Terao 1998), Catalase (CAT), mmol/g FW min (Chaoui 1987), Ascorbate Peroxidase (APX) mmol/g FW min (Nakano and Asada 1987), Peroxidase (PRX), OD470/gFW min (Abeles and Biles (1991), Relative Water Content (RWC), mg (Dispersed), Chlorophyll (CHA¡CHB¡ CHT), mg/g^{-1}FW (Lichtenthaler and Wellurn 1983) and Fresh and Dry Biomass (SFW-SDW) mg. To study the relationships among tested durum genotypes, measured traits factor analysis and hierarchical cluster analysis (HCA) were performed by NTYSYS sps 2.02 software (Rohlf 1998). The mean values of durum wheat genotypes were compared through the Duncan's method for measured physiological traits under optimal and stress conditions.

Results and discussion

Evaluation of genetic variability of in-vitro traits under normal and drought stress

Analysis of variance results for measured traits in callus culture revealed that there was a significant difference between genotypes in terms of all traits at probability level of 1%. In fact, the diversity among genotypes in terms of callus traits indicated the dependence of these traits on genotype. In a study on embryonic callus induction of wheat varieties, Ozgen et al. (1996) reported that callus water content, callus induction percentage and callus dry and fresh weights were influenced by genotype. In present study, the environmental effect was significant on all traits except the callus relative water content. The genotype × environment interaction was significant for callus size and callus fresh weight. This indicated that both environmental and genotypic factors do not function independently for these two traits and their effects pertain together. In fact, some genotypes indicated better situation in one environment than the others. The results obtained in this study are consistent with a previous study in sugar beet monogerm hybrids *in-vitro* for drought tolerance (Ghafari et al. 2014). Chaghakaboodi et al. (2012) reported that the canola genotypes were significantly different in terms of all traits at probability level of 1% in a study of callus culture. Also, there was a significant difference observed between stress levels in all traits. Furthermore, the genotype × drought interaction was significant in terms of these traits. It reflected the dependence of these two factors in effect on the measured traits *in-vitro* (Chaghakaboodi et al. 2012). Present study showed a high level of variation among genotypes. The minimum and maximum mean values and the values of genotypic (s^2g) and phenotypic variance (s^2p) for all traits under normal and stress conditions are given in Table 2.

The existence of genetic variation among breeding materials is very important for success of any crop breeding programmes. High levels of these parameters indicated that sufficient diversity can be useful in the selection process. Mahto et al. (2002) and Vashistha et al. (2013) reported similar results in corn in terms of agro-morphological traits. The evaluation of GCV indicated the total value genotypic variance which transmitted from parents, to offspring and reflected by heritability. According to Deshmukh et al. (1986), amount of PCV and GCV nearly over 20% were considered as high, whereas the amount of less than 10% was considered as low and values

Table 2. Range, means and genotypic (s^2g), phenotypic (s^2p) variance for *in-vitro* traits of 17 durum wheat genotypes under normal (N) and stress (S) conditions

Trait	Min value		Max value		Mean value		s^2g		s^2p	
	N	S	N	S	N	S	N	S	N	S
Callus size	3.62	2.10	10.5	5.10	5.95	3.03	1.18	0.47	1.77	0.50
Fresh weight	0.02	0.011	0.29	0.070	0.078	0.038	0.001	$3.27E^{-4}$	$2E^{-3}$	$3.43E^{-4}$
Dry weight	0.0018	0.0020	0.0353	0.019	0.0104	0.0049	$2.22E^{-5}$	$7.74E^{-6}$	$5.53E^{-5}$	$9.02E^{-6}$
RWC	56.75	60.9	98.5	92.85	85.92	86.04	6.41	54.27	54.25	58.45

between 10 and 20% counted as moderate. In present study, the GCV was high for callus size under stress condition, for fresh weight in both normal and stress conditions and dry weight in both normal and stress conditions. It was moderate for callus size in normal condition. The values were low for relative water content in both normal and stress conditions (Table 3). Yuce et al. (2008) and Wolie et al. (2013) reported similar results in millet. In present study, the heritability ranged from 11.81% for RWC under normal condition to 66.61% for callus size, and from 85.80% for dry weight to 95.33% for fresh weight under stress condition. High heritability levels indicated the low effect of environment on the studied traits. According to Singh (2001), if heritability of one trait be high, then it can be easily selected to achieve good efficiency. The callus size, fresh weight and dry weight had high heritability under stress conditions, whereas callus size and fresh weight have moderate heritability under normal conditions. In low-heritability traits, the selection may have considerably poor efficiency. General heritability indicated both additive and non-additive genetic effects. Genetic advance (GA) under selection refers to improvement of traits in genotypic values for new population compared to the base population under a selection cycle under certain selection intensity. The maximum amount of genetic advance as percent of the mean (GAM) at selection intensity of 5% was estimated to be 108.16% for callus dry weight and 92.89% for callus fresh weight under stress condition (Table 3).

Accurate selection can lead to genetic improvement for traits with moderate to high heritability together with moderate genetic advance. Hence, plant material can more easily be selected for these traits through examination. Heritability along with genetic advance will be more effective in prediction of efficiency in selection (Johnson et al. 1955). This reflects the degree of efficiency in a trait obtained under a specific selection pressure. Therefore, genetic advance is another important parameter assisting the plant breeders in selection program as demonstrated in barley double haploids (Dyulgerova and Valcheva 2014) and millet (Sabiel et al. 2014). Accordingly, the present study demonstrated that efficient selection can be practiced for callus fresh and dry weights and relatively better for callus size (Table 3) when the genotypic and phenotypic variance, heritability and genetic advance are high.

Mean comparison of callus in durum genotypes under normal and drought stress

In the present study, the results of mean comparison of durum genotypes showed the average callus size decreased under stress conditions as compared to normal conditions (Table 4). The maximum callus size was observed under normal conditions in G9 followed by G1, G6 and G13, whereas the minimum value was recorded in G17. Under stress conditions, the maximum callus size was found in G9 followed in G5, G6 and G13 (Fig. 1).

The results also indicated that some genotypes, namely, G6, G9 and G13 maintained their ability to generate larger callus than the other genotypes in both normal and stress conditions. Previous studies suggested that larger callus size lead to more resistant embryos. Hence, the selection of genotypes with this character will enable the plant to become more stress resistant (Ghafari et al. 2014). The average callus fresh weight decreased in stress conditions as compared to normal conditions (Table 4). The maximum callus fresh weight under normal conditions was found in G9 followed by G1, G6 and G11, while G4 showed the minimum value. Under stress conditions, the

Table 3. Estimates GCV and PCV coefficient of variation, heritability (h^2) and GA as percent of mean for *in-vitro* traits of 17 durum wheat genotypes under normal and stress conditions

Trait	GCV(%)		PCV(%)		h^2(%)		GAM	
	N	S	N	S	N	S	N	S
Callus size	18.26	22.62	22.37	23.33	66.61	94	30.67	44.73
Fresh weight	40.54	47.58	57.33	48.73	50	95.33	52.82	92.89
Dry weight	45.30	56.77	71.50	61.29	40.14	85.80	58.65	108.16
RWC	2.94	8.56	8.57	8.88	11.81	92.84	2.08	16.98

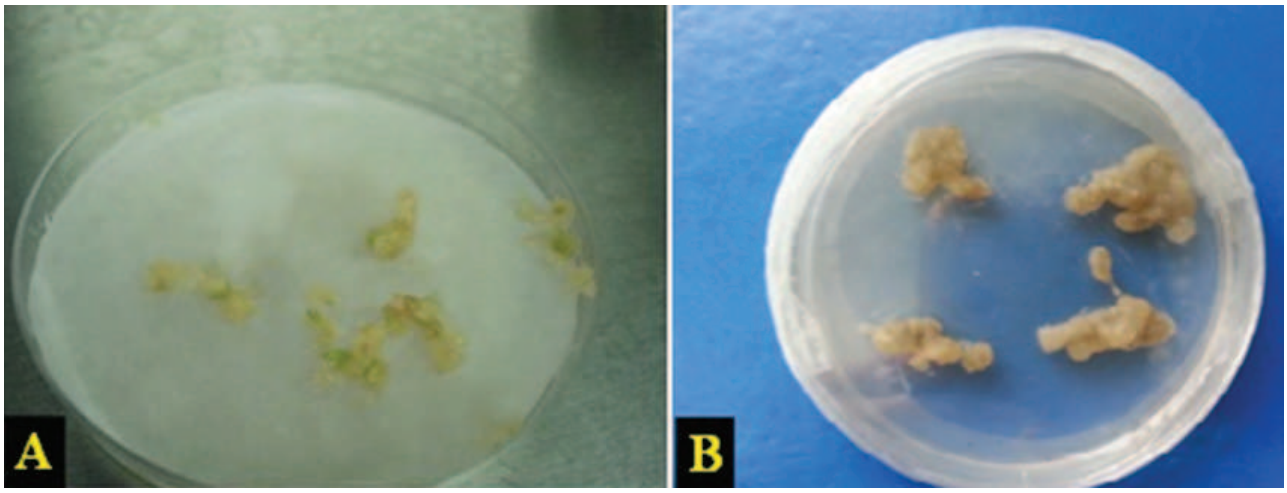

Fig. 1. (A) Callus growth under stress conditions; (B) Callus growth under non-stress conditions

maximum callus fresh weight was found in G1 and G9 and then G6 and G11. The minimum values were found in G4 and G7, respectively. As depicted in Table 4, that some genotypes maintained their capacity for more callus fresh weight than other under both normal and stress conditions. The average callus dry weight

Table 4. Mean comparison of durum wheat genotypes for callus trait

Genotype	Callus size		Fresh weight		Dry weight		RWC	
	N	S	N	S	N	S	N	S
Callus size	18.26	22.62	22.37	23.33	66.61	94	30.67	44.73
1	6.62^{ce2}	3.12^{de}*	0.12^{c}	0.062^{h}	0.01^{ab}	0.005^{b}	89.52^{ab}	91.65^{cg}
2	6.47^{ce}	3.23^{e}	0.09^{c}	0.041^{ef}	0.01^{ab}	0.004^{b}	89.00^{ac}	88.25^{ce}
3	5.96^{ad}	2.36^{ab}	0.08^{ac}	0.032^{c}	0.01^{a}	0.003^{ab}	89.95^{bc}	89.87^{cg}
4	4.83^{ab}	2.81^{cd}	0.03^{a}	0.019^{ab}	0.01^{a}	0.002^{a}	85.89^{ac}	86.22^{c}
5	6.18^{be}	3.55^{f}	0.08^{ac}	0.042^{ef}	0.02^{b}	0.09^{c}	75.43^{a}	77.46^{b}
6	7.46^{e}	3.96^{g}	0.10^{bc}	0.047^{fg}	0.01^{ab}	0.005^{b}	87.63^{ac}	88.73^{cg}
7	6.19^{be}	3.11^{de}	0.04^{ab}	0.012^{a}	0.01^{a}	0.004^{ab}	76.86^{ab}	63.25^{a}
8	5.25^{ac}	2.42^{ab}	0.06^{ac}	0.040^{df}	0.01^{ab}	0.004^{ab}	85.10^{ac}	88.62^{cg}
9	9.24^{f}	4.95^{h}	0.20^{d}	0.062^{h}	0.03^{c}	0.014^{d}	84.16^{ac}	77.85^{b}
10	4.92^{ab}	2.80^{cd}	0.07^{ac}	0.036^{ce}	0.01^{a}	0.003^{ab}	90.37^{ac}	90.22^{dg}
11	5.26^{ac}	2.84^{cd}	0.10^{c}	0.054^{g}	0.01^{ab}	0.004^{ab}	91.46^{c}	92.32^{g}
12	5.48^{ad}	2.53^{ac}	0.07^{ac}	0.038^{ce}	0.01^{a}	0.003^{ab}	88.33^{ac}	91.39^{eg}
13	6.93^{de}	3.76^{fg}	0.07^{ac}	0.033^{cd}	0.01^{a}	0.003^{ab}	88.83^{ac}	89.17^{cg}
14	5.67^{ad}	2.65^{ac}	0.07^{ac}	0.025^{b}	0.01^{ab}	0.004^{ab}	84.53^{ac}	80.91^{b}
15	5.39^{ac}	2.43^{ab}	0.07^{ac}	0.039^{ce}	0.02^{ab}	0.005^{b}	79.46^{ac}	86.35^{cd}
16	4.78^{ab}	2.68^{bc}	0.04^{ab}	0.023^{b}	0.01^{a}	0.002^{a}	85.00^{ac}	88.33^{cf}
17	4.59a	2.31^{a}	0.06^{ac}	0.032^{c}	0.01^{a}	0.002^{a}	89.26^{bc}	92.23^{fg}
Mean	5.95	3.03	0.078	0.038	0.0104	0.0049	85.92	86.04

*The mean values in each column have been represented by similar alphabetical letters indicating no significant differences at probability level of 1%. Suffix letters abcdefg have been used to show abbreviation of mean comparison. For example mean comparison of each genotype that had been abcd, is considered as ag

decreased in stress condition as compared to normal condition. The maximum callus dry weight under normal and stress conditions was recorded in G9 and G5. The calluses fresh and dry weights were comparable in the normal and stress conditions. The calluses of G4 and G16 lost less water and performed better in terms of responding to stress under stress conditions. The above results were consistent with the findings of Ghafari et al. (2014) who observed that callus fresh and dry weights decreases in stress conditions as compared to normal conditions. The average RWC decreased in normal conditions as against the stress conditions. The maximum value of RWC was recorded in G11, G3 and G10 under normal conditions, whereas the minimum was in G5. Under stress conditions also, the RWC was recorded high in G11 and the G7 recorded low value. The comparison of mean values reflected that G6 and G9 achieving higher levels of callus size and RWC under both normal and stress conditions as compared to other genotypes. Moreover, G4 and G16 had minimum difference between fresh and dry weights in normal and stress conditions and therefore, these genotypes possess higher genetic potential for response to drought stress *in-vitro*. A similar outcome has been reported by Farshadfar et al. (2016) as the response of twenty genotypes of bread wheat had high callus induction *in vitro* under drought stress. They also reported significant differences among the genotypes for callus diameter (CD), percentage of callus induction (PCI), fresh weight (FW), dry weight (DW) and relative water content (RWC).

Study on physiological traits at seedling stage under drought and normal conditions

The principal coordinate analysis was conducted on genetic diversity of 17 durum wheat genotypes in terms of 11 physiological traits measured in seedling stage under normal and stress conditions. The first four components explained 68.61% of total variance, based on which cluster analysis was performed to categorize the genotypes. The clustring of genotypes concerning the physiological traits, the correlation between the distance and cophenetic matrices obtained was r=0.6, which indicated a high correlation between the distance and cophenetic matrices. Cut of dendrogram in 2.74 clustered 17 genotypes into 4 groups. G1 and G7 in I cluster; G5, G15, G6, G11 and G17 were placed in cluster II; G2, G4, G8, G13 and G12 were clustered in III, while G3, G16, G9, G10 and G14 were placed group IV. Under stress condition, the first four components explained 73.87% of total data variance and the value r=0.7 indicated a high correlation between the distance and cophenetic matrices. Cut of dendrogram in 2.70 categorized 17 genotypes into 3 groups. Cluster I containing G1, G13, G4, G14, G6, G15, G17, G11, G5, G2, G8, G7 and G16; G3, G9 and G10 were placed in II group, whereas and G12 in a separate group III. However, considering the normal and stress conditions, the different genotypes were clustered into three clusters. Both conditions managed to categorize the two sets of genotypes in terms of 11 physiological traits. The placement of the genotypes has been different in different studies depending on the nature of the materials and the traits studied (Ozgen et al.1996; Razmjoo et al.2015; Sabiel et al. 2014). The correlation matrix of traits was examined in normal and stress conditions separately against the molecular data, where the correlation was insignificant.

Comparison of means of physiological traits in durum genotypes under normal and drought stress

The mean comparison for 17 durum wheat genotypes in respect of 11 physiological traits is presented in Table 5. The production chlorophyll contents in the durum genotypes did not vary under both normal and stress conditions but under stress, the values for all chlorophylls (a, b and T) were decreased. Chlorophyll stability recognized as an indicator of drought stress, and thus implies that the effect of stress on the plant is low providing the plant having better access to light. Ahmadi et al. (2013) showed that the chlorophyll content in bean leaf decreased under drought stress and was directly linked to biomass production. The variations in the concentration of chlorophylls a and b were adopted as a short-term response to stress and a measure of ability to maintain the source power in drought stress conditions. Therefore, the chlorophyll content in leaves is one of the key factors determining the rate of photosynthesis and dry matter production. Drought stress leads to a significant decrease in the amount of chlorophyll a during the spike development and 20 days after flowering in wheat, even though its effect on chlorophyll b is significant only in the first stage (Ahmadi et al. 2013). In present study, G7 and G12 produced greater amounts of chlorophyll a in comparison to other genotypes under stress conditions, while G1, G2, G4 and G7 experienced no chlorophyll loss in stress conditions as compared to normal. Further, the genotypes, G4, G5 and G6 produced greater amounts of chlorophyll b in comparison to other genotypes under stress conditions, while 11 genotypes experienced no chlorophyll loss under stress conditions as compared

Table 5. Comparison of mean values for 17 durum wheat genotypes in terms of 11 physiological traits measured under normal (N) and stress (S) conditions

Genotype	Adjusted mean											
	APX		CAT		CHA		CHB		CHT		MDA	
	N	S	N	S	N	S	N	S	N	S	N	S
1	15.34^{DG3}	17.10	3.98	4.82^{EG}	2.86	2.55	3.21	3.11	6.08	5.67	4.82	11.55
2	13.70^{EG}	14.86	4.10	4.42^{G}	3.14	3.22	3.34	3.18	6.49	6.41	3.82	14.10
3	18.19^{BG}	14.94	6.02	6.36^{AE}	3.33	1.95	1.02	1.34	4.35	3.30	4.90	24.17
4	8.95^{G}	6.92	5.24	5.92^{AG}	2.42	2.08	5.06	4.00	7.48	6.08	3.89	16.17
5	25.69^{AD}	24.52	6.84	7.28^{AC}	1.47	0.61	6.06	4.62	7.53	5.23	4.41	10.75
6	23.19^{AF}	22.28	5.54	5.92^{AG}	2.87	1.13	3.11	4.17	5.99	5.30	4.69	16.45
7	15.60^{AG}	16.50	6.10	6.22^{AF}	3.90	3.65	1.76	1.81	5.67	5.47	4.75	21.85
8	21.19^{BG}	24.30	6.97	7.56^{AB}	3.73	2.49	1.78	2.23	5.51	4.73	4.87	10.01
9	22.22^{BF}	26.37	6.26	6.85^{AD}	4.61	3.02	0.26	0.11	4.34	3.14	3.94	16.48
10	29.22^{AC}	34.40	6.25	6.62^{AE}	4.04	2.46	0.29	0.75	3.74	3.22	4.66	5.64
11	29.47^{AB}	35.09	5.97	6.49^{AE}	3.97	2.87	3.01	1.26	6.98	4.14	4.25	19.81
12	29.73^{AB}	33.45	4.05	4.33^{FG}	5.18	3.23	2.74	2.77	7.93	6.00	4.33	22.39
13	24.21^{AE}	34.92	6.68	6.81^{AD}	3.77	2.77	1.28	1.39	5.06	4.16	3.55	15.17
14	26.28^{AE}	33.97	5.18	5.87^{AG}	3.30	2.32	2.51	2.87	5.81	5.17	4.09	12.07
15	34.65^{A}	38.97	4.69	6.05^{AG}	2.94	2.20	4.16	3.00	7.11	5.21	4.71	11.84
16	24.90^{AE}	30.77	5.03	6.04^{AG}	4.18	1.83	1.28	1.87	5.46	3.70	3.68	22.24
17	29.22^{AC}	32.41	6.60	6.94^{AD}	4.19	2.85	1.64	2.32	5.83	5.17	4.15	16.97

Genotype	Adjusted mean									
	PRX		RWC		SDW		SFW		SOD	
	N	S	N	S	N	S	N	S	N	S
1	1.72^{J}	5.70	80.33	80.86	0.46^{GI}	0.45^{GI}	4.78^{EK}	4.26^{BG}	56.07	48.54
2	1.65^{J}	6.66	81.75	80.67	0.52^{EI}	0.51^{CI}	3.98^{K}	3.56^{G}	40.02	33.36
3	3.05^{AC}	6.29	82.86	82.72	0.63^{BF}	0.59^{AF}	5.38^{BI}	5.26^{AC}	49.08	39.26
4	2.61^{BF}	6.29	73.75	73.09	0.61^{CF}	0.59^{AF}	5.58^{AF}	5.26^{AC}	32.20	30.33
5	1.94^{GJ}	5.92	78.99	74.98	0.72^{AC}	0.63^{AC}	5.48^{AH}	5.06^{AD}	49.93	38.65
6	1.65^{J}	6.14	72.22	70.89	0.57^{DH}	0.54^{BG}	4.88^{DK}	4.46^{BG}	50.74	42.91
7	1.80^{IJ}	6.59	77.20	77.65	0.40^{I}	0.39^{HJ}	4.18^{JK}	3.96^{DG}	57.93	50.47
8	2.64^{BE}	6.35	86.82	86.86	0.56^{DH}	0.52^{CH}	4.78^{FK}	4.10^{CG}	30.35	24.66
9	1.90^{HJ}	6.20	86.29	83.39	0.73^{AC}	0.67^{AB}	5.88^{AD}	4.90^{AE}	41.44	35.49
10	2.34^{DI}	5.91	74.08	72.71	0.79^{A}	0.68^{A}	6.38^{AB}	5.90^{A}	41.15	36.14
11	2.49^{CG}	6.20	77.48	75.94	0.62^{CF}	0.57^{AG}	4.88^{EK}	4.00^{DG}	64.85	49.82
12	3.15^{AB}	5.61	82.75	77.00	0.43^{HI}	0.39^{IJ}	5.78^{AE}	4.70^{BG}	29.41	23.24
13	2.05^{FJ}	5.83	78.39	77.91	0.52^{EI}	0.46^{FI}	4.38^{IK}	3.90^{DG}	34.70	25.12
14	3.22^{A}	5.83	84.44	83.18	0.78^{AB}	0.63^{AC}	6.48^{A}	5.00^{AD}	64.67	36.46
15	2.34^{DI}	6.13	77.92	77.13	0.60^{CG}	0.55^{BG}	4.98^{DK}	4.10^{CG}	37.90	30.22
16	3.08^{AB}	6.94	81.43	81.93	0.65^{BE}	0.61^{AE}	4.68^{FK}	4.20^{CG}	58.21	44.92
17	0.43^{K}	5.91	78.92	76.25	0.61^{CG}	0.51^{CH}	4.98^{CK}	3.80^{EG}	48.66	43.74

to normal. In relation to the total chlorophyll content the genotypes gave comparable values.

The physiological traits of the plant, including the stomata closure, variation in the pattern of growth regulators and accumulation of metabolites indicate the adaptation to stress conditions. Hence, the effects of drought stress can be examined based on enzymes to more quickly identify the resistant plant. That is because there is a strong correlation between tolerance to environmental stresses and variations in the concentration of antioxidant enzymes in photosynthetic plants. Since the synthesis of every substance in cells are controlled by genes, thus breeders can handle to produce the drought resistant plants quickly by identifying the genes responsible for synthesis of these substances and transfer of these genes to another plants. In conjunction with ascorbate peroxidase (APX), there was no significant difference between genotypes in stress conditions (Table 5). Higher levels of APX were produced in genotypes G10, G11, G13 and G15 under stress conditions. The ROS sweepers neutralize the toxic effects of reactive oxygen, which might be an outcome of continuous and simultaneous activity of multiple enzymes. Plant protects cells against oxidative damage through a sweeping free radical system such as ascorbic acid. One of the functions of ascorbic acid is to enhance the ascorbate cycle in the mitochondria and peroxisomes as an outcome of higher H_2O_2 sweepers and subsequently increase in catalase activity, so plants can counter with oxidative stress (Dixit et al. 2001). Significant differences between the genotypes were observed with regards to catalase under stress conditions, the G8 giving the maximum value of catalase (7.56) and under normal condition also G8 performed well (6.97). Dadnia (2012) also recoded significant differences in catalas activity in sunflower. As one of the most important H_2O_2 sweepers, catalase functions by converting H_2O_2 to water and O_2 as well as ascorbate peroxidase a H_2O_2 sweeper, regulating the level of H_2O_2 in cells of *Pisum sativum* (Dixit et al. 2001). It has been well established that increase in drought condition can increase the antioxidant enzymes. For instance, drought condition and high temperatures can increase the activity of SOD, APX, and CAT in adapted wheat genotypes (Sairam et al. 2001). Previous studies have shown that increasing of catalase activity for decreasing the effects of peroxide play an important role to resistance in plants under stresses in wheat, oats, soybeans and peas (Kafi et al. 2000). Statistically significant differences between some genotypes in respect of peroxidase were observed under normal condition however, there was no significant difference under stress conditions. Generally, peroxidase is produced at higher levels in genotypes under stress conditions. Two genotypes, G2 and G16 produced larger amounts of peroxidase under stress conditions. The mechanisms that reduce oxidative stress including peroxidase activity play an important role in resistance of plant to stress (Sreenivasulu et al. 1999). There was no difference observed between the genotypes with regards to RWC under both normal and stress conditions however, G8 achieved maximum value for RWC under both conditions and maintained its capacity under stress. The genotypes with high water maintenance ability have more drought resistance. In terms of fresh and dry biomass in both normal and stress conditions, there was a significant difference between some genotypes and the trend changes under drought stress condition. The lowest difference between fresh biomass under normal and stress conditions were observed in G3, G4 and G7, whereas the lowest difference between dry biomass under normal and stress conditions was observed in G1, G2, G4 and G7. With respect to the enzyme, superoxide dismutase, no difference between the genotypes in both normal and stress conditions was recorded but the amount produced was reduced under stress conditions. Plants are adopted enzymatic and non-enzymatic antioxidant mechanisms to cope with oxidative stress arising from active oxygen radicals. The non-enzymatic antioxidants include glutathione and ascorbic acid, while the enzymatic antioxidanta include catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and glutathione reductase as studied by Hsu and Kao (2003) in rice. Among the enzymatic antioxidants, SOD catalyzes the reaction of change superoxide radicals (O_2^-) into hydrogen peroxide (H_20_2), thus playing a protective role in plants. SOD enhances the stability of membrane in plant cells under drought conditions (Jose et al. 1999). Through a non-enzymatic mechanism, SOD can counteract with kind of dangerous hydroxyl radicals and H_2O_2 which could destroy phospholipids (Fridovich 1995). The production amount of malondialdehyde (MDA) increased under stress, and the maximum amount was found in G3, G12 and G17. Such increase could be associated with incidence of environmental stresses in soybean (Althabegoiti et al. 2008). In this regard, the present results were consistent with the findings of other workers. MDA is a product from peroxidation of polyunsaturated fatty acids in phospholipids. The level of lipid peroxidation has been used as a sign of

free harmful radicals to cellular membrane under stress conditions. Therefore, MDA can be adopted as a reagent for evaluation of membrane injuries amount under stress conditions as observed in *Arabidopsis* (Katsuhara et al. 2005). The germplasm evaluation based on physiological traits showed different levels of variation among genotypes for some antioxidant enzyme activities and biomass of wheat genotypes under both normal and stress conditions. The results revealed high genetic diversity among the genotypes under experiment, which can be applied in durum wheat breeding programs.

Authors' contribution

Conceptualization of research (AE,PH); Designing of the experiments (RA, AE, PH); Contribution of experimental materials (AE, PH); Execution of field/ lab experiments and data collection (RA, PH); Analysisof data and interpretation (RA, PH); Preparationof the manuscript (PH, RA, MKH).

Declaration

The authors declare no conflict of interest.

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