

# The Effect of Water Deficit and Nitrogen on the Antioxidant Enzymes' Activity and Quantum Yield of Barley (*Hordeum vulgare* L.)

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## Abstract

The effects of water deficit and nitrogen fertilizer were studied on antioxidant enzymes activity and quantum yield of barley. An experiment carried out in greenhouse in factorial subject based on a completely randomized design with three replications. Irrigation schedules imposed at three levels of 85%, 60% and 35% field capacity (FC), and nitrogen were applied in quantities of 40, 80 and 120 kg N ha<sup>-1</sup>. We determined Catalase (CAT), Peroxidase (POX) Polyphenol oxidase (PPO) activities, proline, chlorophyll and carotenoid content, quantum yield and grain yield. The results showed that severe stress (35% FC) increased the activities of CAT, POX and PPO enzymes and proline content, whereas the carotenoids, chlorophyll *a* and chlorophyll *b* decreased. Water deficiency caused the reduction in the quantum yield and the grain yield by 34%. Application of 80 kg of N during stress treatments resulted in higher enzyme activity and proline content. High amount of nitrogen reduced carotenoids, chlorophyll *a*, chlorophyll *b*, and in contrast, enhanced quantum yield. Application of 120 kg N ha<sup>-1</sup> increased the yield up to 37% under mild stress (35% FC). Correlation coefficient and path coefficient showed that, grain yield was affected directly by amount of carotenoids and quantum yield.

**Keywords:** catalase, grain yield, peroxidase, polyphenol oxidase, proline

## Introduction

Barley (*Hordeum vulgare* L.) is a resistant plant to salinity and water deficit and has a high application in human nutrition and malt industry. The water shortage is the main factor of crop yield reduction in semi-arid areas (Goodroad and Jellum, 1988). Water deficiency stress led to increase in amounts of reactive oxygen species (ROS) such as superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (HO<sup>•</sup>) and singlet oxygen (O<sub>2</sub>) in plant cell. These active molecules, as a single molecule, cause severe damage to macro molecules and the cell structure or induce some defensive responses at the different molecular, cellular and/or physiological levels (Ohe *et al.*, 2005; Ariano *et al.*, 2005). Drought reduces cells division and inhibits the cells growth due to the reduction of turgor potential and it, also decreases their water content. Also this stress can damage pigments and plastids, reduce chlorophyll *a*, chlorophyll *b* and other carotenoids, hydrolyze proteins and prevalent photochemical reactions in most plants (Reddy *et al.*, 2004; Valladares and Percy, 1997).

The enzymatic and non-enzymatic mechanisms are involved in plant defensive strategies to face reactive oxygen

species. The enzymes are including superoxide dismutase, glutathione reductase, peroxidase, and catalase and ascorbate peroxidase. In general, the adaptation to drought conditions depends on the amount of reactive oxygen species that are neutralized by the antioxidant system to be kept on relatively low level (Mascher *et al.*, 2005). The cooperation of these components forms the important cycles such as ascorbate-glutathione, and xanthophylls (Mittler *et al.*, 2004). The components of these cycles as a defensive mechanism enable the cells to inhibit the production of ROS or scavenge them and reduce their harmful effects (Asada, 2000). There are some studies on antioxidant defensive systems facing aging and environmental adverse conditions, but the results are very different. While some antioxidant activity in some species can be reduced, the antioxidant activity in other species is increased or remained unchanged (Hodges and Forney, 2000). Ohe *et al.* (2005) reported that the aging reduced oxidizing enzymes including superoxide dismutase, ascorbate peroxidase and catalase in spinach and tobacco leaves. Proline accumulation is the most important contributor osmolytes involved in osmo regulation (Cattivelli *et al.*, 2008). It increases during the stress in the

cytoplasm, and protects the intercellular macro molecules structure, cause osmotic adjustment and maintained the cell turgor potential (Bokhari and Trent, 1985). The aim of this study was to investigate the effects of nitrogen consumption on some physiochemical traits of barley under water deficit condition.

### Materials and methods

This study was conducted as a factorial experiment was carried out based on a completely randomized design (CRD) with three replications under greenhouse condition in 2012. Irrigation schedules imposed at three levels (85%, 60% and 35% FC), and nitrogen applied at 40, 80 and 120 kg N ha<sup>-1</sup>.

#### Quantum yield

The quantum yield was measured by the uppermost fool expanded leaf using a fluorometer (chlorophyll fluorometer; Optic Science-OS-30 USA). For this purpose, the plants adapted to darkness for 20 minutes by using one special clamp then the fluorescence amounts were measured in 1000 ( $\mu\text{M photon m}^2\text{s}$ ), and calculation was performed using following formula (Arnon, 1949):

$$\Phi_{\text{PSII}} = (F_m - F_0) / F_m$$

$\Phi_{\text{PSII}}$ : quantum yield amount of photosystem II,  $F_m$  or maximum fluorescence after a saturated light pulse on plants adapted to darkness and  $F_0$ , the minimal fluorescence in the light adapted, which was determined by illumination with far-red light.

#### Photosynthetic pigment content

Chlorophyll content measured in 0.2 g fresh leaf tissue, which gradually worn with 80% acetone and the solution volume was brought 20 ml using acetone 80%. Then it was centrifuged for 10 minutes at 400 rpm and the absorbance at 645, 663 and 470 nm was recorded by a spectrophotometer. Chlorophyll and carotenoids were obtained based on the following equations (Arnon, 1949):

$$\text{Chlorophyll } a = (19.3 \times A_{663} - 0.86 \times A_{645}) V / 100 W$$

$$\text{Chlorophyll } b = (19.3 \times A_{645} - 3.6 \times A_{663}) V / 100 W$$

$$\text{Carotenoid} = (1000 A_{470} - 1.82 C_a - 85.02 C_b) / 198$$

#### Determination of enzyme activities

Enzyme activity was measured three times during the day (pre-dawn, midday and evening), and every time, 0.2 g of fresh tissue was used for the enzyme activity. In order to extract the protein, 0.2 g of fresh leaf was pulverized in a mortar using liquid nitrogen and 1 ml of Tris -HCl (0.05 M, pH = 7.5) buffer was added. The mixture centrifuged for 20 minutes at 13,000 rpm, at 4 °C and the supernatant were used for determining the enzyme activity (Sudhakar *et al.*, 2001). CAT activity was assayed according to Karo and Mishra (1976). The 60  $\mu\text{l}$  protein extraction was added to Tris buffer (50 mM, pH = 7) and 0.3 ml H<sub>2</sub>O<sub>2</sub> 5 mM in the ice bath, then the absorbance curve was considered at 240

nm. The enzyme activity was obtained for (OD mg<sup>-1</sup> protein min<sup>-1</sup>) from fresh tissue. POX activity was measured as described by Karo and Mishra (1976). The 50  $\mu\text{l}$  protein extracts was added to 2.5 ml extraction buffer, containing 100  $\mu\text{M}$  Tris buffer 100 mM and H<sub>2</sub>O<sub>2</sub> 5 mM and 10 mM pirogalol in the ice bath and absorbance was read at 425 nm. Furthermore, PPO enzyme activity was measured by Karo and Mishra (1976) method, as it follows: 100  $\mu\text{l}$  protein extract was dissolved in 1.5 ml Tris 0.2 M and 0.3 ml pirogalol 0.02 M and the resulting composition was placed in the bain-marie bath at 25 °C for 5 minutes and then the absorbance at 420 nm was recorded. Also, the evaluation of protein carried out by Bradford (1976) method, 0.2 g of plant tissue was squashed with 0.6 ml extraction buffer and was centrifuged at 11,500 rpm for 20 minutes at 4 °C. The supernatant was transferred to the new tubes and centrifuged for 20 minutes at 4,000 rpm. To measure the protein amount, 10  $\mu\text{l}$  of obtained extract was added to 5  $\mu\text{l}$  Bradford solution and 290  $\mu\text{l}$  extraction buffer and the absorbance rate was read at 595 nm.

#### Determination of proline content

In order to measure proline, 0.5 g of plant fresh tissue was crushed in 10 ml sulpho acetic acid solution to obtain a homogeneous mixture. Then, the solution was smoothed using whit-man no. 2 and 2 ml dimenhydrinate reagent and 2 ml glacial acetic acid were added. The extract was mixed and stirred on bain-marie at 100 °C for one hour and then 4 ml toluene added and the extract was vortexed to form two separate phases. The supernatant was read at 520 nm by a spectrophotometer (Batesetal, 1973).

#### Grain Yield

Grain yield was calculated by weighting the grain per plant (g plant<sup>-1</sup>).

#### Statistical Analysis

An experimental design was arranged in factorial in greenhouse, based on a completely randomized design (CRD) with three replications. Means were compared using LSD at Pvalue=5%. Correlation coefficients and path coefficient for chlorophyll *a* and *b*, carotenoid, quantum yield, proline accumulation, CAT, PPO, POX activity and grain yield were analyzed by the method of Dewey and Lu (1959).

### Results and discussion

The water limitation and nitrogen fertilizer significantly affected the photosynthetic pigment content. The highest content of chlorophyll *a* (2.14 mg g<sup>-1</sup> Fw) and chlorophyll *b* (0.95 mg g<sup>-1</sup> Fw) was obtained in non-stress condition (85% FC or control) and 120 kg N ha<sup>-1</sup> respectively. The lowest chlorophyll *a* (1.57 mg g<sup>-1</sup> Fw) and chlorophyll *b* (0.40 mg g<sup>-1</sup> Fw) were determined within severe stress (35% FC) and minimum N (40 kg N ha<sup>-1</sup>). Furthermore, additional nitrogen enhanced the chlorophyll content under stress condition, therefore 120 kg N ha<sup>-1</sup> significantly increased

the chlorophyll content (80% chlorophyll *a* and 33% chlorophyll *b* during 35% FC) in comparison with 40 kg N ha<sup>-1</sup>. Water limitation caused the reduction in carotenoids and PSII quantum yield, while the application of nitrogen increased them. The highest content of carotenoids (9.98 mg g<sup>-1</sup> Fw) and quantum yield (0.83) was observed at 85% (FC) and 120 kg N ha<sup>-1</sup>. The lowest amount of carotenoids (7.11 mg g<sup>-1</sup> Fw) and quantum yield (0.75) was achieved under severe stress treatment (35% FC) and 40 kg N ha<sup>-1</sup> (Tab. 1). Water stress reduced chlorophyll and carotenoids content. Photosynthetic pigments were lost because water

shortage and it might due to the reduction in essential compounds synthesis such as chlorophyll pigment (Arnon, 1949). Degradation of pigment protein complex and oxidative complex causes damage to the chloroplast lipids, pigments and proteins (Tambussi *et al.*, 2005). Application of nitrogen increased the chlorophyll and carotenoids contents, which indicates the nitrogen impact in relieving stress effect. Moran *et al.* (2000) indicated that nitrogen application enhances chlorophyll *a* and *b* and total chlorophyll content. Such results were reported by Singh *et al.* (2012).

Tab. 1. The effect of water deficit and nitrogen on carotenoid, chlorophyll content and quantum yield of barley leaves (data are means of three replications ± standard error)

Treatment		Carotenoid (mg g <sup>-1</sup> Fw)	Chlorophyll <i>a</i> (mg g <sup>-1</sup> Fw)	Chlorophyll <i>b</i> (mg g <sup>-1</sup> Fw)	Φ <sub>PSII</sub>
WD*	N**				
85%	40	7.78±0.055	2.05±0.025	0.68±0.035	0.81±0.001
	80	8.24±0.229	2.13±0.017	0.89±0.003	0.82±0.009
	120	9.98±0.084	2.14±0.052	0.95±0.028	0.83±0.009
60%	40	7.11±0.136	1.95±0.038	0.65±0.023	0.77±0.001
	80	7.58±0.153	2.02±0.020	0.75±0.027	0.79±0.004
	120	8.67±0.27	2.13±0.033	0.87±0.017	0.80±0.004
35%	40	7.11±0.142	1.57±0.037	0.40±0.044	0.75±0.002
	80	7.25±0.173	1.59±0.020	0.48±0.015	0.80±0.001
	120	8.46±0.136	1.84±0.007	0.58±0.029	0.80±0.003
LSD <sub>0.05</sub>		0.49	0.09	0.11	0.013

\*WD; water deficit (FC %), \*\*N; nitrogen (kg ha<sup>-1</sup>). Difference between mean difference treatments significant differences (LSD test, P < 0.05)

Nitrogen as a crucial element in plant growth plays a paramount role in the grain protein content, chlorophyll content, cell protoplasm and leaf photosynthetic activity (Delfin *et al.*, 2005). Application of nitrogen can adjust stomatal movements and it increases the green area duration (Yang and Zhang, 2006). Gene's expression of ABA synthesis and its activity was affected by nitrogen (Yang *et al.*, 2001). Probably, chlorophyll, exopeptidases and endopeptidases enzymes involved in chlorophyll degradation, influenced by ABA and the reduction of their amount caused the increasing of chlorophyll and carotenoids contents via nitrogen consumption (Hortensteiner and Feller, 2002). Our results showed that, chlorophyll and carotenoids were influenced indirectly, by proline accumulation. Proline maintains turgor potential and protects the intercellular macromolecules structure and thereby is presence affected the amount of chlorophyll and carotenoids. Water stress reduced the quantum yield, which indicates photosystem II efficiencies. The loss of this trait represents the occurrence of light inhibitory effect by environmental stresses (Kocheva *et al.*, 2004). During electron transport to photosystem I, drought stresses may cause interferences in Hill's reaction and thereby quantum yield of photosystem II can be reduced (Zelatev and Yordanov, 2004). Nitrogen increases fluorescence quantum efficiency through the development of leaf area, assimilates distribution and keeps up the photosynthetic efficiency (Arduiniet *et al.*, 2006). Similar results have also been reported by Huang *et al.* (2004).

The antioxidant enzymes activity was altered by the lack of water and nitrogen application. CAT activity was affected by water deficiency and nitrogen used at different times during the day. The highest CAT activity (0.109 OD mg protein min<sup>-1</sup>) was recorded in severe stress (35% FC) and 80 kg N in the afternoon, and the lowest value (0.014 OD mg protein min<sup>-1</sup>) at (85% FC) and 120 kg N at midday. The enzyme activity changed at different times during the day, so that the maximum and minimum activity was observed at PM and AM respectively. The CAT activity decrease reached to 54.4% during the different daytimes, under non-stress condition (85% FC) and low N access (40 kg N ha<sup>-1</sup>). In contrast, by adding 80 kg of N, the enzyme activity upraised to 90% at PM, while using up to 120 kg ha<sup>-1</sup> N diminished it to 9.5%. Increase stress intensity and N deficiency showed less effect on the enzyme activity at different daytimes. The enzyme activity's was lower during midday than in the morning and PM (Tab. 2). PPO activity significantly changed under water deficit treatment and nitrogen application during different times of the day. The highest activity (0.426 OD mg protein min<sup>-1</sup>) was determined under severe stress treatment (35% FC) and 40 kg N ha<sup>-1</sup> in the sunrise and the lowest activity (0.036 OD mg protein min<sup>-1</sup>) under mild stress (60% FC) and 40 kg N ha<sup>-1</sup> at midday. The results showed that PPO enzyme activity exhibited no clear relationship with stress despite the significant enzyme activity. As the enzyme activity was significantly lower than control during mild stress (60% FC), its increasing trend was observed under severe stress (35% FC). The water stress and nitrogen application

increased PPO activity. Highest (0.35 OD mg protein min<sup>-1</sup>) and lowest (0.09 OD mg protein min<sup>-1</sup>) enzyme activity, were observed under severe stress (35%) with 120 kg N ha<sup>-1</sup> and non-stress (85% FC) and consumption of 40 kg N ha<sup>-1</sup> respectively, which represented 74% variation in the enzyme activity (Tab. 2).

POX activity changed during the daytime. Its activity was observable (0.18 OD mg protein min<sup>-1</sup>) in the morning, but, with time, it reached to 0.17 OD mg protein min<sup>-1</sup> at

noon (5% reduction) and in the evening it raised again to 23.5% (from 0.17 to 0.21 OD mg protein min<sup>-1</sup>) (Tab. 3). The water deficient stress and nitrogen had an impact on the POX activity and it increased their activity. Highest (0.35 OD mg protein min<sup>-1</sup>) and lowest (0.09 OD mg protein min<sup>-1</sup>) enzyme activity, were observed under severe stress (35%) with 120 kg N ha<sup>-1</sup> and non-stress (85% FC) and consumption of 40 kg N ha<sup>-1</sup> respectively, which represents 74% variation in the enzyme activity (Tab. 3).

Tab. 2. Effect of water deficit and nitrogen application on CAT, and PPO activity of barley

Treatment		CAT (OD mg protein min <sup>-1</sup> )			PPO (OD mg protein min <sup>-1</sup> )		
WD*	N**	Daytime			Daytime		
		Morning	Noon	Afternoon	Morning	Noon	Afternoon
85%	40	0.046±0.014	0.037±0.009	0.021±0.004	0.043±0.008	0.079±0.012	0.075±0.012
	80	0.026±0.006	0.014±0.001	0.040±0.018	0.096±0.009	0.113±0.008	0.127±0.024
	120	0.015±0.002	0.014±0.001	0.019±0.004	0.150±0.055	0.128±0.021	0.161±0.042
60%	40	0.058±0.009	0.024±0.002	0.039±0.003	0.068±0.004	0.036±0.008	0.078±0.008
	80	0.042±0.010	0.039±0.006	0.033±0.003	0.095±0.015	0.090±0.036	0.190±0.016
	120	0.033±0.006	0.030±0.007	0.034±0.003	0.184±0.040	0.105±0.048	0.181±0.017
35%	40	0.061±0.016	0.067±0.004	0.083±0.025	0.426±0.165	0.181±0.058	0.365±0.065
	80	0.070±0.016	0.051±0.008	0.109±0.016	0.168±0.044	0.139±0.013	0.21±0.034
	120	0.059±0.011	0.050±0.002	0.067±0.010	0.210±0.057	0.190±0.015	0.241±0.062
LSD <sub>0.05</sub>		0.016			0.126		

\*WD; water deficit (FC %) \*\*N; nitrogen (kg ha<sup>-1</sup>), CAT; catalase, and PPO; poly phenol oxidase. Difference between mean difference treatments significant differences (LSD test, P < 0.05).

Tab. 3. Effect of nitrogen on proline content, POA activity and grain yield of barley underwater deficit

Treatment		POX	Proline	Grain yield
WD*	N**	(OD mg protein min <sup>-1</sup> )	(µg g)	(g plant <sup>-1</sup> )
85%	40	0.09±0.005	0.11±0.004	1.00±0.146
	80	0.12±0.007	0.20±0.036	1.16±0.085
	120	0.14±0.006	0.14±0.006	1.54±0.036
60%	40	0.15±0.009	0.32±0.030	0.63±0.022
	80	0.16±0.010	0.62±0.044	0.68±0.030
	120	0.17±0.008	0.41±0.080	0.77±0.055
35%	40	0.21±0.017	1.76±0.063	0.34±0.031
	80	0.28±0.017	2.54±0.239	0.40±0.040
	120	0.35±0.022	2.11±0.031	0.47±0.024
LSD <sub>0.05</sub>		0.029	0.265	0.191

\*WD; water deficit (FC %), \*\*N; nitrogen (kg ha<sup>-1</sup>), POX; Peroxidase; Difference between mean difference treatments significant differences (LSD test, P < 0.05).

The results showed that the POX enzyme has the most indirect effects on the quantum yield. This enzyme acts as a catalyst for a quinone from phenols in the presence of oxygen (Yilmaz *et al.*, 2003). Perhaps its effect on the photosynthetic cycle and electron transport chain is observable in the photosynthesis process, during the photosynthetic light cycle when it reduces the quantum yield. The antioxidant enzyme activity (CAT and POX) increased during water deficit stress. Simirnof and Colombe (2000) reported that CAT, POX and SOD enzymes in barley increased under drought stress, these results are consistent with Bai and Sui (2006) results on

maize. Indeed, the increasing of CAT and POX activity during the stress is a defensive mechanism, in plant, against free oxygen radical, causing detoxification and decomposition of hydrogen peroxide produced by the cells and it prevents the breakdown of plant proteins (Ariano *et al.*, 2005). Huang *et al.* (2004) showed that nitrogen deficiency decreased CAT and POX activity in rice over time. We observed that CAT activity is modified by changing the time of the day so that the highest activities were observed at PM. Drought could have been exacerbated during mid-day with high radiation, which increased the evapotranspiration of plants. In addition, POX exhibited

the most direct impact on CAT, which affected indirectly the quantum yield. PPO activity was increased under water deficit treatments and nitrogen application, which caused irregular changes on PPO. PPO in most of the higher plants exists and acts as a catalyst for quinone from phenols in the presence of O<sub>2</sub>. The enzyme activity depends on plant genotype and growth conditions and plays a role in adventitious root formation, root development organization (Yilmaz *et al.*, 2003) cell division and primary differentiation (Huystee and Cairns, 1982). Proline accumulation was increased with nitrogen application and water deficit as one of the physiological responses to tolerance to the biotic and abiotic stress (Geravandi *et al.*, 2011).

Proline has significantly changed during water deficient stress and nitrogen consumption. By increasing the stress intensity, proline content increased, whereas in all the water treatments, mild applications of N (80 kg) lead to the highest value from this view points. The highest content of proline (2.54 µg g) was observed under severe stress (35% FC) and 80 kg ha<sup>-1</sup> N (it indicates of 220 fold increasing compared with control). Applying 120 kg ha<sup>-1</sup> N in comparison with 80 kg ha<sup>-1</sup> reduced the proline content

(Tab. 3). During the drought stress, water potential is reduced and the reduction of turgor pressure causes proline accumulation in the cytoplasm (Misra and Gupta, 2006). Proline protects the intercellular macro molecules structure and proteins, neutralizes free radicals, reduces cytoplasmic pH and maintain the proper ratio of NADP<sup>+</sup>/NADPH in metabolism and increase different enzymes activities (Szabados and Savoure, 2009) and it is also used as a combined storage of organic nitrogen, during reconstruction (Sairam and Tyagi, 2004).

The grain yield also was affected by irrigation schedules and nitrogen application. Water stress reduced yield to 66% at 35% FC. Nitrogen increased the yield during stress. Results showed that the highest grain yield (1.535 g plant<sup>-1</sup>) was obtained at 85% FC and 120 kg N ha<sup>-1</sup> and the lowest (0.341 g plant<sup>-1</sup>) at 35% FC and 40 kg ha<sup>-1</sup> (Tab. 3). The grain yield was correlated with all traits. Chlorophyll *a*, chlorophyll *b*, carotenoids and quantum yield, were all positively correlated with yield, while the antioxidant enzyme activities and proline content showed a negative correlation. Furthermore, chlorophyll *a* and *b* had a negative correlation with the enzymes and proline (Tab. 4).

Tab. 4. Correlation coefficient of water deficit and nitrogen

	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Carotenoid	Ø <sub>PSII</sub>	Proline	CAT	PPO	POX	Grain yield
Chlorophyll <i>a</i>	1.00	0.848**	0.499**	0.127	-0.870**	-0.723**	-0.508**	-0.636**	0.666**
Chlorophyll <i>b</i>		1.000	0.572**	0.224	-0.838**	-0.776**	-0.408*	-0.635**	0.679**
Carotenoid			1.000	-0.004	-0.357	-0.370	0.037	-0.263	0.592**
Ø <sub>PSII</sub>				1.000	-0.406*	-0.475*	-0.319	-0.652**	0.668**
Proline					1	.815**	.687**	0.859**	-0.751**
CAT						1	.534**	0.797**	-0.728**
PPO							1	0.738**	-0.423*
POX								1	-0.791**
Grain yield									1

\* Significant at the 5% level, \*\* Significant at 1% level

Tab. 5. Path analysis coefficient for grain yield under water deficit and nitrogen fertilizer

Trait	Direct Effects	Indirect Effects							
		Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Carotenoid	Ø <sub>PSII</sub>	Proline	CAT	PPO	POX
Chlorophyll <i>a</i>	0.313	-	0.236	-0.093	0.176	0.835	0.145	0.991	-0.869
Chlorophyll <i>b</i>	-0.123	-0.252	-	0.426	0.237	0.454	-0.372	-0.219	0.199
Carotenoid	0.426	0.010	0.015	-	0.141	0.342	-0.079	0.157	-0.226
Ø <sub>PSII</sub>	0.529	0.330	0.099	0.205	-	-1.089	-0.594	-0.588	1.209
Proline	-0.115	0.469	-0.223	-0.463	-0.109	-	0.738	0.111	-0.466
CAT	-0.002	-0.149	-0.202	-0.100	0.122	0.038	-	0.209	0.200
PPO	-0.011	0.132	-0.055	-0.115	-0.447	-0.201	-0.001	-	-0.267
POX	-0.069	-0.379	-0.212	-0.440	0.066	-1.081	1.233	0.349	-

The path coefficient results showed that the carotenoids content and quantum yield had the most pronounced direct effects and antioxidants activity, while CAT, POX and PPO had the less noticeable direct effects on the grain yield, so it could be mentioned that in one-unit increment of carotenoids and quantum yield, the grain yield increased with 0.42 and 0.529 units respectively. While increasing each unit in CAT, POX and PPO activity the grain yield was reduced 0.002, 0.09 and 0.011 respectively. Carotenoids content was

negatively correlated with chlorophyll *a* and *b*, also changing each unit of them indirectly altered 0.010 and 0.015 of carotenoids (Tab. 5). Quantum yield was negatively correlated with POX and CAT activity and proline content. It seems that each trait has indirect effects on other traits ultimately and may affect grain yield. Chlorophyll *a* and *b* both affect carotenoid content (0.010 and 0.015) respectively and this affects yield with 0.42. Therefore, chlorophyll *a* and chlorophyll *b* had an indirect impact on the yield via

carotenoids 0.004 and 0.00. Likewise, other traits influenced the yield by interfering and affecting each other (Tab. 5).

Peroxidase activity and quantum yield had the highest indirect effects on proline amount. The reduction of photosynthesis and plant production due to the decrease in quantum yield can affect the production of proline. Grain yield reduction under drought stress may be attributed to accelerated phenological stages that permit the plants to escape from drought effects or loss of reservoir capacity (Turner *et al.*, 2001). Gadallah (2000) noted that the reasons of grain yield reduction under severe water stress are due to higher sensitivity of plants at the reproductive growth than vegetative growth, stage which results in the reduction of assimilate allocation to grain. It seems that the yield reduction at different levels of drought stress is the result of differences between quantum yield and decreased membrane stability as well. Stress prevents the plant from producing desired substrates and energy use via disturbances in the electron transfer pathway and destruction of tissue associated with photosynthesis (Paknejad *et al.*, 2007). Also, damages from reactive oxygen species on the membrane lipids and the peroxidation of membrane polyunsaturated fatty acids were reported as the main reason of the decrease in grain yield during stress (Blokhin *et al.*, 2003).

### Conclusions

The results showed that the water deficit stress reduced photosynthesis and chlorophyll content of the plants, which ultimately reduced grain yield. Our results suggested that plants apply defensive mechanisms, such as antioxidant enzymes and proline to overcome and mask negative effects of stress. Nitrogen application was determined as an optimum strategy for most desirable traits that reduce stresses effect. Among the measured traits, quantum yield had the greatest impact on the grain yield.

### Acknowledgment

The authors wish to thank Dr. Saber Zahri, Dr. Hossain Haidari Sharif Abad for providing necessary funds and research facilities required for this investigation.

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