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Physiological improvement for alleviation of salt stress tolerance in wheat

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Abstract
Salinity is the dangerous constraints facing agriculture in present day. Some biological and chemical process is being pursued to cope with soil salinity. The issue of different pre-sowing seed operations (hydro priming, halo priming, ascorbate priming and pre-sowing chilling operations on seed germination, vigor, antioxidants and entirety soluble protein substance was investigated in 2 wheat (*Triticum aestivum* L.) Salt sensitive & salt tolerant are below saline (20 days m^{-1}) or non-saline (6 days m^{-1}) situation. All pretreatments of seeds, halo priming followed by hydro priming was the mainly powerful in alleviating the adverse outcomes of salinity by improving germination and seedling improvement of both cultivars. Salinity significantly improved leaf protein substance in both cultivars, but the magnitude of enhance in protein substance was greater in Auqab-2000 as compared to that in MH-97. Very pre-sowing seed operations significantly improved Superoxide dismutase movement in MH-97 as priming with $CaCl_2$. $2H_2O$ and as corbate were very capable in Auqab-2000 during stress situation. The tolerant of salt cultivar Auqab-2000 had a superior protection beside reactive oxygen kind as evidenced by increased Superoxide dismutase, substance, and catalase actions underneath salt stress. In conclusion, halo priming and hydro priming successfully improved the seed performance in both cultivars whereas priming with ascorbate was only effective in tolerance cultivar under salinesituation. This advantage was attributed to early and synchronized germination, vigorous stand establishment, and decreased oxidative broken due to improved antioxidant scheme.

Keywords: salt tolerance, oxidative stress, seed priming, wheat

Introduction
Soil salinity is big problem adversely affecting physiological operations, eventually diminishing increase and yield (Ashraf & Harris, 2004). Salinity is one in all major abiotic stresses limiting soil fertility and plant manufacturing increase reduction due to salinity is likewise attributed to ion toxicity and nutrient imbalance. Salt strain similarly to the known components of osmotic stress and ion toxicity, is likewise manifested as an oxidative strain (Guetadahan *et al.* 1998) [12]. Salinity influences the availability of vitamins and water, lowers the pleasant of arable lands, and alters the shape of ecological communities. It induces osmotic stress, the physiological drought, which usually reduces increase and photosynthesis in flowers (Pasternak, 1987). Powerful confirmation exists that during distinct plants salt pressure can result in accumulation of reactive oxygen species consisting of hydroxyl radical, hydrogen peroxide and singlet oxygen superoxide (Lee *et al.* 2001). (ROS) Reactive oxygen species assault proteins, lipids and nucleic acids, and the diploma of damage depends at the balance among formation of ROS and its elimination by using the antoxidative scavenging systems and it appears to represent an important strain-tolerance trait. Expression of antioxidant defense genes could, in turn, be prompted to shield the cellular against oxidative damage (Menezes- Benavente *et al.* 2004). removal of Reactive oxygen species is specifically completed by using antioxidant compounds which include ascorbic acid, glutathione, thioredoxine and carotenoids, and by means of ROS scavenging enzymes e.g., Superoxide dismutase, glutathione peroxidase and catalase (Noctor& foyer, 1998). This shows that oxidative strain tolerance is genetically controlled and it affords a huge scope for crop development the use of traditional breeding and selection, and trans gens manufacturing (Hung *et al.* 2005) or adopting physiological strategies. However, ion content and salt tolerance are not often correlated and several studies indicate that acquisition of salttolerance may also be a consequence of improving resistance to oxidative stress (Hernandez *et al.* 2001) [14]. However, ion substance and tolerance of salt are not often interconnected and numerous studies show that acquisition of salt tolerance can also be an effect of enhancing resistance to oxidative pressure (Hernandez *et al.* 2001) [14]. Seed hydration in solutions containing inorganic solute, hormones or antioxidant compounds might be capable of explicit antioxidant defense genes which would, in turn, be caused to defend the cell against oxidative harm. Therefore, the primary purpose of this have a look at became to signify the impact of presowing seed remedies on plant defense device at some stage in salinity strain.

Materials and Methods
Experimental Material
Solid seeds of 2 regularly developed wheat (*Triticum aestivum* L.) cvs. in Pakistan particularly MH-97 (salt sensitive) and Auqab-2000 (salt tolerant) have been surface sanitized with five% sodium hypochlorite respond in due order regarding 3 mins to keep away from fungal invasion followed with the aid of repeated washings with sterilized refined water. The two

cultivars were chosen because of their recognized response to salinity.

Estimation of ascorbate and solvent protein substance

The ascorbic acid concentration in shoots become determined the usage of the approach as described with the aid of Kampfenkel *et al.* (1995). The assay mixture contained 150 µL sample, 1.8 mL distilled water and 1.2 mL DCIP solution. The absorbance was measured at 530 nm via the use of spectrophotometer (Spectronic 21 D, Milton Roy). Quantitative protein estimation in shoots become done following Bradford (1976) the usage of bovine serum albumin as well-known.

Pre-sowing seed treatments

Following pre-sowing seed treatments were optimized during preliminary studies (Afzal *et al.* 2005) [3]. For hydro priming, seeds were soaked indistilled water for 12 h (Bennett and Waters, 1987). For halo priming, seeds were soakedin solution of 50.0 mM $CaCl_2.2H_2O$ for 12 h at 20 °C in the dark (Basra *et al.* 2005) [3]. 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 presowingchilling, seeds were sealed in polythene bags and placed in a refrigerator at -19±2ºC for 48 h (Basra *et al.* 2005b). During hydration treatments continuous fresh air was supplied

Determination of antioxidant enzyme activities

Catalase activity in the leaf samples was estimated by the method as described by Beers and Sizer (1952) [4]. 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 photo reduction 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 %.

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 is greater than 0.05). Values in the figures indicate mean values ± S.E.

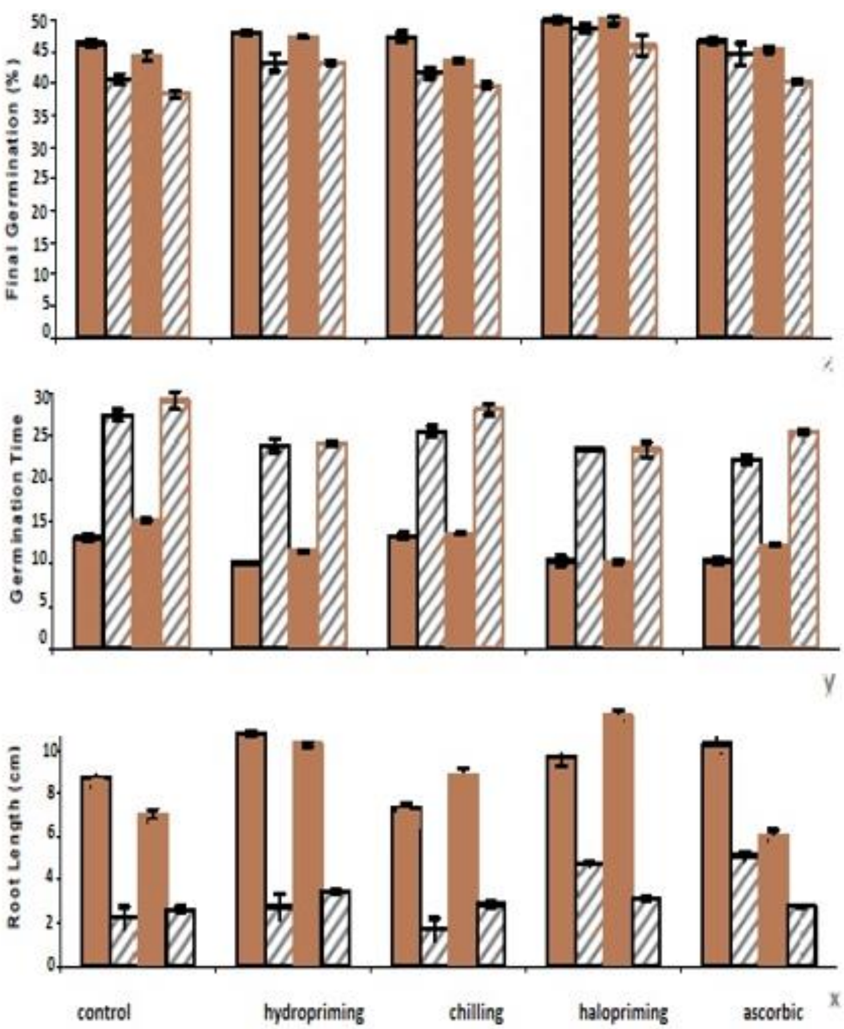

Fig 1: Effect of different pre-sowing treatments on germination and seedling vigor of two wheat cultivars

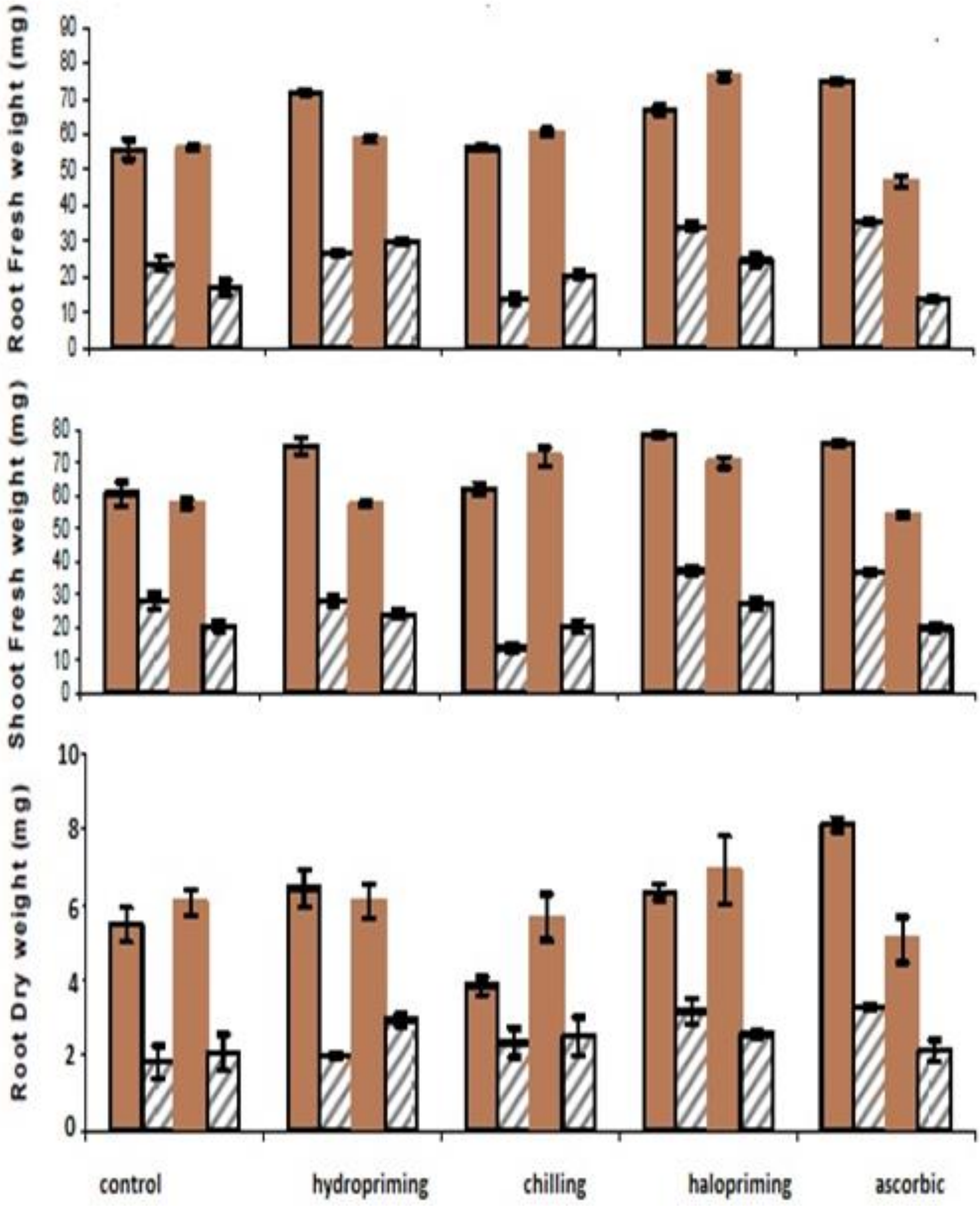

Fig 2: Effect of different pre-sowing treatments on seedling fresh and dry weights of two wheat cultivars

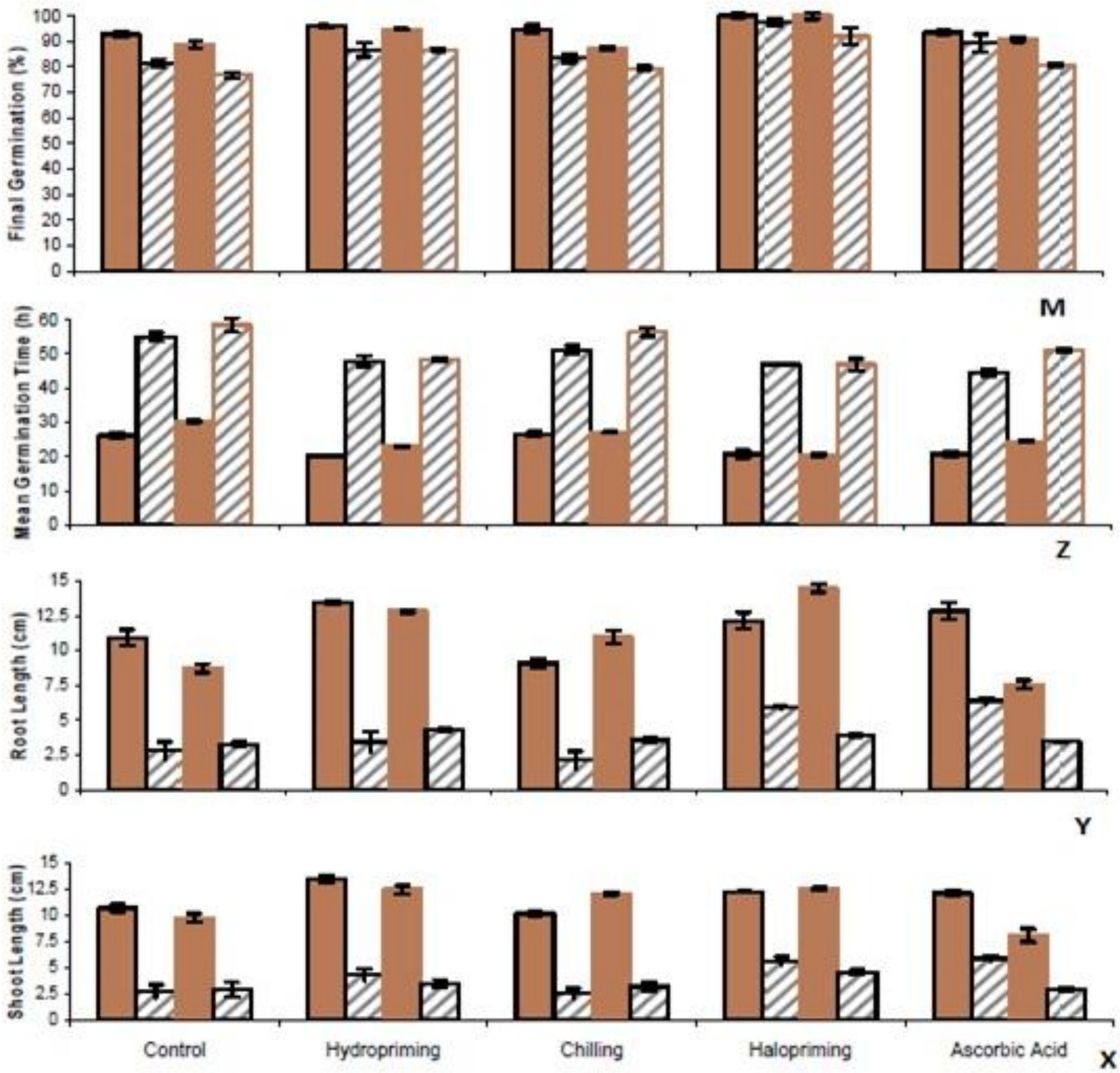

Fig 3: Changes in antioxidant enzymes of two wheat cultivars Auqab-2000 and MH-97 under non-saline

Result and Discussion

Salinity significantly reduced germination and seedling vigor of both cultivars during the present study, however, halo priming followed by hydro priming 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) [7]. 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) [1, 3]. 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) [13]. 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) [11]. 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) [1]. 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 hydro priming was observed in the present study which confirms the findings of Srinivasan & Saxena (2001) [15] who reported that CAT activity was not increased after hydro priming in radish. On the other hand, a greater body of evidence strongly suggestsan 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-sowingseed treatments. Therefore, it is likely that enhanced antioxidant enzyme activity in wheat cultivars due to halo priming 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 genotypeshas 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) [2] 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) [8]. 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 halo priming followed by hydro priming 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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