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RESPONSE OF PROLINE, SOLUBLE SUGARS AND ANTIOXIDANT ENZYMES IN WHEAT (*TRITICUM AESTIVUM* L.) TO DIFFERENT IRRIGATION REGIMES IN GREENHOUSE CONDITION

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ABSTRACT - To evaluate the response of proline and soluble sugars content, catalase (CAT) and ascorbate peroxidase (APX) activity in wheat leaves to different irrigation regimes at two growth stages, a greenhouse factorial experiment was conducted in a completely randomized design (CRD) with three replications. The factors consisted of four different irrigation regimes as 100% (I_0), 75% (I_1), 50% (I_2) and 25% (I_3) of field capacity and growth stages: 50% emergence to booting stage (GS_1) and booting stage to physiological maturity (GS_2). Fresh leaf tissues were used to determine proline and soluble sugars content, CAT and APX activity. According to the results, irrigation regimes had significant effect on proline content, soluble sugars and APX activity, but no significant differences were detected among irrigation regimes for catalase (CAT) activity. Limited irrigation increased proline concentration and total soluble sugars in leaves. None of studied traits were affected by growth stages. Interaction between irrigation regimes and growth stages was not significant for all studied traits. Results indicated that the highest proline content (12 μmol/g DW), total soluble sugars (49 mg/g DW) and APX activity (42 μmol min^{-1} g^{-1} FW) were related to irrigation at 25% of field capacity (I_3). It was concluded that proline and soluble sugar levels were increased in wheat leaves under deficit irrigation regimes.

Key words: Wheat; Drought stress; Proline content; Soluble sugars content; Antioxidant enzymes.

INTRODUCTION

Water is imperative for plant growth and development. Water deficit stress, permanent or temporary, limits the growth and distribution of natural vegetation and the performance of cultivated plants more than any other environmental factor (Kramer, 1983). Water stress is

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characterized by reduction of water content, turgor, total water potential, wilting, closure of stomata, and decrease in cell enlargement and growth. Severe water stress may result in arrest of photosynthesis, disturbance of metabolism, and finally dying (Mckersie and Leshem, 1994). Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. It involves the accumulation of a range of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, glycinebetaine, organic acids, calcium, potassium, chloride ions, etc. (Farooq *et al.*, 2009). The production of reactive oxygen species (ROS) is linear with the severity of drought stress, which leads to enhanced peroxidation of membrane lipids and degradation of nucleic acids, and both structural and functional proteins. Various organelles including chloroplasts, mitochondria and peroxisomes are the seats as well as first target of reactive oxygen species produced under drought stress (Farooq *et al.*, 2009). Stomatal closure, reduction of photosynthesis and osmotic adjustment are typical responses of plants to water stress at the first stage (Tanaka *et al.,* 1990).The photosynthetic apparatus is sensitive to water deficit. Closure of stomata and direct inhibition of Calvin cycle enzymes result in exposure of cells to excess excitation energy (Smirnoff, 1995). This excess energy may be diverted to activate molecular oxygen. Excess accumulation of reactive oxygen species damages various macromolecules, resulting in lipid peroxidation and enzyme inactivation (Elstner, 1982). The active oxygen-induced damage to the cell may be minimized or prevented by increased antioxidant activity (Doulis, 1994). The severity of this damage largely depends on the status of antioxidant systems, since plants develop antioxidants to remove toxic reactive oxygen species and protect the plant cells from lipid peroxidation and inactivation of enzymes that occur under stress (Smirnoff, 1993).

The objective of the present work was to determine the effect of irrigation regimes on proline, soluble sugars and antioxidant enzymes in Wheat (*Triticum aestivum* L. cv. Sardari) in greenhouse condition.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L. cv. Sardari) plants were sown in individual pots (15 cm diameter and 25 cm height, filled with 12 kg soil) grown in a greenhouse at 25-30 °C. The soil moisture content was measured gravimetrically on every alternate day immediately befor each irrigation. Until 40 days after planting, pots were watered equally then, irrigation treatments were applied as: 100% of field capacity (FC) (I_0), 75% of field capacity (0.75 FC) (I_1), 50% of field capacity (0.5 FC) (I_2), and 25% of field capacity (0.25 FC) (I_3). Pots were weighed daily and water added to each pot as lost weight of pots. Growth stages consisted of 50% emergence to booting stage (GS_1) and booting stage to physiological maturity (GS_2). All treatments were repeated three times in

experimental pots in a factorial form based on complete randomized design.

Enzyme extraction and assay. One-tenth g of fresh foliar tissue (uppermost leaves taken at the end of two growth stages) was analyzed for enzymatic assays. Catalase activity (µmol H_2O_2 min^{-1} g^{-1} FW) was assayed by measuring the initial rate of hydrogen peroxide disappearance (Chance and Maehly, 1959). The reaction mixture contained 2.5 ml of 50 mM potassium phosphate buffer (pH 7.4), 0.1 ml of 1% hydrogen peroxide and 50 µl of enzyme extract. The homogenate was centrifuged at 15000 g for 15 min at 4 ºC and the supernatant was immediately used for the enzyme assay. The decrease in hydrogen peroxide was followed as a decline in optical density at 240 nm and the activity was calculated using the extinction coefficient of 36 mM cm^{-1} for hydrogen peroxide. Ascorbate peroxidase (µmol.g^{-1} FW.min^{-1}) activity was determined as described by Asada (2001). The reaction mixture contained 2.5 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 0.2 ml of 1% hydrogen peroxide and 0.1 ml enzyme extract. The homogenate was centrifuged at 15000 g for 15 min at 4ºC and the supernatant was used to measure enzyme activity. The hydrogen peroxide-dependent oxidation of ascorbate was followed by monitoring the decrease in absorbance at 290 nm, using the extinction coefficient of 2.8 mM cm^{-1}. Determination of proline and soluble sugars content to determine the proline content, 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. Above phase of filtrate was separated and its sediments were washed by 5 ml of 70% ethanol for two times and its above phase added to the previous over compartment. The mixture was centrifuged at 3500 g for 10 min at 4ºC and the supernatant was recovered and alcoholic extract kept in refrigerator at 4 ºC (Paquin and Lechasseur, 1979). One ml of alcoholic extract was diluted with 10 ml of distilled water and 5 ml of ninhydrin (0.125 g ninhydrin, 2 ml of 6 mM NH_3PO_4, 3 ml of glacial acetic acid) and 5 ml of glacial acetic acid added then mixture placed in boiling water bath for 45 min at 100ºC. The reaction was stopped by placing the test tubes in cold water. The samples were rigorously mixed with 10 ml benzene. The light absorption of benzene phase was estimated at 515 nm using a PD-303 model spectrophotometer. The proline concentration was determined using a standard curve. Free proline content was expressed as µmol g^{-1} DW of leaves (Irigoyen *et al.,* 1992). To measuring the content of soluble sugars, 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. One-tenth ml of alcoholic extract preserved in refrigerator mixed with 3 ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The samples placed in boiling water bath for 10 minutes. The light absorption of the samples was estimated at 625 nm using a PD-303 model spectrophotometer. Contents of soluble sugar were determined using glucose standard and expressed as mg g^{-1} DW of leaves.

RESULTS AND DISCUSSION

Statistical analysis showed that proline content and soluble sugars content in leaves was highly affected (P< 0.01) by irrigation regimes, but not affected by growth stages and the interaction between growth stages and irrigation regimes *(Table 1).*

Table 1 - Variance analysis for proline, soluble sugars content, ascorbate peroxidase (APX) and catalase (CAT) activity as effected by different irrigation regimes and growth stages

Source of variation	df	Proline	CAT	APX	Soluble sugars
Irrigation regime (I)	3	18.32**	124	0.99**	188.2**
Growth stage (Gs)	1	0.42	16	0.005	42.44
I × Gs	3	3.1	9.19	0.003	12.45
Error	12	1.8	8.33	0.4	82

** Significant at P<0.01

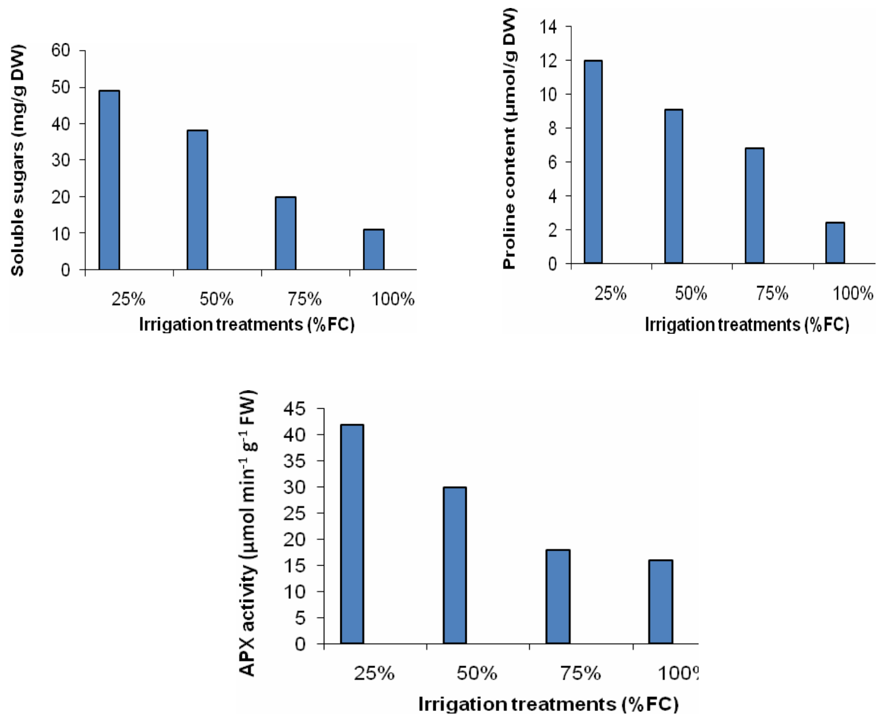

Figure 1 - Effect of irrigation regimes on proline content, soluble sugars content and ascorbate peroxidase (APX) activity in the leaves of *T. aestivum* L. cv. Sardari. Shoot samples were taken and measured at the end of two growth stages. Values are the means of three replicates.

The maximum proline content (12 µmol g^{-1} DW) is related to irrigation regime 25% (I_3) of field capacity, whereas the lowest one (2.4 µmol/g DW) is related to irrigation regime 100% (I_0) of field capacity *(Fig. 1)*. The lowest amount of soluble sugars content (11 mg/g DW) is related to irrigation regime 100% (I_0) of field capacity, whereas the highest one (49 mg/g DW) is related to irrigation regime 25% (I_3) of field capacity *(Fig. 1)*. Our results were fortified by those of Irigoyen *et al.*(1992), Sanchez *et al.*(1998) and Izanloo *et al.*(2008). It was documented proline content accumulated intensively in all stressed organs of plants espicially in leaves as a consequence of increasing breakdown of proteins with simultaneous decline in its synthesis in addition to conversion some of amino acids as ornithin, arginine and glutamic to proline (Chaitante *et al.,* 2000). Osmotic adjustment is accomplished with the accumulation of compatible solutes. Of these, proline is one amongst the most important cytosolutes and accumulates in plants during the adaptation to various types of environmental stress, such as drought, salinity, high temperature, nutrient deficiency, and exposure to heavy metals and high acidity (Oncel *et al.,* 2000).

Also Statistical analysis showed that ascorbate peroxidase activity (APX) increased with the decrease of irrigation levels ($P < 0.01$) but catalase activity remains unchanged *(Table 1)*. Interaction effects between irrigation levels and growth stages were not significant for all traits in this study *(Table 1)*. Reactive oxygen species (ROS) are a group of very reactive, short-lived chemicals produced during normal metabolism or after an oxidative reaction. ROS include superoxide anion, hydrogen peroxide, hydroxyl radical (Sun, 1990). The limitations of carbon dioxide exchange and metabolic fixation result in exposure of chloroplasts to excess excitation energy. Some of excitation energy may be diverted to activate molecular oxygen and excess reactive oxygen species, will be produced and accumulated in the cell (Walker, 1992). All these species (e.g. superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen) are reactive and may damage cells by causing lipid peroxidation and inactivation of enzymes (Winston, 1990). Many reports show the deleterious effects of reactive oxygen species, whose production is stimulated under water stress (Blokhina et al., 2003). The transcript of some of the antioxidant genes such as glutathione reductase or ascorbate peroxidase was higher during recovery from a water deficit period and appeared to play a role in the protection of cellular machinery against damage by reactive oxygen species (Ratnayaka *et al.,* 2003). Plants develop various antioxidants to cope with oxidative stress. Ascorbate peroxidase activity (APX) and catalase (CAT) are important antioxidants which protect plants by

suppressing oxidative injury. In general, antioxidant enzymes content of leaves increased with the decline in irrigation water, suggesting that the production of antioxidant enzymes is probably a common response of plants under drought conditions. The activities of antioxidant enzymes are generally increased during abiotic stress conditions and correlate with enhanced cellular protection

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