Cereal Research Communications 42(3), pp. 450–462 (2014)
DOI: 10.1556/CRC.2013.0066
First published online 21 February 2014

Chemo-priming with Mannose, Mannitol and H_2O_2 Mitigate Drought Stress in Wheat

A. HAMEED* and N. IQBAL

Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang Road, Faisalabad, Pakistan

(Received 12 April 2013; Accepted 21 August 2013;
Communicated by A. Pécsváradi)

Drought is a worldwide problem, getting more serious with global climate change. Among various strategies, seed priming is the simplest approach for improving drought tolerance in crop plants. Seed priming treatments were applied by soaking seeds in aerated solution of 1% mannose (56 mM) and 10 mM mannitol for 8 h while 100 µM H_2O_2 for 5 h. Seeds soaked in aerated water (hydropriming) and non-primed seed were used as controls. Drought stress significantly reduced the seedling fresh weight and leaf relative water content. Pre-sowing seed treatment with mannitol significantly increased the seedling, root and shoot fresh as well as dry weights under non-stress condition. Moreover, H_2O_2 increased the root length; seedling and root dry weights while mannose increased the shoot dry weight under drought stress. Leaf relative water content (RWC) improved after mannitol and H_2O_2 priming under drought and non-stressed conditions. Hydropriming increased the root and shoot fresh weights, shoot dry weight and RWC under non-stress condition while seedling, root, shoot fresh weights and shoot dry weight along with raised TSP, MDA, reducing sugars under drought stress. Drought stress raised the total soluble protein (TSP), protease, APX and POD activities, MDA and reducing sugars in leaves. Mannitol and H_2O_2 confiscate the drought-induced increase in TSP while H_2O_2 significantly increase it under non-stress condition. Drought stress reduced the catalase activity in leaves while H_2O_2 and mannitol priming brought it back to control level. Drought stress elevated the MDA in leaves and H_2O_2 treatment prevented this increase. Only mannose priming rose the reducing sugars in leaves under non-stress condition. Under drought, mannose and mannitol priming raised the reducing sugars in the leaves as a tactic for osmotic adjustment. In conclusion, seed priming treatments ameliorated the drought tolerance in wheat by elevating the level of antioxidants, reducing oxidative damage of biomolecules and accumulating more reducing sugars for osmotic adjustments.

Keywords: mitigation strategies, adaptation, drought stress, seed priming, antioxidants

Introduction

Among abiotic stresses, drought stress is the most important that adversely influences the crop productivity. Plants live simultaneously under the effect of multiple stress factors in natural habitats or fields. Along with water scarcity, high temperature can limit productiv-

* Corresponding author; E-mail: amjad46pk@yahoo.com

ity in several important crops (Hameed et al. 2012). One way of increasing productivity in stressful environments is to breed crops that are more tolerant to stress. However, success in breeding for tolerance has been limited. Among various strategies, pre-sowing treatment and priming of seeds are easy, low cost, low risk and effective approaches to overcome the environmental stress problems in crop plants (Afzal et al. 2009; Ahmad et al. 2012). Priming has been developed and used extensively to improve seed germination and seedling emergence in a wide range of crop species (McDonald 2000). Better synchronized germination is crucial for achieving an optimal crop stand and better productivity, but several environmental constraints are great impediments (Wahid et al. 2008). To induce pre-germination changes, various priming strategies include osmopriming, halopriming, hormonal priming or hydropriming etc. involving treatment of seeds with osmotica, inorganic salts, hormones or water, respectively. Chemo-priming, i.e. seed treatment with economic and safe chemicals is an attractive approach for improvement of germination and stress tolerance in crop plants.

Much of the injury on plants under abiotic stresses like water deficit is linked to oxidative damage at the cellular level and antioxidant defense response (Hameed et al. 2011). The beneficial effects of priming are associated with the repair and build up of nucleic acids, the increased synthesis of proteins as well as the repair of membranes (McDonald 2000). Seed priming and salinity induced variations in wheat (*Triticum aestivum* L.) leaf protein profiles have been reported (Hameed et al. 2010). Seed priming induces the mobilization and solublization of globulins and the synthesis of late embryogenesis abundant proteins (Gamboa-de Buen et al. 2006). Priming also enhances the activities of antioxidants in treated seeds (Wang et al. 2003). Antioxidant enzymes including superoxide dismutase, catalase, and glutathione reductase have been reported to express during seed priming (Bailly et al. 2000). Among other pre-germination metabolic changes, seed priming decreased the level of malondialdehyde (Bailly et al. 1998, 2000), changed saturated and unsaturated fatty acids (Walters et al. 2005) and induced α-amylase to increase the soluble sugar pool, thereby enhancing seedling emergence and other related attributes (Farooq et al. 2006). In this view, the research objectives was to access whether seed priming with H_2O_2, mannitol and mannose could improve the drought tolerance in the wheat seedlings with special emphasis on antioxidant defense potential.

Material and Methods

Experiment was conducted using wheat (*Triticum aestivum* L.) genotype FD-83. Chemo-priming treatments used in the study were soaking seeds in aerated solution of 1% mannose (56 mM) for 8 h, 10 mM mannitol for 8 h and 100 μM H_2O_2 for 5 h. Seeds soaked in aerated water (hydropriming) and non-primed seed were used as controls. On completion of the treatment time, seeds were dried near to original weight with forced air under shade.

Primed and non-primed seeds were grown under optimum conditions in plastic pots filled with equal quantity of soil. In control pots water was maintained at soil water holding capacity throughout the experiment period. While to induced drought or water deficit stress, pots were irrigated once only before sowing and then no irrigation was provided to

these pots during rest of experimental periods. At 12th day after sowing water in non-irrigated pots (drought stressed) dropped to approximately 20% of soil water holding capacity indicating a severe drought stress. On this day, leaf samples for biochemical analysis and data for seedling growth response studies were collected from control and stressed pots. For comparison of growth, fresh weight of seedlings was recorded immediately after harvesting to avoid any evaporation. For dry weight estimations, seedlings were kept at 90ºC till drying. Seedling, root and shoot dry weights were measured after complete drying when there was no further decrease in weight. Root and shoot length was measured by spreading them on a scale calibrated in cm.

For biochemical studies, collected leaf samples were immediately stored at –40ºC till further analysis. For extraction of enzymes, fresh leaves (0.5 g) were ground in extraction buffer specific for different enzymes and centrifuged at 15,000 × *g* for 20 min at 4ºC. The supernatant was separated and used for the determination of different enzyme activities. Enzyme activities were expressed on fresh weight basis. Details of methodologies are given below.

Superoxide dismutase (SOD)

For the estimation of SOD activity, leaves were homogenized as described by Dixit et al. (2001). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) following the method of Giannopolitis and Ries (1977).

Catalase (CAT) and peroxidase (POD)

For the estimation of POD, leaves were homogenized 50 mM potassium phosphate buffer (pH 7.0) and activity was measured using the method of Chance and Maehly (1955) with some modification. CAT was estimated by the following method described by Beers and Sizer (1952). Total soluble protein concentration was measured by dye binding assay as described by Bradford (1976).

Protease and ascorbate peroxidase (APX)

Protease activity was determined by the casein digestion assay described by Drapeau (1976). For the estimation of APX activity, the rate of H_2O_2 dependent oxidation of ascorbic acid was determined (Chen and Asada 1989).

Malondialdehyde (MDA) content

The level of lipid peroxidation in the leaf tissue was measured in terms of MDA content determined by the thiobarbituric acid reaction using method of Heath and Packer (1968).

Total phenolic content and reducing sugars

A micro-colorimetric method described by Ainsworth and Gillespie (2007) was used for total phenolics (gallic acid equivalents) assay, which utilizes Folin-Ciocalteu reagent. Reducing sugars were determined by dinitrosalycylic acid method (Miller 1972).

Statistical analysis of data

All experiments were conducted in triplicates and F-test was applied. The significance of differences between means was measured using Student's *t*-test (two tailed), at 0.01 or 0.05 significance level. All the statistical calculations were performed using computer software Microsoft Excel 2002.

Results

Growth response

Pre-sowing seed treatments with mannitol and H_2O_2 enhanced the fresh weights of seedlings as compared to the non-treated control (Fig. 1a). Highest increase in the fresh weight of the seedlings was observed in case of mannitol treatment. Drought stress significantly reduced the seedling fresh weight. Simple seed socking in the water replenished the drought induced decrease in the seedling fresh weight. In case of mannitol treatment, seedling fresh weight was further reduced under drought stress. All pre-sowing seed treatments enhanced the root fresh weight under non-stress condition (Fig. 1b). Highest

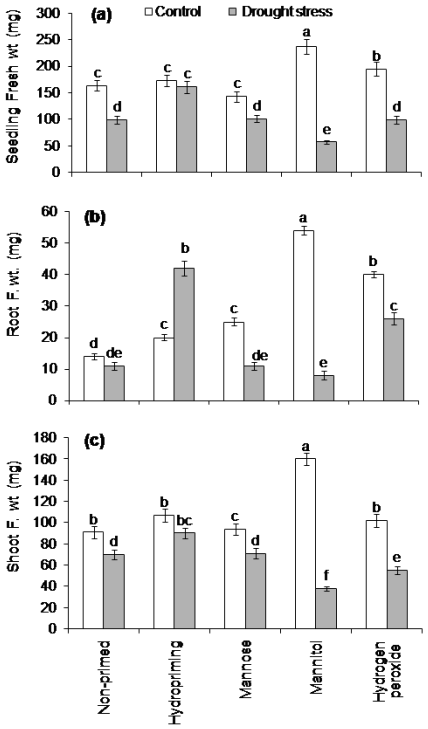

Figure 1. Effect of seed priming on wheat seedling (a), root (b) and shoot (c) fresh weights under control and drought stress conditions

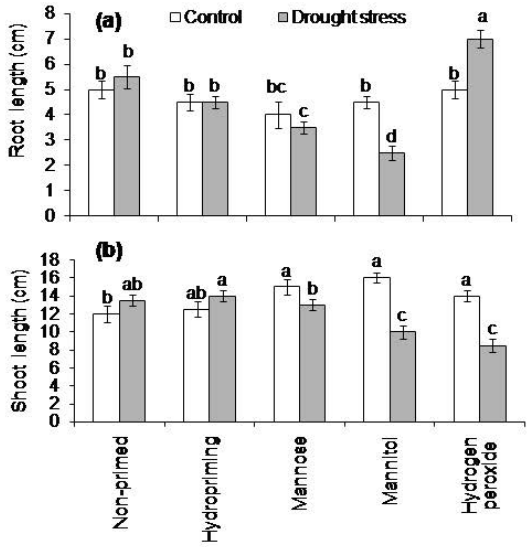

Figure 2. Effect of seed priming on root (a) and shoot lengths (b) under control and drought stress conditions

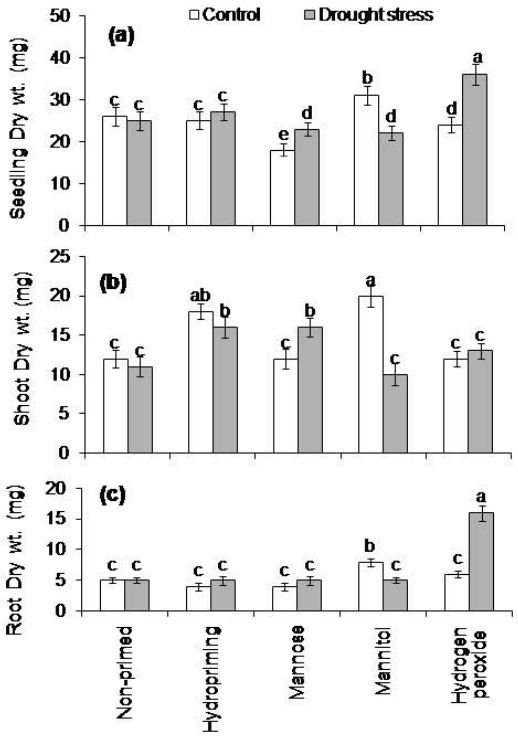

Figure 3. Effect of seed priming on wheat seedling (a), shoot (b) and root (c) dry weights under control and drought stress conditions

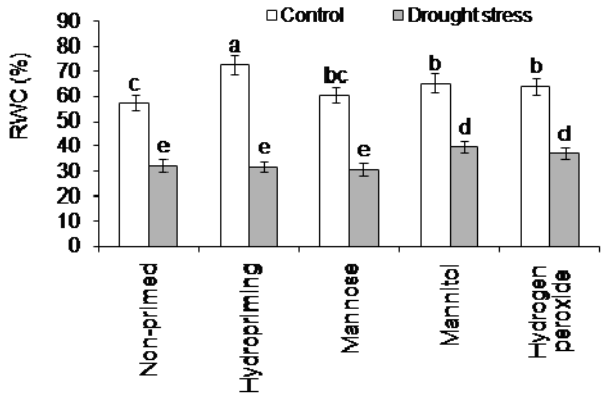

Figure 4. Effect of seed priming on wheat leaf relative water content under control and drought stress conditions

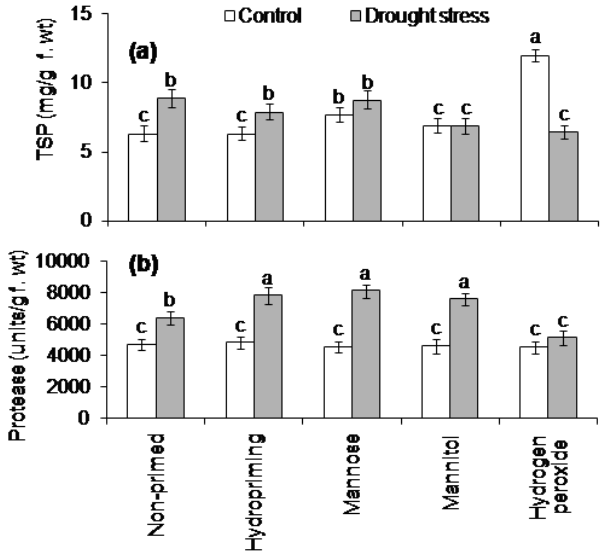

Figure 5. Effect of seed priming on wheat leaf total soluble protein (a) and protease (b) activity under control and drought stress conditions

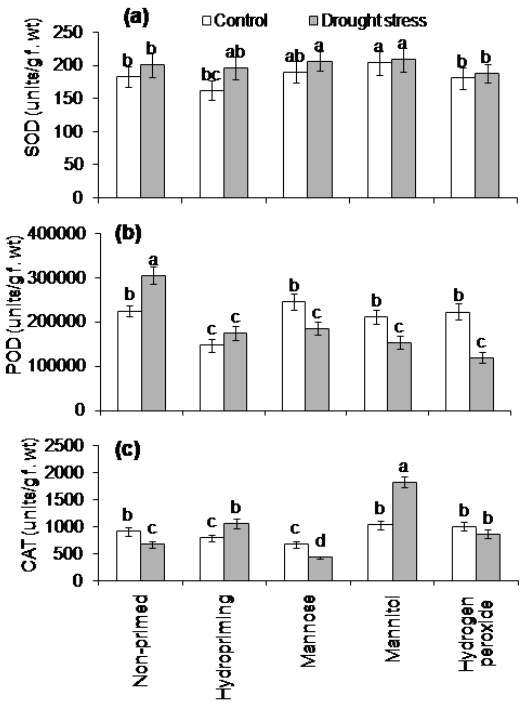

Figure 6. Effect of seed priming on wheat leaf SOD (a), POD (b) and CAT (c) activities under control and drought stress conditions

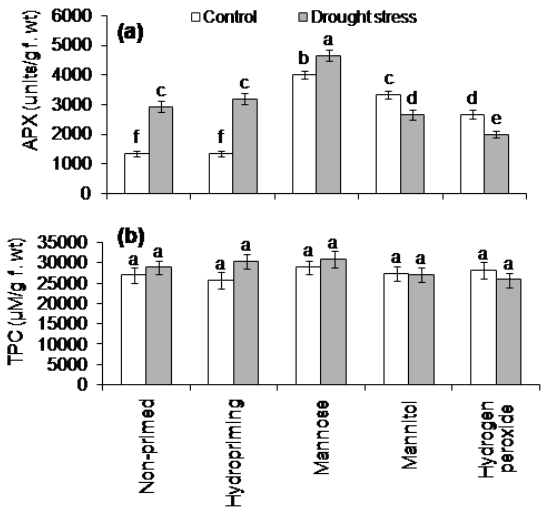

Figure 7. Effect of pre-sowing seed treatments on wheat leaf APX activity (a) and TPC (b) under control and drought stress conditions

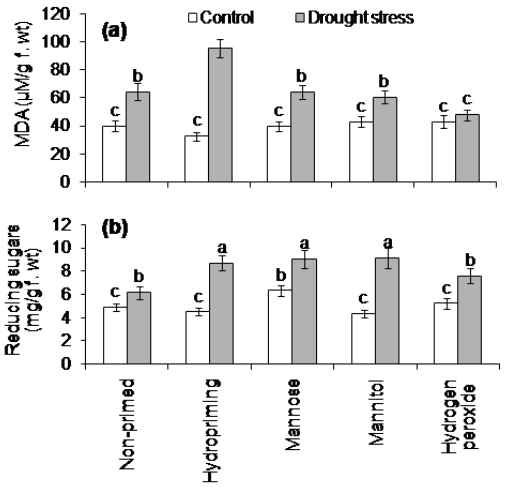

Figure 8. Effect of seed priming on wheat leaf MDA content (a) and reducing sugars (b) under control and drought stress conditions

increase in root fresh weight was induced by mannitol seed priming. However, under drought stresses, only seed priming with H_2O_2 increased the root fresh weight as compare to control. Shoot fresh weight significantly increased by mannitol seed priming under non-stress condition (Fig. 1c) compared with non-treated control. Under drought stress, only hydropriming was able to raise the shoot fresh weight while it was decreased by mannitol and H_2O_2 seed priming.

Pre-sowing seed treatment with H_2O_2 increased the root length under drought stress (Fig. 2a). However, mannitol treatment reduced the root length under drought stressed condition. Pre-sowing seed treatment with mannose, mannitol and H_2O_2 increased the shoot length under non-stressed condition (Fig. 2b). Seedling dry weight was increased by mannitol treatment under non-stressed conditions (Fig. 3a). Only H_2O_2 treatment was able to increase the seedling dry weight under drought stress. Shoot dry weight was enhanced by mannitol priming when seedlings grown without stress (Fig. 3b). Treatment with mannose was able to increase the shoot dry weight under stress. Root dry weight was enhanced by mannitol treatment under non-stressed condition (Fig. 3c). Only H_2O_2 treatment increased the root dry weight under drought. Increase in root dry weight was many folds as compared to non-treated control. Leaf relative water content was improved after mannitol and H_2O_2 treatments (Fig. 4). Under drought stress, leaf relative water content was reduced significantly. Pre-sowing seed treatments with mannitol and H_2O_2 improved the leaf relative water content under drought.

Biochemical response

Pre-sowing seed treatment with H_2O_2 raised the level of leaf soluble proteins under non-stressed conditions (Fig. 5a). Drought stress raised the soluble protein content in wheat leaves. Mannitol and hydrogen peroxidase treatments confiscate the drought in-

duced increase in protein content by maintaining the level at non-stressed control level. Under non-stress condition, no treatment altered the protease activity in leaves (Fig. 5b). Leaf protease activity was elevated under drought stress. Mannose, mannitol and water soaking further increased the protease activity above control under drought stress. However, H_2O_2 priming triumphs over the drought induced increase in leaf protease activity by maintaining it to the level of non-stressed control.

Leaf SOD activity remain unaffected under drought stress. Except mannitol, seed priming treatments did not influence the SOD activity under drought or non-stressed conditions (Fig. 6a). Seed priming with mannitol raised the SOD activity under drought and non-stress condition.

Under drought stress, POD activity raised promptly (Fig. 6b). POD activity was generally lower after treatments as compared to non-treated control under drought stress. Catalase activity decreased by mannose treatments whereas remained almost unchanged after other treatments under non-stressed condition (Fig. 6c). Drought stress reduced the catalase activity in leaves. This stress induced decrease in catalase activity was replenished after H_2O_2 seed priming treatment. While seed priming with mannitol raised the catalase activity above control under drought stress and level was even higher as compared to that in non-treated control under non-stressed condition. Catalase activity decreased by mannose treatment under drought.

All chemo-priming treatments raised the APX activity in leaves under non-stressed condition (Fig. 7a). APX activity was also raised under drought stress. Seed priming with mannose increased the APX activity as compared to non-treated control under drought stress. In case of treatment with H_2O_2 and mannitol, APX activity was lower as compared to non-treated control under drought stress. TPC level did not alter by treatments under non-stressed condition and also by drought stress (Fig. 7b). Drought stress elevated the lipid peroxidation (MDA) in leaves (Fig. 8a). Pre-sowing seed treatment with H_2O_2 was able to prevent the drought stress induced increase in lipid peroxidation in leaves. Reducing sugar level was increased in the leaves under drought stress. Pre-sowing seed treatment with mannose raised the level of reducing sugars in the leaves under non-stress condition (Fig. 8b). All pre-sowing seed treatments except H_2O_2 raised the reducing sugars in the leaves under drought stress.

Discussion

Growth response

Decreased wheat (*Triticum aestivum* L.) seedling growth (mg per seedling) as affected by drought and salinity stresses is a well-known phenomenon (Soltani et al. 2006). In our study, drought stress significantly reduced the seedling fresh weight. Decreased seedling growth has also been reported under drought in wheat (Soltani et al. 2006). Root length is an important trait against drought stress in plant varieties; in general, varieties with higher root growth have ability for resistance to drought (Leishman and Westoby 1994). In our studies, pre-sowing seed treatment with H_2O_2 increased the root length under drought

stress. The superior root and shoot mass following drought stress have been proposed as reliable drought selection criteria for different plant species, including wheat (Yang et al. 1991; Basal et al. 2005). Drought tolerance has been reported to be associated with the increase in root dry matter in cowpea (Matsui and Singh 2003). In our studies, seedling dry weight was increased by mannitol treatment under non-stressed condition. H_2O_2 treatment was able to increase the seedling dry weight under drought stress. Shoot dry weight was enhanced by mannitol treatments when seedlings grown without stress. Treatment with mannose was able to increase the shoot dry weight under stress. Root dry weight was enhanced by mannitol treatment under non-stress condition. H_2O_2 treatment increased the root dry weight under drought. Our finding regarding H_2O_2 treatment are in line with previous ones reporting that seed treatment with H_2O_2 enhanced almost all the growth parameters of wheat seedlings (Wahid et al. 2007). Highest shoot fresh and dry weight was recorded from the seedlings receiving 80 and 120 mM H_2O_2 followed by water control (Wahid et al. 2007). El-Tayeb (2006) reported that drought caused a decrease in RWC in *Vicia faba* cultivars. Similarly, drought stress significantly reduced leaf relative water content in present study. Decrease in RWC with the negative effect of PEG has been related to low water uptake by germinating seed (Yagmur and Kaydan 2008). In our study, leaf relative water content was improved after mannitol and H_2O_2 treatments. Pre-sowing seed treatments with mannitol and H_2O_2 improved the leaf relative water content under normal and drought stress conditions. Hydropriming also improved the RWC under normal condition. Previously, two priming techniques KH_2PO_4 and hydropriming have been reported to increase relative water content of shoot compared to untreated seeds under control, PEG and NaCl induced stresses (Yagmur and Kaydan 2008).

Biochemical response

Protein breakdown and recycling, which depend on the levels of proteolytic enzymes, are essential parts of the plant response to environmental stress (Hieng et al. 2004). Proteases are involved in protein maturation, degradation and protein rebuilding in response to different external stimuli and to removal of abnormal, misfolded proteins (Grudkowska and Zagdanska 2004). Drought stress raised the soluble protein content in wheat leaves along with elevated protease activity. This drought induced rise in proteases may be to remove abnormal and misfolded proteins. Mannitol and hydrogen peroxidase treatments confiscate the drought induced increase in protein content by maintaining the level at non-stressed control level. This means these treatments reduced the stress effect on protein patterns. Pre-sowing seed treatment with H_2O_2 raised the level of soluble leaf protein content when seedlings grown under non-stressed conditions. This increase in the protein content may be due to enhanced synthesis of proteins required for faster seedling growth.

Oxidative damage in the plant tissue is prevented by a concerted action of both enzymatic and non-enzymatic antioxidant systems. Among enzymatic mechanisms, superoxide dismutase plays an important role, and catalyzes the dismutation of two molecules of superoxide into O^{-2} and H_2O_2; the first step in reactive oxygen species scavenging systems (Farooq et al. 2009). In our study, drought stress did not induce any effect on SOD activity in leaves. However, drought stress reduced the catalase activity in leaves. Previ-

ously, drought induced oxidative conditions in rice anthers leading to down-regulation of antioxidants has been reported (Nguyen et al. 2009). Drought induced decrease in catalase activity in present study was replenished after H_2O_2 treatment. While seed priming with mannitol raised the catalase activity above control under drought stress and level was even higher as compared to that in non-treated control under non-stressed condition. This clearly indicates that drought induced down regulation of antioxidant activities can be replenished by seed priming treatments.

Plants detoxify reactive oxygen species (ROS) by upregulating antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) (Hameed et al. 2009). Under drought stress, POD activity raised promptly. APX activity was also raised under drought stress. These observations on increased activities of H_2O_2 scavenging enzymes and unaffected SOD activity point out that during drought; oxidative stress is produced through generation of H_2O_2. That is why POD activity was generally lower after pre-sowing seed treatments as compared to non-treated control under drought stress. Actually, ascorbate peroxidase is a key antioxidant enzyme in plants (Orvar and Ellis 1997). Moreover, all priming treatments raised the APX activity in leaves under non-stressed condition, possibly to quench the H_2O_2 produced during germination.

Peroxidation of membrane lipids requires active uptake of oxygen. Excess ROS triggers phytotoxic reactions such as lipid peroxidation, protein degradation and DNA mutation (McCord 2000). In the present study, drought stress elevated the lipid peroxidation in leaves. This indicates that excess ROS are produced during the drought stress and even go beyond a controllable level causing peroxidation of membrane lipids. Interestingly, pre-sowing seed treatment with H_2O_2 was able to prevent the drought stress induced increase in lipid peroxidation in leaves. Possibly, enhanced expression of antioxidants by this treatment prevented/quenched the excessive ROS and intern protect membranes from damage. Actually, in contrast to higher anti-oxidative activities, lower levels of malondialdehyde (MDA) display higher anti-oxidative ability, reflecting higher drought tolerance (Dhanda et al. 2004).

In our study, reducing sugar level was increased in the wheat leaves under drought stress obviously as a tactic for osmotic adjustment under stressed condition. Pre-sowing seed treatment with mannose raised the level of reducing sugars in the leaves under normal and drought stress conditions. All other pre-sowing seed treatments raised the reducing sugars in the leaves only under drought stress. These pre-sowing seed treatments were thus able in osmotic adjustment by accumulating higher level of sugars in the leaves under drought stress. Sugars levels can be determinant for the osmotic adjustment that the leaf uses in drought conditions (Di-Fonzo et al. 2000). Drought tolerance seems to be the most important trait in stay-green phenotype and can also represent the trait of interest in many applied breeding programmes. An important aspect in a stay-green phenotype is also related to the sugar contents in the leaf (Di-Fonzo et al. 2000).

In conclusion, drought stress adversely affected the anti-oxidative processes in the leaves leading to increased damage to membrane lipids and proteins. Tested pre-sowing seed treatments were able to ameliorate the drought tolerance by elevating the level of antioxidants, preventing damage to membranes and osmotic adjustment. Hydropriming

alone proved better development under drought stress, although with the highest MDA content. H_2O_2 priming has stimulatory effect mostly in the roots, but not in the leaves. Mannitol priming was most effective in enhancing the seeding growth attributes under non-stress condition. Mannose and mannitol priming was more effective in improving the most of biochemical processes in wheat leaves.

Acknowledgement

Authors are thankful to Pakistan Science Foundation (PSF) for financial support through project No. P-NIAB-Bio (353).

References

Afzal, I., Munir, F., Ayub, C.M., Basra, S.M.A., Hameed, A., Nawaz, A. 2009. Changes in antioxidant enzymes, germination capacity and vigour of tomato seeds in response of priming with polyamines. Seed Sci. and Technol. **37**:765–770.

Ahmad, I., Khaliq, T., Ahmad, A., Basra, S.M.A., Hasnain, Z., Ali, A. 2012. Effect of seed priming with ascorbic acid, salicylic acid and hydrogen peroxide on emergence, vigour and antioxidant activities of maize. African J. of Biotech. **11**:1127–1137.

Ainsworth, E.A., Gillespie, K.M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols **2**:875–877.

Bailly, C., Benamar, A., Corbineau, F., Come, D. 1998. Free radical scavenging as affected by accelerated ageing and subsequent priming in sunflower seeds. Physiologia Plantarum **104**:646–652.

Bailly, C., Benamar, A., Corbineau, F., Come, D. 2000. Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. Seed Sci. Res. **10**:35–42.

Basal, H., Smith, C.W., Thaxton, P.S., Hemphill, J.K. 2005. Seedling drought tolerance in upland cotton. Crop Sci. **45**:766–771.

Beers, R.F., Sizer, I.W. 1952. A spectrophotometeric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195**:133.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.

Chance, B., Maehly, A.C. 1955. Assay of catalase and peroxidase. Method Enzymol. **2**:764–775.

Chen, G.X., Asada, K. 1989. Ascorbate peroxidase in tea leaves: Occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant and Cell Physiol. **30**:987–998.

Dhanda, S.S., Sethi, G.S., Behl, R.K. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. J. Agron. Crop Sci. **190**:6–12.

Di-Fonzo, N., Flagella, Z., Campanile, R.G., Stopelli, M.C., Spano, G., Rascio, A., Russo, M., Trono, D., Padalino, L., Laus, M., De Vita, P., Shewry, P.R., Lawlor, D., Troccoli, A. 2000. Resistance to abiotic stresses in durum wheat: Which ideotype? Options Mediterraneenes, Series A **40**:215–225.

Dixit, V., Pandey, V., Shyam, R. 2001. Differential antioxidative response to cadmium in roots and leaves of pea. J. Exp. Bot. **52**:1101–1109.

Drapeau, G.R. 1976. Protease from *Staphylococcus aureus.* Methods in Enzymology **45**:469–475.

El-Tayeb, M.A. 2006. Differential response of two *Vicia faba* cultivars to drought: Growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agron. Hung. **54**:25–37.

Farooq, M., Barsa, S.M.A., Wahid, A. 2006. Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. Plant Growth Regul. **49**:285–294.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A. 2009. Plant drought stress: Effects, mechanisms and management. Agron. Sustain. Dev. **29**:185–212.

Gamboa-de Buen, A., Cruz-Ortega, R., Martinez-Barajas, E., Sanchez-Coronado, M.E., Orozco-Segovia, A. 2006. Natural priming as an important metabolic event in the life history of Wigandia urens *(Hydrophyllaceae)* seeds. Physiol. Plant. **128**:520–530.

Giannopolitis, C.N., Ries, S.K. 1977. Superoxide dismutases occurrence in higher plants. Plant Physiol. **59**:309–314.

Grudkowska, M., Zagdanska, B. 2004. Multifunctional role of plant cysteine proteinases. Acta Biochem Pol. **51**:609–624.

Hameed, A., Afzal, I., Iqbal, N. 2010. Seed priming and salinity induced variations in wheat (*Triticum aestivum* L.) leaf protein profiles. Seed Sci. and Technol. **38**:236–241.

Hameed, A., Bibi, N., Akhter, J., Iqbal, N. 2009. Mannose-induced modulations in antioxidants, protease activity, lipid peroxidation, and total phenolics in etiolated wheat leaves. J. Plant Growth Regul. **28**:58–65.

Hameed, A., Bibi, N., Akhter, J., Iqbal, N. 2011. Differential changes in antioxidants, proteases, and lipid peroxidation in flag leaves of wheat genotypes under different levels of water deficit conditions. Plant Physiol. and Biochem. **49**:178–185.

Hameed, A., Goher, M., Iqbal, N. 2012. Heat stress-induced cell death, changes in antioxidants, lipid peroxidation, and protease activity in wheat leaves. J. Plant Growth Regul. **31**:283–291.

Heath, R.L., Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. **125**:189–198.

Hieng, B., Ugrinovic, K., Sustar, V.J., Kidric, M. 2004. Different classes of proteases are involved in the response to drought of *Phaseolus vulgaris* L. cultivars differing in sensitivity. J. Plant Physiol. **161**:519–530.

Leishman, M.R., Westoby, M. 1994. The role of seed size in seedling establishment in dry soil conditions – Experimental evidence from semi-arid species. J. Ecol. **82**:249–258.

Matsui, T., Singh, B.B. 2003. Root characteristics in cowpea related to drought tolerance at the seedling stage. Experimental Agriculture **39**:29–38.

McCord, J.M. 2000. The evolution of free radicals and oxidative stress. Am. J. Med. **108**:652–659.

McDonald, M.B. 2000. Seed priming. In: Black, M., Bewley, J.D. (eds), Seed Technology and Its Biological Basis. Sheffield Academic Press, Sheffield, UK, pp. 287–325.

Miller, G.L. 1972. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. **31**:426–428.

Nguyen, G.N., Hailstones, D.L., Wilkes, M., Sutton, B.G. 2009. Drought induced oxidative conditions in rice anthers leading to a programmed cell death and pollen abortion. J. Agron. Crop Sci. **195**:157–164.

Orvar, B.L., Ellis, B.E. 1997. Transgenic tobacco plants expressing antisense RNA for cytosolic ascorbate peroxidase show increased susceptibility to ozone injury. Plant J. **11**:1297–1305.

Soltani, A., Gholipoor, M., Zeinali, E. 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environ. Exp. Bot. **55**:195–200.

Wahid, A., Perveen, M., Gelani, S., Basra, S.M.A. 2007. Pretreatment of seed with H_2O_2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. J. of Plant Physiol. **164**:283–294.

Wahid, A., Noreen, A., Basra, S.M.A., Gelani, S., Farooq, M. 2008. Priming-induced metabolic changes in sunflower *(Helianthus annuus)* achenes improve germination and seedling growth. Botanical Studies **49**:343–350.

Walters, C., Landre, P., Hill, L., Corbineau, F., Bailly, C. 2005. Organization of lipid reserves in cotyledons of primed and aged sunflower seeds. Planta **222**:397–407.

Wang, H.Y., Chen, C.L., Sung, J.M. 2003. Both warm water soaking and solid priming treatments enhance anti-oxidation of bitter gourd seeds germinated at sub-optimal temperature. Seed Sci. Technol. **31**:47–56.

Yagmur, M., Kaydan, D. 2008. Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. African J. of Biotechnol. **7**:2156–2162.

Yang, R.C., Jana, S., Clarke, J.M. 1991. Phenotypic diversity and association of some potentially drought-responsive characters in durum wheat. Crop Sci. **31**:1484–1491.