INVESTIGATION OF PHYSIOLOGICAL RESPONSES OF SARDARI WHEAT UNDER SALT STRESS CONDITION

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Salinity is a major problem which negatively affects a multitude of metabolic processes of plants resulting in reduced growth and yield of most crops. In this study the physiological parameters on wheat under salt stress (30 and 90 mM NaCl) were conducted. Experiments revealed that MDA and proline, accumulated under salt stress but photosynthetic pigments such as chlorophyll a, b, total and cartenoides reduced. Other parameters such as sugars and protein contents decreased under salt stress. Also, salt stress causes accumulation of AOS and the wheat physiological response is enhancement of the activity of anti-oxidative enzymes such as guaiacol peroxidase.

Keywords: chlorophyll, hydrogen peroxide, proline, salt stress, Sardari wheat.

INTRODUCTION

Wheat is a major crop in many parts of countries and it is commonly known as king of cereals. It belongs to Germaine family and globally after maize, wheat is the second most produced food among the cereal crops, while rice ranks third. Salinity of soil is contemplated as a major problem which negatively affects a multitude of metabolic processes of plants resulting in reduced growth and yield of most crops (Ahmad, 2011). High substrate salinity is a major limiting factor for plants in coastal habitats that germination being one of the most critical periods in the life cycle of halophytes (Rubio-Casal *et al*., 2003). Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on plant species (Ungar, 2005). It was reported that maximum germination of the seeds of halophytic plants occurred in distilled water or under reduced salinity (Khan *et al*., 2003) and it has been found that germination percentage was reduced with a high NaCl concentration (Tobe *et al*., 2001). Salt stress may provoke osmotic or water-deficit effect which causes reduction of water and nutrient uptake and ion-excess effect resulting from altered K^+/Na^+ ratios and/or accumulation of toxic levels of Na^+ and Cl^-. Also salt tolerance in higher plants is regulated by a number of different physiological and biochemical processes. The aim of this study was conducted to evaluate salt tolerance physiological responses. Therefore, properties

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such as photosynthetic pigments, MDA, solution sugars, proline and proteins were measured. To determine the antioxidative physiological response the hydrogen peroxide and guaiacol peroxidase will be measured.

MATERIAL AND METHODS

Sardari Seeds were sown in plastic pots (15 cm diameter) under greenhouse condition for two weeks. Then treatments of salt stress (NaCl) were started in three groups for two weeks. G1: 0 mM NaCl (control). G2: 25 mM NaCl and G3: 100 mM NaCl. For salinity treatments, 50 ml of brine samples were added each three days. Finally the necessary samples of leaves were taken for each experiment. Malondialdehyde contents were measured using a thiobarbituric acid reaction (Heath and Packer, 1968). About 0.6 to 1.2 g of tissue was homogenized in 5 ml of 5% (w/v) tricloroacetic acid and the homogenate was centrifuged at 12,000 g for 15 min at room temperature. The supernatant was mixed with an equal volume of thiobarbituric acid (0.5% in 20% [w/v] trichloroacetic acid) and the mixture was boiled for 25 min at 100 °C, followed by centrifugation for 5 min at 7,500 g to clarify the solution. Absorbance of the supernatant was measured at 532 nm and corrected for non-specific turbidity by subtracting the Abs600. MDA contents were calculated using an extinction coefficient of 155 M^{-1} cm^{-1}. The Chlorophylls and Carotenoids content were determined according to procedure described by (Lichtenthaler, 1987). Leaf tissues (500 mg) were homogenized in acetone 80%. The homogenate was centrifuged. Then the absorbance was measured. Proline was estimated according to the method of (Bates *et al*., 1973). Five gram of leaves was homogenized with 3% sulfosalicylic acid and the content was centrifuged at 10,000 g. A volume of 2 ml of glacial acetic acid and 2 ml of acid ninhydrin was added to 2 ml of tissue homogenate and incubated for 1 h in boiling water bath followed by cooling in ice bath. About 4 ml of toluene was then added and mixed vigorously. The chromophore containing toluene was aspirated from aqueous phase and the absorbance was measured at 575 nm. Solution sugars content was measured based on acidic hydrolyzed of sugars with sulfuric acid and production of colored furfural complex according to the method of (Roberts *et al*., 1959). Leaf tissues were homogenized in ice bath with 5 ml water. The homogenate was centrifuged at 12,000 g for 15 min. One ml of phenol 5% and 3 ml of sulfuric acid were mixture with supernatant. The reaction was carried out for 1 h in darkness and absorbance was measured at 485 nm. The amount of sugars content was calculated using a standard curve prepared with known concentrations of sugar. Hydrogen peroxide content was measured spectrophotometrically after reaction with potassium iodide (KI) according to the method of (Alexieva *et al*., 2001). Leaf tissues (500 mg) were homogenized in ice bath with 5 ml 0.1% TCA. The homogenate was centrifuged at 12,000 g for 15 min. The reaction mixture consisted of 0.5 ml of supernatant, 0.5 ml

of 100 mM potassium phosphate buffer (pH 7.0) and 2 ml reagent 1 M KI in fresh double-distilled water. The blank probe consisted of 0.1% TCA in the absence of leaf extract. The reaction was carried out for 1 h in darkness and absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of hydrogen peroxide. To measure the protein content, (Bradford, 1976) method was used. Guaiacol peroxidase activity measurement was performed using (Upadhyaya *et al*., 1985). The reaction mixture contains 2.5 ml of phosphate bufer 50 mM (pH 6.1) 1 ml 1% hydrogen peroxide, 1 ml guaiacol 1% and 20 µl enzyme extract and increase the absorption at 420 nm was measured over one minute. Each biochemical indicator was replicated for three times. All results were reported as the mean ± standard error (SE) and were analyzed by an analysis of variance ANOVA. If significance was found in ANOVA, group means were compared using Duncan's test. Differences were considered significant when ($p \leq 0.05$).

RESULTS AND DISCUSSION

When the seedlings were exposed to salinity stress photosynthetic pigments decreased. Results showed that the 30 and 90 mM NaCl (G2, G3) decreased chlorophyll a, b, total and cartenoides to compare control G1. The differences between G2 and G3 at chlorophyll a, b and cartenoides are significant. Decrease in chlorophyll content caused by salinity has been reported by (Hernandez, 1995) who suggested chlorophyll was one of the best parameters indicating salt tolerance in crop plants. Salinity significantly reduces the total chlorophyll content and the degree of reduction in total chlorophyll depending on salt tolerance of plant species and salt concentrations. In salt tolerant species, chlorophyll content increased, while in salt-sensitive species it decreased. It was also reported carotenoids content reduced under salinity stress (Abd el Samad, 1993), as carotenoids were responsible for quenching of singlet oxygen (Knox and Dodge, 1985). When the seedlings were exposed to salt stress, Active Oxygen Species accumulate in different cell compartment. One of the indications of AOS injuries to cell membrane lipids is production of MDA. The results show that the sugar solution decreased in salt stress and salt stress has a different effect on carbohydrate contents. Also salt stress decreased protein synthesis and the amount of proteins in G2 and G3 reduced to compare control. Amino acids such as proline, asparagines and amino butyric acid, can play important roles in osmotic adjustment of plant under saline conditions. Proline accumulation might be used as an indicator in selection for withstanding saline stress through the involvement in osmoregulation (Haroun, 2002 and Ueda, *et al*., 2007). Salt stress increased Hydrogen peroxide in seedlings. Under salinity conditions, hydrogen peroxide content induced significantly. Differences between G1, G2 and G3 are significant. AOS can limit with production of guaiacol peroxidase.

As it was shown in Fig. 3, the activity of guaiacol peroxidase increased in G2 and G3 significantly. Higher activity of phenolic compounds could be due to the greater H-donating ability and radical stabilization than a variety of other antioxidant metabolites (Rice-Evans, *et al*., 1996).

CONCLUSION

A lot of research is being conducted these days to elucidate the role of various antioxidant metabolites in plant stress tolerance. Biological and antioxidant properties of phenolic compounds among other metabolites have been studied to a great extent. Higher activity of phenolic compounds could be due to the greater H-donating ability and radical stabilization than a variety of other antioxidant metabolites.

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