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ORIGINAL ARTICLES

Antioxidative Protection and Electrolyte Leakage in Durum Wheat under Drought Stress Condition

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ABSTRACT

Induction of oxidative stress and possible involvement of antioxidants in durum wheat (*T. durum*) leaves after in vivo treatment against drought stress on the 37 genotypes were investigated. The activities of Superoxide Dismutase (SOD), Catalase (CAT), yield and index of damage (ID) of stress were studied after exposure to drought stress phases after 30 days and in control condition. The results analysis showed significant differences between genotypes and stress condition in terms of SOD, and CAT enzyme activity and ID parameters. The mean comparison showed that the activity of SOD and CAT decreased in susceptible landraces, where as in resistant landraces SOD and CAT remained unchanged and in some cases they showed an increase under stress condition. In these genotypes (resistant) increasing SOD and CAT accompanied with ID decrease in the membrane. Cluster analysis on the basis of ID (20% PEG), ID (30% PEG) and on the basis of SOD, CAT and yield in the stress conditions classified resistant and other landraces into different groups. Therefore, these characters can be used as an indirect selection criterion for screening drought-resistant plant materials.

Key words: Durum wheat, SOD, CAT, drought stress.

Introduction

Drought, one of the environmental stresses, is the most significant factor that restricting plant growth and crop productivity in the majority of agricultural fields of the world (Tas and Tas, 2007; Abedi and Pakniyat, 2010). Wheat is essential nourishment for more than 1/3 of the world population and crop yield will be considerably influenced by global climate changing and limitation of water resources in the environment (Simova-Stoilova, *et al*., 2008; AL-Ghamdi, 2009). Drought exacerbates the effect of the other stresses to which plants are submitted (abiotic or biotic) and several different abiotic stresses result in water stress (like salt and cold stresses) (Helena Cruz de Carvalho, 2008).Survival under this stressful condition depends on the plant's ability to perceive the stimulus, generate and transmit the signals and initiate various physiological and chemical changes (Sayar, *et al*., 2008; Tas and Tas, 2007). The antioxidant defenses appear to provide crucial protection against oxidative damages in cellular membranes and organelles in plants grown under un-favorable conditions (AL-Ghamdi, 2009). Active oxygen species were considered to be important damaging factors in plants which exposed to stressful environmental conditions such as drought (Badawi, *et al*., 2004), and pathogen attack (Tertivanidis, *et al*. 2004) as well as to chemical treatment such as parquet (Perl, *et al*., 1993; Chatzidimitriadou, *et al*., 2009). Acclimation of plants to drought is considered to promote antioxidants defense systems to face the increased levels of activated oxygen species (AOS), which in turn, cause membrane damage by lipid peroxidation and indicated by malondialdehyde (MDA) content, which is one of the main parameters for evaluating membrane oxidation extent and are toxic for cells (Shao, *et al*., 2005).

Plants functioning in an aerobic environment are often subjected to continuous threat from molecular oxygen which is due to toxic reactive oxygen species (ROS) like superoxide radical ($O2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), alkoxyl radical ($RO^{\bullet}$) and singlet oxygen ($^{\bullet}O2$) (Baek, and Skinner, 2003)

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Toxic hydrogen peroxide is the product of peroxisomal and chloroplast oxidative reactions and can act both as an oxidant and reductant. It is the most stable form of the ROS and is capable of rapid diffusion across cell membrane (Upadhyaya, *et al*., 2007). The ROS are strong oxidizing agents that cause oxidative damage to bimolecular such as lipids and proteins and eventually lead to cell death (Gunes, *et al*., 2006; Sai Kachout, *et al*., 2009). The active oxygen species which are produced during stress can damage many cellular components including lipids, proteins, carbohydrates and nucleic acids (Kruk, *et al*., 2005; Sai Kachout, *et al*., 2009). In addition, they play an important role in the antioxidative system and protect membranes from oxidative damage during response to environmental stresses (Kuznetsov *et al*., 2006; Shevyakova *et al*., 2006), but the mode of their involvement in these actions is not fully understood (Cakmak and Atici, 2009).

Mechanisms for the generation of ROS in biological systems are represented by both non-enzymatic and enzymatic reactions. There is evidence that the tolerance of plants is correlated with increasing amounts of antioxidants and increasing activity of radical scavenging enzymes. The antioxidant defense system in the plant cell includes both enzymatic (antioxidants), such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) (Sai Kachout, *et al*., 2009; Malik, *et al*., 2010) and -Non-enzymatic antioxidants including β-carotenes, ascorbic acid (AA) (Pignocchi and Foyer, 2003) α-tocopherol (α-toc) (Müller, et. al., 2006), reduced glutathione (GSH) (Xu *et al*., 2008; Abedi and Pakniyat, 2010). As a major scavenger SOD catalyzes the dismutation of superoxide (O-2) to hydrogen peroxide (H2O2) and oxygen (O2) (Chatzidimitriadou, *et al*., 2009). Transformation of many plant genera for useful traits, such as oxidant-resistance, is now routine (Tertivanidis *et al*., 2004). Recently the involvement of H2O2 and SOD in regeneration of plants has also been proposed (Zheng *et al*., 2005). Hydrogen peroxide is commonly taken as an indicator of oxidative stress, because it is induced by AOS and also influencing the level of lipid peroxidation (Mittler, 2002; AL-Ghamdi, 2009). However, H2O2 is also toxic to cells and has to be further detoxified by CAT and/or peroxidase (POD) to water and oxygen, (Zhu, *et al*., 2004; Joanny, 2005; Sai Kachout, et. al., 2009).

Different studies have confirmed that the production of H_2O_2 under the action of SOD is the triggering factor in the natural antioxidant defense mechanisms. SOD therefore seems to be the key enzyme in the natural defense against free radicals (Joanny, 2005). Catalases and superoxide dismutases are the most efficient antioxidant enzymes. The expression of specific catalase isoenzymes is important and critical against oxidative stress induced by a given environmental stress (Bakalova, *et al*., 2004). When plants are subjected to environmental stresses, oxidative damage may result because the balance between the production of ROS and their detoxification by the antioxidative system is altered (Sai Kachout, et. al., 2009). Tolerance of damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify activated oxygen species (Foyer, *et al*., 1994; Sai Kachout, et. al., 2009). Furthermore, the reactions of the plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Dacosta and Huang, 2007; Abedi and Pakniyat, 2010). In plants, changes in antioxidants are also correlated with water deficit (Schwanz, *et al*., 1996). Plants with higher levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage (Boo and Jung, 1999). The response of plants' antioxidative systems has been studied under drought (Aroca, *et al*., 2003), low temperature (Iannelli, *et al*., 1999), and salinity (Acevedo, *et al*., 2006). It has been reported that in different tolerant species an increase in the amount of enzymatic antioxidants under stress conditions protects membranes from damage. Therefore, acclimation to drought is generally correlated with keeping ROS levels relatively low through the antioxidant system (Zaefyzadeh, *et al*., 2009).Usually enhanced anti-oxidative protection is related to better drought resistance (Sairam and Srivastava, 2001; Simova-Stoilova, *et al*., 2008).

Cell membranes are one of the first targets of many plant stresses (Yamazaki, *et al*., 2003; Candan, and Tarhan, 2003) and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells. The method is based on an in vitro stress of leaf tissues by a PEG solution and a subsequent measurement of electrolyte leakage into an aqueous medium (Sullivan and Ross, 1979; Bajji, *et al*., 2001). Common plant symptoms to water deficit are stunted growth, limited CO2 diffusion to chloroplasts by stomatal closure, reduced photosynthesis rate, and accelerated leaf senescence. Moreover, water stress can increase reactive oxygen species synthesis (ROS), increasing proteins, membrane lipids and photosynthetic pigments degradation and cell membrane damages (Beltrano *et al*., 1997; Clua, *et al*., 2009). It has an enduring appeal-because it requires readily available and inexpensive equipment, it is not destructive of whole plants, is easily used on plant material from a variety of cultural systems and it is suitable for analysing large number of samples. Such a technique has also been applied to quantify damages to cell membranes in various abiotic stress conditions such as low temperatures (Vainola and Repo, 2000; Arvin and Donnelly, 2008),high temperatures (Arvin and Donnelly, 2008), air pollution (Garty *et al*. 2000), salt stress (Sreenivasulu *et al*., 2000; Arvin and Donnelly, 2008) acid conditions (Spencer and Ksander, 1999), heavy metals (De and Mukherjee, 1996) and drought stress

(PEG) (Arvin and Donnelly, 2008; Bajji, *et al*., 2001) or even in response to biotic stresses such as wheat leaf rust (Adam *et al*., 2000) or rice sheath blight (Sriram *et al*., 2000). However, despite its many advantages, electrolyte leakage was found to be markedly influenced by various experimental parameters, especially washing time of collected samples before PEG exposure (Blum and Ebercon, 1981; Premachandra and Shi-mada, 1987), intensity and duration of the PEG treatment (Vasquez-Tello *et al*., 1990) and duration of the rehydration period (Bandurska *et al*., 1997).

In this paper, we evaluated the effect of drought stress on superoxide dismutase (SOD), catalas (CAT) activity under drought stress and effect of different PEG6000 concentrations on the leaf electrolyte leakage during rehydration in durum wheat (*Triticum durum* Desf) landraces.

Materials and methods

Experiments were undertaken on 37 durum wheat (*Triticum durum* Desf.) landraces. Seed's samples are representing a single plant which is collected from Northwest Iran and the Azerbaijan Republic (Table. 1) and they were grown under normal (irrigated) and drought (dry cultivation) conditions in the research station of Azad University. Ten leaf samples were collected from each genotype. Seedlings were sampled on days 30, 35, and 39 of water stress.

Enzyme extraction and Determination of Enzymes activity:

For enzyme extracts and assays of leaf's tissues were sampled, frozen under liquid nitrogen, then ground to fine powder, and kept at -20°C. The frozen powder (0.5 g) was homogenized in 10 ml potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA. The homogenate was centrifuged at 4 °C for 15min at 20,000 rpm, and the supernatant was immediately used as an enzyme source (Sairam, *et al*., 1998). The activity of SOD was measured according to the method of Giannopolities and ries (1977). Catalas was assayed by the method of Chance and Maehly (1955). The enzyme activities were expressed in terms of specific activity (Unit/mg Fresh Weight).

The stress tolerance index (STI) suggested by Fernandez (1992) was used to measure drought resistance levels of genotypes via the following formula:

$STI = (Y_{Pi} \times Y_{Si})/Y_p^2$
Y_{si} = the yield of a given genotype in a drought environment;
Y_{Pi} = the yield of a given genotype in a non-stress (irrigated) environment;
Y_p = mean yield in a non-stress environment.

Optimisation of the electrolyte leakage measurement for the estimation of cell membrane stability in durum wheat:

Five leaves per genotype were collected, immediately weighed and cut into segments (cut in 1 cm segments), Segments originating from the same leaf were put into 20 ml of deionised water in a test tube and washed slowly using a rotary shaker (100 rpm) at room temperature to remove solutes from both leaf surfaces and damaged cells due to cutting and then exposed either to 0% (control) or to 20% and 30% PEG 6000 for 15 h in the dark. Electrolyte leakage was then measured before (ECi) and after (ECf) 4 h of rehydration and ultimately after autoclaving (ECt). Cell membrane injuries were expressed as an index of injury calculated as Id= [(Rs-Rc)/(1-Rc)] ×100, where Rs and Rc represent (ECf−ECi)/(ECt−ECi) for control or PEG-treated tissues, respectively (Bajji, *et al*., 2001).

Results and discussion

Analysis of variance showed presence of considerable variability among all genotypes as mean squares of catalas and superoxide dismutase were highly significant ($P < 0.01$) and ID was also significant ($P < 0.05$). That demonstrator genetic diversity between genotypes, this subject consists of height potential value in breeding wheat. Environment mean squares were significant for all the traits studying, showing that the water stress has significant effect on all traits. G x E interaction was significant for SOD showing variation of genotypes over environments (Table. 2), and non significant for CAT and ID. This could provide scope for breeding for traits studied, under drought stress conditions.

Table 3 showed that magnitude of mean performance for SOD and CAT increased in water stress environment. Mean value of SOD and CAT density increase 35.6 and 3.1%, respectively. Water stress is

inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O_2^- and H_2O_2 in chloroplasts, mitochondria, and peroxisomes (Abedi and Pakniyat, 2010). H_2O_2, which resulted from the action of SOD, is toxic to cells. Therefore, it is important that H_2O_2 be scavenged rapidly by the antioxidative defence system to water and oxygen (Guo *et al*. 2006). The ability of plants to overcome oxidative stress partly relies on the induction of SOD activity and subsequently on the upregulation of other downstream antioxidant enzymes (Alscher *et al*. 2002). In our experiment, the results showed significantly enhanced SOD and CAT activity in durum wheat genotypes exposed to water stress (Table 3). Our results are consistent with other studies reporting the increased SOD activity in response to drought stress in sunflower (Gunes *et al*. 2008), poplar (Xiao *et al*. 2008), cowpea (Manivannan *et al*. 2007), liquorice (Pan *et al*. 2006), wheat (Bakalova *et al*. 2004; Csiszar *et al*. 2005), and Oilseed Rape (Abedi and Pakniyat, 2010). According to our results, the maximum increase in the SOD and CAT activity were observed in the 3, 28, 15, 24, 2, 16, 5, 1, 29, 23, 12, 25, 10, 6, 37, 7, 22, 26, 14, 4, 30 and 27 genotypes (table. 4), which might lead to their higher protection against water deficit. Considering this result express with drought stress, in durum wheat genotypes increase catalase and superoxide dismutase activity enzyme. So selection for higher SOD, CAT will be beneficial for select drought resistant genotypes, this subject accordance whit result of experiments: Zaefyzadeh, *et al*., (2009), Ervin, *et al*., (2004), AL-Ghamdi, (2009) and Abedi and Pakniyat, (2010)

Increase the osmotic stress level, due to increase ID (44.87%). Bajji, *et al*., (2001) evaluated effects of different levels osmotic stress with PEG on three cultivar durum wheat, observed significant different between levels of PEG and reported the drought tolerant cultivar, had high stability membrane and conduction electric in drought stress.

Mean comparison of Genotypes in term of catalse activity showed that genotype 5 was maximum activity and genotype 13 was the lowest activity of this trait in non-stress condition. Genotypes 3, 28, 15, 24, 2, 16, 5, 1, 29, 23, 12, 25, 10, 6, 37, 7, 22, 26, 14, 4, 30 and 27 were maximum activity of this trait in stressed condition (Table. 4). The highest SOD activity was determined in genotypes 3, 11, 10, 1, 24, 8, 4, 14, 17 and 31 in non-stressed. Under stressed condition, all genotypes, excluding 17, 22, 36, 31, 19, 32, 18, 20, 16 and 6 had the highest SOD activity (Table. 4). It is known that plants have a well-organized defense system against ROS under stress conditions and SOD constitutes the first line of defense via detoxification of superoxide radicals. Higher increments in the SOD activity in the above-mentioned landraces might have decreased the possible toxic concentration of $O2^{\cdot-}$ radicals more efficiently than in other genotypes. The reduction in SOD activity under drought may have been due to either reduced synthesis or enhanced degradation of the enzyme.

Genotypes 1, 3, 2, 28 and 26 had the highest Yield in non- stressed condition. Genotypes 2, 15, 26, 29 and 28 had the highest Yield in stress condition (Table. 5).

The possibility of using electrolyte leakage measurement to evaluate water stress tolerance in durum wheat has been studied here jointly with seedling Growth of 37 durum wheat genotypes following a period of water shortage. Mean comparison of Genotypes in term of ID showed that genotype 12 highest electrolyte leakage and at other diction had the lowest stability membrane, Genotypes 28 had the lowest electrolyte leakage or other diction had the highest stability membrane in osmotic stress at 20% PEG (Table. 4). Water stress can increase reactive oxygen species synthesis (ROS) that produce Proteins, membrane lipids and photosynthetic pigments degradation with a loss of cell membrane stability (Navari-Izzo *et al*, 1997; Beltrano *et al*., 1997). Tolerance drought strategy could be associated to integrity cell membrane preservation and its rapid reparation (Oliver, 1991).

Stress tolerance index:

STI calculated on the basis of SOD and CAT is given in Table 5. On the basis of STI for SOD, the most resistant genotypes were 11, 4, 3, 24, 14, 1, 28, 10, 5, 2, 12, 23, 26, 21, 15 and 34, whereas these genotypes in STI for CAT also were of resistant genotypes Therefore, SOD STI and CAT STI could be used as criteria for selecting resistant varieties. This subject also accordance whit result of experiments: Zaefyzadeh, *et al*., (2009).

Correlations among characteristics:

The Pearson correlation between physiologic traits has been evaluated for drought condition (Table. 6). SOD and CAT under drought condition showed positive and significant correlation with yield. Activity SOD showed positive and significantly correlation with activity CAT. These results are also in agreement whit findings of other workers (Sairam, *et al*., 2001; Lascano, *et al*., 2001). Shao, *et al*., (2005) reported in tolerant genotypes wheat, more activity antioxidant enzymes than sensitive genotypes. Considering to positive and significant correlation coefficient between SOD activity and CAT activity may express high activity SOD with

brush-off superoxide radicals, plant product high H_2O_2 in stress condition, likely activity CAT provoke for remove H_2O_2.

These results can be used as practical biochemical parameters for selection of drought tolerant durum wheat genotypes when selecting drought tolerant genotypes for breeding in arid regions. In addition, Drought resistance is defined as a higher relative yield of a certain genotype compared with another at the same stress intensity. It appears likely that a better antioxidative protection plays a role in drought resistance at the reproductive stage in the field. Drought tolerance of crop's plants is a genetically determined character but interaction with environment determines the expression of the plant traits. Lascano *et al*. (2001) observed no clear correlation between water-stress tolerance and antioxidant system behavior between drought-tolerant and susceptible wheat cultivars under field conditions.

The ID (20% PEG) showed negative and significant correlation with yield for two conditions. That ID (30% PEG) showed negative and significant correlation with yield in stress condition and showed negative and significant correlation with ID (20% PEG) (Table. 6). These results are also in agreement whit findings of the studying of Sairam, *et al*., (2001), Zarei, *et al*., (2007), Garcia Del Moral, *et al*., (2003) and Franca, *et al*., (2000) were significant relations between stability membrane and resistances at drought.

The STI for SOD, showed positive and significant correlation with CAT (r = 0.364*) and SOD (r = 0.691**). That STI for CAT, showed positive and significant correlation with SOD (r = 0.401*) and CAT (r = 0.896**) (Table. 6). STI selects genotypes with high performance under both stress and normal conditions (Fernandez, 1992). The positive significant correlation of STI with CAT and SOD indices in this research and classification of resistant and susceptible genotypes by this index shows that these indices can be used for screening drought resistant sources in durum wheat varieties. It is required for more detailed researches on existing drought resistant native plants and the use of these genotypes as sources of germplasm in breeding for resistance to environmental stress.

Table 1: Origin and taxonomy of durum wheat landraces tested.

NO.	Landraces	Origin	Name	NO.	Landraces	Origin	Name1
Korifla	Control	Korifla		20	Ardabil-samrein	Iran	Apolicum(1)
2	chakmak	Control	chakmak	21	Ardabil	Iran	Apolicum(2)
3	Zardak	Control	Zardak	22	Germi-moghoan	Iran	Hordeiforme(1)
4	Haurani-1	Control	Haurani-1	23	Germi-langin	Iran	Melasnopus(1)
5	Omrabi-5	Control	Omrabi-5	24	Naxcevan	Azerbaijan	Boeufii(2)
6	Germi-langin	Iran	Niloticum	25	Naxcevan	Azerbaijan	Africanum(3)
7	Ardabil-samrein	Iran	Albobscurum	26	Naxcevan	Azerbaijan	Leucumelan(1)
8	Germi-langin	Iran	No-name	27	lerik	Azerbaijan	Leucumelan(2)
9	Germi-langin	Iran	Riechenbachii(G1)	28	Naxcevan	Azerbaijan	Leucurum(3)
10	Germi-moghoan	Iran	Riechenbachii(G2)	29	xanlar	Azerbaijan	Murciense(2)
11	kordgheshlaghi	Iran	Albiprovinciale(1)	30	Guba	Azerbaijan	Hordeiforme(2)
12	Germi-langin	Iran	Albiprovinciale(2)	31	xatmaz	Azerbaijan	Murciense(3)
13	Germi-langin	Iran	Melaleucum	32	Naxcevan	Azerbaijan	Boeufii(3)
14	Ahar	Iran	Leucurum(1)	33	Gux	Azerbaijan	Leucurum(4)
15	Ardabil-bagh oliya	Iran	Leucurum(2)	34	Ardabil	Iran	Hordeiforme(3)
16	Germi-boldash	Iran	Murciense(1)	35	Ardabil	Iran	Melasnopus(2)
17	Germi-langin	Iran	Boeufii(1)	36	Shamaxi	Azerbaijan	Hordeiforme(4)
18	Germi-langin	Iran	Africanum(1)	37	Naxcevan	Azerbaijan	Leucurum(5)
19	sari boghda	Iran	Africanum(2)				

Table 2: Mean squares of components 37 durum wheat genotypes under normal irrigation and drought stress condition.

		M.S		
S.O.V	df	CAT	SOD	ID
Condition	1	***	***	***
Error 1	4	9.5 × 10-9	0.059	15.131
Genotype	36	**	**	0
C × G	36	Ns	0	Ns
Error 2	144	1.3 × 10-8	0.022	2.478

***, **,* and Ns, significant at P < 0.0001, 1%, 5% level of probability and non-significant, respectively

Table 3: Range, mean, percentage decrease, under water stress (C2) compared with control conditions (C1) in durum wheat genotypes.

Traits	Condition	Rang	Mean ± S.E.M	% difference
CAT	C1	5.18 × 10-4	0.00473 ± 9.3 × 10-6	3.1
	C2	6.16 × 10-4	0.00488 ± 1.32 × 10-5	
SOD	C1	0.9440	0.475± 0.018	35.6
	C2	1.286	0.738± 0.027	
ID	20% PEG	49.84	17.41± 0.93	44.87
	30% PEG	70.31	31.59± 1.54	

Table 4: Mean of SOD, CAT and ID each genotype in normal irrigation and drought stress condition.

NO.	landraces	SOD(Unit/Gfw)[1]		CAT(mM/Gfw/Min)[2]		ID	
		Normal	Stress	Normal	Stress	20%	30%
1	Korifla(control)	0.665 A-C	0.719 A-F	0.00481 A-C	0.00496 A-D	28.78 B-E	41.81 A
2	chakmak(control)	0.531 A-E	0.828 A-F	0.00475 A-D	0.00498 A-D	5.30 GH	19.55 A
3	Zardak(control)	0.732 A	0.833 A-F	0.00479 A-D	0.00501 A	7.42 E-H	27.84 A
4	Haurani-1(control)	0.628 A-D	1.021 A-C	0.00483 AB	0.00488 A-D	18.78 B-H	37.27 A
5	Omrabi-5(control)	0.430 A-F	0.979 A-D	0.00488 A	0.00496 A-D	29.84 B-D	44.31 A
6	Germi-langin	0.434 A-F	0.591 B-F	0.00478 A-D	0.00492 A-D	6.24 F-H	26.79 A
7	samrein	0.376 A-F	0.765 A-F	0.00478 A-D	0.00491 A-D	20.47 B-H	34.13 A
8	Germi-langin	0.630 A-D	0.714 A-F	0.00474 A-D	0.00475 D	12.97 C-H	36.30 A
9	Germi-langin	0.399 A-F	0.771 A-F	0.00471 A-D	0.00479 CD	9.70 D-H	33.95 A
10	moghoan	0.666 A-C	0.690 A-F	0.00471 A-D	0.00492 A-D	11.69 D-H	34.65 A
11	kordgheshlaghi	0.687 AB	1.025 A-C	0.00478 A-D	0.00483 B-D	35.52 B	35.30 A
12	Germi-langin	0.499 A-F	0.749 A-F	0.00471 A-D	0.00494 AB	54.08 A	27.47 A
13	Germi-langin	0.560 A-E	0.707 A-F	0.00461 D	0.00474 D	11.44 D-H	31.19 A
14	Ahar	0.627 A-D	0.800 A-F	0.00474 A-D	0.00489 A-D	27.57 B-F	43.63 A
15	bagh oliya	0.427 A-F	0.856 A-F	0.00474 B-D	0.00505 A-D	4.48 GH	30.27 A
16	Germi-boldash	0.462 A-F	0.564 C-F	0.00475 A-D	0.00497 A-D	19.16 B-H	29.75 A
17	Germi-langin	0.592 A-D	0.386 F	0.00469 A-D	0.0048 B-D	18.23 B-H	27.93 A
18	Germi-langin	0.295 D-F	0.535 C-F	0.00465 A-D	0.00473 D	11.20 D-H	27.56 A
19	sari boghda	0.391 A-F	0.487 D-F	0.00478 A-D	0.00474 D	5.40 GH	26.42 A
20	samrein	0.568 A-E	0.546 C-F	0.00471 A-D	0.00479 CD	19.48 B-H	42.21 A
21	Ardabil	0.456 A-F	0.792 A-F	0.00478 A-D	0.00481B-D	18.46 B-H	34.00 A
22	Germi	0.441 A-F	0.434 EF	0.00477 A-D	0.00491 A-D	14.71 B-H	31.57 A
23	Germi-langin	0.487 A-F	0.801 A-F	0.00470 A-D	0.00494 A-D	33.87 BC	32.18 A
24	Naxcevan	0.640 A-D	0.874 A-F	0.00469 B-D	0.00502 A-C	5.14 GH	24.65 A
25	Naxcevan	0.435 A-F	0.623 A-F	0.00465 B-D	0.00493 A-D	21.44 B-H	25.05 A
26	Naxcevan	0.365 B-F	1.100 AB	0.00471 A-D	0/00490 A-D	11.66 D-H	40.00 A
27	lerik	0.312 C-F	0.970 A-D	0.00464 B-D	0.00487 A-D	23.03 B-H	33.78 A
28	Naxcevan	0.389 A-F	1.130 A	0.00473 A-D	0.00505 AB	3.55 H	23.32 A
29	xanlar	0.212 EF	0.621 A-F	0.00477 A-D	0.00495 A-D	16.46 B-H	27.76 A
30	Guba	0.343 B-F	0.926 A-E	0.00481 A-C	0.00488 A-D	17.16 B-H	30.26 A
31	xatmaz	0.575 A-D	0.483 D-F	0.00465 B-D	0.00483 B-D	16.06 B-H	25.16 A
32	Naxcevan	0.445 A-F	0.534 C-F	0.00463 CD	0.00476 CD	25.97 B-G	29.08 A
33	Gux	0.384 A-F	0.970 A-D	0.00472 A-D	0.00476 CD	9.77 D-H	22.47 A
34	Ardabil	0.518 A-E	0.730 A-F	0.00474 A-D	0.00482 B-D	6.12 F-H	39.02 A
35	Ardabil	0.437 A-F	0.598 A-F	0.00476 A-D	0.00481 B-D	7.56 E-H	33.57 A
36	Shamaxi	0.390 A-F	0.467 D-F	0.00473 A-D	0.00484 B-D	5.87 F-H	27.50 A
37	Naxcevan	0.155 F	0.700 A-F	0.00475 A-D	0.00492 A-D	8.14 D-H	31.22 A

Values with the same superscript letters are non significantly different at $P < 0.05$.
1.Unit/ Gram fresh weight, 2.mili mol/Gram fresh weight/minut

Table 5: STI for CAT and SOD of landraces and yield in normal irrigation and drought stress condition.

NO.	landraces	STI for				YIELD(ton h^{-1})	
		SOD	Rank	CAT	Rank	Normal	Stress
1	Korifla(control)	2.14 A-F	(6)	1.06 A-C	(5)	5.587 A	3.806 D-J
2	chakmak(control)	1.95 A-G	(10)	1.05 A-C	(6)	5.52 AB	4.990 AB
3	Zardak(control)	2.77 A-C	(3)	1.08 A	(1)	5.543 A	3.332 H-J
4	Haurani-1(control)	3.00 AB	(2)	1.05 A-D	(9)	4.283 E-L	3.258 IJ
5	Omrabi-5(control)	1.96 A-G	(9)	1.08 AB	(2)	3.923 I-L	3.313 H-J
6	Germi-langin	1.03 D-G	(29)	1.04 A-D	(13)	3.8 J-L	3.65 D-J
7	samrein	1.22 C-G	(24)	1.04 A-D	(11)	3.8 J-L	3.70 D-J
8	Germi-langin	1.81 A-G	(13)	1.00 C-E	(31)	3.513 L	3.242 IJ
9	Germi-langin	1.45 B-G	(21)	1.00 C-E	(30)	4.35 E-L	4.25 B-G
10	moghoan	1.97 A-G	(8)	1.03 A-E	(19)	4.6 A-K	4.30 B-G
11	kordgheshlaghi	3.27 A	(1)	1.02 A-E	(20)	4.99 A-H	3.460 F-J
12	Germi-langin	1.91 A-G	(11)	1.03 A-E	(17)	3.556 L	3.50 E-J
13	Germi-langin	1.89 A-G	(12)	0.97 E	(37)	4.98 A-H	3.728 D-J
14	Ahar	2.19 A-E	(5)	1.03 A-E	(18)	5.046 A-H	3.692 D-J
15	bagh oliya	1.67 A-G	(18)	1.06 A-C	(3)	5.3 A-E	5.00 AB
16	Germi-boldash	1.10 C-G	(26)	1.05 A-D	(7)	4.4 D-L	3.55 D-J
17	Germi-langin	0.96 D-G	(30)	1.00 C-E	(32)	4.3 E-L	3.00 J
18	Germi-langin	0.65 E-G	(35)	0.98 DE	(35)	4.25 F-L	3.60 D-J
19	sari boghda	0.79 D-G	(33)	1.01 B-E	(27)	5.0 A-H	3.680 D-J
20	samrein	1.27 C-G	(23)	1.00 C-E	(29)	4.112 H-L	3.807 D-J
21	Ardabil	1.72 A-G	(16)	1.02 A-E	(22)	4.30 E-L	3.58 D-J
22	Germi	0.82 D-G	(32)	1.04 A-E	(14)	5.256 A-F	3.435 G-J
23	Germi-langin	1.79 A-G	(14)	1.03 A-E	(16)	3.613 K-L	3.202 IJ
24	Naxcevan	2.47 A-D	(4)	1.04 A-D	(10)	4.85 A-I	4.45 B-D

Table 5: Continue

25	Naxcevan	1.04 D-G	(28)	1.02 A-E	(23)	4.70 A-J	4.10 C-I
26	Naxcevan	1.75 A-G	(15)	1.02 A-E	(21)	5.423 A-C	4.90 A-C
27	lerik	1.33 B-G	(22)	1.00 C-E	(28)	5.40 A-D	4.35 B-F
28	Naxcevan	1.99 A-G	(7)	1.06 A-C	(4)	5.50 A-C	5.39 A
29	xanlar	0.40 G	(37)	1.05 A-D	(8)	4.85 A-I	4.80 A-C
30	Guba	1.47 B-G	(20)	1.04 A-D	(12)	4.50 C-L	4.20 B-H
31	xatmaz	1.13 C-G	(25)	1.00 C-E	(33)	4.18 G-L	3.423 G-J
32	Naxcevan	1.04 D-G	(27)	0.98 DE	(36)	4.931 A-I	4.40 B-E
33	Gux	1.67 A-G	(17)	1.00 C-E	(34)	4.76 A-J	4.433 B-D
34	Ardabil	1.61 A-G	(19)	1.02 A-E	(26)	5.16 A-G	3.047 J
35	Ardabil	0.93 D-G	(31)	1.02 A-E	(25)	4.506 B-L	3.651 D-J
36	Shamaxi	0.77 D-G	(34)	1.02 A-E	(24)	4.367 E-L	4.10 C-I
37	Naxcevan	0.47 F-G	(36)	1.04 A-E	(15)	4.20 G-L	3.80 D-J

Values with the same superscript letters are non significantly different at $P < 0.05$.

Table 6: Correlation between antioxidant enzymes activity, ID, STI for CAT and SOD with yield in two condition.

	Condition	CAT	ID 20% PEG	ID 30% PEG	Yn	Ys	STI for SOD	STI for CAT
Sod	Stress	0.379*	0.087^{NS}	0.194 ^{NS}	0.264 ^{NS}	0.367*	0.691**	0.401*
CAT	Stress		0.0007 ^{NS}	-0.128 ^{NS}	0.284 ^{NS}	0.362*	0.364*	0.896**
ID	20% PEG			0.334*	-0.33*	-0.352*	0.244 ^{NS}	0.014 ^{NS}
	30% PEG				-0.083^{NS}	-0.348*	0.263 ^{NS}	0.065 ^{NS}
STI for	SOD							0.351*

**,* and Ns, significant at 1%, 5% level of probability and non-significant, respectively

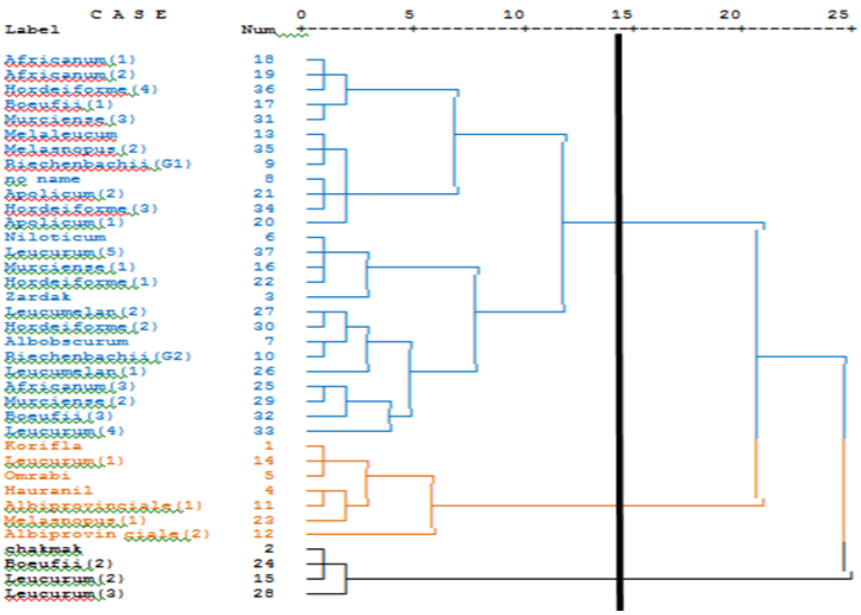

Fig. 1: Dendogram of cluster analysis of durum wheat genotypes classified according to SOD, CAT, ID and Yield in drought condition.

Grouping of genotypes:

Cluster analysis on the basis of ID (20% PEG), ID (30% PEG) and on the basis of SOD, CAT and yield in the stress conditions classified genotypes into 3 main clusters (Figure. 1). As seen in the dendrogram, genotypes 2, 24, 15 and 28 were located in the third cluster, that these genotypes in mean comparison also in activity antioxidant enzyme, yield and STI for SOD and CAT were the best genotypes; also genotype 28 had the height stability membrane. So may this group recommend as superior groups. The cluster analysis supported mean comparison, too. Therefore, we can use these traits for classification of genotypes for drought resistance. The existence of a significant interaction between genotype and environmental conditions (drought and irrigated conditions) in terms of SOD showed that the response of genotypes in terms of amount of SOD activity in these two conditions were different.

Results of this experiment showed that in order to screening of drought tolerance genotypes we can used physiological character and antioxidant activity such as SOD and in the primary stage of the stress condition.

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