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Study of Bread Wheat Genotype Physiological and Biochemical Responses to Drought Stress

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Abstract

This study investigates the impacts of drought-induced stress on the responses of ten bread (common) wheat genotypes to the physiological parameters and activities of POX, catalase CAT, and SOD under irrigation and stress conditions. The results of variance and mean comparison analysis indicated that the drought stress increased the activity of CAT while significantly decreased POX and SOD levels. In comparison with the irrigated plants, the photochemical efficiency of photosystem II (Fv/Fm) didn't change significantly; however, the concentration of chlorophyll and the relative water content in the flag leaves decreased in response to harsh environmental conditions. The observations included a significant enzyme activity in cultivar's interactions with the environment, which indicates various biochemical reactions of bread wheat plants under stress and non-stress environmental conditions.

Keywords

Antioxidant Enzymes, Bread Wheat, Drought, Physiological Traits, RWC.

Introduction

Western Iran has been the center of bread wheat agriculture using either relatively modern or rain-fed irrigation methods for thousands of years. The main application of hexaploid bread wheat (T. Aestivum, L.), or as some call it 'hard wheat' is in the food sector where it is used as a base material to make a various range of flours and wheat-based products such as the so-called pasta or spaghetti. However, although few farms are allocated to this plant, its disease-resistant and stress-tolerant properties are remarkable, especially under drought conditions (Khayatnezhad & Zaeifizadeh et al., 2011). Sadly, drought and difficult access to water resources either for the agriculture or the urban sector is a worldwide challenge as many countries deal with drought or related conditions. Also, the shortage of water causes severe damages to the overall yields all over the world. Based on the findings of a research study, the stress caused by drought has been identified with an estimated average loss of 17 to 70% in grain yield (Ahmadizadeh & Shahbazi et al., 2011), and (Khayatnezhad, 2012). This study used the morphological, agronomic, and physiological properties of wheat because of their especially positive impacts on the overall yield. Hence, these properties were utilized for identifying commercial varieties with suitable resistance to seasonal drought stress (Ahmadizadeh & Nori et al., 2011), and (Khayatnezhad & Gholamin, 2012). The parameters investigation of the chlorophyll fluorescence phenomenon is easily measurable through a quick, non-destructive technique. Since the photochemical application potency of raised energy in F0 is at its maximum, the photochemical reduction of fluorescence is also at a maximum level. Fm, being the highest amount of F0 value increased, in reaction to the sufficiency of light conditions. (Gholamin and Khayatnezhad 2011). High levels of reactive oxygen species (ROS) caused by abiotic stresses can severely damage lipids, carbohydrates, proteins, and DNA as ROSs are highly reactive and toxic. They can ultimately cause oxidative stress. The ROS comprises both free radical (O2$^{\bullet -}$, superoxide radicals; OH$^{\bullet}$, hydroxyl radical; HO2$^{\bullet}$, perhydroxy radical and RO$^{\bullet}$, alkoxy radicals) and non-radical (molecular) forms (H2O2, hydrogen peroxide, and ^{1}O2, singlet oxygen). 1O2 and O2$^{\bullet -}$ are mainly produced in PSI and PSII in chloroplasts. Various factors, including exposure to harsh environmental conditions like temperature fluctuations, heavy metals, torrid conditions, air pollutants, nutrient deficiency, or salt stress can enhance ROSs production. Plant cells and its organelles like chloroplast, mitochondria, and peroxisomes have antioxidant defense mechanisms against these toxic oxygen intermediates. In the protection against various types of cellular stress, the induction of the cellular antioxidant machinery is plays a vital role (Mittler, Vanderauwera et al., 2004), (Tuteja, 2007), (Singh & Anjum et al., 2008), and (Gill,Tuteja & Hirt, 2010), (Gill & Khan et al., 2011) (Fig.1). The antioxidant defense system contains enzymatic and non-enzymatic antioxidant components. While enzymatic antioxidants include SOD, CAT, APX, MDHAR, DHAR, and GR, non-enzymatic antioxidants are AAA, GSA, carotenoids, and tocopherols (Mittler, Vanderauwera et al., 2004, Gill and Tuteja 2010). This study was an attempt to evaluate some physiological properties and biochemical responses of bread wheat genotypes under terminal drought stress.

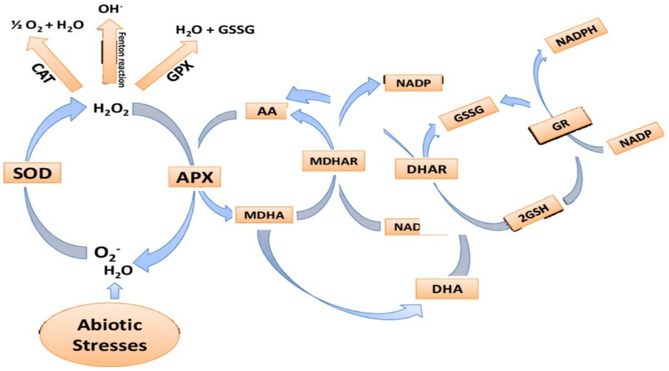

Figure 1: ROS and Antioxidants Defence Mechanism [9].

Materials and Methods

The experiment was conducted in the fall of 2017-2018 at the Islamic Azad University in Ardebil. The field used for the experiment was located at 48° 30" E and 38° 15" N and a height of 1350 m above the sea level. This study used a randomized block design as the empirical design with three replications. The planting layout designed for this research consisted of 1.5 × 4 (m) areas with 5 rows, the distance between which was 20 cm, where four hundred grain seeds were planted per square meter. The fertilizer used consisted of farm manure with 41 kg ha^{-1} N and 46 kg ha^{-1} P_2O_5 content. Finally, the study abided by the local relevant soil recommendations regarding pre-cultivation sowing. The irrigation process of the non-tensioned was conducted. The identification of chemical and physical properties of soil samples required sample collection before the beginning of the soil preparation process. The study collected the samples from different levels of the soil with various deepness levels (0-30, 30-60cm) to send to The University of Yangzhou for water and soil content laboratory investigations, the results of which are included in Table 2. It should be noted that this examination was solely for soil unity evaluation and limiting false data production. Figure. 2 depicts the rain outcome reports for the 2015- 2018 period. (WWO 2018).

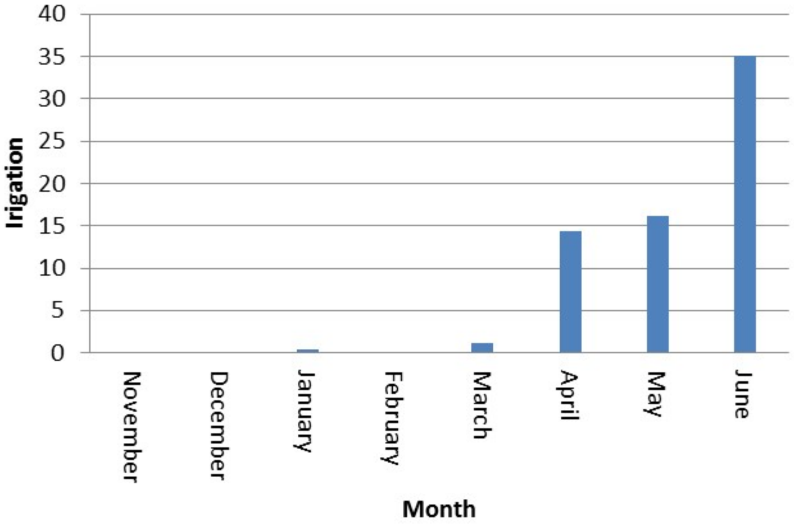

Figure 2: Rain for Statistics for 2017-2018 Crop Years

Photosynthetic Parameters

The study used a chlorophyll meter device to measure the chlorophyll contents of flag leaves (CCI-200). This device was a product of Opti-Sciences Inc. used as an index of leaves' chlorophyll content. The study used BioMonitor SCI AB, which is a device designed for plant stress to determine the quantity of chlorophyll fluorescence within one month after flowering. The study stored the leaves in dark for about forty minutes and then, attached the clamps to the machine's optic fiber, and opened the valves. As the gadget began to work, the 695 nm modulated light was radiated through the optic fiber toward the leaf. Next, the readings on the device concerning several fluorescence param-

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eters were recorded: the parameters included F0, Fm, FV, and the Fv/Fm ratio (Fracheboud, 2006). The measurement was conducted at 11:00 am to keep the impacts of dew and air humidity on the results to a minimum level.

Enzyme Assays

The study used iceboxes to store leaf samples and protect them from damage before sending them to the laboratory. Leaves were then rinsed with distilled water and floor moisture also was wiped out. The study used ice-cold mortar and pestle to extract enzymes from leaf tissues. Using the Beauchamp and Fridovich approach, the study managed to determine superoxide dismutase (EC 1.15.1.1) pastime (Beauchamp and Fridovich 1971). It was found that one unit of SOD could cause 50% inhibition of nitroblue tetrazolium (NBT) so it was defined as the inhibiting quantity of enzyme. Once recorded at 570 nm using a spectrophotometer, the consumption of hydrogen peroxide helped estimate the pastime of Catalase (CAT, EC 1.11.1.6). Also, guaiacol oxidation helped measure the activity of Peroxidase (EC 1.11.1.7) through an increased level of absorbance (470nm). Finally, the reaction combination consisted of 32 mM potassium phos¬phate buffer, pH 7.0, 0.1% H2O2, 0.25% guaiacol, and the extract (Khamssi and Najaphy 2018).

Grain Yield Measurement

When ripened, one-meter long plants in two middle rows of each plot were manually harvested and the grain yield per unit area (GY) for each treatment at each replication was determined.

Table 1: List and Pedigree of 10 Bread Wheat Genotypes Grown Under the Rain-fed and Irrigated Trials.

Code	Genotype	Code	Genotype
1	Anza	6	Inia 66
2	YecoraRojo	7	G4838
3	G4840	8	G4843
4	G4836	9	Naphal
5	G4842	10	G869

Determination of Relative Water Content (RWC)

The study measured the fresh weight (FW) of wheat's fresh-cut flag leaves. Then, they were soaked in distilled water for 24 hours at room temperature under low-light conditions. To determine the turgid weight (TW), the leaves were thoroughly dried using filter paper. Samples' dry weight (DW) was calculated after oven-drying for 72 hours at 70 ○C. Then, the RWC was calculated using Tambussi's equation [25]:

RWC (%) = [(FW - DW)/(TW -DW)] ×100.

Statistical Analysis

This study used MStatC and SPSS25 package (SPSS, 2018) to calculate, conduct, and evaluate the relationship among various methods; variance and gene sequence group analysis; an average comparing following the Euclidean distance. Furthermore, the study implemented PCA was utilized to categorize the screening procedures as well as the genome sequence. Based on the results of the PCA, the study also utilized a biplot method in Minitab 16 to evaluate genome sequence and tolerance.

Results

The findings, including the combined data variance analysis, as depicted in Table 2, identified the environment as the major factor causing significant variation ($P < 0.01$) in the activity of CAT, POX, RWC, and SOD enzymes. Various bread wheat genotypes had different (P < 0.01or 0.05) levels of chlorophyll concentration (Chl t), grain yield (YG), CAT, POX, and SOD, which proves the substantial genotypic diversity of bread wheat cultivars. Table 1 represents the significant ($P < 0.01$) interaction of environment and genotype for all the enzymes. This research had an estimated stress intensity of 0.1, which means indicating a moderate water deficit stress. While the total chlorophyll content under non-stress irrigation conditions changed significantly, it only decreased insignificantly under rain-fed stress conditions. Table 3 represents the relevant reports. There were no significant changes in the photochemical efficiency of PSII (Fv/Fm) averaged across genotypes by comparing the two conditions. Grain yield reduction due to the terminal water deficit was about ten percent. As depicted in Table 3, the stress caused by drought significantly decreased peroxidase and superoxide dismutase activities and significantly increased catalase activity and RWC (Table 3). Various genotypes represented a different level of change.

Table 4 represents the simple correlation coefficients between the studied properties. The results indicate positive correlation of RWC with Cht (r=0.51*), Fv/Fm (r=0.57*), GY (r=0.83**). Also, they indicated its negative correlation with SOD (r=-0.51*). While the correlation between CAT, SOD (r-0.67*), and GY and Fv/Fm (r=0.66*) was positive, the correlation between SOD and was negative GY (r=-0.6*).

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**	**RWC**
Environment (E)	1	7.23**	0.002	635212**	1.8**	42658.5**	2152467**	4251.5**
R/E	4	1.82	0.05	65289	0.004	4215.5	512035	3262.4*
Genotype (G)	9	8.01**	0.02	785216**	0.31**	61542.5**	3021548**	1256
G×E	9	7.51**	0.006	706514**	0.65**	12156.7**	8795462**	22152*
Error	36	3.01	0.014	16485	0.018	1986.5	54986	1452
CV (%)		13.1	15.8	30.1	25.6	27.6	24.9	21.6

** And *: important at 0.01 and 0.05 possibility stages.

Table 3: Effect of Terminal Water Deficit Stress on different Traits of Bread Wheat.

Traits	**Irrigated (non-stress)**	**Rain-fed (stress)**	**Difference (%)**
Total Chlorophyll (mg g-1 fresh leaf)	13.07	12.71	-2.75ns
Photochemical Efficiency of PSII	0.71	0.79	+11.26ns
Grain Yield (g m-2)	602.7	579.4	-3.86ns
Catalase (U g-1 mg-1 sol/protein)	2795.4	3592.7	+28.52**
Peroxidase (U g-1 mg-1 sol/protein)	200.4	138.6	-30.83*
Superoxide dismutase (U g-1 mg-1sol/protein)	0.71	0.48	-32.39*
Determination of relative water content (RWC %)	80.1	43.2%	-46.6**

Table 4: Coefficients of Correlation for different Traits under Water Deficit Stress Conditions.

Traits	**Chl t**	Fv/Fm	**GY**	**SOD**	**POX**	**CAT**	**RWC**
Chl t	1						
Fv/Fm	0.42	1					
GY	0.51	0.66*	1				
SOD	-0.24	-0.32	-0.6*	1			
POX	0.16	0.41	-0.11	0.08	1		
CAT	0.57	-0.15	-0.27	0.67*	-0.21	1	
RWC	0.51*	0.57*	0.83**	-0.51*	0.34	0.24	1

As depicted in Table 5, the results of (PCA) indicated that the first three components were responsible for 79.95% of the total variation under the irrigated environment. PC1, which caused about 34.96% of the variation, was mostly impacted by SOD, CAT, and Chl t. On the other hand, though, Fv/Fm had a negative impact. Among the traits of PC2, YG, Fv/Fm, and CAT were the most effective. Finally, PC3 was mostly associated with POX and RWC activity. Under rain-fed conditions, three principal components explained 85.64% of the total variability. Determining 41.02% of the total variance, PC1 correlated with POX, SOD, and partly YG. The third component had high correlations with YG and CAT activity and explained 20.6% of the total variability (Table 5).

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	0.38	0.35	0.11	0.18	-0.48	-0.17
Fv/Fm	-0.32	0.51	0.05	-0.32	0.48	0.21
GY	0.31	-0.48	0.31	0.57	0.15	0.51
SOD	0.48	0.17	0.27	0.49	0.12	0.34
POX	-0.17	0.27	0.72	0.61	0.34	0.12
CAT	0.36	0.41	-0.33	0.27	0.54	-0.42
RWC	0.21	0.11	0.64	0.18	0.07	0.13
Eigen Value	2.21	1.87	1.12	2.11	1.04	1.32
Cumulative Variance (%)	34.96	63.59	79.95	41.02	61.25	85.64

Discussion

Arguably, the shortage of water and the associated stress is one major antagonistic environmental 'crisis' that can damage crops overall yield and, in response, various species of plants have developed particular physiological and biochemical properties to protect themselves and tolerate such environmental stress. This study imposed on bread wheat plants an intense terminal (intensity = 0.1) water shortage. Significant variations were observed among the genotypes for all the characters except the photochemical efficiency of PSII (Fv/Fm). Genotypes responded to the water deficit differently and stress conditions did not significantly change the total chlorophyll and Fv/Fm as compared to the non-stress conditions. Similarly, in another research concerning the photochemical efficiency of PSII in wheat and coffee, the environmental stress caused by drought had no significant (Saeidi et al., 2015) and (Lima et al., 2002).

The stress caused by the shortage of water remarkably impacted the activates of antioxidant enzymes. The observations indicated that in response to a decrease in POX and SOD, CAT increased. These observations provide various reports concerning the decrease or increase of enzyme activities in water deficit conditions (Lima & DaMatta et al., 2002), and (Gill & Khan et al., 2011). Arguably, these 'various reports' can be associated with plant species, stress intensity, and the timespan of water deficit occurrence. (Kahrizi, Maniee, Khamssi, & Golezani et al., 2010).

The study used the PCA method to determine independent components using common properties to identify the correlation between relevant components. The high correlation between a component and a trait indicates the association of the trait with data variability in the experiment. The results of PCA for the drought stress conditions demonstrated that water deficit respectively impacts the activities of antioxidant enzymes, including chlorophyll content, RWC, photosynthesis, and grain yield. In relevant research concerning the application of stress to the amount of F0, the amount of chlorophyll and the ratio of Fv/Fm, and the overall yield declined, indicating the insignificant impact of the drought stress on the chlorophyll parameters (Gholamin and Khayatnezhad 2011). The observations in the study suggested that the greater the chlorophyll levels, the higher the stress tolerance of plants. Thus, since single pass genotype has the highest amount of chlorophyll, it was selected as the tolerant genotype (Yang & Chen et al., 2006), (Maniee & Kahrizi et al., 2009), and (Mohammadi, Armion et al., 2012), (Naeem-ud-Din & Naeem et al., 2012). In the end, the findings suggested that the high correlation between the ratio of Fv/Fm, leaf chlorophyll, RWC, and SOD allows their application in stress levels assessment and selecting a genotype with optimal stress tolerance.

Conclusion

The conventional breeding methods of identifying and developing crops with drought-resistant properties are impractical as they are both costly and time-taking, and above all, the process is complicated. Thus, investigating existing varieties to identify cultivars with optimal drought-tolerance by screening under arid conditions seems to be a viable solution. The observations included a significant enzyme activity in cultivar's interactions with the environment, which indicates various biochemical reactions of bread wheat plants under stress and non-stress environmental conditions.

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