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CONTEMPLATION OF WHEAT GENOTYPES FOR ENHANCED ANTIOXIDANT ENZYME ACTIVITY

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Abstract

Wheat (*Triticum aestivum* L.) is leading cereal crop in Pakistan but its yield is highly affected due to various abiotic factors especially drought stress, which affects the metabolism of plants. The present study was conducted at Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, using thirty three genotypes during 2011 to investigate the response of anti oxidative enzymes. Seedlings were subjected to stress condition with 30 % PEG 6000 solution along with control (irrigated with water) under *in vitro* conditions. The experiment was conducted in pots following Complete Randomized Design in Laboratory. Results revealed that under control conditions the maximum values for Guaiacol peroxidase were found in Punjab-96 and Auqab-2000 (2.523), for superoxide in C-273 (0.294), for ascorbate peroxide in PAK-81 (2.523) and for catalase in Kohsar-95 (0.487). Under moisture stress condition the maximum value for Guaiacol peroxidase were recorded for Kohsar-95 (2.699), for superoxide in Kohsar-95 (1.259), for ascorbate peroxide in Pak-81, SA-75, Mexipak-65 and PARI-73 (3.000) and for catalase in Mexipak-65 (0.640). The genotypes which showed higher antioxidant enzyme activity under drought stress have the ability to perform better under adverse soil moisture condition. Such potential genotypes can be utilized in the future breeding programs and also in improving the wheat varieties against drought stress.

Key words: Antioxidant enzyme, Catalase, Drought, Peroxidase, Superoxide, *Triticum aestivum* L.

Introduction

Wheat is an important cereal crop after rice and maize. As the population is increasing day by day, demand for food also increases and to feed large number of population yield of wheat must be increased. Pakistan produced 25.28 million tons of wheat from 9.039 million hectares during 2013-2014 (Anon., 2014).

Water deficit condition (commonly known as drought) can be defined as the absence of sufficient moisture necessary for a plant to grow normally and complete its life cycle (Zhu, 2002). The lack of adequate soil moisture occurs commonly in rainfed areas leading to water stress, brought about by infrequent rains and poor irrigation (Wang *et al.*, 2005).

Drought eaffects various plant metabolism, extent of damage varies from plant to plant. Wheat grown in rainfed condition suffers from drought due to increased rate of transpiration or low level of water in the root zone. Every stage of wheat growth is important but if the drought occurs at anthesis and maturity stage, decrease in yield is observed. Abiotic stresses affect the normal morpho-physiological processes of many important plant species (Jan *et al.*, 2016 [a, b]; Narusaka *et al.*, 2003; Nakashima *et al.*, 2000; Shinwari *et al.*, 1998).

Drought basically causes the oxidative damage disrupting the antioxidant mechanism through production of reactive oxygen species (Qiu *et al.*, 2008, Wang & Huang, 2004), which is very reactive and cause serious damage to all organelles like cell membrane, chloroplast etc. (Smirnoff, 1993 and Foyer *et al.*, 1994). Reactive oxygen species, produced under water stress conditions, oxidize photosynthetic pigments, membranes, lipids, proteins, and nucleic acids (Smirnoff, 1993; Alscher *et al.*, 1997; Yordanov *et al.*, 2000). Thus synthesis of antioxidants such as carotenoids, ascorbate, α-tocopherol, glutathione and flavonoids, as well as antioxidant enzymes viz. peroxidases, superoxide dismutase and catalase can be triggered to protect the plant cells (Tanaka *et al.*, 1990). The main objective of present study was to identify wheat genotypes with enhanced antioxidant enzymes using enzymes activities determination.

Materials and Methods

The present study was carried out at the Laboratory of Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, during 2011. Thirty three diverse wheat genotypes were grown for collection of leaf samples. This germplasm was studied and investigated for drought tolerance under *in vitro* condition. Wheat seedlings were subjected to stress condition with 30% Polyethylene Glycol 6000 solution along with control irrigated with water. The experiment was conducted in small plastic pots (3" dia) in laboratory.

Sample preparation: Seeds of different genotypes were sown in small plastic pots and placed in the growth chamber under a 12 h photoperiod, 70 % relative humidity and 25°C/18°C (day/night). The pots were watered regularly to provide ample soil moisture for proper germination. When the seedlings were 12 days old, they were treated with 20 ml of 30% (w/v) PEG 6000 solution whereas 20 ml of water was applied to control treatment for 8 days. The leaf samples were taken from each treated and control pot after 2, 4, 6 and 8 days of drought stress respectively for determination of enzyme activity.

Enzyme activity determination: Frozen leaves (0.2 g) were taken and put them in a mortar, homogenized the sample with the help of a pestle, after adding 2 ml of 50 mM ice-cold phosphate buffer of pH 7.8 containing 1mM EDTA. The homogenate was centrifuged at 4°C for 15

min to get pellet without any debris. The supernatant was taken and stored at -80ºC. The supernatant was used to determine the activities of SOD, POD, CAT and APX.

Guaiacol peroxidase: Analysis of guaiacol peroxidase (POD) activity was done by using oxidation of guaiacol and for this hydrogen peroxide was used (Zhang & Kirkham, 1994). The enzyme extract (0.01 mL) was added to the reaction mixture containing 0.02 mL guaiacol solution and 0.01 mL hydrogen peroxide solution in 3 mL of 50mM potassium phosphate buffer solution (pH 7.0). To initiate the reaction enzyme extract was added in the tubes containing reaction mixture and the formation of tetraguaiacol was measured through spectrophotometer (SPEKOL 1300). The increase in absorbance was recorded at the wavelength of 470 nm for a time span of 5 mins against the blank containing reaction mixture without H_2O_2. The Enzyme activity was quantified by the amount of tetraguaiacol formed using its molar extinction coefficient which is 26.6 mM^{-1} cm^{-1}.

Superoxide dismutase: To determine the activity of superoxide dismutase (SOD), nitro blue tetrazolium (NBT) was used and ability of SOD to inhibit the photoreduction of NBT was measure as described by Giannopolitis & Ries (1976). The quantity of reaction mixture for each sample was 3 ml. The reaction mixture was prepared by adding 50 mM phosphate buffer having pH 7.8 containing 0.1 mM of EDTA, 130 mM of methionine, 0.75 mM of NBT and 0.02 mM of riboflavin. Riboflavin was the last constituent to be added. Add 0.1 ml of the enzyme extract in the reaction tubes containing reaction mixture. Then reaction tubes were placed under two 20 W fluorescent lamps to initiate the reaction. The reaction was terminated after 10 mins by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. Absorbance of the reaction mixture was recorded at 560 nm wavelength against the blank containing non-illuminated reaction mixture except sample. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo-reduction rate and the results expressed as U mg^{-1} of fresh weight (FW).

Ascorbate peroxidase: The activity of ascorbate peroxidase (APX) was quantified according to Nakano & Asada (1981). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2. Add 0.1 mL enzyme extract in the tubes containing reaction mixture. The reaction was initiated by the addition of H_2O_2 and then ascorbate oxidation was measured at 290 nm wavelength using spectrophotometer (Spekol 1300) for time span of 3 mins against the blank containing reaction mixture without enzyme extract. Enzyme activity was then quantified by using the molar extinction coefficient for ascorbate which is 2.8 mM^{-1} cm^{-1}.

Catalase: The activity of Catalase (CAT) was assayed by using the method of Cakmak & Marschner (1992) with minor modifications. The reaction mixture for each sample was 3 mL in quantity. The reaction mixture consisted of 100 mM of phosphate buffer having pH 7.0 containing 0.1 mM of EDTA and 0.1% H_2O_2. Add 0.1 mL of enzyme extract in the tubes containing reaction mixture. The reaction was initiated by adding the enzyme extract in the reaction mixture. The decrease of H_2O_2 was monitored at 240 nm wavelength against the blank containing reaction mixture and enzyme extract except hydrogen peroxide using spectrophotometer (SPEKOL 1300) and quantified by its molar extinction coefficient (36 M^{-1} cm^{-1}).

Results and Discussion

Guaiacol peroxidase: Guaiacol peroxidase (POD) plays important role in biosynthesis of lignin, plant development, organogenesis, cell wall lignifications, cell wall stiffening, auxin metabolism and root elongation. POD is tissue specific and it converts Hydrogen peroxide into water. A perusal of Table 1 revealed that two wheat genotypes i.e., SA-75 and Punjab-96 had higher POD activities under treatment T_1 i.e., 2nd day control, whereas wheat genotype (C-273) has minimum POD activity in control. The values of POD activity varied from 0.058 to 2.301 under controlled condition. Four genotypes have a higher POD activity under treatment T_2 (4th day control) and these genotypes are Pak-81, Punjab-96, Kohistan-97 and GA-2002. LLR-13 has the lowest POD activity with the range of 2.398 to 0.164 in T_2. In T_3 i.e. 6th day control, WC-2 showed a minimum POD activity whereas Parwaz-94 showed a maximum POD activity and the values ranged from 0.2 to 2.398. Punjab-96 and Auquab-2000 had the highest POD activity under treatment T_4 i.e. 8th day control whereas WC-4 has a minimum POD activity. The values varied from 0.087 to 2.523 under controlled condition.

Table 1 showed that Parwaz-94 and Punjab-96 had the highest POD activity under treatment T_1 i.e. 2nd day after treatment under treated condition whereas LLR-13 had the lowest activity. The values for POD activity varied from 2.301 to 0.271after treatment. Under treatment T_2 i.e. 4th day after treatment Inqilab-91 showed the minimum POD activity whereas LLR-12 showed the minimum activity. The value varied from 2.301 to 0.139 under treated condition. The two wheat genotypes *i.e.*, Auqab-2000 and GA-2002 had maximum POD activity while LLR-14 showed minimum activity under treatment T_3 i.e. 6th day after treatment. The values varied from 2.39 to 0.2. The genotype Kohsar-95 showed maximum activity under treatment T_4 i.e. 8th day after treatment where as the minimum POD activity was found in WC-1. The values of T_4 after treatment lie between 2.699 and 0.316.

Guaiacol peroxides is known for their capability to reduce hydrogen peroxide to water. Genotypes having more POD activity are considered to be resistant against stress. Many researchers have reported the increase in peroxidase activity under water stress indicating the formation of large part of H_2O_2 during water stress. An increase of POD activity was also observed in other studies under drought stress (Badiani *et al.*, 1990, Zhang & Kirkham, 1994).

Table 1. POD activity of wheat genotypes at various intervals under stress and controlled conditions.

Genotypes	Control					Treated				
	2d c	4d c	6d c	8d c	Mean ± S.E.	2d t	4d t	6d t	8d t	Mean ± S.E.
Mexipak-65	1.553	1.387	1.367	1.143	1.363±0.140	1.886	0.559	1.678	0.971	1.274±0.123
Chenab-70	1.921	1.721	1.187	1.921	1.688±0.140	1.824	0.983	2.222	0.640	1.417±0.123
Blue silver	1.658	1.638	1.602	2.222	1.780±0.140	1.097	1.252	2.155	0.873	1.344±0.123
PARI-73	1.796	2.046	2.000	2.301	2.036±0.140	1.398	1.119	2.097	2.398	1.753±0.123
Lyalpur-73	2.097	1.509	2.222	2.097	1.981±0.140	1.244	0.951	2.222	2.046	1.616±0.123
SA-75	2.301	2.097	2.097	2.398	2.223±0.140	1.897	1.745	2.000	2.523	2.041±0.123
Yecora	2.155	2.301	1.921	2.301	2.170±0.140	1.854	1.137	2.222	1.174	1.597±0.123
Pak-81	1.745	2.398	1.377	2.222	1.936±0.140	1.367	1.041	1.886	2.398	1.673±0.123
Kohinoor-83	1.678	1.824	2.097	2.097	1.924±0.140	1.328	1.409	1.102	2.097	1.484±0.123
Chakwal-86	1.602	2.301	2.046	2.222	2.043±0.140	2.097	1.699	2.222	2.222	2.060±0.123
Pasban-90	1.796	2.155	2.000	2.097	2.012±0.140	2.000	1.721	2.222	2.301	2.061±0.123
Inqilab-91	2.097	1.721	1.328	2.222	1.842±0.140	1.509	2.301	2.301	2.398	2.127±0.123
Parwaz-94	2.000	2.155	2.398	2.398	2.238±0.140	2.301	0.631	2.097	2.301	1.833±0.123
Kohsar-95	1.310	1.797	1.770	2.155	1.758±0.140	1.481	1.041	2.155	2.699	1.844±0.123
Punjab-96	2.301	2.398	2.301	2.523	2.381±0.140	2.301	2.047	2.097	2.222	2.167±0.123
Kohistan-97	2.097	2.398	2.097	2.301	2.223±0.140	2.000	2.155	2.097	2.398	2.163±0.123
Auqab-2000	0.427	2.301	2.222	2.523	1.868±0.140	2.155	1.638	1.921	2.398	2.028±0.123
GA-2002	2.155	2.398	2.155	2.155	2.216±0.140	2.222	1.585	2.398	2.222	2.107±0.123
Sehar-06	1.228	2.155	2.301	2.046	1.933±0.140	2.097	1.222	2.398	2.046	1.941±0.123
Lasani-08	1.509	2.222	2.097	2.222	2.013±0.140	2.097	1.921	2.301	2.301	2.155±0.123
C-273	0.058	0.537	0.534	0.806	0.484±0.140	0.510	0.652	0.449	1.301	0.728±0.123
Faisalabad-08	0.220	0.563	0.582	1.031	0.599±0.140	0.565	0.658	0.613	1.286	0.781±0.123
LLR-10	0.458	0.453	0.467	0.415	0.448±0.140	0.688	0.291	0.297	1.397	0.668±0.123
LLR-11	0.466	0.327	0.314	0.806	0.478±0.140	0.368	0.293	0.258	0.618	0.384±0.123
LLR-12	0.442	0.274	0.480	0.410	0.402±0.140	0.357	0.293	0.373	0.339	0.341±0.123
LLR-13	0.383	0.164	0.232	0.355	0.284±0.140	0.280	0.139	0.410	0.413	0.311±0.123
LLR-14	0.372	0.366	0.247	0.585	0.393±0.140	0.271	0.237	0.448	0.575	0.383±0.123
WC-1	0.237	0.386	0.205	0.377	0.301±0.140	0.446	0.858	0.200	0.381	0.471±0.123
WC-2	0.253	0.322	0.200	0.342	0.279±0.140	0.432	0.424	0.330	0.316	0.376±0.123
WC-3	0.341	0.448	0.291	0.561	0.410±0.140	0.300	0.389	0.478	0.596	0.441±0.123
WC-4	0.598	0.265	0.229	0.087	0.295±0.140	0.455	0.477	0.431	0.652	0.504±0.123
CB-336	0.569	0.375	0.352	0.347	0.411±0.140	0.410	0.440	0.463	0.440	0.438±0.123
CB-333	0.504	0.481	0.461	0.561	0.502±0.140	0.704	0.362	0.601	0.750	0.604±0.123

Superoxide dismutase: Within a cell, the SOD constitutes the first line of defense against reactive oxygen species. It is the first enzyme in the detoxifying process that converts O_2 (radicals) to Hydrogen peroxide. Table 2 depicted that the wheat genotypes under treatment T_1, 2nd day in control showed that values ranged from 0.095-0.294. (C-273) had maximum SOD activity (0.294) while CB-336 (0.095) had minimum activity under controlled condition. Lasani-08 showed the highest activity in treatment T_2 i.e. 4th day in control and Chakwal-86 showed the lowest SOD activity with the range of values from 0.223 to 0.088. In the treatment T_3, 6th day in control, the maximum (0.223) and minimum (0.088) values observed for Lasani-08 and Pasban-90 respectively. The SOD activity values in T_3 lie between 0.111-0.243. Wheat genotypes under treatment T_4, 8th day control depicted that Sehar-06 had the highest SOD activity while WC-3 had the lowest activity.

The high SOD activity has been associated with stress tolerance in plants because it neutralizes the activity of O2- which over produced under stress (Bowler *et al.*, 1992). The wheat genotype PARI-73 showed maximum while CB-336 showed minimum SOD activity (Table 2). The values varied from 0.303 to 0.062 in treatment T_1, 2nd day treatment. The wheat genotype (LLR-10) had the highest SOD activity in treatment T_2, 4th day treatment whereas Kohistan-97 had maximum activity with the values ranging from 0.032-0.338. Under treatment T_3 i.e. 6th day treatment, Kohsar-95 and WC-2 had maximum (0.338) and minimum (0.032) SOD activity, respectively. The values of T_3 range from 0.111 to 1.259. The genotypes under treatment T_4 i.e. 8th day treatment showed that Chakwal-86 had the lowest activity while LLR-12 had higher SOD activity with the range from 0.082 to 0.213.

Superoxide dismutase is the most important oxygen radical scavenger whose enzymatic action results in production of hydrogen peroxide and oxygen. Increased SOD activity in genotypes is known to confer oxidative stress tolerance. The decrease in SOD activity under extreme water stress indicated that the scavenging ability in the cells of leaves was inhibited under extreme water stress. It is also indicated that species, which showed less extent of decrease in SOD activity as compared to the species which showed higher decrease in enzyme activity, could be better able to tolerate water stress (Pandey *et al.*, 20~~09~~10). Lui & Huang (2000) reported similar findings in Bent grass. The depression of SOD activity in plants also has been observed in other studies (Quartacci & Navari-Izzo 1992, Zhang *et al.*, 1990).

Table 2. SOD activity of wheat genotypes at various intervals under stress and controlled conditions.

Genotypes	Control					Treated				
	2d c	4d c	6d c	8d c	Mean ± S.E.	2d t	4d t	6d t	8d t	Mean ± S.E.
Mexipak-65	0.126	0.095	0.139	0.178	0.135±0.004	0.216	0.113	0.143	0.126	0.150±0.013
Chenab-70	0.145	0.110	0.167	0.188	0.153±0.004	0.283	0.108	0.444	0.177	0.253±0.013
Blue silver	0.180	0.151	0.155	0.157	0.161±0.004	0.282	0.151	0.439	0.112	0.246±0.013
PARI-73	0.143	0.107	0.173	0.120	0.136±0.004	0.303	0.200	0.347	0.131	0.245±0.013
Lylpur-73	0.151	0.111	0.128	0.146	0.134±0.004	0.289	0.067	0.338	0.134	0.207±0.013
SA-75	0.119	0.133	0.145	0.159	0.139±0.004	0.196	0.138	0.222	0.094	0.163±0.013
Yecora	0.142	0.153	0.16	0.181	0.159±0.004	0.274	0.169	0.845	0.144	0.358±0.013
Pak-81	0.098	0.119	0.136	0.149	0.126±0.004	0.254	0.151	0.327	0.111	0.211±0.013
Kohinoor-83	0.131	0.149	0.157	0.166	0.151±0.004	0.188	0.194	0.231	0.134	0.187±0.013
Chakwal-86	0.137	0.088	0.122	0.166	0.128±0.004	0.210	0.074	0.532	0.082	0.225±0.013
Pasban-90	0.206	0.152	0.111	0.127	0.149±0.004	0.271	0.162	0.319	0.142	0.224±0.013
Inqilab-91	0.180	0.149	0.158	0.172	0.165±0.004	0.212	0.158	0.845	0.142	0.339±0.013
Parwaz-94	0.164	0.218	0.139	0.145	0.167±0.004	0.255	0.096	0.754	0.146	0.313±0.013
Kohsar-95	0.175	0.139	0.174	0.196	0.171±0.004	0.290	0.094	1.259	0.120	0.441±0.013
Punjab-96	0.153	0.185	0.165	0.171	0.169±0.004	0.302	0.192	0.452	0.154	0.275±0.013
Kohistan-97	0.101	0.156	0.126	0.119	0.126±0.004	0.192	0.032	0.301	0.116	0.160±0.013
Auqab-2000	0.223	0.202	0.173	0.197	0.199±0.004	0.250	0.039	0.162	0.100	0.138±0.013
GA-2002	0.210	0.180	0.190	0.199	0.195±0.004	0.301	0.169	0.327	0.171	0.242±0.013
Seher-06	0.236	0.212	0.224	0.256	0.232±0.004	0.231	0.095	0.189	0.151	0.167±0.013
Lasani-08	0.180	0.223	0.243	0.255	0.225±0.004	0.182	0.102	0.327	0.159	0.193±0.013
C-273	0.294	0.095	0.174	0.114	0.169±0.004	0.146	0.108	0.161	0.125	0.135±0.013
Faisalabad-08	0.185	0.175	0.206	0.116	0.171±0.004	0.242	0.157	0.213	0.184	0.199±0.013
LLR-10	0.183	0.202	0.220	0.134	0.185±0.004	0.124	0.338	0.190	0.156	0.202±0.013
LLR-11	0.215	0.166	0.152	0.135	0.167±0.004	0.157	0.154	0.142	0.145	0.150±0.013
LLR-12	0.137	0.18	0.175	0.206	0.175±0.004	0.243	0.236	0.126	0.213	0.205±0.013
LLR-13	0.110	0.157	0.202	0.136	0.151±0.004	0.146	0.229	0.139	0.174	0.172±0.013
LLR-14	0.152	0.123	0.210	0.118	0.151±0.004	0.162	0.123	0.170	0.129	0.146±0.013
WC-1	0.165	0.188	0.180	0.115	0.162±0.004	0.173	0.188	0.212	0.111	0.171±0.013
WC-2	0.195	0.100	0.150	0.141	0.147±0.004	0.130	0.144	0.111	0.134	0.130±0.013
WC-3	0.161	0.164	0.161	0.107	0.148±0.004	0.127	0.121	0.138	0.085	0.118±0.013
WC-4	0.229	0.210	0.177	0.138	0.189±0.004	0.120	0.170	0.129	0.097	0.129±0.013
CB-336	0.095	0.201	0.132	0.171	0.150±0.004	0.062	0.177	0.154	0.173	0.142±0.013
CB-333	0.131	0.223	0.162	0.152	0.167±0.004	0.141	0.201	0.138	0.111	0.148±0.013

Ascorbate peroxidase: Ascorbate peroxidase (APX) uses ascorbate as a reducing agent to catalyze the conversion of hydrogen peroxide to water. It plays a key role in regulating hydrogen peroxide level and it's signaling in plant cells. It can be found in many plant tissues even in the absence of any stress. The main function of APX is to protect chloroplast against reactive oxygen species. Table 3 showed that WC-3 had maximum APX activity whereas WC-1 had minimum activity under treatment T_1 i.e. 2nd day control, under controlled condition. The values of APX varied from 0.032 to 0.845. Wheat genotype Chenab-70 had the highest APX activity under 2nd level of treatment T_2 i.e. 4th day control and Lasani-08 showed the lowest activity with values ranging from 0.019 to 0.699. Under the treatment T_3 i.e. 6th day control Pak-81 illustrates maximum activity while C-273 showed minimum APX activity with array of values from 0.051 to 2.523. In treatment T_4 i.e. 8th day control Mexipak-65 and Punjab-96 varieties had extreme and least APX activity respectively. The maximum and minimum values of fourth treatment T_4 lie between 0.019-1.114.

In Table 3 the treated wheat genotypes revealed that Lyalpur-73 has maximum APX activity and CB-336 showed minimum activity under treatment T_1 i.e. 2nd day treatment depicting range of 0.105-0.917. The genotypes under treatment T_2 i.e. 4th day treatment showed that Blue silver and CB-336 had maximum and minimum APX activity respectively. The values ranged between 0.025-0.699. Four genotypes i.e. Mexipak-65, PARI-73, SA-75 and Pak-81 had the highest activity on treatment T_3 i.e. 6th day treatment, whereas WC-1 had the lowest APX activity. Ascorbate peroxidase can convert hydrogen peroxide into water molecules. Genotypes having higher APX activity had ability to resist under soil moisture stress condition

Catalase: Catalase is a member of the peroxidase family that specifically uses hydrogen peroxide as a substrate and decomposes hydrogen peroxide and oxygen. It is located in the cellular organelle called peroxisome. Table 4 depicted that wheat genotypes (Pak-81) had higher CAT activity under treatment T_1 i.e. 2nd day control whereas Parwaz-94 had lower activity. The values of CAT activity ranged from -0.024 to 0.063. In treatment T_2, 4th day control, Lyalpur-73 and Inqilab-91 showed maximum and minimum CAT activity respectively. The values lie between -0.012 and 0.493. The wheat genotype (Chenab-70) had higher CAT activity under treatment T3, 6th day control whereas Blue silver has lower activity with the range of 0.075 to -0.028. The genotypes varied from 0.071 to -0.017 in treatment T_4 i.e. 8th day control. Maximum CAT activity had shown by the Chenab-70 and minimum activity shown by the Lyalpur-73.

Table 3. APX activity of wheat genotypes at various intervals under stress and controlled conditions.

Genotypes	Control					Treated				
	2d c	4d c	6d c	8d c	Mean ± S.E.	2d t	4d t	6d t	8d t	Mean ± S.E.
Mexipak-65	0.380	0.260	2.398	1.114	1.038±0.051	0.471	0.398	3.000	0.415	1.071±0.061
Chenab-70	0.155	0.699	1.921	0.071	0.712±0.051	0.478	0.398	2.523	0.187	0.897±0.061
Blue silver	0.301	0.347	2.046	0.222	0.729±0.051	0.917	0.699	2.699	0.301	1.154±0.061
PARI-73	0.301	0.398	1.959	0.301	0.740±0.051	0.478	0.602	3.000	0.301	1.095±0.061
Lyalpur-73	0.523	0.347	2.155	0.138	0.791±0.051	0.917	0.347	2.097	0.342	0.926±0.061
SA-75	0.398	0.125	2.046	0.138	0.677±0.051	0.419	0.130	3.000	0.301	0.963±0.061
Yecora	0.523	0.087	2.301	0.138	0.762±0.051	0.889	0.109	2.699	0.321	1.005±0.061
Pak-81	0.398	0.060	2.523	0.176	0.789±0.051	0.491	0.083	3.000	0.301	0.969±0.061
Kohinoor-83	0.699	0.106	2.155	0.264	0.806±0.051	0.712	0.131	2.398	0.342	0.896±0.061
Chakwal-86	0.398	0.106	2.155	0.157	0.704±0.051	0.538	0.428	2.097	0.266	0.832±0.061
Pasban-90	0.398	0.06	2.301	0.266	0.756±0.051	0.646	0.106	2.699	0.339	0.948±0.061
Inqilab-91	0.523	0.063	0.155	0.234	0.244±0.051	0.538	0.138	0.097	0.339	0.278±0.061
Parwaz-94	0.301	0.041	0.398	0.357	0.274±0.051	0.412	0.058	0.523	0.357	0.338±0.061
Kohsar-95	0.523	0.145	0.523	0.222	0.353±0.051	0.588	0.212	0.046	0.222	0.267±0.061
Punjab-96	0.359	0.106	2.097	0.019	0.645±0.051	0.646	0.248	2.301	0.301	0.874±0.061
Kohistan-97	0.523	0.106	2.155	0.301	0.771±0.051	0.767	0.131	2.097	0.333	0.832±0.061
Auqab-2000	0.398	0.131	1.921	0.406	0.714±0.051	0.572	0.157	2.155	0.415	0.825±0.061
GA-2002	0.301	0.106	1.959	0.301	0.667±0.051	0.409	0.248	2.699	0.447	0.951±0.061
Seher-06	0.249	0.248	2.155	0.258	0.728±0.051	0.478	0.321	2.097	0.421	0.829±0.061
Lasani-08	0.301	0.019	2.046	0.333	0.675±0.051	0.38	0.106	2.155	0.368	0.752±0.061
C-273	0.067	0.160	0.051	0.339	0.154±0.051	0.114	0.211	0.176	0.682	0.296±0.061
Faisalabad-08	0.243	0.211	0.109	0.128	0.173±0.051	0.301	0.415	0.255	0.33	0.325±0.061
LLR-10	0.447	0.214	0.176	0.167	0.251±0.051	0.544	0.255	0.176	0.282	0.314±0.061
LLR-11	0.192	0.214	0.109	0.125	0.160±0.051	0.368	0.176	0.301	0.479	0.331±0.061
LLR-12	0.105	0.141	0.118	0.271	0.159±0.051	0.301	0.176	0.146	0.398	0.255±0.061
LLR-13	0.105	0.079	0.176	0.105	0.116±0.051	0.67	0.141	0.208	0.301	0.330±0.061
LLR-14	0.105	0.079	0.118	0.032	0.084±0.051	0.145	0.079	0.169	0.094	0.122±0.061
WC-1	0.032	0.025	0.194	0.101	0.088±0.051	0.192	0.051	0.036	0.105	0.096±0.061
WC-2	0.368	0.051	0.319	0.217	0.239±0.051	0.192	0.079	0.284	0.13	0.171±0.061
WC-3	0.845	0.141	0.284	0.184	0.364±0.051	0.243	0.176	0.495	0.1	0.254±0.061
WC-4	0.447	0.051	0.146	0.06	0.176±0.051	0.192	0.051	1.022	0.041	0.327±0.061
CB-336	0.146	0.025	0.225	0.327	0.181±0.051	0.105	0.025	0.419	0.552	0.275±0.061
CB-333	0.105	0.025	0.408	0.034	0.143±0.051	0.243	0.5	0.255	0.199	0.299±0.061

The wheat genotypes having treatment are shown in Table 4. In treatment T_1, 2nd day treatment Lyalpur-73 had higher whereas Parwaz-94 had lower CAT activity. The range of values under treatment T_1 is 0.509 to -0.007. In wheat genotype (Mexipak-65) showed maximum CAT activity while two wheat genotypes that are Kohsar-95 and C-273 showed minimum activity under treatment T_2 i.e. 4th day treatment with the range of 0.64 to -0.009. Under treatment T3 i.e. 6th day treatment WC-2 and Lyalpur-73 showed maximum and minimum CAT activity respectively and the values lie between -0.017-0.09. The wheat genotypes in treatment T_4 i.e. 8th day treatment showed minimum CAT activity for Lyalpur-73 while maximum CAT activity in Chenab-70 with the range -0.017 to 0.071.

Catalase enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen. The genotypes having higher CAT activity can sustain under soil moisture stress condition. The increased activity of catalase might be due to enhance super oxide dismutase activity (Casano *et al.,* 1999). CAT activities declined with progress of water stress in most species, thus favoring the accumulation of H_2O_2. A similar decline of CAT activity also has been reported in water-stressed corn (Zhang *et al.,* 1990), rice (Dwivedi *et al.,* 2010), *Vigna catjang* (Mukherjee & Choudhuri, 1983) and Sunflower (Quartacci & Navari-Izzo, 1992).

Table 4. CAT activity of wheat genotypes at various intervals under stress and controlled conditions.

Genotypes	Control					Treated				
	2d c	4d c	6d c	8d c	Mean ± S.E.	2d t	4d t	6d t	8d t	Mean ± S.E.
Mexipak-65	0.033	0.045	0.006	-0.011	0.018±0.005	0.076	0.640	-0.011	-0.011	0.174±0.006
Chenab-70	0.013	-0.011	0.075	0.071	0.037±0.005	0.001	0.052	0.071	0.071	0.049±0.006
Blue silver	0.001	0.043	-0.028	0.042	0.015±0.005	0.014	0.006	0.042	0.042	0.026±0.006
PARI-73	0.023	0.032	0.013	0.008	0.019±0.005	0.018	0.022	0.008	0.008	0.014±0.006
Lyalpur-73	0.048	0.493	-0.022	-0.017	0.126±0.005	0.509	0.029	-0.017	-0.017	0.126±0.006
SA-75	0.019	0.014	0.019	0.012	0.016±0.005	0.029	0.007	0.012	0.012	0.015±0.006
Yecora	0.027	0.020	0.004	0.027	0.020±0.005	0.059	0.034	0.027	0.027	0.037±0.006
Pak-81	0.063	0.010	0.019	0.024	0.029±0.005	0.028	0.025	0.024	0.024	0.025±0.006
Kohinoor-83	-0.011	0.087	0.042	0.009	0.032±0.005	0.030	0.037	0.009	0.009	0.021±0.006
Chakwal-86	0.032	0.017	0.031	0.041	0.030±0.005	0.023	0.016	0.041	0.041	0.030±0.006
Pasban-90	0.016	0.010	0.030	0.013	0.017±0.005	0.039	0.048	0.013	0.013	0.028±0.006
Inqilab-91	0.028	-0.012	0.044	0.022	0.021±0.005	0.027	0.032	0.022	0.022	0.026±0.006
Parwaz-94	-0.024	0.027	0.005	0.029	0.009±0.005	-0.007	0.006	0.029	0.029	0.014±0.006
Kohsar-95	0.022	0.487	0.014	0.011	0.134±0.005	0.030	-0.009	0.011	0.011	0.011±0.006
Punjab-96	0.019	-0.009	0.005	0.016	0.008±0.005	0.015	0.041	0.016	0.016	0.022±0.006
Kohistan-97	0.030	0.025	0.009	0.013	0.019±0.005	0.007	0.012	0.013	0.013	0.011±0.006
Auqab-2000	0.032	0.017	0.023	0.005	0.019±0.005	0.047	0.007	0.005	0.005	0.016±0.006
GA-2000	0.019	0.003	0.018	0.028	0.017±0.005	0.009	0.021	0.028	0.028	0.022±0.006
Sehar-06	-0.012	0.002	0.011	-0.002	0.000±0.005	0.004	0.035	-0.002	-0.002	0.009±0.006
Lasani-08	-0.014	0.010	-0.012	0.018	0.001±0.005	0.007	0.010	0.018	0.018	0.013±0.006
C-273	0.001	-0.003	0.023	0.022	0.011±0.005	0.005	-0.009	0.023	0.003	0.006±0.006
Faisalabad-08	0.008	0.042	-0.001	0.034	0.021±0.005	0.007	-0.005	0.040	0.021	0.016±0.006
LLR-10	-0.023	0.006	0.013	0.013	0.002±0.005	0.007	-0.003	0.015	0.027	0.012±0.006
LLR-11	0.001	0.020	0.041	-0.005	0.014±0.005	0.023	0.022	0.031	0.007	0.021±0.006
LLR-12	0.017	0.014	0.013	0.025	0.017±0.005	0.01	0.028	0.02	0.015	0.018±0.006
LLR-13	0.011	0.013	0.023	0.024	0.018±0.005	0.013	0.027	0.012	0.039	0.023±0.006
LLR-14	-0.01	0.018	0.006	0.012	0.007±0.005	0.019	0.031	0.038	0.022	0.028±0.006
WC-1	0.023	0.042	0.047	0.042	0.039±0.005	0.056	-0.001	0.064	0.004	0.031±0.006
WC-2	0.043	0.039	0.034	0.038	0.039±0.005	0.026	0.027	0.09	-0.001	0.036±0.006
WC-3	-0.005	0.013	-0.006	0.003	0.001±0.005	0.021	0.004	0.036	0.042	0.026±0.006
WC-4	-0.011	0.03	0.022	0.034	0.019±0.005	0.021	0.037	0.002	0.008	0.017±0.006
CB-336	0.011	0.022	0.021	0.028	0.021±0.005	0.003	0.008	0.021	0.019	0.013±0.006
CB-333	-0.024	0.008	0.038	-0.015	0.002±0.005	0.006	0.019	0.017	0.02	0.016±0.006

Conclusion

In present study the wheat genotypes showed significant response to moisture stress by showing varying level of anti-oxidative enzyme activity. Genotypes; Inqilab-91, Kohinoor-83, WC-3, WC-4, CB-333 and C-273 are identified as highly responsive against moisture stress conditions by showing high anti-oxidative enzyme activity. The genotypes identified through this study may be used in wheat breeding program for the development of drought tolerant material. However, it is recommended that a large number of wheat genotypes should be assessed at various stages of plant growth for anti-oxidative enzyme activity against drought stress conditions.

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