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EFFECTS OF NACL SALINITY ON SEEDLING GROWTH, SENESCENCE, CATALASE AND PROTEASE ACTIVITIES IN TWO WHEAT GENOTYPES DIFFERING IN SALT TOLERANCE

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Abstract

Changes in seedling growth, senescence, protease activities and possible involvement of hydrogen peroxide scavenging enzyme e.g., catalase, in relation to salt tolerance were investigated in two wheat genotypes differing in salt tolerance. The 3days old wheat seedlings were subjected to 5, 10 and 15dSm^{-1} NaCl salinity for 6days. Data showed that salt-stress brought about a reduction of the growth and protein content, particularly at 15dSm^{-1} NaCl salinity. Application of low salinity (5dSm^{-1}) did not show marked effects, but under high NaCl stress growth was suppressed even in tolerant genotype. Overall good growth of wheat cultivar Lu-26 at seedling stage might be due to osmotic adjustment. Leaf senescence or decrease in leaf protein content was only observed at higher (15dSm^{-1}) NaCl stress in salt sensitive wheat cultivar Pak-81. The magnitude of salt induced proteolysis was many folds higher in sensitive wheat genotype Pak-81 at 15dSm^{-1} NaCl salinity. The prominent salt induced senescence in leaves of wheat cultivar Pak-81 was associated with higher salt sensitivity in terms of extensive proteolysis. Severe salt-stress resulted in an inhibition of the antioxidative enzyme catalase as revealed by spectrophotometric assay. Catalase activity was decreased at all salinity levels in both wheat cultivars signifying that high salinity generally reduced the catalase activity irrespective of wheat genotype. The results suggest that cv. Lu-26, exhibits a better protection mechanism against salinity as indicated by lower salt induced proteolysis, higher biomass accumulation and protein content than the relatively sensitive cv. Pak-81.

Introduction

Agricultural crops face different types of biotic and abiotic stresses. Among abiotic stresses, salinity is very harmful and adversely affects the agricultural production. However productivity and internal drainage of saline soils can be restored to some extent by better management practices like combination of physical and chemical treatments (Zia *et al.,* 2006). Osmotic stress induced by soil salinity affects plant growth and development (Sairam & Tyagi, 2004). Salinity alters general metabolic processes and enzymatic activities, causing increased production of reactive oxygen species (Menezes-Benavente *et al.,* 2004).

To counteract the toxicity of reactive oxygen species, a highly efficient antioxidative defense system, including both nonenzymic and enzymic constituents, is present in plant cells. The formation of ROS is prevented by an antioxidant system: low molecular mass antioxidants (ascorbic acid, glutathione, tocopherols), regenerating the reduced forms of antioxidants and ROS-interacting enzymes such as SOD, peroxidases and catalases (Blokhina *et al.,* 2003). Activation of the plant antioxidant system by H_2O_2 plays an important role in induced tolerance against salt stress (Gechev *et al*., 2002).

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Therefore in this work, using two wheat cultivars one relatively salt tolerant (Lu-26) and the other sensitive (Pak-81), the effects of various levels of NaCl (5, 10, 15dSm^{-1}) salinity on activity of hydrogen peroxide scavenger catalase, protease, leaf senescence and early seedling growth were studied for a better understanding of salt tolerance mechanism.

Material and Methods

Two wheat (*Triticum aestivum* L.) genotypes Lu-26 and Pak-81 differing in tolerance to salinity were included is this study. Uniform sized seeds (44.05 ± 3.07mg) of both wheat strains were selected for the experiment. Seeds were germinated in darkness for 24 hours in an incubator at 25±1°C on wet filter paper in Petri dishes. Germinated seeds were then covered with a lid to minimize evaporation and growth was continued for 24 h at 25±1°C. The germination of seeds was tested at 25±1°C. After 72 hours from seed soaking time, different levels of osmotic stress (salinity) were applied. Except for control, water as the medium was changed with 5, 10 and 15dSm^{-1} NaCl salinity and the growth of the seedlings was continued at 25±1°C for day (16Hours) and at 22±1°C for night (darkness) (8hours) for next 6 days.

Growth characteristics

Fresh and dry weight: At least 36 seedlings from each treatment and control were analyzed for different morphological, and biochemical studies. Fresh weights of seedlings (8 days old) were determined immediately after taking out of Petri plates to avoid evaporation. For dry weight estimations, pre-weighted seedlings were kept at 90 ^{o}C till drying. Seedling, root and shoot dry weights were measured after complete drying when there was no further decrease in weight. Some seedlings were stored at -80 ^{o}C for different biochemical studies.

In biochemical studies total soluble protein content, catalase and protease activities were estimated in leaf samples. For enzyme estimations known amount of frozen (-80 ^{o}C) ground plant material was suspended in lying buffer containing 0.05 M Tris HCl, pH 7.5, 0.025 M EDTA and 1% Sodium dodecyl sulfate. The mixture was immediately centrifuged in microcentrifuge machine (Eppendorf 5415c) for 10 min at 14,000rpm at 4^{o}C. The supernatant was separated and used for enzyme estimations.

Catalase activity: For the estimation of catalase activity, leaf sample (0.5g) was homogenized in 5ml medium, composed of 50mM phosphate buffer, pH 7.0 and 1mM dithiothreitol (DTT) as described by Dixit *et al.*, (2001). Catalase activity was assayed in 50mM phosphate buffer, pH 7.0 by following the decreased in absorbance at 240nm (Worthington, 1988).

Proteases activity: Enzyme activity was determined by the casein digestion assay described by Drapeau *et al*., (1974). Briefly by this method, one unit is that amount of enzyme, which releases acid soluble fragments equivalent to 0.001 A_{280} per minute at 37°C and pH 7.8.

Total soluble protein content: Protein concentration was measured in the supernatant using Bradford method (Bradford, 1976).

Statistical analysis: Experiment was conducted with three replications (30 seedlings per replication). The descriptive statistics were applied to analyze and organize the resulting data. The f-test was applied to find differences in variance among samples. The significance of differences between means (for stressed and control) for different parameters was measured using Student's t-Test (two tailed), at 0.05 significance level. All the statistical calculations were performed using computer software Microsoft Excel 2000.

Results

Effects of different level of NaCl salinity on growth, senescence, catalase and protease activities were studied in relatively salt tolerant wheat cv. Lu-26 and sensitive wheat cv. Pak-81.

Growth characteristics: Fresh weight of seedling was significantly decreased at all salinity levels in wheat cv. Pak-81 and at 10 and 15dSm^{-1} NaCl salinity in wheat cv. Lu-26. In salt tolerant wheat genotype Lu-26 fresh weight was not affected at 5dSm^{-1} NaCl salinity. Fresh weight of seedling was also significantly higher ($p<0.05$) in salt tolerant wheat cv. Lu-26 as compared with sensitive wheat cv. Pak-81 at 5dSm^{-1} NaCl salinity. There was non-significant ($p>0.05$) deference in seedling fresh weight in both wheat genotypes under higher NaCl stress levels (Fig. 1a).

Seedling and root dry weights were significantly higher ($p<0.05$) in salt tolerant wheat cv. Lu-26 as compared with sensitive wheat cv. Pak-81 at 5 and 10dSm^{-1} NaCl salinity. However seedling and root dry weights were not significantly different in both wheat genotypes at 15dSm^{-1} NaCl salinity (Fig. 1b, 1d).

Seedling dry weight was unaffected in salt tolerant wheat cv. Lu-26, while significantly ($p<0.05$) decreased in sensitive wheat cv. Pak-81 at 5 and 10dSm^{-1} NaCl salinity. Seedling dry weight was not significantly affected in both cultivars at 15 dSm^{-1} NaCl salinity as compared with that in control (Fig. 1b). Root dry weight was increased at 5 and 10dSm^{-1} NaCl salinity in wheat cv. Lu-26 and at 15 dSm^{-1} NaCl salinity in wheat cv. Pak-81 as compared to that in control (Fig. 1d). A sharp and highly significant ($p<0.05$) decrease in shoot dry weight (Fig. 1c) and length (Fig. 2a) with increasing salt concentration was observed in both cultivars.

Root length was also decreased in both cultivars at 5 and 10dSm^{-1} NaCl salinity (Fig. 2b). Root length and Root/shoot ratio were generally higher in salt tolerant cultivar Lu-26 as compared with salt sensitive cultivar Pak-81 under control and all NaCl stress levels. Root/shoot ratio was significantly ($p<0.05$) increased at 5 and 15 dSm^{-1} NaCl salinity in salt tolerant wheat cv. Lu-26 and only at 5 dSm^{-1} in salt sensitive cultivar Pak-81 (Fig. 2c).

Leaf protein contents: Leaf senescence or decrease in leaf protein content was only observed at higher (15 dSm^{-1}) salinity level in wheat cultivar Pak-81. Leaf protein contents were almost twofold ($p<0.05$) at 5 dSm^{-1} NaCl salinity as compared with control in cv. Pak-81. Similarly a significant ($p<0.05$) increase in protein contents was also observed in salt tolerant cultivar Lu-26 at 10 dSm^{-1} NaCl salinity (Fig. 3a).

Protease activity: There was a non-significant ($p>0.05$) effect on protease activity in salt tolerant wheat cv. Lu-26 at 5 and 10 dSm^{-1} NaCl salinity (Fig. 3b). However at higher salinity level (15 dSm^{-1}) protease activity was increased significantly and was almost double as compared to that in control. On other hand a significant decrease ($p<0.05$) in protease activity at 5 and 10 dSm^{-1} NaCl salinity while a many fold increase in activity at 15 dSm^{-1} NaCl salinity was observed in cv. Pak-81.

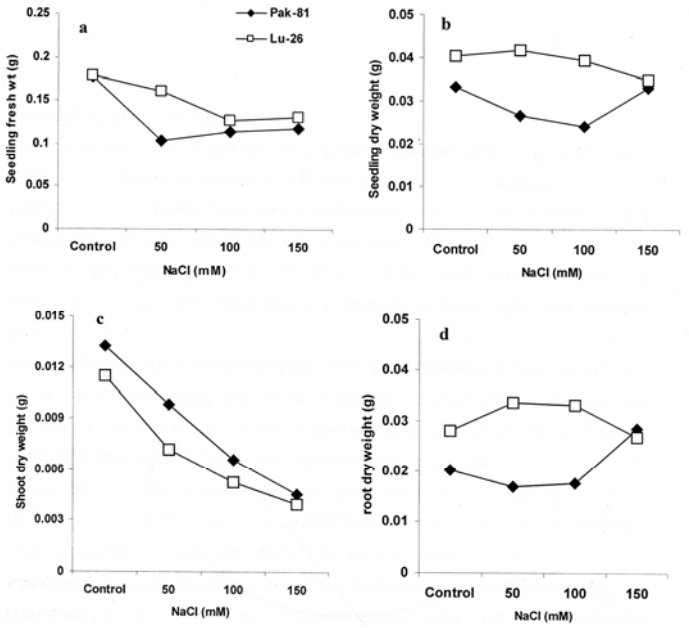

Fig. 1. Effect increasing NaCl salinity on seedling fresh weight (a), dry weight (b), shoot (c) and root dry weight (d) of different wheat varieties.

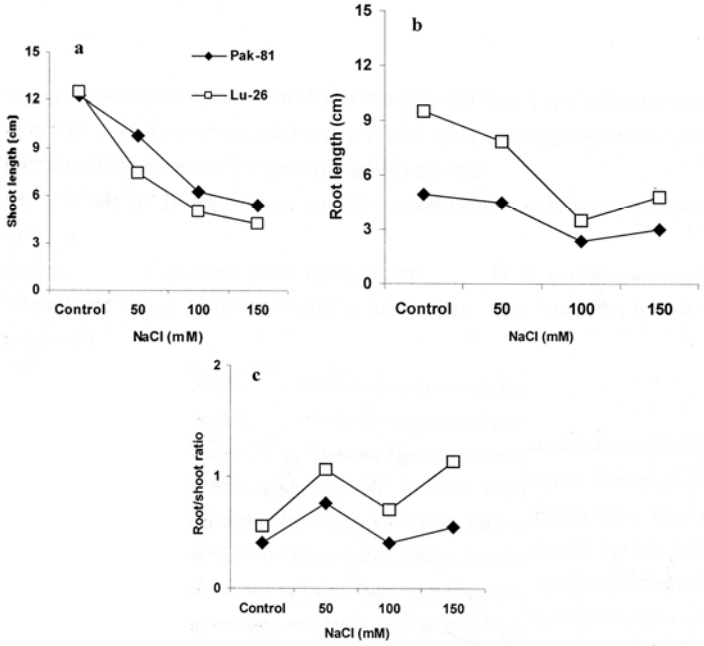

Fig. 2. Effect of increasing NaCl salinity on shoot (a), root length (b) and root/shoot ratio (c) of different wheat varieties.

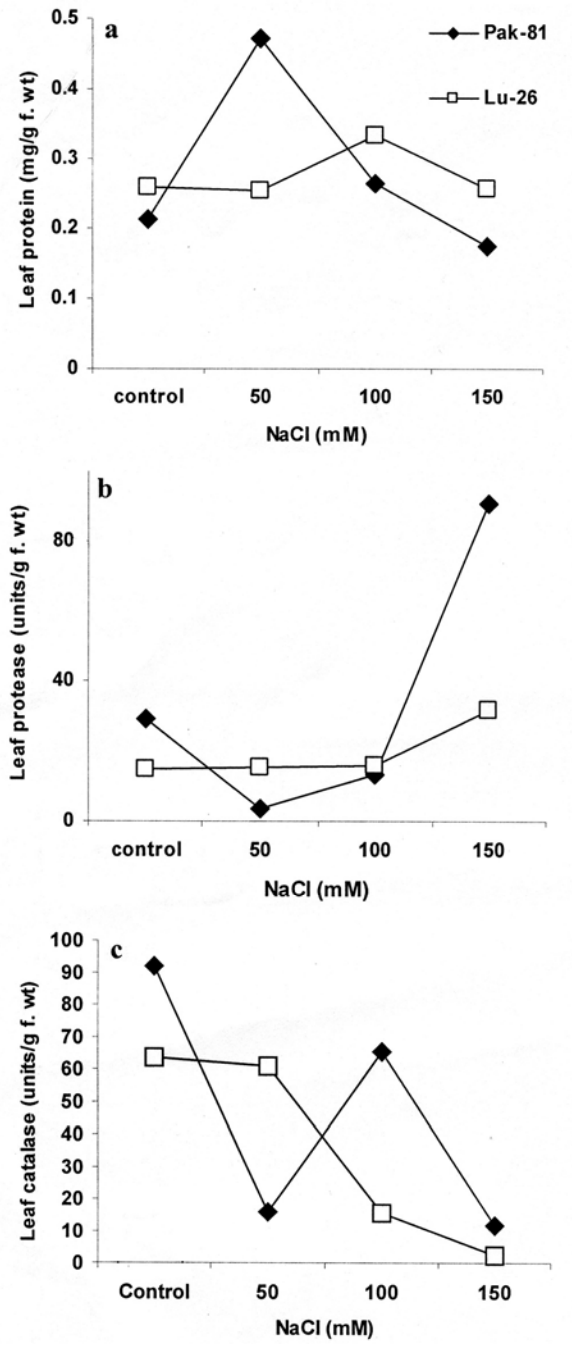

Fig. 3. Effect of increasing NaCl salinity on leaf protein contents (a), protease (b) and catalase activity (c) in different wheat varieties.

Catalase activity: Catalase activity was decreased in both cultivars at all NaCl stress levels and decrease in activity was more pronounced with increasing salt concentration (Fig. 3c). A gradual decrease in catalase activity with increasing salt concentration was observed in cv. Lu-26. Though in cv. Pak-81 catalase activity was decreased sharply at 5 dSm^{-1} NaCl salinity, and then increased at 10 dSm^{-1} NaCl salinity followed by a decrease at 15 dSm^{-1} NaCl salinity.

Discussion

Inhibition of growth by salinity is the most evident effect in all crop plants. Almost all growth related parameters like seedling fresh and dry weights, shoot and then root dry weight and lengths were adversely affected under saline conditions. Fresh weight of seedling in both wheat cultivars was decreased at higher salinity level (15 dSm^{-1} NaCl salinity). Salt tolerant variety gave more fresh weights as compared to other under low salt stress. Fresh weight of seedling was significantly higher in salt tolerant wheat cv. Lu-26 as compared with sensitive wheat cv. Pak-81at 5 dSm^{-1} NaCl salinity. It means higher fresh weight was associated with salt tolerant cultivar.

The shoot dry weight was also decreased at higher salt stress (15 dSm^{-1} NaCl salinity) in both cultivars in the present study. Husain *et al*., (2003) used six durum wheat genotypes with varying Sodium accumulation to assess the effect of Sodium exclusion on biomass production in saline soil. Plant height and dry biomass were measured at 3 salinity levels (1, 75 and 150mM NaCl). At ear emergence, the effects of salinity on biomass were less on low Na^+ than on the high Na^+. At maturity, salinity had a similar effect on biomass of both genotypes at both 75 and 150 mM NaCl. In the present study, at seedling stage the root dry weight was higher in salt tolerant cultivar as compared to control under low (5 dSm^{-1}) and medium (10 dSm^{-1}) salt stress.

Previously Del Zoopo *et al*., (1999) studied the effect of 50 to 200 mM NaCl on two lines (CP with solid stem and CV with hollow stem) of Haynaldoticum sardoum. NaCl significantly reduced shoot length of CP and CV plants. In the present study, shoot length was also decreased in both cultivars with increasing level of salinity.

Root/shoot length ratio was increased as compared to control under 5 and 15 dSm^{-1} NaCl salinity in both cultivars in present study. While at higher salinity (15 dSm^{-1}) root/shoot length ratio was similar to control in Pak-81 and was slightly higher than control in Lu-26. Recently in a study, thirty diverse genotypes of bread wheat were evaluated for root-to-shoot length ratio and osmotic membrane stability under laboratory conditions (Dhanda *et al*., 2004). The root-to-shoot length ratio, increased under osmotic stress. Correlation studies indicated that the osmotic membrane stability and root/shoot length ratio were the important traits, on the basis of their relationships with other traits.

The present study, supported by previous ones indicates that application of low salinity did not show harmful effect on root and shoot growth, but under high saline conditions growth is effected even in tolerant genotypes. Over all good performance of wheat cultivar Lu-26 at seedling stage might be due to its better osmotic adjustment (i.e., by high Na absorption in its shoots), which is considered to be an important adaptation of plant to salt stress (Shirazi *et al.,* 2001). Better osmotic adjustment or plant water status increased the stomatal conductance and thus favored higher CO_2 fixation rate (Raza *et al.,* 2006). Wheat cultivar Lu-26 has proved to be salt-tolerant by producing greater biomass, showing less reduction in NRA, maintaining low sodium (Na^+), and accumulating more K^+ and Ca^{2+} in response to salinity (Naeem *et al.,* 2006).

Leaf protein content was almost twofold ($p<0.05$) as compared with control at 5 dSm^{-1} NaCl salinity in cv. Pak-81 and a significant ($p<0.05$) increase over control was also observed in salt tolerant cultivar Lu-26 at 10 dSm^{-1} NaCl salinity (Fig. 3a). Previously increase in protein content in inter and intracellular leaf compartments of both sensitive and tolerant wheat genotypes under salinity has also been reported (Muhling & Lauchli, 2003).

Leaf senescence or decrease in leaf protein content was only observed at higher (15 dSm^{-1} NaCl salinity) NaCl stress in wheat cultivar Pak-81. Similarly the hypocotyl soluble proteins from sunflower have also been reported to decrease consistently with increasing salt level (Ashraf *et al.*, 2003). It has been suggested in an earlier report that NaCl stress enhances the production of oxygen radicals and H_2O_2, especially in leaves of salt sensitive genotype of wheat (Muhling & Lauchli, 2003) which caused decrease in protein content (senescence) in wheat leaves. This may also be true for the present study. Further decrease in protein contents in Pak-81 provided an evidence for salt sensitivity of this cultivar.

Previously to establish the importance of the salt induced proteolysis and the glutamine synthetase activity on the proline accumulation, cashew (*Anacardium occudentale*) plants were exposed to a short and long term exposure to NaCl (Silveira *et al*., 2003). The leaf proline accumulation was correlated to protease activity, accumulation of free amino acid and ammonia. The leaf protease activity was increased by salt stress. Moreover, the prominent salt induced proline accumulation in leaves was associated with the higher salt sensitivity in terms of proteolysis and salt induced senescence as compared to the roots. In the present study, at higher salinity (15 dSm^{-1}l) protease level was significantly increased in both cultivars. The magnitude of salt induced proteolysis was manifold higher in sensitive wheat genotype Pak-81 at 15 dSm^{-1} salinity. As mentioned earlier salt induced senescence (decrease in protein contents) was also observed in Pak-81 at 15 dSm^{-1} salinity. Therefore the prominent salt induced senescence in leaves of wheat cultivar Pak-81 was associated with higher salt sensitivity in terms of extensive proteolysis.

Salinity alters general metabolic processes and enzymatic activities, causing increased production of reactive oxygen species (ROS) (Menezes-Benavente *et al.*, 2004). Expression of antioxidant defense genes would, in turn, be triggered to defend the cell against oxidative damage. Catalase, which is involved in the degradation of H_2O_2 into water and oxygen, is the major H_2O_2 scavenging enzyme in all-aerobic organisms (Yang & Poovaiah 2002). Catalase is critical for maintaining the redox balance during oxidative stress. Catalase functions as a cellular sink for H_2O_2 (Willekens *et al.*, 1997). Exposure to oxidative stress has been reported to cause a decrease in catalase activity in wheat as well as rice seedlings (Shim *et al*., 2003). Providing further evidence, catalase activity was also decreased at all salinity levels as compared with control in both wheat cultivars in the present study. This indicates that high salinity generally reduces the catalase activity irrespective of wheat genotype.

In two wheat cultivars differing in salt tolerance, overall response to NaCl stress was significantly different. Similar genotype dependent response was also reported recently where it was observed that the effects of three priming agents (i.e. $CaCl_2$, KCl and NaCl) on different plant hormones were different in the two cultivars viz., MH-97 (salt sensitive) and Inqlab-91 (salt tolerant) (Iqbal *et al.*, 2006).

In conclusion, biochemical responses to NaCl stress were significantly different in two cultivars differening in salt tolerance. The leaf senescence in salt sensitive cultivar Pak-81 was due to increase in the salt induced proteolysis.

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