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Original Research

Physiological and biochemical responses of durum wheat under mild terminal drought stress

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Abstract: The effects of mild terminal drought stress on physiological parameters and activities of peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD) were investigated in ten durum wheat genotypes using two field experiments under rain-fed (terminal drought stress) and irrigated (non-stress) conditions. Stress intensity was calculated to be 0.1 indicating mild water deficit stress. Based on combined analysis of variance, the mild terminal drought stress increased the activity of CAT while, POX and SOD content decreased significantly. Rain-fed durum wheat plants showed non-significant increases in photochemical efficiency of PS II (F_v/F_m) as compared to the irrigated plants. Chlorophyll concentration in the flag leaves of the plants under terminal drought decreased slightly than normal conditions. The interaction of cultivar and environment was significant for enzyme activities, indicating different biochemical reaction of durum wheat plants in the two conditions.

Key words: Antioxidant enzymes; Drought; Durum wheat; Physiological traits.

Introduction

Durum wheat (*Triticum turgidum* var. durum) is a cultivated and important food crop in the world. This crop is mainly (>90%) cultivated in the Mediterranean basin, Europe and India (1-3).

Among prevailing abiotic stresses, drought is the most important and severe factor inhibiting crop growth and yield. The water deficit is a worldwide problem seriously constraining global crop production (4, 5) as in world map (Figure 1) demonstrated the drought-prone areas. There is necessary to increase wheat yield worldwide, particularly in developing countries and to improve genetic potential of wheat, it is important to understand the physiological and genetic basis of yield (6). Most of the countries of the world are facing to drought. The water deficit is the principal environmental stress that causes heavy damage to plant products in many regions of the world. It has been estimated that average yield loss of 17 to 70% in grain yield is due to drought stress (7). Morphological, agronomic and physiological traits of wheat have special roles in increasing yield, so these characters were used in breeding programs which led to improving yield and releasing commercial varieties that can withstand seasonal drought stress conditions (8).

Abiotic stresses lead to the overproduction of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to lipids, carbohydrates, proteins and DNA which ultimately result in oxidative stress. The ROS comprises both free radical ($O_2^{\bullet-}$, superoxide radicals; $OH^{\bullet}$, hydroxyl radical; $HO_2^{\bullet}$,

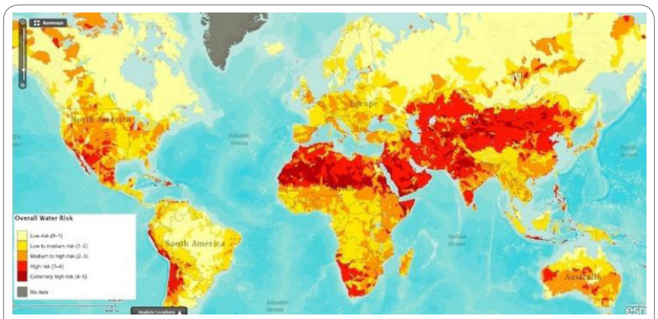

Figure 1. World map demonstrating the drought-prone areas (SPEI 2014).

perhydroxy radical and $RO^{\bullet}$, alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and $^1O_2$, singlet oxygen). Photosystems I and II (PSI and PSII) in chloroplasts are the major sites for the production of $^1O_2$ and $O_2^{\bullet-}$ (9).

Exposure of plants to unfavourable environmental factors such as low and high temperatures, heavy metals, drought, air pollutants, nutrient deficiency, or salt stress can increase the production of ROS e.g., $^1O_2$, $O_2^{\bullet-}$, H_2O_2 and $OH^{\bullet}$. To protect themselves against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defence systems. The induction of the cellular antioxidant machinery is necessary for protection against the various stresses (9-15) (Figure 2). The components of antioxidant defence system are enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants include SOD, CAT, APX, MDHAR, DHAR and GR and non-enzymatic forms are GSH, AA,

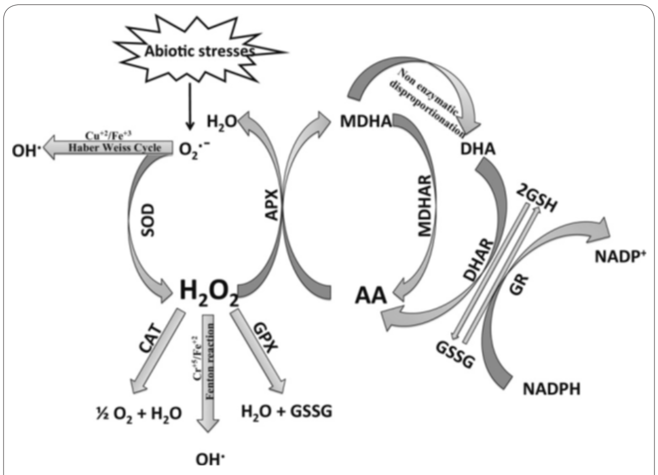

Figure 2. ROS and antioxidants defence mechanism (9).

carotenoids and tocopherols (12, 13).

The present study was carried out for evaluation of some physiological traits and biochemical responses of durum wheat genotypes under terminal drought stress.

Materials and Methods

Site description and plant material

The experiment was carried out in 2012 at the Research Farm of Kermanshah Azad University (latitude 34º20' N, longitude 46º20' E, altitude 1351.6 m above sea level). Kermanshah is located in the west of Iran and has a mean annual temperature of 13.8^{ºC} and an annual rainfall of 478 mm. The soil texture of the research area was sandy-loam. Ten wheat genotypes were planted. List and pedigree of the wheat accessions are presented in Table 1.

Experimental conditions

The experiment was performed based on randomized complete block design with three replications, in two environments (irrigated and rain-fed). The genotypes were sown in four rows of 3 m length, spaced 25 cm apart in early November. All of the phosphorus (50 kg ha-1, P_2O_5) and half of the total nitrogen (45 kg ha^{-1}, N) was applied at sowing time. The other half of the N was splited and given at tillering (as urea) and booting (as ammonium nitrate) stages, respectively. Seeds were pretreated with Mancozeb to minimize the probability of seed- and soil-borne diseases. The density of sowing was about 400 plants per m^2. Experimental plots were hand weeded. Three supplemental irrigations were done in irrigated plots. The study was conducted using 10 durum wheat (*Triticum aestivum* L.) genotypes to provide information about interrelationships of some physiological and biochemical traits with grain yield.

Photosynthetic parameters

The maximum photochemical efficiency of PSII or quantum yield (F_v/F_m) of the leaves was measured by using a portable Plant Stress Meter (PSM; Hansanthech, UK) according to (16). The F_o, F_m, variable fluorescence (F_v) and maximum photochemical efficiency of PSII (F_v / F_m) were measured on 15 flag leaves from each plot immediately after dark-adapted of the leaves for 30 min using leaf clips provided with PSM. The fluorescence transients were measured within 1 s. The data was recorded during the grain-filling period. The maximum photochemical efficiency of PSII (F_v/F_m) measures the efficiency of excitation energy captured by open PSII reaction centres representing the maximum capacity of light-dependent charge separation. The measurement was performed at 10:00 am in order to avoid the effects of dew and air humidity. Total chlorophyll content (Chl t) was measured according to (17).

Enzyme assays

Leaf samples were collected in an ice bucket and brought to the laboratory. Leaves were then washed with distilled water and surface moisture was wiped out. Enzymes were extracted from leaf tissues using an ice-cold mortar and pestle. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the method of Beauchamp and Fridovich (1971). One unit of SOD was defined as the amount of enzyme producing 50% inhibition of nitroblue tetrazolium (NBT). Catalase (CAT, EC 1.11.1.6) activity was estimated by consumption of hydrogen peroxide, which was recorded at 570 nm by a spectrophotometer. Peroxidase (POD, EC 1.11.1.7) activity was determined by monitoring the increase of absorbance at 470 nm due to guaiacol oxidation. The reaction mixture consisted of 32 mM potassium phosphate buffer, pH 7.0, 0.1% H_2O_2, 0.25% guaiacol and the extract (19).

Grain yield measurement

At ripening, plants in 1 m length of two middle rows of each plot were hand harvested and grain yield per unit area (GY) for each treatment at each replication was determined.

Table 1. List and pedigree of 10 durum wheat genotypes grown under the rain-fed and irrigated trials.

Genotype No.	Name/Pedigree	Origin*
1	KC-656	DARSI
2	DENA	DARSI
3	KC-591	DARSI
4	KC-647	DARSI
5	W-C 45587	DARSI
6	TN-12595	DARSI
7	TN-12595	DARSI
8	G-152/ZARDAK	DARSI
9	SAJI	DARSI
10	ZARDAK	DARSI

*DARSI: Dryland Agricultural Research Sub-Institute, Kermanshah, Iran.

Statistical analysis

Data were analyzed using SAS version 9.1 and SPSS 22 statistical software. Environments (rain-fed and irrigated) were considered as fixed effects. Correlation among characters and PCA was performed by SPSS and SAS softwares.

Results

Combined analysis of variance of the data (Table 2) showed that the environment was a significant source of variation ($P < 0.01$or 0.05) for the activity of CAT, POX and SOD enzymes. The durum wheat genotypes differed ($P < 0.01$or 0.05) for chlorophyll concentration (Chl t), grain yield (YG), CAT, POX and SOD, showing considerable genotypic diversity among the durum wheat cultivars. Two-way interaction of environment and genotype was significant ($P < 0.01$) for all the enzymes (Table 2). Stress intensity was estimated to be 0.1, indicating a moderate water deficit stress. Total chlorophyll content decreased slightly and non-significantly under water deficit stress (rain-fed), compared to non-stress (Irrigated) conditions (Table 3). There were no significant changes in the photochemical efficiency of PSII (F_v/F_m) averaged across genotypes by comparing the two conditions. Grain yield reduction due to the terminal water deficit was about ten percent. Drought decreased considerably the activities of peroxidase and superoxide dismutase while significantly increased catalase activity (Table 3). The degree of these decreases or increases was different among the durum wheat genotypes.

Simple correlation coefficients between the studied traits are shown in Table 4. Under water deficit stress, Total chlorophyll was negatively correlated with the photochemical efficiency of PSII (F_v/F_m). POX antioxidant enzyme had positive correlations with CAT and SOD enzymes. Grain yield was positively correlated with SOD (r=0.57) and POX (r=0.50). These two average correlation coefficients were not significant due to the number of the genotypes (n=10) (Table 4). Grain yield had a significant negative correlation with (F_v/F_m) under non-stress environment. There was a significant positive correlation between CAT and SOD enzyme (Table 4).

Principal component analysis (PCA) showed that the first three components explained 82.4% of the total variation under the irrigated environment (Table 5). The first component (PC1) accounting for 35.64% of the variation, mostly affected by SOD, CAT and Chl t. In contrast, the effect of F_v/F_m with a negative sign in the component was in the reverse direction. The most effective traits in the second component (PC2) were YG, F_v/F_m and CAT. This component was negatively correlated with YG and determined 28.5% of the total variance (Table 4). The third component (PC3) was mostly related with the activity of POX. Under rain-fed conditions, three principal components explained 87.3% of the total variability. PC1 determining 39% of the total variance was correlated with POX, SOD and partly YG. PC2 was mostly affected by Chl t and F_v/F_m and accounted for 27.7% of the variation. The third component had high correlations with YG and CAT activity, and explained

Table 2. Combined analysis of variance for some measured traits under irrigated and rain-fed conditions.

		Mean Squares					
SV	df	Chl t	Fv/Fm	GY	SOD	POX	CAT
Environment (E)	1	3.33	0.001	53340	1.2**	41920.3*	11682357**
R/E	4	1.12	0.06	86245	0.006	3759.1	440633
Genotype (G)	9	7.92**	0.01	87564**	0.25**	5467.3*	13192195**
G×E	9	2.06	0.007	13959	0.3**	11533.3**	9054893**
Error	36	2.45	0.012	26561	0.013	2370.9	635071
CV (%)		12.7	16.1	29.3	24.7	28.8	23.9

Table 3. Effect of terminal water deficit stress on different traits of durum wheat.

Traits	Irrigated (non-stress)	Rain-fed (stress)	Difference (%)
Total Chlorophyll (mg g^{-1} fresh leaf)	12.56	12.09	-3.74^{ns}
Photochemical Efficiency of PSII	0.65	0.68	$+4.6^{ns}$
Grain Yield (g m^{-2})	585.5	525.9	-10.18^{ns}
Catalase (U g^{-1} mg^{-1} sol/protein)	2865.0	3747.0	$+30^{**}$
Peroxidase (U g^{-1} mg^{-1} sol/protein)	195.1	142.2	-27.1^{*}
Superoxide dismutase (U g^{-1} mg^{-1}sol/protein)	0.60	0.32	-46.67^{**}

Table 4. Coefficients of correlation for different traits under water deficit stress (above diagonal) and non-stress (below diagonal) conditions.

Traits	Chl t	F_v/F_m	GY	SOD	POX	CAT
Chl t	1	-0.69*	-0.01	0.16	-0.05	-0.18
F_v/F_m	-0.20	1	-0.05	0.24	-0.36	-0.03
GY	-0.13	-0.63*	1	0.57	0.50	-0.11
SOD	0.36	-0.28	0.32	1	0.52	0.09
POX	0.14	0.34	-0.09	0.09	1	0.65*
CAT	0.48	-0.10	-0.22	0.61*	-0.19	1

Table 5. Principal component analysis for the measured traits under rain- fed (stress) and irrigated (non stress) conditions.

	Irrigated			Rain-fed		
Traits	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Chl t	0.40	0.37	0.10	0.19	-0.68	-0.12
F_v/F_m	-0.44	0.48	0.04	-0.37	0.53	0.29
GY	0.27	-0.61	0.34	0.42	0.12	0.59
SOD	0.56	0.12	0.26	0.51	0.02	0.33
POX	-0.14	0.29	0.84	0.57	0.27	-0.20
CAT	0.48	0.40	-0.31	0.26	0.41	-0.64
Eigen Value	2.14	1.71	1.09	2.34	1.66	1.24
Cumulative Variance (%)	35.64	64.12	82.40	39.00	66.66	87.3

20.6% of the total variability (Table 5).

Discussion

Water deficit stress is the most adverse environmental condition that can seriously reduce crop yield. To survive the stress, physiological and biochemical changes occur in various plants species. In this study, a mild terminal water deficit stress (stress intensity= 0.1) was imposed on durum wheat plants. Significant variations were observed among the genotypes for all the characters except photochemical efficiency of PSII (F_v/F_m). The response of the genotypes to the water deficit was different. Total chlorophyll and F_v/F_m didn't change significantly under stress conditions as compared to non-stress environment. Saeidi *et al*. (2015) and Lima *et al*. (2002) reported unchanged photochemical efficiency of PSII after drought stress in wheat and coffee, respectively (17, 20).

Antioxidant enzymes activities were significantly affected by the water deficit stress. POX and SOD decreased while CAT increased. The reports about changes (decrease or increase) in the activity of antioxidant enzymes under water deficit conditions are different (12, 20). These variations are due to the kind of plant, stress intensity and the time that plants encounter water deficit.

Principal component analysis (PCA) was done for determining independent components using correlated traits to clarify association among them. In each component, a high correlation between the component and a trait indicating that the trait is associated with the direction of the maximum or minimum amount of variability in the data set. PCA for the drought stress conditions indicated that antioxidant enzyme activities, chlorophyll content and photosynthesis, and finally grain yield were affected by water deficit, respectively.

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