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Effect of water stress on oxidative damage and antioxidant enzyme activity of bread wheat genotypes

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Abstract: A field experiment as a split plot based on RCB design with four replications in 2008-2009 was carried out to investigate the effect of different irrigation treatments (70, 100, 130 and 160 mm evaporation from the class A pan) on oxidative injuries and antioxidant activity of 10 wheat. Results showed that C-81-10, C-84-9 and C-83-8 were water deficit stress tolerant compared with other genotypes. Water deficit stress significantly increased activities of catalase, superoxide dismutase and glutathione peroxidase. 8-hydroxyl-2-deoxyguanosine, MDA and dityrosine contents also increased significantly at the mild and extreme water deficit. Tolerant genotypes (C-83-8 and C-84-9) had the highest antioxidant activity under water stress. Grain yield had negative and significant correlation with 8-hydroxyl-2-deoxyguanosine, catalase, SOD and glutathione peroxidase. Thus, enzymatic antioxidants played an important role in scavenging harmful oxygen species in tolerated wheat genotypes which resulted in greater grain yield and selection of tolerated genotypes under stressful condition.

Key words: *Antioxidant enzymes; Oxidative injury; Water deficit; Wheat*

1. Introduction

Drought is one of the most severe limitations on the yield of crops. This stress induces various biochemical and physiological responses in plants as a survival mechanism (Tas and Tas, 2007). In general, drought is responsible for several metabolic processes of plants, with photosynthetic apparatus (Nayyar and Gupta, 2006). Besides changes in photosynthesis, such adverse effects on metabolism lead to growth inhibition, stomata closure with consecutive reduction of transpiration, which are considered necessary for coping with osmotic changes in their tissues (Yordanov et al., 2003).

Water stress leads to the formation of Reactive Oxygen Species (ROS), which are extremely harmful to the plants. Generation of ROS also leads to lipid peroxidation (Chen et al. 2000). During water stress, there is considerable potential for increased accumulation of superoxide and hydrogen peroxide resulting from the increased rate of O_2 photo-reduction in chloroplasts (Robinson and Bunce, 2000). Mechanisms of ROS detoxification exist in all plants (Mundree et al., 2002). One of the defense mechanisms against different stresses is the antioxidant enzymes production. Plants to prevent or alleviate injuries from ROS have evolved an antioxidant defense system that includes non-enzymatic compounds, like ascorbate, glutathione, tocopherol, carotenoids, flavonoids and enzymes such as SOD, CAT, POX, APOX, GR and PPO (Gratao et al., 2005).

Changes of antioxidants reflect the impact of environmental stresses on plant metabolism (Herbinger et al., 2002). The level of response depends on the species, the development and the metabolic status of the plant, as well as the duration and intensity of the stress. In addition, the degree of damage by ROS depends on the balance between the product of ROS and its removal by this antioxidant scavenging mechanism (Azooz and Al-Fredan, 2009). On the other hand, it has been reported that membrane of plant cells are subject to rapid damage with increase in water stress. This leakage of membrane is caused by an uncontrolled enhancement of free radical, which cause lipid peroxidation. Damage to fatly acids of membrane could produce small hydrocarbon fragments including Malondialdehyde (MDA). MDA is the final product of plant cell membrane lipid peroxidation and is one important sign of membrane system injury (Cunhua et al., 2010). Mohammadi et al. 2014 reported that barley genotypes under terminal water stress condition were produced greater oxidant and antioxidant enzymes rather than those under full irrigation condition, so tolerant barley genotypes had less oxidant and greater antioxidant enzymes than susceptible barley genotypes. Thus, this research was designed to investigate the genotypes

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for cultivation in environments with limited water availability.

2. Materials and methods

The experiments were conducted in 2008-2009 at the Miyandoab Agricultural and Natural Resource Research Station (Latitude 36˚58' N, Longitude 46˚6' E, Altitude 1314 m). Soil texture at the 30 cm depth was clay loam with pH=7.5-8 and EC= 2 dSm^{-1}. The experiment was arranged as strip-plot based on RCB design with 4 replications. Irrigation was uniform until heading for all plots, then irrigation treatment of 70, 100, 130 and 160 mm evaporation from Class A pan were applied after heading stage as main plot, and 10 wheat genotypes of Zarrin, Shahriyar, Alvand, Sardari, C-80-4, C-81-10, C-81-4, C-83-3, C-83-8 and C-84-9 were allocated as subplots. Each plot consisted of 6 rows with 4 m length and 20 cm apart. Seeds were sown with a density of 450 seeds m^{-2}. Seedbed preparation, fertilizers used, weed control for all treatments were similar. Grain yield for each plot at physiological maturity harvested. Samples for measurement metabolites harvested at 70% of each irrigation treatment. To quantify antioxidant enzymatic activity, fifteen leaf samples were taken randomly. In order to prepare samples for the enzyme assays and protein measurement, the leaves from each plant were washed with distilled water and homogenized in a 0.16M Tris buffer (pH=7.5) at 4°C. Then, 0.5 ml of total homogenized solution was used for protein determination using the Lowry et al. (1951) method. Catalase (CAT), Glutathione peroxidase (GPX) activity using the Paglia and Valentine (1987), Superoxide dismutase (SOD) activity by Dhindsa et al. (1981), Lipid peroxidation (MDA) by Sairam et al. (1998), 8-Hydroxy-2-Deoxyguanosine (8-oHdg) by Bogdanov et al. (1999), Protein damage (dityrosine content) by Amado et al. (1984) method were measured. Analysis of variance was performed using the MSTATC and SPSS programs. Means were compared using the Duncan test at the 5% probability level.

3. Results

3.1. Grain yield

The analysis of variance of data showed significant effects of irrigation and genotype on wheat grain yield. Interaction of irrigation × genotype was also significant (Table 1). The increase in irrigation period from 70 mm (5.927 t/ha) to 100 mm (5.227 t/ha), 130 mm (4.259 t/ha) and 160 mm (4.121 t/ha) decreased grain yield by 12, 28 and 30%, respectively. Under irrigation after 70 and 100 mm evaporation, there was no significant difference among local cultivar and promising lines. Since grain yield of Sardari as a dry land cultivar was lower than other genotypes. Grain yield of promising lines C-81-10 (5.031 t/ha), C-80-4 (4.762 t/ha), C-83-8 (4.764 t/ha) and YET-84-9 (5.202 t/ha) was greater than Zarrin (4.125 t/ha) under irrigation after 130 mm evaporation. Also under 160 mm irrigation treatment, C-83-8 (4.755 t/ha), C-84-9 (4.919 t/ha) and C-81-10 (5.341 t/ha) had greater grain yield in comparison with Zarrin (3.999 t/ha) (Fig.1).

Table 1: Analysis of variance of the effect of different irrigation treatments on wheat genotypes biochemical characteristics

S.O.V	d.f	MS						
		Grain yield	8-oHdg	MDA	Dityrosine	GPX	CAT	SOD
Replication	3	0.9	6.01	108.8	21.02	312.1	99.72	1935.4
Irrigation (I)	3	29.02**	7542**	28164**	3407**	175943**	110607**	4494425**
Error	9	0.04	15.3	25.5	1.51	123.51	171.7	3567.2
Genotype (G)	9	7.5**	2861**	9289**	1727**	15081.5**	9414.8**	454216**
I×G	27	1.06**	83.3 ns	230.2 ns	47.1**	568.2**	849.3**	18331.4**
Error	108	0.27	76.6	165.86	20.5	248.83	248.02	8906.2
C.V (%)	-	27	19.76	14.72	15.34	8.15	10.40	9.45

ns, **: No significant and significant at p≤0.01, respectively.

3.2. hydroxy-2-deoxyguanosine (8-oHdg)

8-oHdg was significantly influenced by irrigation and wheat genotype. However, interaction of irrigation × genotype was not statistically different (Table 1). The highest (63.9 nmol. mg^{-1} protein) and the lowest (63.9 nmol. mg^{-1} protein) 8-oHdg content were observed for severe water deficit (160 mm) and favorable irrigation condition (70 mm), respectively. 8-oHdg content increased as water availability decreased from 70 to 160 mm (Table 2). Among genotypes, C-84-9 and Shahriyar had the highest and the lowest 8-OHdG content, respectively (Table 2).

3.3. Lipid peroxidation (MDA content)

Water stress and genotype had significant effects on MDA content of wheat leaves. Interaction of irrigation × genotype for MDA content yield was not significant (Table 1). Significant increase in MDA content was detected with decreasing water availability. The highest MDA content was obtained for C-80-4 and C-84-9 (Table 2).

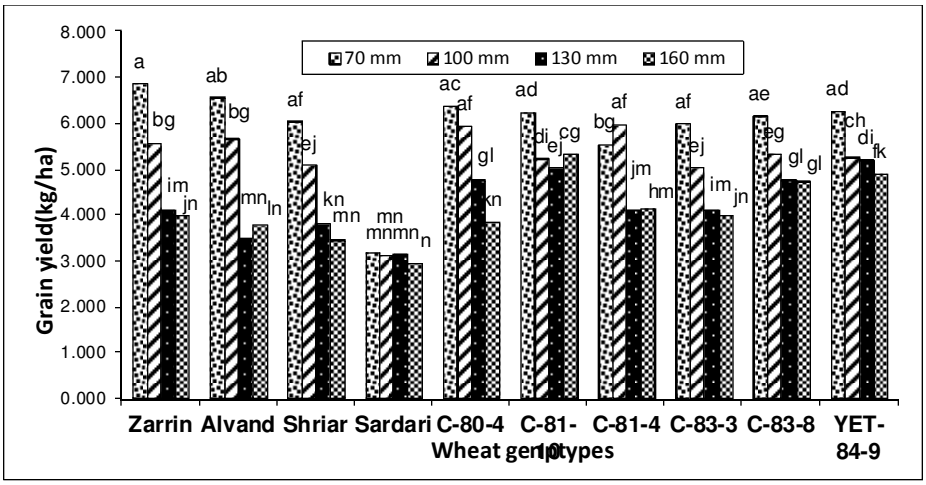

Fig. 1: Mean grain yield of wheat genotypes under different irrigation levels

Table 2: Means of biochemical parameters of wheat genotypes affected by different irrigation treatments

Treatments	(8-OHdG) (nmol. mg^{-1} protein)	(MDA) (nmol. mg^{-1} protein)
Irrigation		
70	33.00 d	63.15 d
100	37.05 c	74.72 c
130	43.17 b	87.80 b
160	63.90 a	124.32 a
Genotype		
Zarrin	58.31 b	97.31 b
Shahriyar	28.31 e	59.62 d
Alvand	28.94 e	62.50 d
Sardari	50.12 cd	96.12 b
C-80-4	33.18 e	117.31 a
C-81-10	47.50 d	61.50 d
C-81-4	31.12 e	66.50 d
C-83-3	44.19 d	85.37 c
C-83-8	55.50 bc	104.43 b
C-84-9	65.62 a	124.31 a

Different letters at each column indicate significant difference at p≤ 0.05

3.4. Protein damage (dityrosine content)

The dityrosine levels were found to be significantly higher under different water deficit treatments (100, 130 and 160 mm) than that under favorable irrigation (70 mm) (Fig.2). Under optimum irrigation condition the highest and lowest dityrosine content were observed for C-80-4 (34.17 nmol.mg^{-1}protein) and Shahriyar (12.83 nmol.mg^{-1} protein), respectively. With increasing water deficit severity from 70 to 100 and 130 mm, the greatest increase recorded for C-81-10 with 21% and 41%, respectively. C-83-3 under severe water deficit (160 mm) showed the highest increase (60%) of dityrosine content.

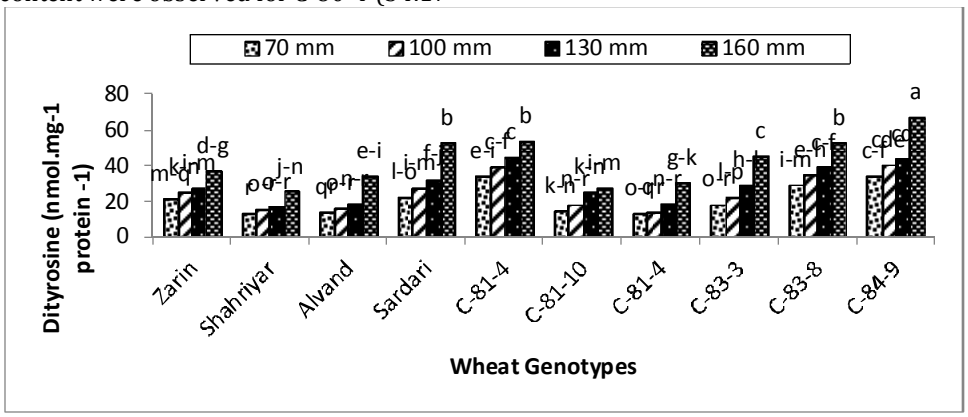

Fig. 2: Dityrosine content of wheat genotypes affected by different irrigation treatments

Average increase of dityrosine of all genotypes under different water deficit (100, 130 and 160 mm) compared with 70 mm were 14%, 25% and 50, respectively (Fig.2).

3.5. Antioxidaive enzyme activities

Under favorable irrigation condition (70 mm), difference among genotypes in point of GPX content was 55 nmol. mg^{-1} protein (Shahriyar and C-81-10 with 123 nmol.mg^{-1} protein, and the highest C-84-9 with 178 nmol.mg^{-1}protein). As the severity of water deficit was increased, the activity of GPX increased markedly in all ten genotypes. The highest increase in GPX activity was obtained for Sardari (331 nmol. mg^{-1} protein) and C-84-9 (349 nmol. mg^{-1} protein). But the highest increase in GPX content under severe water deficit (160mm) compared to 70 mm, belonged with Alvand (54%), Sardari (54%), C-81-4 (54%), C-83-3 (53%) and C-81-10 (51%) (Fig.3).

Water stress had different effect on studied genotypes. Under irrigation after 70, 100, 130 and 160mm, the greatest CAT content recorded for C-84-9 with 144, 166, 176 and 317 nmol.mg^{-1} proteins, respectively. With increasing the severity of water stress, the highest increase in CAT content under irrigation after 100, 130 and 160mm compared with 70 mm, obtained for C-81-10 (17%), C-81-4 (28%) and Sardari (57%), respectively. With deduction of water availability from 130 to 160 mm, Sardari and C-80-4 genotypes showed the highest increase in CAT content (45%), while this increase for C-81-10 was 22% (Fig.4).

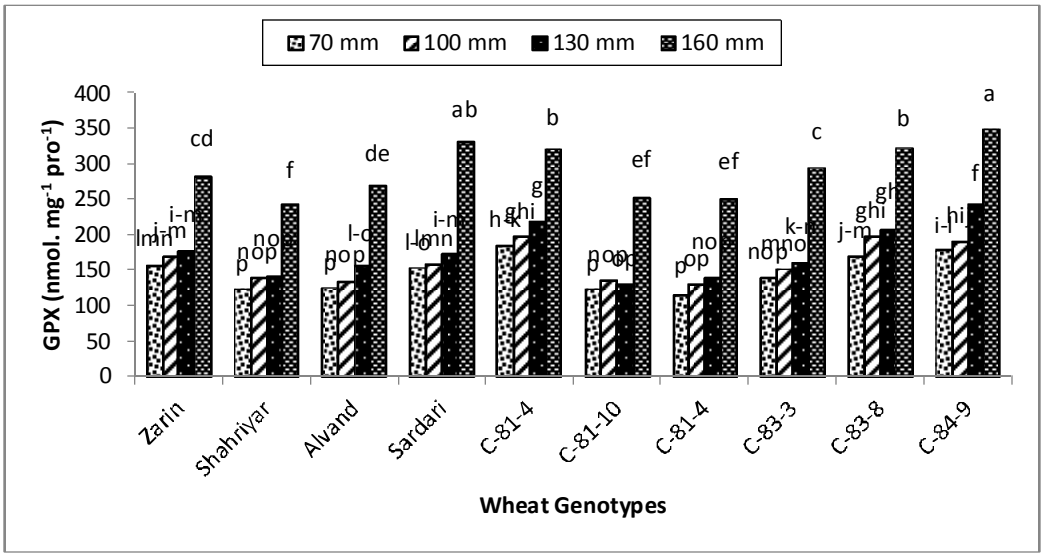

Fig. 3: Glutathion peroxidase (GPX) activity of wheat genotypes under different irrigation treatments

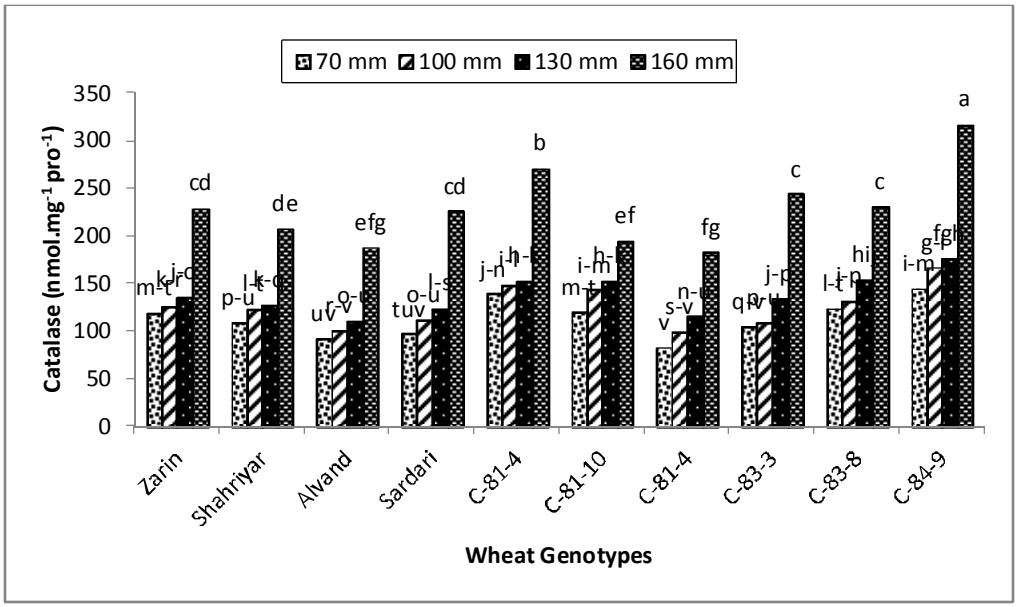

Fig. 4: Catalase (CAT) activity in 10 wheat genotypes affected by different irrigation treatments

The highest SOD activity under favorable irrigation treatment (70 mm) were achieved by C-80-4 (933 nmol. mg^{-1}protein) and C-84-9 (943 nmol. mg^{-1}protein) and under severe water limitation (160mm), the highest SOD activity belonged with C-84-9 (1897 nmol.mg^{-1}protein) and Sardari (1712 nmol.mg^{-1}protein) (Fig.5). The highest increase in SOD content under different water stress (100, 130 and 160 mm) in comparison with favorable watering (70 mm) was achieved by C-81-4 (18%), C-81-4 (28%), C-84-9 (28%), and Alvand (55%), respectively.

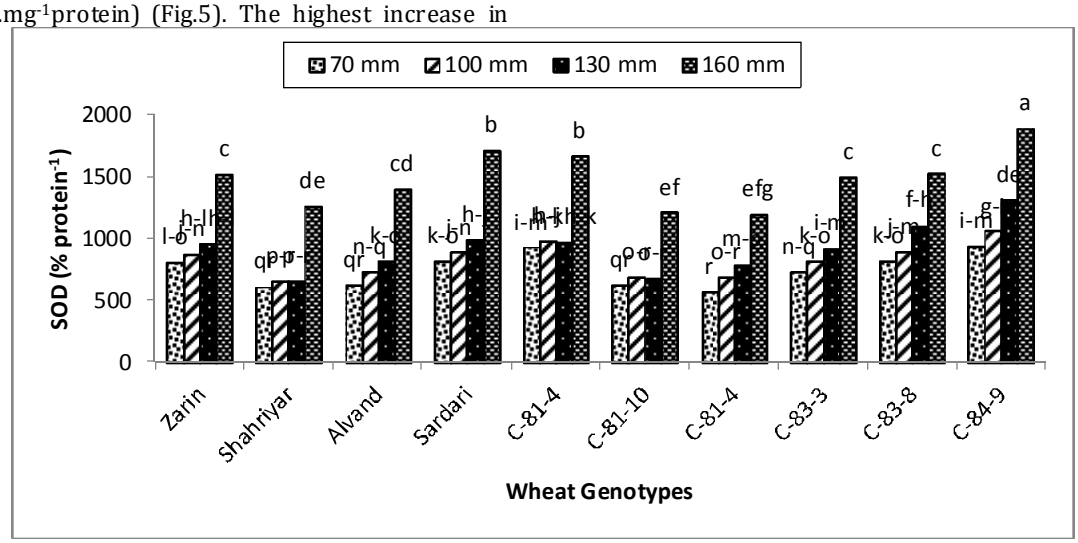

Fig. 5: Superoxide dismutase (SOD) activity of wheat genotypes under different irrigation treatments

8-oHdg, dityrosine content, MDA content, GPX, CAT and SOD activities had significant and positive correlations with each other. However, all these traits showed negative correlations with grain yield. GPX and SOD activities showed the highest negative correlations with grain yield (Table 3).

Table 3: Correlation coefficients among some biochemical parameters of wheat

Traits	1	2	3	4	5	6	7
1. 8-oHdg (nmol. mg-1 pro^{-1})	1						
2. Dityrosine (nmol. mg-1 pro^{-1})	0.675**	1					
3. MDA (nmol. mg-1 pro^{-1})	0.742**	0.951**	1				
4. GPX (nmol. mg-1 pro^{-1})	0.743**	0.867**	0.918**	1			
5. CAT (nmol. mg-1 pro^{-1})	0.748**	0.810**	0.840**	0.921**	1		
6. SOD (%pro^{-1})	0.774**	0.871**	0.925**	0.982**	0.922**	1	
7.Grain yield (Kg/ha)	-0.260**	-0.278**	-0.327**	-0.360**	-0.265**	-0.400**	1

**: significant at p≤0.01

4. Discussion

Greater grain yield of C-83-8, C-84-9 and C-81-10 (Fig.1) shows that these genotypes could be cultivated in regions that face to terminal water stress. Evaluation 10 genotypes based on tolerance indices showed that C-81-10, C-84-9 and C-83-8 were water deficit tolerant compared with the other genotypes. Water deficit can cause oxidative damages. Therefore, plant cells need different mechanisms that will enable the detoxification of excess ROS and keep the formation and removal of ROS in balance. CAT is the principal enzyme that scavenges harmful oxygen species in plants (Pereira et al., 2002). The slight increment in activity of antioxidant enzymes under mild water stress conditions (100 and 130 mm) can be explained by the very low affinity of them for free radicles; they become active at relatively high free radicles concentrations (Gechev et al., 2006). The antioxidant activity of GPX, CAT and SOD in particular play protective role for drought tolerance in all genotypes especially in Sardari, C-81-4 and C-84-9 (Fig.2, 3 and 4).

The data indicated that the responses of some physiological parameters symptomatic for oxidative stress and the related enzymes strongly depend on the severity of water deficit stress. Also, our results clearly demonstrated a wide variation in water deficit tolerance in wheat genotypes. They did differ significantly for water deficit stress injury in their lipid and protein oxidation (MDA and dityrosine content), antioxidant enzymes (CAT, SOD, GPX), and 8-hydroxy-2-deoxyguanosin contents at moderate and extreme levels of water deficit stress (100, 130 and 160 mm). The increase in MDA levels under drought stress suggests that water stress could cause the formation of membrane lipid peroxidation by means of ROS production (Sairam et al., 2000). When plants are subjected to environmental stresses

involving drought, ROS production overcomes antioxidant system capacity, and oxidative stress occurs, which results in cytotoxic protein damage, DNA damage, and lipid peroxidation (Yazici et al., 2007).

Under water deficit conditions (100, 130 and 160 mm), antioxidant enzyme increased in all of the genotypes. Results indicated that, activities of the antioxidants (CAT, SOD, GPX) were increased in all of the genotypes and all levels of water deficit stress, but antioxidants content in tolerant genotypes(C-83-8 and C-84-9) were greater than the susceptible genotypes (Shahriyar and C-81-4). Induction of oxidative stress in drought-stressed plants reported in the previous studies (Borrmann et al., 2009; Manavalan et al., 2009). They showed that enzymatic antioxidants content played an important role in scavenging harmful oxygen species and the activities of antioxidant enzymes were altered when plants were subjected to stress. Results of our research also showed that, the content of antioxidants were higher at level of extreme water deficit stress than moderate (160>130>100mm). This subject would be explained such away, when crops are exposed in sever water deficit stress conditions, their antioxidant defensive mechanism is activated and the content of antioxidants will rise in them. Results indicate the same trend, too. Previously, an increase in the level of antioxidants was reported with an increase in stress intensity in maize and soybean by Vasconcelos et al. (2009) and Jiang and Zhang (2002) which might be attributed to inhibitory effects of water stress on protein turnover causing depletion of antioxidants.

High and significant correlations of GPX and SOD activities with grain yield (Table 3) indicate that these parameters can be used to estimate potential field performance of wheat under different irrigation conditions. These results showed that dityrosine and antioxidants CAT, GPX and SOD simultaneously increased, also when severity of water deficit stress increased (100, 130 and 160 mm), all the oxidants and antioxidants increased. Enzymatic antioxidants content in tolerant genotypes (C-83-8 and C-84-9) were greater than the other genotypes, these results showed enzymatic antioxidants content played an important role in scavenging harmful oxygen species and caused that tolerant genotypes had greater ability in point of biomass and grain yield under water deficit stress. Moreover, Lee et al. (2009) reported a positive and significant correlation between CAT, SOD and ascorbate peroxidase (APX) under both conditions of well irrigated and water deficit stress conditions. The MDA and dityrosine contents in leaves increased markedly to a higher extent in all of the genotypes at all levels of water deficit stress. Among genotypes and in the extreme water deficit (160 mm), the lowest dityrosine contents were observed in Shahriyar. This might explain lower lipid and protein oxidation of this cultivar relative to other cultivars. In other words, this cultivar appeared to have experienced less oxidative damage as compared to other cultivars, which is perhaps due to its superior capacity to counter the oxidative stress as well as higher water content. The same results reported by Dolatabadian et al. (2008), who showed that salt stress increased lipid peroxidation (MDA content) in canola cultivars.

5. Conclusion

Our results clearly demonstrate that all tested wheat genotypes responded positively with respect to antioxidant enzymes to water stress conditions. In all of wheat genotypes, enzymatic antioxidant defense systems were activated in response to the increase in intensity of water deficit stress by a significant increase in antioxidants content. The elevation of MDA and dityrosine in our experiment could be a direct reflection of an oxidative injury of the cells after water deficit stress. Furthermore, it was observed that, genotypes with higher lipid and protein oxidation had higher levels of antioxidant activities. This may be due to the protective effect of antioxidant enzymes on the membranous structure in cells. Tolerant genotypes (C-83-8 and C-84-9) had greater grain yield and enzymatic antioxidant especially in saver water deficits stress. Finally, results showed that antioxidant enzymes of CAT, GPX and SOD could be used in selection of tolerant wheat genotypes to water deficit stress.

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