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PHYSIOLOGICAL ENHANCEMENTS FOR ALLEVIATION OF SALT STRESS IN WHEAT

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Abstract

Increased salinity is a severe problem to crop production while pre-sowing seed treatments can effectively induce salt tolerance in plants. The effect of different pre-sowing seed treatments (hydropriming, halopriming (50 mM $CaCl_2.2H_2O$), ascorbate priming (50 mg L^{-1}) and pre-sowing chilling treatment (-19°C) on seed germination, vigor, antioxidants and total soluble protein content was investigated in two wheat (*Triticum aestivum* L.) cultivars Auqab-2000 (salt tolerant) and MH-97 (salt sensitive) under saline (15 dS m^{-1}) or non-saline (4 dS m^{-1}) conditions. Of all the seed pretreatments, halopriming followed by hydropriming was the most effective in alleviating the adverse effect of salinity by improving germination and seedling growth of both cultivars. In addition, the effect of ascorbate priming was more pronounced in salt tolerant cultivar as revealed from curtailed mean germination time, improved seedling vigor and enhanced ascorbate contents and catalase (CAT) activity. Salinity significantly increased leaf protein content in both cultivars but the magnitude of increase in protein content was higher in Auqab-2000 as compared to that in MH-97. All pre-sowing seed treatments significantly enhanced superoxide dismutase (SOD) activity in MH-97 while priming with $CaCl_2.2H_2O$ and ascorbate were very effective in Auqab-2000 during stress conditions. The salt-tolerant cultivar Auqab-2000 had a better protection against reactive oxygen species (ROS) as shown by increased SOD amd CAT activities under salt stress. In conclusion, halopriming and hydropriming successfully improved the seed performance in both cultivars whereas priming with ascorbate was only effective in salt tolerant cultivar under saline conditions. This benefit was attributed to early and synchronized germination, vigorous stand establishment, and decreased oxidative damage due to enhanced antioxidant system.
Keywords: salt tolerance; oxidative stress; seed priming; wheat.

Introduction

Soil salinity is considerable problem adversely affecting physiological and metabolic processes, finally diminishing growth and yield (Ashraf & Harris, 2004). Salinity affects the availability of nutrients and water, lowers the quality of arable lands, and alters the structure of ecological communities. It induces osmotic stress, the physiological drought, which typically reduces growth and photosynthesis in plants (Pasternak, 1987). Growth reduction due to salinity is also attributed to ion toxicity and nutrient imbalance. Salt stress in addition to the known components of osmotic stress and ion toxicity, is also manifested as an oxidative stress (Guetadahan *et al.*, 1998). However, ion content and salt tolerance are not often correlated and several studies indicate that acquisition of salt tolerance may also be a consequence of improving resistance to oxidative stress (Hernandez *et al.*, 2001).

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A strong evidence exists that in different plants salt stress can induce accumulation of reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen (Lee *et al.*, 2001). Reactive oxygen species (ROS) attack proteins, lipids and nucleic acids, and the degree of damage depends on the balance between formation of ROS and its removal by the antioxidative scavenging systems and it appears to represent an important stress-tolerance trait. Expression of antioxidant defense genes would, in turn, be triggered to defend the cell against oxidative damage (Menezes-Benavente *et al.*, 2004). Elimination of ROS is mainly achieved by antioxidant compounds such as ascorbic acid, glutathione, thioredoxine and caroteniods, and by ROS scavenging enzymes e.g., superoxide dismutase, glutathione peroxidase and catalase (Noctor & Foyer, 1998). This shows that oxidative stress tolerance is genetically controlled and it provides a wide scope for crop improvement using conventional breeding and selection, and transgens production (Hung *et al*., 2005) or adopting physiological approaches (Agarwal *et al*., 2005; Azevedo Neto *et al.,* 2005).

Of various strategies, pre-sowing seed treatments (seed priming) are simple, low cost and effective approaches for induction of salt tolerance in wheat. Priming is a controlled hydration process followed by redrying that allows all pregerminative metabolic activities but prevents radicle protrusion (Khan *et al.,* 1992). Concerted attempts have been made to improve salinity tolerance in wheat by hydropriming (Basra *et al.*, 2005a), pre-sowing chilling treatment (Basra *et al.,* 2005b) and halopriming (Ashraf *et al.*, 1999; Kamboh *et al.*, 2000). Recently, there are reports that priming resulted in a strong increase in SOD and CAT activities in plants (Basra, 2004). Seed hydration in solutions containing inorganic solute, hormones or antioxidant compounds might be able to express antioxidant defense genes which would, in turn, be triggered to defend the cell against oxidative damage.

Therefore, the primary aim of this study was to characterize the influence of pre-sowing seed treatments on plant defense system during salinity stress.

Materials and Methods

Experimental material: Healthy seeds of two commonly grown wheat (*Triticum aestivum* L.) cvs. in Pakistan namely MH-97 (salt sensitive) and Auqab-2000 (salt tolerant) were surface sterilized with 5% (v/v) sodium hypochlorite solution for 3 minutes to avoid fungal invasion followed by repeated washings with sterilized distilled water. The two cultivars were chosen due to their known response to salinity.

Plant growth conditions: Germination of the wheat seeds was assessed in accordance with the International Rules for Seed Testing (ISTA, 1985). Four replicates of 25 seeds each were germinated in 12 cm diameter Petri dishes on Whatman No.1 filter paper at 25°C in a growth chamber (Type 8194, VINDON). A sufficient volume (5 mL) of salt solution with 15 dS m^{-1} electrical conductivity (EC) was added to submerge the seeds partially under saline environment, whereas water having 4 dS m^{-1} EC was provided to the seeds grown under normal conditions. Water and saline solution requirements were checked daily and topped-up according to necessity. A seed was scored germinated when coleoptile and root lengths reached 2-3 mm. Counts of germinating seeds were made every 6 h, and terminated when maximum germination was achieved. During this, mean

germination time was calculated according to the equation of Ellis and Roberts (1981) and expressed as

$$MGT\ (h) = \Sigma\ (hn)/\ \Sigma n$$

where *h* is the number of hours from the beginning of the germination test and *n* is number of seeds germinating on hours *h*.

Seedlings were harvested after ten days and washed with deionized water after harvest. Five washed seedlings from each replication were separated into root and shoot for the determination of their fresh and dry weights. Dry weight was determined after oven drying the samples at 65 ^{o}C. Remaining seedlings were placed in ultra low freezer at -80 °C for further use in biochemical analysis.

Pre-sowing seed treatments: Following pre-sowing seed treatments were optimized during preliminary studies (Afzal *et al*., 2005). For hydropriming, seeds were soaked in distilled water for 12 h (Bennett and Waters, 1987). For halopriming, seeds were soaked in solution of 50.0 mM $CaCl_2.2H_2O$ for 12 h at 20 °C in the dark (Basra *et al.,* 2005a). The osmotic potential of aerated solution was –1.25 MPa. For ascorbate priming, seeds were soaked in 50 mg L^{-1} ascorbate solution for 12 h (Sundstrom *et al.,* 1987). For pre-sowing chilling, seeds were sealed in polythene bags and placed in a refrigerator at -19±2 ^{o}C for 48 h (Basra *et al.,* 2005b). During hydration treatments continuous fresh air was supplied.

After respective priming treatment for specific period, seeds were washed with distilled water (Khan *et al.,* 1992). The seeds were dried back near to original weight on laboratory benches with forced air under shade for 48 hours. The laboratory temperature during the drying period was 27±2 ^{o}C. These seeds were packed in polythene bags and stored in a refrigerator 7±2 for further studies (Basra *et al.,* 2005b).

Determination of antioxidant enzyme activities: Catalase (units/mg of protein) activity in the leaf samples was estimated by the method as described by Beers and Sizer (1952). Catalase activity level was determined by following the decrease in absorbance at 240 nm for 3 min by using spectrophotometer (Spectronic 21 D, Milton Roy). SOD activity in units/mg of protein was assayed by using the photochemical NBT method as described by Dixit *et al*. (2001). The photoreduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was prepared against different volumes of extract. One unit of SOD was defined as that being present in the volume of extract that caused inhibition of the photo-reduction of NBT by 50 %.

Estimation of ascorbate and soluble protein contents: The ascorbic acid concentration in shoots was determined using the method as described by Kampfenkel *et al.* (1995). The assay mixture contained 100 µL sample, 1.9 mL distilled water and 1mL DCIP solution. The absorbance was measured at 520 nm by using spectrophotometer (Spectronic 21 D, Milton Roy). Quantitative protein estimation in shoots was performed following Bradford (1976) using bovine serum albumin as standard.

Statistical analysis: All the experiments were performed in triplicate by using a completely randomized design. Data recorded each time were pooled for statistical analysis to determine the significance of variance ($P<0.05$). Values in the figures indicate mean values ± S.E.

Results

All the pre-sowing seed treatments had a significant ($P < 0.05$) effect on germination and seedling vigor of both cultivars under non-saline and saline conditions (Fig. 1-2). Most of priming agents were effective in improving FGP and decreasing MGT in both cultivars under saline conditions. However, halopriming followed by hydropriming resulted in higher FGP and lower MGT in both cultivars. In addition, ascorbate priming was also effective in improving FGP in salt tolerant cultivar under saline environment. Among varietal comparison, salt sensitive cv. MH-97 took more time to germinate as compared to the salt tolerant cultivar (Fig. 1).

Salt medium caused a reduction in the root and shoot lengths of both cultivars (Fig. 1c and 1d). Except chilling, all the priming agents significantly increased root and shoot lengths of salt tolerant cultivar under saline medium. Similarly, fresh weight of seedlings was significantly increased in all the plants raised from treated seeds except chilled seeds but ascorbate priming did not increase fresh weight of both root and shoot in MH-97. Hydropriming improved fresh weight of seedlings of both cultivars to the maximum limit.

A significant reduction in dry weight of seedlings of both cultivars was observed under saline conditions. However, most of the pre-sowing seed treatments were effective in improving dry weight of seedlings under saline medium. The cultivars did not differ significantly due to dry weight of root under salinity stress. Halopriming was found to be most effective in increasing dry weight of seedlings in both cultivars under salt stress. Except chilling, all the pre-sowing seed treatments were effective in increasing dry weight of seedlings under saline conditions (Fig. 2).

More increase in CAT activity was recorded in wheat cv. Auqab-2000 than that in MH-97 under saline conditions (Fig. 3a). Among all the pre-sowing seed treatments, halopriming maximally increased CAT activity in the plants of both cultivars under saline conditions while ascorbate priming was only effective in case of salt tolerant cultivar. The present studies also reveal that there was a significant decrease in SOD activity in MH-97, while a significant increase in Auqab-2000 was observed under salinity (Fig. 3b). All the treatments were effective in increasing SOD activity in wheat cv. MH-97 under stress conditions. Among seed treatments, maximal rate of increase in SOD activity was observed in plants of both cultivars raised from seeds primed with $CaCl_2$ and ascorbate under stressful environment.

A significant increase in leaf protein content was observed under salinity stress in both wheat cultivars. The magnitude of increase in protein contents under saline condition was higher in Auqab-2000 as compared with that in MH-97. Protein contents were increased after all other treatments except chilling under salinity stress particularly in Auqab-2000. All pre-sowing seed treatments significantly decreased protein contents in MH-97 under saline conditions whereas the protein content was increased remarkably by hydropriming, halopriming and ascorbate priming under normal conditions (Fig. 3c).

Pre-sowing seed treatments significantly ($P < 0.05$) affected ascorbate in both wheat cultivars under normal and saline conditions. Ascorbate was significantly higher in wheat cv. Auqab-2000 as compared to that in MH-97 under saline conditions. Under saline conditions, ascorbate contents were significantly higher in those seedlings of both cultivars that raised from seeds primed with ascorbate as compared to the non-primed seeds (Fig. 3d).

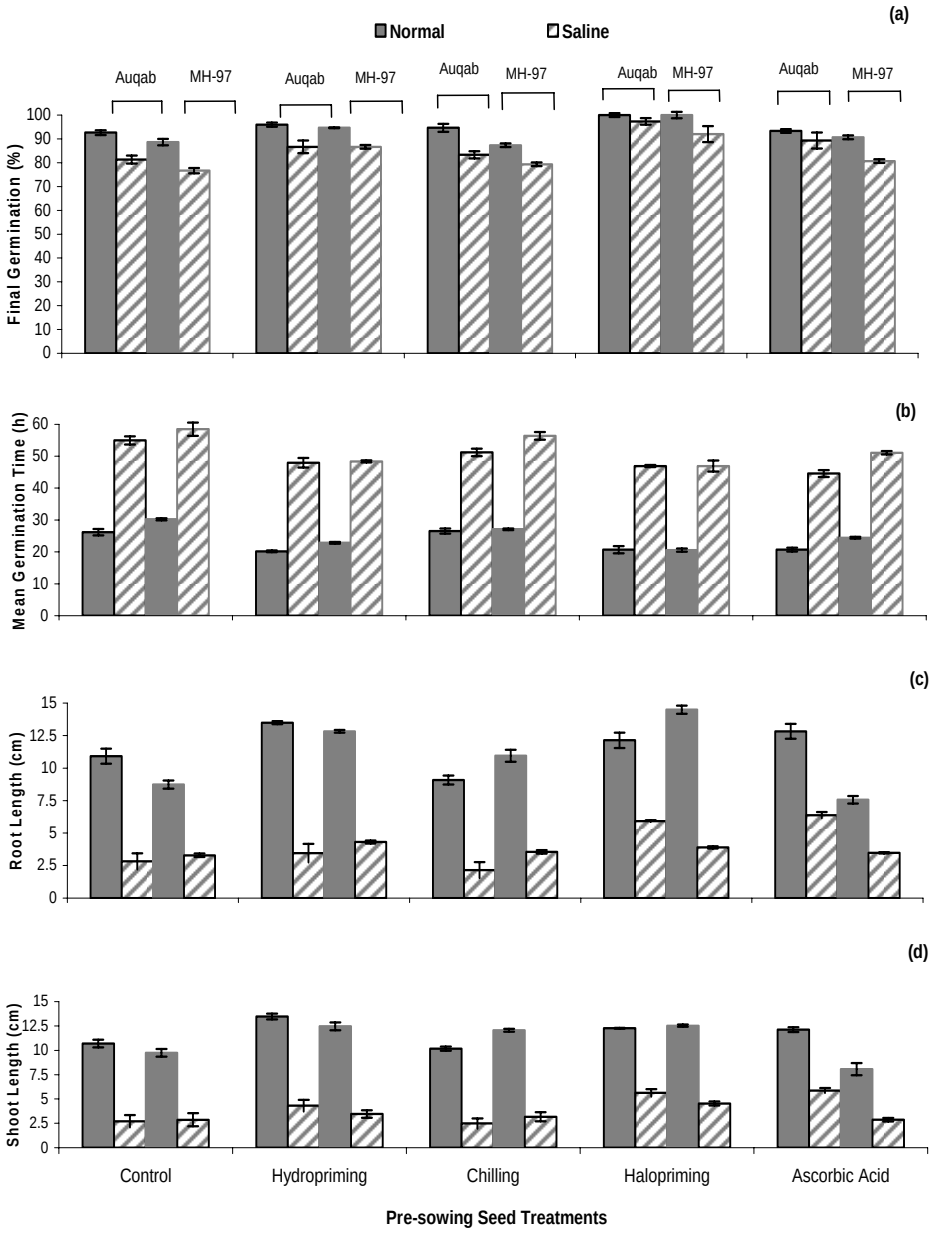

Fig. 1. Effect of different pre-sowing treatments on germination and seedling vigor of two wheat cultivars Auqab-2000 and MH-97 grown under non-saline (4 dS m^{-1}) and saline (15 dS m^{-1}) conditions. Data are the means±SE.

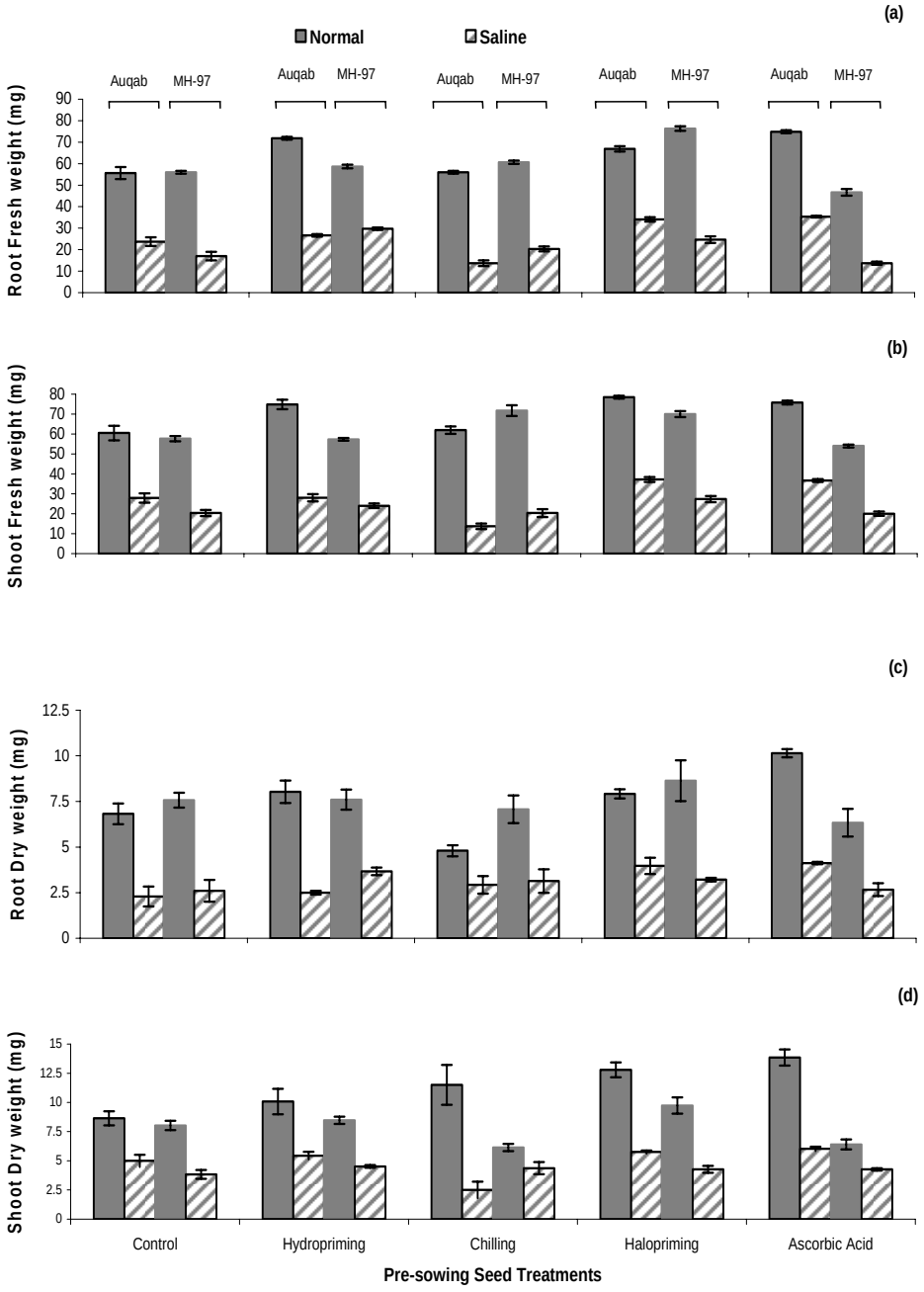

Fig. 2. Effect of different pre-sowing treatments on seedling fresh and dry weights of two wheat cultivars Auqab-2000 and MH-97 growing under non-saline (4 dS m^{-1}) and saline (15 dS m^{-1}) conditions during germination test. Data are the means±SE.

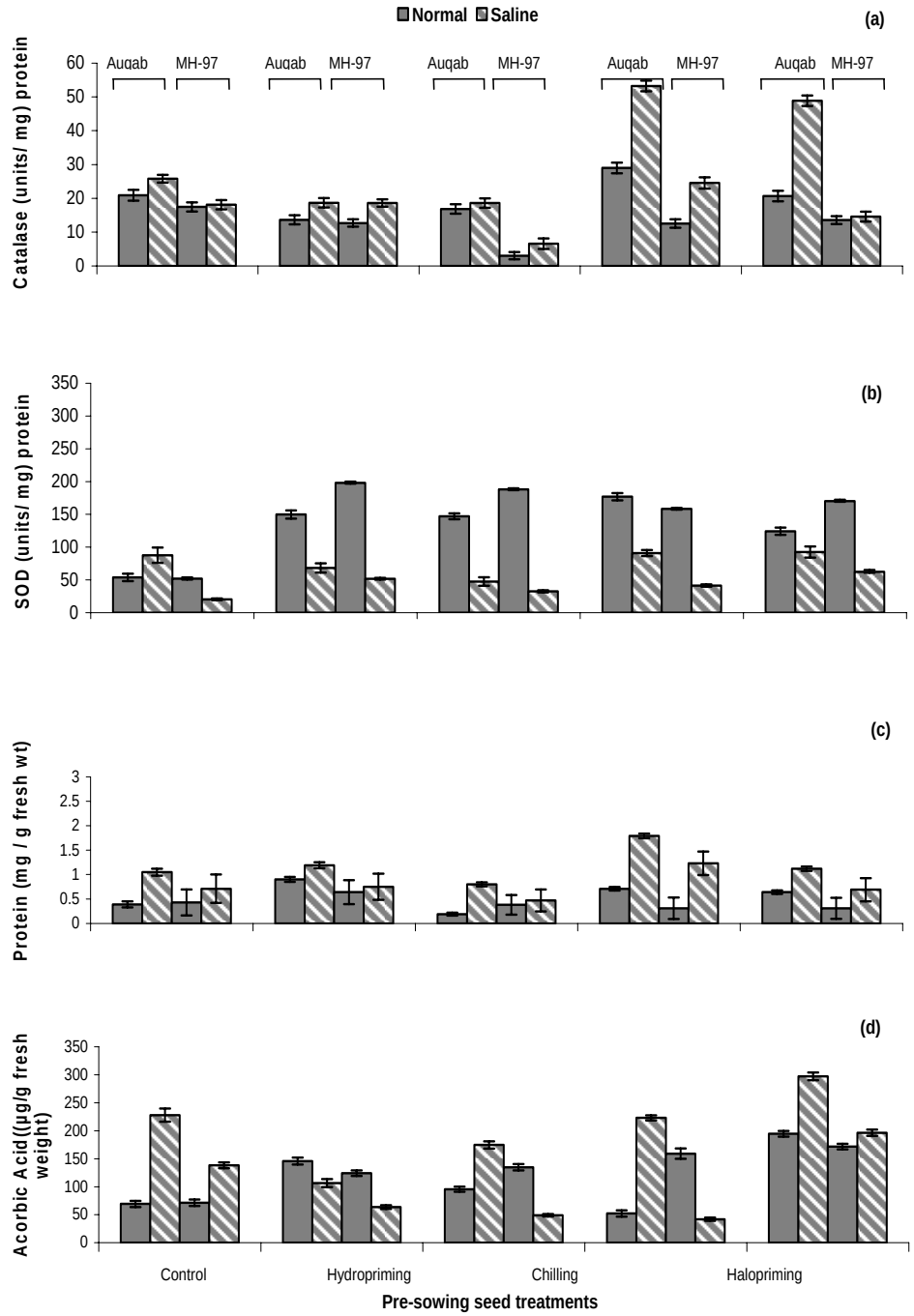

Fig. 3. Changes in antioxidant enzymes of two wheat cultivars Auqab-2000 and MH-97 under non-saline (4 dS m^{-1}) and saline (15 dS m^{-1}) conditions after different pre-sowing seed treatments. Data are the means±SE.

Discussion

Salinity significantly reduced germination and seedling vigor of both cultivars during the present study, however, halopriming followed by hydropriming enhanced earlier and synchronized germination compared with that of control (non-primed seeds) as described by lower MGT and higher FGP, root and shoot length as well as fresh and dry weight of seedlings in both cultivars (Fig. 1-2). Early germination (lower MGT) by various priming tools under saline conditions was due to enhanced pre-germination metabolic activities during priming and resulted in triggering germination (Soon *et al*., 2000). This earlier synchronized and faster emergence might have been due to the enhanced synthesis of DNA, RNA and protein during priming (Bray *et al.,* 1989). Similar results have been earlier reported for improving germination and seedling vigor in wheat cultivars by seed priming under saline conditions (Harris *et al.,* 1999; Kamboh *et al.,* 2000; Basra *et al.,* 2003; Basra *et al.,* 2005a). It was also found that primed seeds had higher vigor levels (Ruan *et al.,* 2002), which resulted in earlier and uniform germination (Hampton and Tekrony, 1995). Pre-sowing chilling that resulted in less germination and seedling vigor might have been due to membrane rupture during the chilling treatment (Farooq *et al*., 2004). Reduction in germination and seedling vigor in wheat seeds might have been the result of taking up more Na^+ and/or Cl^- from the salt solution, hence leading to the toxic effect as earlier suggested by Bradford (1995) and Basra *et al.* (2003).

It is evident that priming can increase free radical scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase in seeds (Chiu *et al.*, 1995; Chang & Sung, 1998). Similarly, pretreatment of seeds with antioxidant compounds such as ascorbic acid and tochopherol improved seed vigor and seed storage of rice (Bhattacharjee and Bhattacharyya, 1989), maize and mustard (Dey & Mukherjee, 1988) and sunflower (Bhattacharjee & Gupta, 1985). In the present study, it was examined that most of priming treatments were effective in improving seedling vigor and the improved seedling vigor of primed seeds, therefore, may be attributed to the counteraction of free radicals and synthesis of membrane-bound enzymes as in other non-primed seeds (Saha *et al.,* 1990). Thus, priming the seeds increased the activity of scavenging enzymes and improved the seedling vigor as indicated by increase in SOD and CAT activities in the leaves of both wheat cultivars with the application of halopriming and ascorbate priming under saline conditions (Fig. 3a & 3b). Here, it also shows that priming with ascorbic acid increased ascorbate contents in both wheat cultivars during salinity stress (Fig. 3d) and hence reduced ROS levels. This supports the hypothesis that increased ROS is the primary cause of the seedling lethality under these stressing conditions. The results of present study are in accordance with the findings of Borsani *et al.* (2001) who reported that the addition of 2 m*M* ascorbate improved the germination and growth of Arabidopsis seedlings in 100 m*M* NaCl and hence reversed the toxic effect caused by NaCl suggesting that changes in the redox state take place under NaCl stress and ultimately are helpful in preventing the formation of ROS under salinity stress.

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. A decrease in CAT activity after hydropriming was observed in the present study which confirms the findings of Srinivasan & Saxena (2001) who reported that CAT activity was not increased after hydropriming in radish. On the other hand, a greater body of evidence storngly suggests an increase in SOD activity of plants under salt stress conditions. Yet the enhancement

was shown to be closely related to the genetic background of cultivars (sensitive/tolerant), level of salt stress (NaCl concentration and duration) and pre-sowing seed treatments. Therefore, it is likely that enhanced antioxidant enzyme activity in wheat cultivars due to halopriming and ascorbate priming is a key component against tolerance to NaCl stress.

As in the present study a significant decrease in SOD activity in MH-97, while a significant increase in Auqab-2000 was observed under salinity. These results suggest that salt-tolerant cultivar Auqab-2000, may have a better protection against reactive oxygen species (ROS) by increasing the activity of antioxidant enzymes (SOD and CAT) under salt stress. Similar observation has also been reported for salt tolerant and sensitive cultivars of potato (Rahnama & Ebrahimzadeh, 2005).

An increase in protein contents of both salt-tolerant and sensitive wheat genotypes has been reported during salinity stress (Karl & Läuchli, 2000). In the present study, both salt stress and seed priming with different priming agents caused an increase in leaf protein. However, this effect was more in cv. Auqab. While working with wheat Al-Hakimi & Hamada (2001) found that seed priming with ascorbic acid counteracted adverse effects of salt stress by increasing leaf soluble proteins, which protect the membrane and membrane bound enzymes (Jeng & Sung, 1994). Thus, increased in leaf protein due to seed priming was one of the reasons that contributed in improved growth of both wheat cultivars under saline conditions, particularly in cv. Auqab.

Finally it can be concluded that halopriming followed by hydropriming proved to be the most effective means of alleviating salt stress in both wheat cultivars while ascorbate priming was more effective in the salt tolerant cultivar. On the other hand, pre-sowing chilling treatment failed to improve salt tolerance in both wheat cultivars.

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