



Physio-biochemical Responses of Wheat Genotypes under Drought Stress

Ali Aliakbari-Sadeghabad¹, Ali Dadkhodaie^{1*} and Hojjat Hasheminasab²

¹Department of Crop Production and Plant Breeding, Shiraz University, Fars Province 71441-65186, Iran.

²Department of Crop Production and Plant Breeding, Razi University, Kermanshah, Kermanshah Province, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AAS and AD designed the study, wrote the protocols, performed the experiment and wrote the first draft of the manuscript. Author HH analyzed the data. All authors read and approved the final manuscript.

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ABSTRACT

Tolerance strategies under water deficit conditions involve many physiological and biochemical responses which help crops to cope with drought stress. To study wheat response to stress, twenty Iranian wheat genotypes were evaluated in two separate field experiments during 2009-2010 growing season. Each experiment was conducted as randomized complete block design with three replications. The moisture level in one experiment was 100% field capacity while 45% field capacity was applied at the second experiment. Several biochemical (catalase, peroxidase, superoxide dismutase, ascorbate peroxidase and lipid peroxidation) and physiological indices (relative water content, relative water protection, stomatal resistance, chlorophyll and canopy temperature depression) were measured. Drought stress increased activities of lipid peroxidation, relative water protection, relative water content, stomatal resistance, chlorophyll and canopy temperature depression significantly at the pre-anthesis stage (Z34). Higher antioxidant activities, relative water content, relative water protection, chlorophyll, stomatal resistance and canopy temperature depression, and lower lipid peroxidation were observed in tolerant genotypes. The results

*Corresponding author: E-mail: dadkhodaie@shirazu.ac.ir;

demonstrated positive correlation of antioxidants with relative water content, relative water protection, chlorophyll, stomatal resistance and canopy temperature depression, and negative correlation with lipid peroxidation. Relative water protection and superoxide dismutase were suitable indices for screening and classifying tolerant genotypes. The results suggested that a combination of traits such as higher antioxidant activity, stomatal resistance and water retention capacity lead to wheat drought tolerance and the cultivars Kavir and Bahar were the most and least tolerant genotypes, respectively.

Keywords: Antioxidants; *Triticum aestivum*; *Triticum durum*; physiological traits; water deficit.

1. INTRODUCTION

Global climate change has led to severe drought and consequently dwindling water resources for crop production systems. Such conditions lead to detrimental effects in plants which can be mitigated by introducing tolerant crops. In response to drought, changes occur in crops, which contribute to their adaptation to unfavorable conditions [1-2].

When subjected to abiotic stresses, plants produce reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($\cdot O_2^-$), singlet oxygen (1O_2) and hydroxyl ($\cdot OH$) radicals during photosynthetic and respiration processes [3-4]. These ROS can cause damage to cell components and consequently cause plant death [5]. However, plants have developed efficient enzymatic and non-enzymatic defense systems such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and catalase (CAT) to protect their cells and subcellular systems by scavenging of ROS [6-7].

SOD is an enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Both POD and APX play critical roles in scavenging H_2O_2 which is produced through dismutation of O_2^- catalyzed by SOD. As the main enzyme in eliminating H_2O_2 in mitochondria and microbodies, CAT helps in ameliorating the detrimental effects of oxidative stress [8-9]. The relationships between drought stress and these antioxidant systems have been the subject of many studies in different plant species [10]. For example, Dong et al. [11] reported that plant tolerance to drought stress is correlated with its antioxidant activity which reduces drought effects by balancing production and elimination of ROS at the intracellular level.

Researchers such as Karimizadeh and Mohammadi [12] and Hasheminasab et al. [13] have also reported changes in relative water content (RWC), relative water protection (RWP), stomatal resistance (Sr), and canopy

temperature depression (CTD) in drought tolerant crops. These physiological traits indicate plants' water status in relation to water stress and reflect the metabolic activity and osmotic regulation in their leaf tissues. Chlorophyll content (Chl) is the major chloroplast component and its relative content has a positive relationship with photosynthetic rate [9]. Decrease in RWC and Chl content, and increase in RWP, Sr and CTD in response to drought stress have been reported [14]. Recently, Anjum et al. [9] associated osmotic regulation with cell membrane stability under drought stress.

Wheat (*Triticum aestivum* L.) as an important crop, is adapted to different climatic conditions. However, this crop often experiences drought stress during growth in arid and semi-arid regions of Iran. Thus, improving cultivars suitable for these areas is a major challenge in breeding programs. Therefore, the present study was conducted to: (i) investigate the effects of drought stress on antioxidant defense systems, oxidative damage and physiological traits of tolerant, intermediate and sensitive wheat genotypes (ii) identify efficient traits for screening tolerant genotypes (iii) determine the relationships among physio-biochemical traits and yield stability index (YSI) using different statistical approaches.

2. MATERIALS AND METHODS

Eighteen Iranian bread wheat genotypes including six drought tolerant ones (Azar 2, Pishtaz, Toos, Chamran, Kavir and Koohdasht; group 1), six intermediate (Roshan, Alvand, Tabasi, Niknejad, Cross Adl and Darab 2; group 2) and six sensitive (Shiraz, Shiroudi, Flat, Bahar, Zarin and Almut; group 3) and 2 durum wheat genotypes (Seimare and Yavarus) were used in two separate field experiments in 2009-2010 growing season at the Experimental Station, School of Agriculture, Shiraz University, Iran ($52^{\circ}46' E$, $29^{\circ}50' N$, altitude 1,810 m above sea level). Soil of the experimental site was sandy-clay with $EC = 0.563 ds \cdot m^{-1}$ and $pH = 7.6$.

The experiment was conducted as a randomized complete block design in three replications. Each plot consisted of six 4-m long rows spaced 30 cm apart. The moisture level in one of the experiments was optimum (100% FC) while drought stress (45% FC) was applied in the second experiment at pre-anthesis stage (Z34). The required irrigation water for each treatment was determined using the following equation [15]:

$$I \text{ (cm)} = (FC - pb) / 100 \times d \text{ (g*cm}^{-3}\text{)} \times D \text{ (cm)}$$

Where I indicates irrigation depth, FC shows soil water content at field capacity (% weight), pb is soil water content at the irrigation day (% weight), d and D represent bulk density and soil depth, respectively.

2.1 Biochemical Assay

Flag leaves collected at flowering stage to evaluate biochemical characteristics. The leaf samples (0.5 g) were homogenized with 2 ml of 50 mM phosphate buffer using a pre-chilled mortar and pestle (1 mM EDTA, 1 mM PMSF and 1% PVP-40). Subsequently, the homogenates were centrifuged in 4°C at 15,000xg for 15 minutes and the supernatant was collected for enzyme assays.

SOD (EC 1.15.1.1) activity was measured using its ability to inhibit the photo reduction of nitroblue tetrazolium (NBT) by Chance and Maehly [16]. The reaction mixture contained 50 mM phosphate buffer (pH 7.8), 0.66 mM EDTA, 10 mM L-methionine, 33 µM NBT, 3.3 mM riboflavin and 50 µL of diluted enzyme. The method of Nakano and Asada [17] was used to determine APX (EC 1.11.1.11) activity. The reaction mixture contained 36.6 mM phosphate buffer (pH 6.0), 36.6 µM EDTA, 1.0 mM H₂O₂, 0.5 mM ascorbate and 300 µL enzyme extract. CAT (EC 1.11.1.6) activity was assayed by following the reduction of H₂O₂ ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) at 240 nm [4-6]. The reaction mixture consisted of 86 mM phosphate buffer (pH 7.0), 86 µM EDTA, 20 mM H₂O₂ and 150 µL enzyme extract. POD (EC 1.11.1.7) activity was determined according to the method of Chance and Maehly [16]. The reaction mixture contained 47.5 mM phosphate buffer (pH 7.0), 47.5 µM EDTA, 5.0 mM guaiacol, 15.0 mM H₂O₂ and 50 µL enzyme extract.

Lipid peroxidation (LPO) rate was determined by measuring malondialdehyde (MDA, $\epsilon = 155 \text{ mmol}^{-1} \text{ cm}^{-1}$) in plant tissues according to the

method of Heath and Packer [18] and was expressed as µmol of MDA formed using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.2 Physiological Assay

Fresh weight (FW) of ten young leaves from each plant was measured. The leaves were then floated on distilled water in Petri dishes for four hours and the turgid weight was recorded (TW). After that, the leaf tissues were dried in an oven at 80°C for 24 hours and their dry weight was measured (DW). RWC was calculated according to the following formula $RWC = (FW - DW)/(TW - DW)$ [19].

Relative water protection (RWP) was determined using the following equation [14] where withering weight (W_w) was measured when leaves wilted at 25°C for 8 h: $RWP = (W_w - D_w)/(F_w - D_w)$.

Five days after applying stress, canopy temperature (CT) was measured using a hand-held infrared thermometer (Kane May Model In fratrace 800, USA).

Canopy temperature depression (CTD) was calculated using the following formula [11]: $CTD = AT - CT$ where AT represents ambient temperature.

Stomatal resistance (Sr) was measured with a portable photosynthesis system (IQRA model LCA4-ADC, Hoddesdon, UK) on three random plants per plot.

Chl was determined as $18.42 A_{646.8} - 2.05 A_{663.2}$ where $A_{646.8}$ and $A_{663.2}$ show the absorbance of supernatant at 646.8 nm and 663.2 nm for Chl, respectively [20].

2.3 Yield Stability Index

Yield under stress (Ys) and non-stress (Yp) conditions was determined and yield stability index (YSI) was measured as the ratio of Ys to Yp [21].

2.4 Statistical Analyses

Analysis of variance (ANOVA), simple correlation coefficients, cluster analysis, principal component analysis (PCA) and biplot analysis were performed by SPSS 16 (SPSS 2007). Mean comparisons were made using least significance difference (LSD) test at $P < 0.05$. To compare the effects of stress and non-stress conditions, and genotype by moisture condition interactions, combined analysis of variance was used.

3. RESULTS AND DISCUSSION

3.1 Biochemical Responses

The results showed significant variations among genotypes for antioxidant enzyme activity under drought stress conditions (Table 1). As it can be seen, SOD activity increased significantly under water stress condition. The highest SODs observed in group 1 (Azar 2, Pishtaz, Roshan, Tabasi, Cross Adl, Darab 2, Niknejad and Yavarus) and the lowest values were measured in group 3 (Bahar, Flat, Shiraz, Zarin, Alvand, Alamut and Shiroudi) while group 2 (Chamran, Kavir, Koohdasht, Toos and Seimare) showed intermediate SOD activities under stress condition. Of these, the genotypes Seimare (from group 2) and Alamut (from group 3) had the highest ($578 \text{ U}\cdot\text{g}^{-1} \text{ FW}$) and lowest ($338 \text{ U}\cdot\text{g}^{-1} \text{ FW}$) SOD activity under stress. These present an increase of 79 and 20% in SOD respectively, compared to normal conditions. SOD detoxifies $\text{O}_2^{\cdot-}$ free radicals by forming H_2O_2 [4-8]. Thus, higher SOD activity in these genotypes can explain the efficient system of $\text{O}_2^{\cdot-}$ scavenging under drought conditions which help them to tolerate drought. Similarly, Hasheminasab et al. [13] reported significant differences in SOD activities of wheat genotypes under drought and found close associations between SOD with drought tolerance. Some researchers have also demonstrated that SOD up regulation has a critical role in the survival of plants [4-21].

The effect of drought stress on CAT, APX and POD activities was also significant and these enzymes had higher activities under drought stress than normal conditions. On average, drought stress increased CAT, APX and POD activities by 2.0, 27.3 and 24.6%, respectively. The differences in antioxidant activity of genotypes were significant ($P < 0.01$) under both stress and non-stress conditions (Table 1). Yavarus had the highest CAT activity under both normal ($39 \text{ U}\cdot\text{g}^{-1} \text{ FW}$) and stress conditions ($46 \text{ U}\cdot\text{g}^{-1} \text{ FW}$). Luna et al. suggested that CAT is a less susceptible scavenging enzyme than other antioxidant regarding oxidative stress [21].

All these three enzymes (CAT, APX and POD) play key roles in scavenging H_2O_2 , which is produced through dismutation of $\text{O}_2^{\cdot-}$ catalyzed by SOD [20]. Our results are consistent with the findings in mulberry [22], rose [10] and wheat [20]. High concentrations of H_2O_2 also result in lipid peroxidation (LPO) and membrane injury [23]. Therefore, LPO has been widely used to

measure stress-induced damage using MDA content. Under water stress conditions, LPO increased significantly in all genotypes ($P < 0.05$), except the tolerant ones; Chamran, Koohdasht, Seimare and Pishtaz (Table 1). Under this condition, the highest and the lowest LPOs were observed in Alamut ($10.04 \mu\text{mol}\cdot\text{g}^{-1} \text{ FW}$) and Koohdasht ($6.42 \mu\text{mol}\cdot\text{g}^{-1} \text{ FW}$), respectively. As can be seen in Table 1, the lowest LPOs were observed in tolerant genotypes (group 1) while the highest values were detected in susceptible ones (group 3) under stress condition. Increased levels of LPO have also been reported by various studies [13]. Similar to wheat [20], higher antioxidant activity, and lower LPO and H_2O_2 have also been reported in drought tolerant genotypes of *Mentha pulegium* [7], and Mulberry [22].

3.2 Physiological Responses

Relative water content decreased significantly under drought stress ($P < 0.01$), but the decrease was not significant in tolerant genotypes ($P < 0.05$; Table 2). The highest and lowest RWC were observed in group 1 and group 3 respectively, under stress condition while intermediate values were observed in group 2. The highest and lowest RWCs were observed in Pishtaz (0.71%) and, Flat and Shiroudi (0.59%), respectively under stress condition. These are equivalent to 10 and 24% decrease from non-stress to stress. Yadav and Bhushan reported a close relation between RWC and crop yield [24].

Values of canopy temperature depression (CTD) ranged between 3.90 for Darab 2 to 8.64 for Yavarus with an average of 5.78 over all genotypes in stressed conditions. Under irrigated conditions, CTD varied from 0.88 for Bahar to 2.92 for Roshan with an average of 1.99 over all genotypes tested (Table 2). Drought stressed plants displayed higher canopy temperatures than well-watered plants [25]. High CTD has been used as a selection criterion to improve tolerance to drought and heat [26]. Hasheminasab et al. [13] reported that higher CTD and Sr during grain filling period in wheat were associated with higher tolerance of water stress. Likewise, Karimizadeh and Mohammadi [12] demonstrated that CTD provides an efficient method to monitor plant response to water stress. Similarly, De Micco and Aronne, reported that durum wheat under conditions of drought condition enables the closure of stomata and limits the intensity of transpiration by plants [27].

Table 1. Mean of enzymatic antioxidants in twenty wheat genotypes under non-stress and stress conditions

Group	Genotypes	SOD (U*g ⁻¹ FW)		CAT (U*g ⁻¹ FW)		APX (U*g ⁻¹ FW)		POD (U*g ⁻¹ FW)		LPO (μmol*g ⁻¹ FW)		YSI (%)
		Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	
3	Bahar	317	373	35	40	113	135	49	58	5.25	9.73	0.49
3	Flat	339	382	33	37	102	147	47	56.7	6.94	8.87	0.72
3	Zarin	337	406	35	40	124	135	51	61	5.33	9.84	0.57
3	Alamut	282	338	32	36	108	136	67	73	6.09	10.04	0.49
3	Shiraz	370	401	38	43	127	141	51	56	5.35	9.00	0.49
3	Shiroudi	336	393	37	41	118	133	59	61	5.08	9.11	0.74
2	Koohdasht	330	539	37	45	116	157	49	73	5.02	6.42	0.78
2	Kavir	299	522	36	46	114	165	36	71	4.96	7.58	0.81
2	Toos	298	496	32	44	119	145	68	81	5.07	7.42	0.84
2	Chamran	329	525	34	44	127	172	48	86	5.34	7.72	0.76
1	Azar 2	328	443	32	45	104	166	46	82	4.48	8.11	0.86
1	Pishtaz	378	530	36	40	130	147	61	82	5.34	6.87	0.74
2	Seimare	323	578	34	43	128	152	41	70.95	6.62	7.97	0.88
1	Roshan	292	462	32	42	116	152	51	71	4.96	9.25	0.79
3	Alvand	277	398	35	37	119	131	69	77	5.29	8.24	0.72
1	Tabasi	282	480	33	41	114	160	46	71	3.95	8.40	0.73
1	Cross Adl	333	492	31	43	110	153	34	59	4.11	7.63	0.63
1	Darab 2	368	471	37.2	45	116	146	53	69	6.14	7.28	0.65
1	Niknejad	367	472	35	42	123	147	64	73	4.27	8.05	0.72
1	Yavarus	282	461	39	46	119	160	51	69	5.07	8.25	0.82
2	Seimare	323	578	34	43	128	152	41	70.95	6.62	7.97	0.88
	LSD (0.05)	22.45	13.67	3.98	4.23	14.06	7.66	12.11	3.82	1.73	2.61	16.70

FW-fresh weight; SOD-superoxide dismutase; CAT-catalase; APX-ascorbate peroxidase; POD-peroxidase; LPO-lipid peroxidation; YSI-yield stability index;
LSD-least significance difference test (probability level of 5%)

Table 2. Mean of physiological traits in twenty wheat genotypes under non-stress and stress conditions

Group	Cultivars	RWC (%)		RWP (%)		CTD (°C)		Sr (mol*m ⁻² s ⁻¹)		Chl (mg*g ⁻¹ FW)		GY (Kg*ha ⁻¹)	
		Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress	Non-stress	Stress
3	Bahar	0.81	0.64	0.47	0.59	0.88	4.09	8.3	18.9	25.5	15.2	7896	3856
2	Chamran	0.75	0.69	0.63	0.69	2.12	6.22	12.1	20.6	16.4	14.6	5573	4190
1	Cross Adl	0.83	0.62	0.57	0.67	2.51	5.78	11.4	27.8	23.9	16.7	8130	5016
3	Shiraz	0.80	0.63	0.54	0.65	1.29	5.29	5.3	18.6	17.9	17.6	784	3707
2	Kavir	0.78	0.69	0.65	0.72	1.56	6.13	6.59	26.0	19.5	16.8	6692	5419
3	Shiroudi	0.78	0.59	0.54	0.60	2.19	6.65	11.1	26.1	24.3	16.8	5981	4291
2	Koohdasht	0.80	0.70	0.59	0.73	2.54	6.23	12.6	33.3	21.4	16.3	4440	3297
1	Darab 2	0.79	0.66	0.57	0.66	1.97	3.90	10.1	26.0	18.0	15.8	6544	4229
2	Seimare	0.85	0.68	0.60	0.69	2.70	6.89	7.8	28.2	20.5	18.0	6573	5756
3	Flat	0.78	0.59	0.60	0.60	1.84	5.23	6.3	22.3	19.4	12.4	7178	5092
1	Niknejad	0.83	0.65	0.63	0.68	2.33	4.38	9.8	25.4	24.1	13.3	5788	3952
1	Yavarus	0.81	0.69	0.67	0.71	2.41	8.64	12.7	33.7	17.3	14.9	5444	4460
1	Roshan	0.77	0.65	0.62	0.68	2.92	6.21	12.6	32.6	18.2	15.4	7354	5774
1	Azar 2	0.76	0.64	0.60	0.69	2.32	6.14	13.7	48.3	16.3	14.1	5436	4666
1	Tabasi	0.74	0.66	0.60	0.65	2.72	4.54	13.0	25.3	18.8	12.2	5805	4151
3	Zarin	0.81	0.60	0.58	0.59	2.21	6.10	8.2	21.0	22.8	13.6	781	4361
3	Alamut	0.84	0.60	0.60	0.60	0.81	5.54	11.3	23.4	19.2	14.3	7985	3860
2	Toos	0.80	0.65	0.59	0.69	1.60	6.42	7.9	35.0	22.9	17.1	6704	5392
1	Pishtaz	0.79	0.71	0.61	0.71	1.25	5.73	10.9	44.4	18.3	15.6	5939	4322
3	Alvand	0.75	0.61	0.57	0.65	1.66	5.62	10.9	30.7	19.9	16.8	7788	5396
	LSD(0.05)	0.075	0.074	0.078	0.083	1.27	1.43	3.55	9.63	5.24	4.72	2559	1168

FW-fresh weight; *RWC*-relative water content; *RWP*-relative water protection; *CTD*-canopy temperature depression; *Sr*-stomatal resistance; *Chl*-chlorophyll content and *GY*-grain yield; *LSD*-least significance difference test (probability level of 5%)

The genotypes in group 1 had high RWP under drought stress while the cultivars in groups 2 and 3 showed intermediate and low values, respectively. In previous studies, it has been shown that leaves of drought tolerant cultivars retain higher leaf water content under drought stress [21]. Tetraploid wheat genotypes (Seimare and Yavarus) had higher CTDs (6.89, 8.64), Sr (28.2, 33.7) and YSI (0.88, 0.88) compared to bread wheat under stress although their corresponding figures were not the highest under normal conditions.

Chlorophyll decreased significantly under water stress conditions ($P < 0.01$, Table 2). Based on the results, it was revealed that tolerant genotypes, had the highest chlorophyll content so that, genotypes in group 1 which are classified as tolerant, significantly had the highest chlorophyll content under drought stress. The cultivar Flat which is classified as sensitive had lowest chlorophyll content. The cultivars Kavir and Tabasi had 19.5 and 18.8 mg Chl under non-stress while this figure was 16.8 and 12.2 under stress, respectively. This indicates a reduction of approximately 14 and 35%, respectively. Chlorophyll maintenance is essential for photosynthesis and its decline under stress conditions is a typical symptom of oxidative stress. A reason for such a decrease as affected by water deficit is that drought or heat stress by producing reactive oxygen species (ROS) such as O_2^- and H_2O_2 , can lead to lipid peroxidation and consequently, chlorophyll destruction [3]. Some researchers have also reported damage to leaf pigments as a result of water deficit [9-10].

3.3 Simple Correlation Analysis

Correlations of all variables were obtained under drought stress condition (Table 3). YSI had positive correlations with all enzymatic antioxidants and physiological traits. Thus, indicating their effectiveness in screening drought tolerant genotypes. However, its correlation with Chl was not significant (0.16) and had a negatively significant correlation with MDA (-0.57). This correlation denotes that yield stability index increases when there is no damage to the crop.

Some reports have suggested that drought tolerance is often correlated with a more efficient antioxidative system. Jagtap and Bhargava [28] reported that SOD activity increased in drought-tolerant cultivars. Lichtenthaler and Wellburn [20] stated that adaptation to drought stress is related to plants' ability to detoxify superoxide radical by antioxidant enzymes. Particularly, SOD and CAT play a key role in protecting plants from oxidative stress by increasing their activities. Hasheminasab et al. [13] reported that SOD activity coincides with an increase in APX, POD and CAT activities. These enzymes convert $O_2^{\bullet-}$ and H_2O_2 to molecular oxygen (O_2) and water, thus prevent cellular damage.

Negative and significant correlations were observed among oxidative stress factors and all antioxidant enzymes (Table 3). SOD (-0.80**), CAT (-0.57**), APX (-0.52*) and POD (-0.50*) significantly correlated with MDA. These results clearly indicate the efficient role of antioxidant

Table 3. Pearson correlation coefficients between traits in wheat genotypes under drought stress condition

	RWC	RWP	CTD	Sr	Chl	SOD	CAT	APX	POD	MDA
RWP	0.83**									
CTD	0.19 ^{ns}	0.38 ^{ns}								
Sr	0.33 ^{ns}	0.56**	0.36 ^{ns}							
Chl	0.26 ^{ns}	0.39 ^{ns}	0.29 ^{ns}	0.05 ^{ns}						
SOD	0.81**	0.85**	0.26 ^{ns}	0.36 ^{ns}	0.34 ^{ns}					
CAT	0.64**	0.71**	0.32 ^{ns}	0.26 ^{ns}	0.35 ^{ns}	0.65**				
APX	0.68**	0.73**	0.29 ^{ns}	0.31 ^{ns}	-0.02 ^{ns}	0.66**	0.68**			
POD	0.52*	0.59**	0.18 ^{ns}	0.59**	0.00 ^{ns}	0.47*	0.22 ^{ns}	0.44*		
MDA	-0.70**	-0.82**	-0.10 ^{ns}	-0.50*	-0.32 ^{ns}	-0.80**	-0.57**	-0.52*	-0.50*	
YSI	0.48*	0.69**	0.53*	0.61**	0.16 ^{ns}	0.66**	0.48*	0.60**	0.55*	-0.57**

RWC-relative water content; RWP-relative water protection; CTD-canopy temperature depression; Sr-stomatal resistance; Chl-chlorophyll content; SOD-superoxide dismutase; CAT-catalase; APX-ascorbate peroxidase; POD-peroxidase; LPO-lipid peroxidation and MDA-malondialdehyd. * and ** indicate significance at the 0.05 and 0.01 probability levels, respectively. ns = Non-significant

defense machinery in protecting cells against oxidative damage. MDA showed a significant correlation with RWP (-0.82**), RWC (-0.70**) followed by Sr (-0.50*). Hasheminasab et al. [13] obtained association between drought tolerance and the ability of leaves to retain water. Dong et al. [11] reported that higher RWC reduces stomatal conductance and transpiration rate. Several authors have also provided evidence on the relationship between osmotic adjustment (OA) and cell membrane stability under water-limited conditions [9-14].

3.4 Cluster Analysis

Cluster analysis based on physio-biochemical traits under drought stress condition categorized the genotypes into three groups consisting of 8, 5 and 7 genotypes (Fig. 1). The genotypes in the first group except Yavarus and Azar 2 showed an intermediate YSI. Genotypes in the second group had the highest YSI (Table 2) and were identified to be superior in tolerating drought while all genotypes in the third group except Alvand had low YSI indicating their sensitivity to drought. The highest distance was observed between Darab 2 and Alamut, while the lowest distance was obtained between the cultivars Chamran and Kavir. This is because Alamut and Darab were drought tolerant and sensitive, respectively but both Chamran and Kavir are drought tolerant. The results of this analysis explicitly suggest that physio-biochemical traits could be useful in classifying genotypes for drought tolerance. Our findings are consistent with results reported by other researchers [13-29] who reported physiological and biochemical characters could classify genotypes into to drought-tolerant and drought-sensitive.

3.5 Principal Component Analysis (PCA)

The results of principal component analysis indicated that the first three components with eigenvalue greater than one explained 81.19% of variations (Table 4). The first PC (PC1) accounted for 54.83% of variation and had a high positive correlation with LPO and negative correlations with YSI, RWC, RWP, Sr, SOD, CAT, APX and POD under stress condition. Therefore, it was named as stress susceptible component with low yield in a stressful environment. The second component (PC2) explained 14.71% of the total variation and was positively correlated with YSI, CTD and Sr. Based on these results, genotypes with low PC1 and high PC2 are suitable for stress conditions.

The third PC (Chl) accounted for 11.64% of the total variation in the dependent structure and therefore was named as Chl factor. PC3 can screen genotypes with high Chl under stress. The results of PCA rank variables that affect YSI based on their importance. Therefore, RWP, SOD, APX, RWC, CAT, LPO, POD, CTD, Sr and Chl have been ranked from the most to the least important. Similar results were obtained by Naroui Rad et al. [30,31] when PCA classified eight physio-biochemical variables into 3 main groups accounting for 77 % of the total variation. This implies that the potential candidate breeding materials could be selected from genotypes in first and second principal components [11].

Table 4. Principal component analysis of measured traits under drought stress condition

Trait	Dimension		
	1	2	3
RWC	-0.825	-0.411	-0.118
RWP	-0.974	0.02	-0.049
CTD	-0.302	0.83	0.273
Sr	-0.562	0.59	-0.208
Chl	-0.176	0.059	0.918
SOD	-0.919	-0.16	0.01
CAT	-0.811	-0.331	0.231
APX	-0.837	-0.25	-0.02
POD	-0.692	0.223	-0.458
LPO	0.785	0.141	-0.203
YSI	-0.816	0.377	-0.004
Eigen value	6.032	1.619	1.281
Proportion percentage of variance	54.832	14.715	11.644
Cumulative percentage of variance	54.832	69.547	81.191

RWC-relative water content; RWP-relative water protection; CTD-canopy temperature depression; Sr-stomatal resistance; Chl-chlorophyll content; SOD-superoxide dismutase; CAT-catalase; APX-ascorbate peroxidase; POD-peroxidase; LPO-lipid peroxidation and YSI-yield stability index

Biplot identifies superior genotypes for both stress and non-stress conditions better than simple correlation analysis, because genotypes are compared simultaneously for all attributes [32]. Based on the relationships between traits, the genotypes Bahar (1), Shiraz (4), Shiroudi (6), Flat (10), Zarin (16), Alamut (17) and Alvand (20) were placed in a region of the plot which had the lowest RWC, RWP, CAT, APX and SOD and therefore were classified as sensitive. According

to these results, the cultivar Bahar (1) was identified as the most sensitive genotype. The genotypes Chamran (2), Koohdasht (7), Seimare (9), Yavarus (12), Roshan (14), Pishtaz (18) and

Toos (19) were placed in a region of the plot which had the highest RWC, RWP, CAT, APX and SOD, and are suitable for drought conditions.

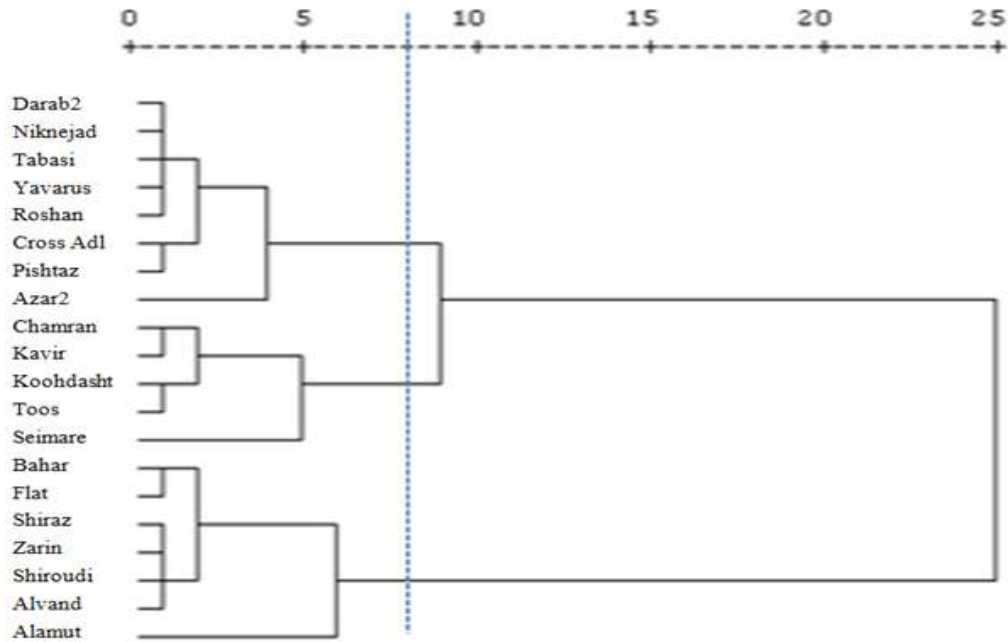


Fig. 1. Dendrogram of cluster analysis of twenty wheat genotypes based on physio-biochemical traits

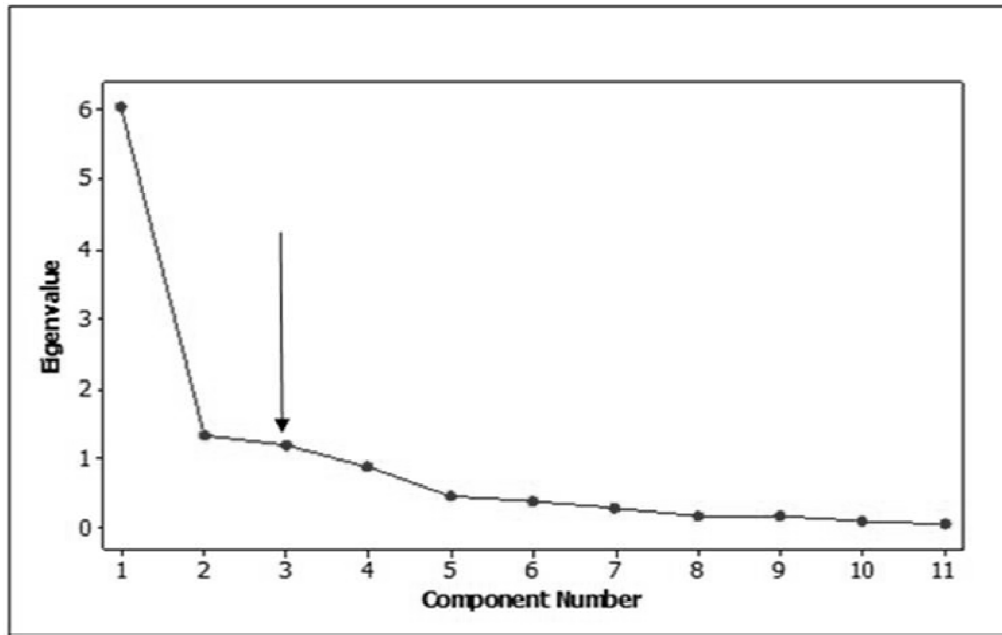


Fig. 2. Eigen values in response to number of components for the estimated variables

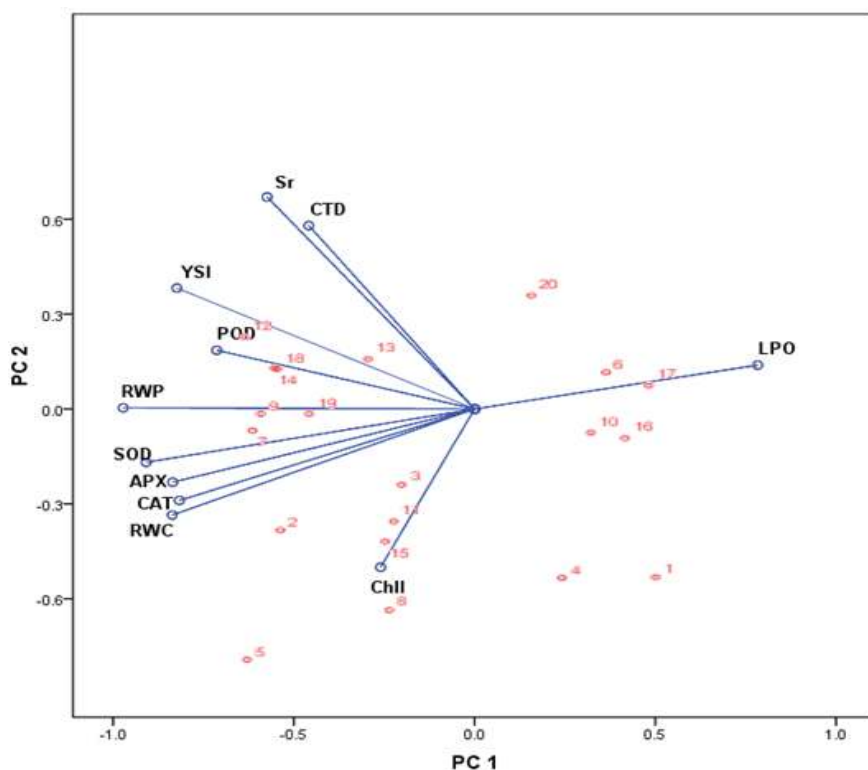


Fig. 3. Biplot analysis of physio-biochemical traits and 20 wheat genotypes. RWC-relative water content; RWP-relative water protection; CTD-canopy temperature depression; Sr-stomatal resistance; Chl-chlorophyll content; SOD-superoxide dismutase; CAT-catalase; APX-ascorbate peroxidase; POD-peroxidase; LPO-lipid peroxidation and YSI-yield stability index

4. CONCLUSION

Significant differences were detected among the genotypes for all physio-biochemical traits under drought stress condition. Drought stress increased enzymatic antioxidant activities; LPO, RWP, Sr and CTD, while decreased RWC and Chl in all genotypes. Drought tolerant genotypes showed antioxidant activities, RWC, RWP, Chl, Sr as well as CTD, and lower LPO under drought stress condition. Genotypes with intermediate drought tolerance showed a moderate response in terms of these traits. Also, durum wheat genotypes had similar behavior to those of the first group under drought stress. The results obtained from different statistical methods demonstrated that enzymatic antioxidants had positive correlations with RWC, RWP, Chl, Sr and CTD, and negative with LPO. Thus, it can be concluded that these traits are appropriate for screening drought tolerant genotypes under stress condition. Generally, our results indicated that higher antioxidant activity results in lower oxidative stress and pigment photo-oxidation,

higher Sr and greater water retention capacity. Furthermore, RWP and SOD are appropriate for selecting drought tolerant genotypes and the cultivars Kavir and Bahar were the most tolerant and susceptible genotypes, respectively. Regarding the results, further investigation is required to compare these two cultivars in terms of RWP, SOD, proteomics and genomics to draw a clear image of their response under drought stress conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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