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ANTIOXIDATIVE AND BIOCHEMICAL RESPONSES OF WHEAT TO DROUGHT STRESS

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ABSTRACT

Drought stress is considered as an effective parameter in decreasing crop production. Present study was investigated to understand the effect of drought stress on wheat (*Triticum aestivum*) seedlings under controlled condition. The seeds of wheat were subjected to five levels of water potential. 0 MPa (as control) and -2, -4, -6 and -8 MPa (as treatments) and germination percentage, mean germination time, proline and sugar amounts, chlorophyll contents, maximum photochemical efficiency of PSII and electron transport rate (ETR) have been examined. In addition enzymatic and non-enzymatic response of wheat seedlings to drought have been explored. Germination percentage and mean germination time were affected by different osmotic potentials. The least germination percentage and MGT were obtained from -8 osmotic potential. Drought caused significant losses in relative water content. Total chlorophyll content was reduced in all studied treatments. Drought levels higher than -2 MPa resulted in significant decrease of F_V/F_M value. ETR, however, observed no significant changes in drought treated seedlings. MDA, AsA and GSH contents increased in relation to the drought period. Activities of enzymatic antioxidants, such as SOD, CAT, APX, POD and GR increased to manage the oxidative stress.

Keywords: antioxidant; drought stress; *triticum aestivum*; wheat

INTRODUCTION

Regarding to the FAO organization the wheat (*Triticum aestivum*) crop accounts for about 21% of food and 200 million hectares of farmland worldwide (available from: http://www.fao.org). Developing countries produce and utilize 81% of wheat they consume. In the period leading up to 2020, demand for wheat for human consumption in developing countries is expected to grow at 1.6% each year. The global average wheat yield must be increase during the coming 25 years from 2.6 to 3.5 tones ha^{-1}.

Plants in nature are continuously exposed to many biotic and abiotic stresses. Among these stresses, drought stress is one of the most adverse factors of plant growth and productivity and considered a severe threat for sustainable crop production in the conditions on changing climate. Future climate scenarios suggest that global warming may be beneficial for the wheat crop in some regions, but could reduce productivity in zones where optimal temperatures already exist. Global warming, however, may negatively affect wheat grain yields which potentially increasing food insecurity [1]. High temperature is often accompanied with low water supply, so the primary aim of cereal breeding must be to develop cultivars tolerating both types of stresses [2].

Drought, the result of low precipitation or high temperature, is one of the most important factor limiting yield by restricting most stages of crop growth in arid and semiarid areas [3]. Drought affects about 32% of 99 million hectares under wheat cultivation in developing countries and at least 60 million hectares under wheat cultivation in developed countries [4]. Drought stress can reduce grain yield, therefore, it has been estimated that average yield loss of 17 to 70% in grain yield is due to drought stress [5]. Wheat yields are reduced by 50-90% of their irrigated potential by drought on at least 60 million hectare in the developing world [6].

Drought triggers a wide variety of plant responses, ranging from cellular metabolism to changes in growth rates and crop yields. Understanding the biochemical and molecular responses to drought is essential for a holistic of perception plant resistance mechanisms to water-limited conditions [7].

Chlorophyll which is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation [7]. Photosynthesis is one of the most sensitive processes to drought stress [8]. The inhibitory effects of drought on photosynthesis may be associated with low CO_2 availability due to low stomatal and mesophyll conductances [9] and/or impairments in carbon assimilation metabolism [10]. Stomatal closure is an early response to drought and an efficient way to reduce water loss in water-limiting environments. Biochemical limitation of photosynthesis also plays an important role under prolonged periods of drought stress [9].

Relative water content (RWC), leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. Relative water content is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures. RWC related to water

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uptake by the roots as well as water loss by transpiration. [7]. During optimal conditions, the balance between reactive oxygen species (ROS) formation and consumption is tightly controlled by antioxidant enzymes and redox metabolites [11]. These include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and redox metabolites such as ascorbic acid and glutathione. Most environmental stresses, including drought induce enhanced production of ROS [12]. Antioxidant enzymes are important components in the mechanism of drought and desiccation tolerance [13-14]. Drought stress leads also to increased accumulation of reactive oxygen species (ROS) in plants. Various subcellular organelles such as chloroplast, mitochondrion and peroxisome are the common sites of ROS production. Increased levels of ROS cause damage to various cellular mechanisms, such as enzyme inhibition, protein degradation, DNA and RNA damage, and membrane lipid per-oxidation, which ultimately culminate in cell death [15-16]. Oxidative stress can lead to inhibition of the photosynthesis and respiration processes and, thus, plant growth. As the key process of primary metabolism, photosynthesis plays a central role in plant performance under drought, via decreased CO_2 diffusion to the chloroplast and metabolic constraints [17]. Modulation in the activities of antioxidant enzymes may be one of the important factors in tolerance of various plants to environmental stress [18]. When molecular O_2 undergoes reduction, it gives rise of ROS such as superoxide (O_2•−), hydrogen peroxide (H_2O_2) and the hydroxyl radical (•OH). Singlet oxygen ($1O_2$), which may arise due to reaction of O_2 with excited chlorophyll molecules, is also considered as one of the potential ROS.

SOD (EC 1.15.1.1) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide, H_2O_2. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. CAT (EC 1.11.1.6) is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second [19]. POD (EC 1.11.1.7) can be coupled to other proteins via its amino groups, as well as its carbohydrate moiety. POD also protects the cells against the destructive influence of H_2O_2 by catalyzing its decomposition through oxidation of phenolic and endiolic cosubstrates [20]. APX (EC 1.11.1.11) is enzyme that detoxifies peroxides such as hydrogen peroxide using ascorbate as a substrate. The reaction they catalyse is the transfer of electrons from ascorbate to a peroxide, producing dehydroascorbate and water as products [21]. GR (EC 1.8.1.7) is an enzyme that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant [22]. The peroxidases then scavenged the produced H_2O_2 by and by oxidation of co-substrates such as phenolics or other antioxidants [23].

The aim of this study was to investigate the effects of drought stress on pigment composition, photosynthetic efficiency of PS II, Relative water content and changes in enzymatic and non-enzymatic antioxidants of leaves from wheat (*Triticum aestivum* L.).

MATERIALS AND METHODS

Experiments were laid out in a complete randomized block design with 5 replications. Wheat (*Triticum aestivum* L., cv. Shahryar) seeds were surface-sterilized with 30% sodium hypochlorite for 10 min and were thoroughly washed with redistilled water. Seeds were then soaked in sterile deionized water at room temperature for 6 h and then subjected to five stress levels at 0 bars, deionized water, as control, and at -2, -4, -6 and -8 bars to test germination and seedling growth. Osmotic stress (-2, -4, -6 and -8 bars) was created using different concentrations of polyethylene glycol (PEG) 6000 at 25°C according to the Equation-1 described by Michel and Kaufmann [24].

Equation-1

$$\psi_s(\text{Water potential, bar index})(MPa) = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

Determination of germination percentage and MGT

Forty healthy and uniform surface-sterilized seeds were transferred in Petri dishes on two sheets of sterile filter paper moistened with deionized water, as control (0 bars), and four stress levels at -2, -4, -6 and -8 bars to test germination The Petri dishes were kept at 24 ± 1°C and the number of seeds germinated in each Petri dish recorded daily for 8 days. Seeds that presented approximately 2 mm of root length were considered germinated. The germination percentage and mean germination time (MGT) were calculated. MGT was determined using Equation-2 as described by Sadeghi *et al*., [25].

Equation-2

$$MGT = \Sigma Dn / \Sigma n$$

Where n is the number of seeds, which were germinated on day D, and n is the number of days counted from the beginning of germination.

Relative water content

To determine relative water content (RWC), four plants from each treatment were randomly selected and the method described by Turner [26] was fallowed. About 0.1 g leaf sample was cut into smaller pieces and weighed to determine initial weight (W_i). The leaf samples were then floated in freshly de-ionized water for 12 h and weighed thereafter to determine fully turgid weight (W_f). The sample was oven-dried at 80˚C for 3 days and the dry weight was obtained (W_d). The relative water content

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(RWC) was determined using the following formula: RWC = (W_i - W_d) (W_f - W_d)$^{-1}$ × 100.

Leaf pigment content

Fresh leaves sample (0.1 g) were analyzed for pigment contents. The leaves were sliced and ground to a fine powder in liquid nitrogen using a pestle and mortar. Pigments were extracted in 3 ml cold 80% acetone as described by Brouers and Michel-Wolwertz [27]. Acetone extracts were centrifuged at 3000 g for 10 min and the resulting pellets were extracted in cold 80% acetone. This operation was repeated three times. The successive supernatants were pooled and centrifuged at 4000 g for 5 min for clarification. The absorbance of the acetone extracts was recorded at 626, 647, 664 nm and (for background correction) 720 nm using a Perkin Elmer Lambda 900 UV/VIS spectrophotometer (Perkin-Elmer Corp. Norwalk, CT, USA). The amounts of Chl were calculated according to Brouers and Michel-Wolwertz [27] by putting the obtained value on the following formulas:

Chl a (µg/ml) = 12.68 OD_{664} - 2.65 OD_{647} + 0.30 OD_{626}
Chl b (µg/ml) = - 4.23 OD_{664} + 23.62 OD_{647} - 3.26 OD_{626}

Fluorescence measurements

The Maximal photochemical efficiency of PS2 (Fv/Fm) and the rate of linear electron transport (ETR) were determined by chlorophyll fluorescence, measured with a chlorophyll fluorometer (PAM-2000©; Heinz-Walz, Effeltrich, Germany) according to the manufacturer's instructions. The experimental protocol of Genty *et al*., [28] was basically used in this experiment. Following 30 min of dark adaptation, the minimum chlorophyll fluorescence (F_0) was determined using a measuring beam, which was sufficiently low (<0.1 µmol m^{-2} s^{-1}) not to induce any significant variable change in fluorescence. To obtain the maximum fluorescence in the dark-adapted state (F_M), a saturation pulse (8000 µmol m^{-2} s^{-1}) was used and the quantum efficiency of PSII open centers in dark-adapted seedlings (F_V/F_M) was determined. The leaves were then illuminated with 300 µmol m^{-2} s^{-1} light. Results are the average of 5 replicates.

Leaf proline content

Proline was extracted and the content assayed spectrophotometrically according to the method of Bates [29]. Leaves (0.5 g) from plants of each of the treatments were harvested. Leaves were ground in liquid nitrogen to a fine powder for the determination of proline content. The powder were homogenized with 10 ml of 3% (w/v) sulphosalicylic acid and passed through whatman No. 2 filter paper. 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid were added to 2 ml of the filtered extract. The mixture was incubated in 100°C water bath for 1 h. The reaction mixture was placed on ice and extracted with 4 ml toluene. Absorption of chromophore was read at 520 nm using a Perkin Elmer Lambda 900 UV/VIS spectrophotometer (MC USA). Toluene was used as blank. The proline concentration was calculated using L-proline corresponding on the standard curve.

Sugar estimation

Shoots were oven dried at 70°C for 24 h, homogenised in 80% ethanol and placed in water bath at 80°C for 30 min. After centrifugation at 3000·g for 5 minutes, samples were washed twice with H_2O at room temperature. Each sample was resuspended with 3 ml H_2O and boiled for 2 hours. Contents of total sugars were estimated calorimetrically using phenol sulphuric acid method described by Dubois [30].

Lipid peroxidation assay

Thiobarbituric acid (TBA) concentrations were determined in four leaves per treatment. The TBA test, which determines malondialdehyde (MDA) as an end product, was used to analyses lipid peroxidation. The MDA content was calculated using its molar extinction coefficient of 155 mM^{-1} cm^{-1} and and the results expressed as nmol MDA g^{-1} FW.

Extraction and determination of antioxidative enzymes

Fresh leaf tissues of control and treated plants were used for determination of antioxidative enzymes. Fresh leaves (0.2 g) were sliced and ground to a fine powder in liquid nitrogen using a pestle and mortar. The powder was homogenized in 0.5 ml extraction buffer containing 50 mM Na-phosphate (pH 7.0), 0.25 mM EDTA, 2% (w/v) polyvinylpyrrolidone-25, 10% (w/v) glycerol, and 1 mM ascorbic acid. The homogenate was then centrifuged at 15, 000g for 20 min at 0°C. The supernatant (soluble fraction) was used as the crude extract for the superoxide dismutase (SOD), catalase (CAT), peroxidase (APX) and glutathione reductase (GR) assay [31].

Superoxide dismutase (SOD, EC:1.15.1.1) was assayed on the basis of its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT), according to the methods of Beauchamp and Fridovich [32] and Beyer and Fridovich [33]. The reaction mixture contained 50 mM phosphate buffer (pH = 7.8), 13 mM methionine, 75 mM NBT, 100 µM EDTA, 200 mL of enzyme extract and 2 mM riboflavin. The reaction mixture was read at 560 nm. The increase in absorbance in the absence of enzyme was taken as 100 and 50% initial was taken an equivalent to 1 unit of SOD activity.

Catalase (CAT, EC 1.11.1.6) activity was measured by the method of Tan *et al*., [34]. Enzyme extract and 4 ml of 50 mM phosphate buffer (pH 7.0) were mixed and incubated at 30°C for 10 min. The reaction was started by adding 1 ml of 50 µM H_2O_2, and terminated after 1 minute by adding 2 ml of 10% H_2SO_4. CAT activity was then determined by estimating the residual H_2O_2 in the reaction solution using 10 mM $KMnO_4$ titration to pink. CAT activity was expressed as U mg-1 FW.

Ascorbate peroxidase (APX; E.C.1.11.1.11) activity was measured according to Panchuk [35]. The

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reaction mixture contained 25 mM Na-phosphate (pH 7.0), 0.1 mM EDTA, 1 mM H_2O_2, 0.25 mM ascorbic acid (AsA), and protein extract in a total volume 1 ml. The oxidation rate of AsA was assayed photometrically using a UV/Visible Spectrophotometer by monitoring the decrease in A290 after 1 minute of incubation following the addition of protein extract. A fall in absorbance at 290 nm was measured as ascorbate was oxidized. APX activity (unit/g FW) was calculated using an extinction coefficient of 2.8 mmol/l/cm for ascorbate at 290 nm [36].

Glutathione reductase (GR; E.C. 1.6.4.2) activities were measured following previously published techniques [37]. The assay mixtures were composed of (final concentrations): 1.20 ml buffer; 0.10 ml extract (or 0.10 ml buffer in controls); 0.05 ml NADPH (83 μM) made from a 2.5 mM work solution in 0.1% $NaHCO_3$, and kept on ice. The total assay volume was 1.5 ml. After 10 min incubation (25°C), 0.15 ml of 1 mM GSSG (Sigma) was added, and the decrease of NADPH absorption was monitored for 3 minutes at 340 nm using a UV/Visible Spectrophotometer; the cuvette light path was 1 cm. The NADPH concentration change [μmol NADPH (ml extract)$^{-1}$ min^{-1}] was calculated on the basis of the molecular extinction coefficient of NADPH at 340 nm (ε_{340}= 6.22 mM^{-1} cm^{-1}).

Statistical analysis

Statistical analysis was performed using SPSS 16. The data represent means calculated from five replicates. The analysis of variance procedure (ANOVA) was used to compare the effect of high temperature and statistical significance was set at P<0.05.

RESULTS

Variance analysis and mean comparison results displayed that germination percentage and mean germination time were affected by different osmotic potentials (Table-1). The least germination percentage and MGT were obtained from -8 osmotic potential. Germination percentage decreased from 99.5% in control to 58.86 in -8 bars osmotic stress, while MGT decreased from 16.8 to 5.20 under -8 bars osmotic stress. Generally less germination percentage and MGT were attained from seed priming treatment than control (Table-1).

Drought caused significant losses in relative water content (Table-1). The decrease in leaf RWC was found to be lowest, 54.35%, when the water potential was -8. RWC has been affected in all water deficit levels compared to the control.

Pigment content was changed in all studied treatments (Figure-1). The minimum chlorophyll content was observed at -8 MPa treatments. Under water stress, the chlorophyll *b* content of leaves was more affected than the chlorophyll *a* content at low stress treatments. But at high level stresses the amount of chl *a* was more sensitive. Chl *a, b* and total chl contents of the leaves did not decreased significantly with -2 MPa water potential but they decreased significantly with changing water potential to -4 MPa and less.

Exposing wheat seedlings to different levels of drought stress resulted in changes of the chlorophyll fluorescence parameters. Increasing drought stress to -2 MPa did not cause any significant changes of photochemical efficiency of PS2, F_V/F_M. However, higher levels of stress resulted in significant decrease of F_V/F_M $_{value}$. The F_V/F_M value of the control was about 0.85 (Figure-2). F_V/F_M ratio decreased by increasing drought. Significant decrease of F_V/F_M was shown when osmotic stress was at -4 MP or less.

As Figure-2 shows ETR observed no significant changes in drought treated seedlings.

The difference among the water stress treatments for proline and sugar were highly significant at 5% level of probability (Figure-3) and there was a progressive increase in proline and sugar contents with decreased water potential. Proline content increased from 0.98 in control to 4.82 mg/g in -8 MPa osmotic stresses, which is about 2.97 times more than the control. The sugar content increased about 2.4 times mor than the control when the osmotic stress was -8 MPa.

Malondialdehyde (MDA) content changes in relation to the drought period (Table-1).

Figure-4 shows the changes in AsA concentration under drought stress. AsA concentration was significantly (P ≤ 0.05) affected when treated with all treatment compared. AsA increased to 1.7 fold compared to the control. Differences between results of treatment -4, -6 and -6, -8, however, were not significant.

Changes in GSH concentrations under drought stress are shown in Figure-4. GSH concentration showed a significant (P<0.05) alternation at all treatments.

To manage the oxidative stress, the cells activate several enzymatic antioxidants, such as SOD, CAT, APX, POD and GR. To evaluate the oxidative stress generated by water stress, antioxidative enzymes were determined.

The activity of SOD was not significantly increased at -2 MPa. Higher levels of drought stress, however, resulted in increase of SOD activity (Figure-5). The activity of catalase (Figure-5) increased at all treatments. Activity increase at -8 MPa was about 2.6 fold more than the control. Ascorbate peroxidase activity showed increase with rise in osmotic stress (Figure-5). The activity of APX had, however, no significant changes at -2 MPA. At -8 MPA, APX activity showed 5.8 times more than the control. The activity of POD in seedlings under all treatments had significant differences compare to the control (Figure-5). With rise in level of water stress, the activity of glutathione reductase elevated (Figure-5). At -8 MPA, there was a great increase in GR activity, which was about 3 fold more than the activity of control.

DISCUSSIONS

An inverse relationship was observed between osmotic stress level and germination percentage and MGT. One of critical stages of plant life is germination. Water stress at this stage can result in delayed and reduced germination or may prevent germination completely [38]. It has been suggested that once a seed attains a critical

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level of hydration it will precede without cessation toward full germination. However, physiological changes do occur at hydration levels below this critical level that can cause an inhibition of germination. Reduction in germination percentage can result from PEG treatments that decrease the water potential gradient between seeds and their surrounding media [39]. Our findings which revealed that water stress has an impact on the final germination percentages are consistent with that of Qayyum *et al*., [38].

The results showed a decrease in the RWC in salt treated seedlings. RWC is an important characteristic that influence plant water relations. RWC is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as a most meaningful index for dehydration tolerance. A decrease in the RWC in response to drought stress has been noted in wide variety of plants [16, 40] that when leaves are subjected to drought, leaves exhibit large reductions in RWC and water potential. These results support the results of present study.

Resuls of present study showed a dcrease in the amount of chlorophylls. Loss of chlorophyll contents under water stress is considered a main cause of inactivation of photosynthesis. Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast membranes [7]. The reduction in chlorophyll concentration of leaves from plants subjected to water deficit may be an additional strategy of photo-protection reducing light absorption and thus decreasing energetic pressure at the PSII leve [8]. Decreased chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought [41]. Drought stress is a reason of a large decline in the chl *a, b* and the total chlorophyll content in different sunflower varieties [42].

ETR can be an indicator to evaluate the photosynthetic activity of plant leaves [43]. ETR is the actual rate of electron flow, is derived from the quantum yield of PSII and considered as one of the photosynthetic parameters which show the efficiency of photosynthesis [44]. There is a relationships between the electron transport rate (ETR) measured by pulse amplitude modulated (PAM) fluorometer and the rate of O_2 production and C fixation. A remarkable linear relationship was reported between the rate of O_2 production and C fixation to ETR [45]. Thus changes in ETR shows that O_2 production and C fixation has been affected by drought. Measurements did not, however, show any significant ETR changes on used seedlings affected by salinity.

Proline and sugar contents increased at all treatments. Although the precise role of proline accumulation is still debated, proline is often considered as a compatible solute involved in osmotic adjustment, which accumulates in majority of plants under stress The induction of proline accumulation may be due to an activation of proline synthesis through glutamate pathway involving γ-glutamyl phosphate reductase, glutamyl phosphate reductase and P5CR Δ'-pyrroline-5-carboxylate activities. It has been shown that accumulation of proline is a common response to a wide range of biotic and abiotic stresses such as salt [46], drought [47] and high temperature [48]. These results are in accordance with the results of some other author who reported that wheat sugar and proline content increased under drought stress conditions [49]. Higher proline content in wheat plants after water stress has also been reported by Vendruscolo *et al*., [50] and Qayyum *et al*., [38].

The results of present study showed that MDA level was significantly increased. MDA is considered as a suitable marker for membrane lipid peroxidation. A decrease in membrane stability reflects the extent of lipid peroxidation caused by ROS. The ROS generated by drought stress-induced oxidative stress can directly attack membrane lipids and increase lipid peroxidation [51-52]. Drought-induced overproduction of ROS increases the content of MDA which has been considered as a suitable marker for membrane lipid peroxidation [53]. Lipid peroxidation has also been associated with damages provoked by a variety of environmental stresses [54].

Ascorbic acid content of wheat seedlings increased significantly in all treatments. A maximum of increase was noted -8MPa which was about 77% more than the control. Ascorbic acid is readily oxidised to monodehydro ascorbic acid as part of its antioxidant function [55-56]. Similar results have been previously reported by Jaleel *et al*., [57]. GSH concentration increased progressively during drought stress, concomitant with high level of GR activity, indicating that GSH plays an important role in scavenging the ROS by ascorbate-glutathione cycle. GSH is an ideal biochemical to protect plants against stress including oxidative stress, and takes part in the control of H_2O_2 levels [58]. Ascorbate acid and glutathione are key nonenzymatic antioxidants which participate in redox regulation in different cell compartments [59].

The generation of reactive oxygen species (ROS) is one of the earliest biochemical responses of eukaryotic cells to biotic and abiotic stresses. The production of ROS in plants, known as the oxidative burst, is an early event of plant defense response to water-stress and acts as a secondary massager to trigger subsequent defense reaction in plants. The leading role in protecting the plants from ROS belongs to antioxidant enzymes. The expressions of the antioxidant enzyme-related genes were up-regulated by water stress treatments. SOD activity directly modulates the amount of ROS. This suggests that drought adaptation improved the antioxidant capacity, which may effectively decrease ROS injury during heat stress. Similar results were seen in the case of CAT activity also. SOD and CAT activities have been reported to be negatively correlated with the degree of damage of plasma membrane, chloroplast, and mitochondrial membrane systems, and positively related to the indices of stress resistance [60]. It is reported that the APX found in organelles is believed to scavenge H_2O_2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H_2O_2 that is produced in the cytosol

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or apoplast and that which has diffused from organelles. In the chloroplast, H_2O_2 can be detoxified by the ASA-GSH-NAPDH system that has been catalyzed by APX [61]. POD plays a role in decreasing H_2O_2 content accumulation, eliminating MDA resulting cell peroxidation of membrane lipids and maintaining cell membrane integrity [34, 62].

Table-1. Effect of water stress on height, fresh weight, dry weight and relative water content (RWC), of *Catharanthus roseus* seedlings leaves. Data are the mean value ± SD of five individual experiments.

	Water potential (MPa)				
	0 (control)	**-2**	**-4**	**-6**	**-8**
Germination %	99, 49 ± 0, 36	94, 53 ± 2, 12	80, 83 ± 2, 64	70, 20 ± 3, 72	58, 86 ± 2, 79
MGT	16, 82 ± 1, 13	13, 20 ± 0, 83	10, 71 ± 1, 12	7, 35 ± 0, 74	5, 20 ± 0, 68
RWC (%)	91, 03 ± 4, 01	81, 15 ± 3, 29	71, 45 ± 4, 02	61, 81 ± 4, 88	54, 36 ± 6, 57
MDA (nmol/g FW)	4, 01 ± 0, 02	3,29 ± 0,03	4,02 ± 0,03	4, 88 ± 0, 03	6, 57 ± 0, 05

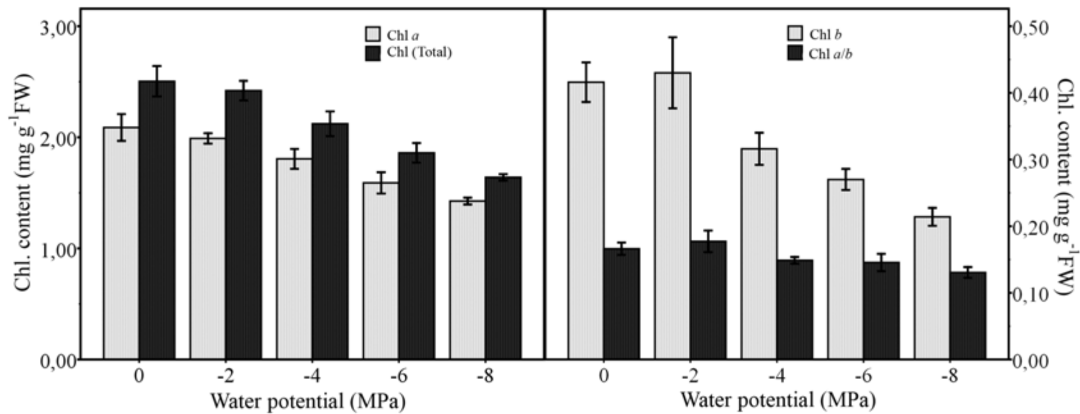

Figure-1. Effect of water stress on amount of chlorophyll *a*, *b*, total chlorophyll and ratios of chlorophyll *a* to *b* in wheat seedlings. Data are the mean value ± SD of five individual experiments.

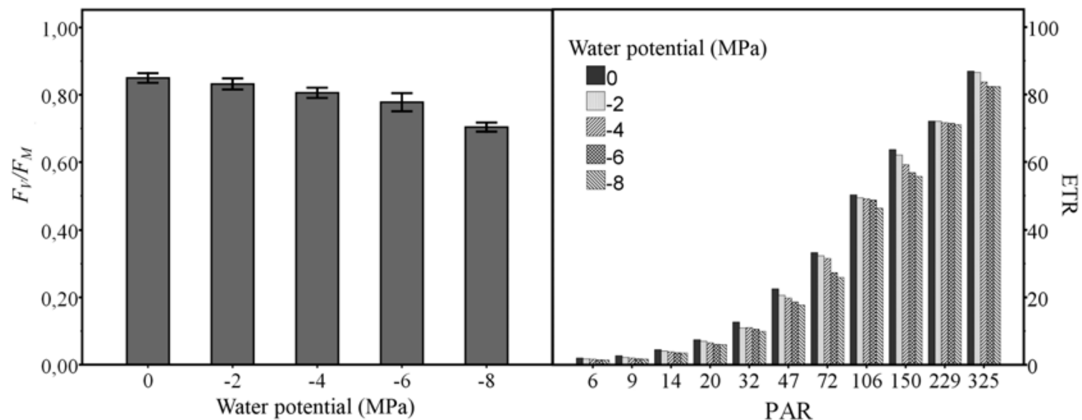

Figure-2. Effect water stress on photochemical parameters of wheat seedlings, maximum photochemical efficiency of PSII (F_v/F_m) and electron transport rate. The results are presented as means ± SD of five individual experiments.

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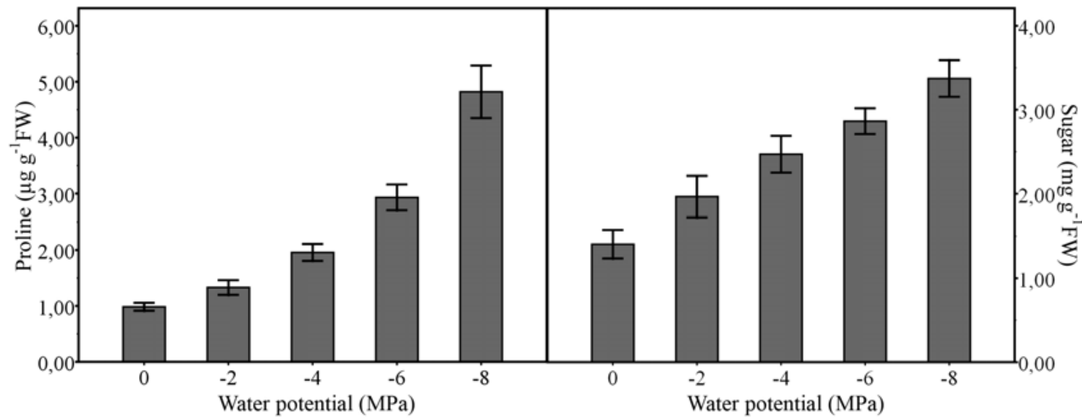

Figure-3. Effect of water stress on proline and sugar contents of wheat seedlings exposed to different levels of drought. Data are the mean value ± SD of five individual experiments.

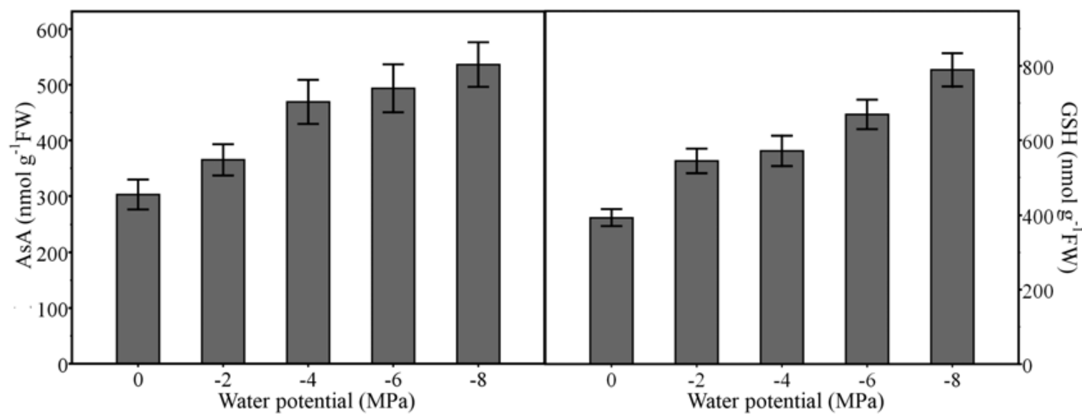

Figure-4. Effect of drought stress on nonenzymatic antioxidants, ascorbic acid and glutathionecontents of wheat seedlings exposed to different levels of drought. Data are the mean value ± SD of five individual experiments.

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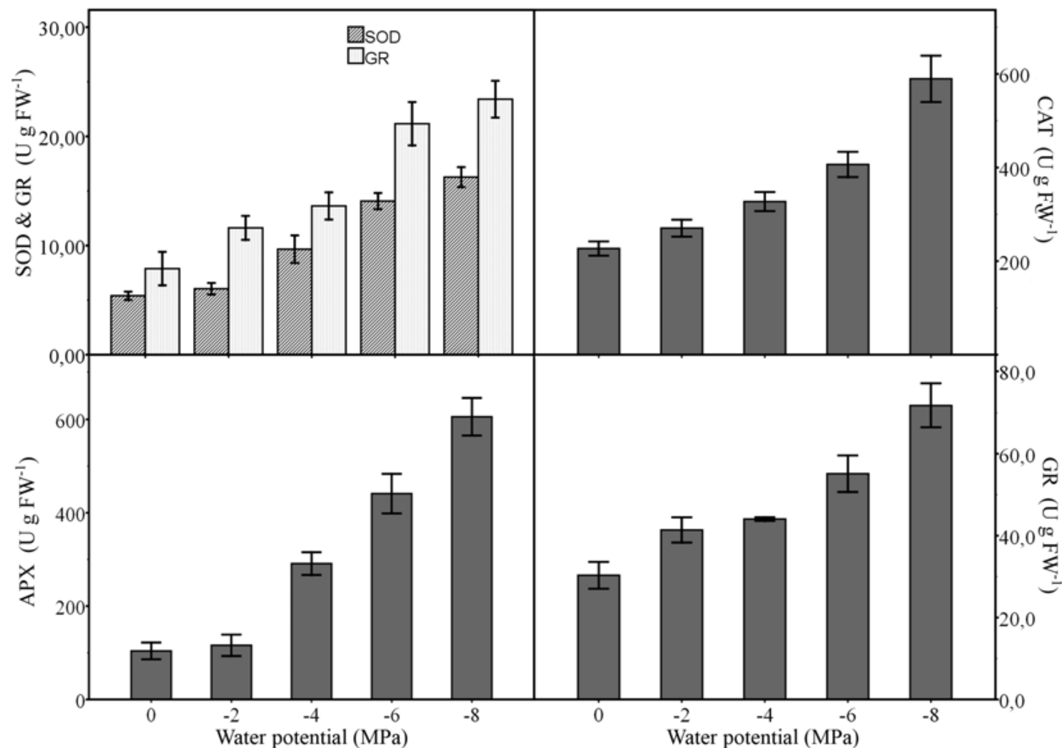

Figure-5. Effect of drought stress on enzymatic antioxidants, superoxide dismutase (SOD), glutathione reductase (GR), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) activities of wheat seedlings leaves. Data are the mean value ± SD of five individual experiments.

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