Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress

I. Afzal[1], S. Rauf[2], S.M.A. Basra[1], G. Murtaza[3]

[1]*Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan*
[2]*Department of Plant Breeding, University of Agriculture, Faisalabad, Pakistan*
[3]*Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan*

ABSTRACT

The present study was conducted to investigate whether salt tolerance may be induced in wheat at germination stage by halopriming with different inorganic salts ($CaCl_2$, NaCl and $CaSO_4$), and how far these salts affect the mobilization of different nutrients to different parts of seedlings. Seeds of two wheat cultivars (Inqlab-91 and SARC-1) were primed in 50 mmol solutions of $CaCl_2$, NaCl or $CaSO_4$ for 12 h separately and germinated under non-saline and saline (125 mmol NaCl) conditions. All seed treatments hastened germination under saline and non-saline conditions as compared to those of non-primed seeds. However, priming with $CaSO_4$ enhanced germination of both cultivars under saline conditions more than any other treatment. Maximum root length and fresh and dry weights were obtained in plants raised from seeds primed with $CaSO_4$ followed by $CaCl_2$. Concentrations of Na^+ and K^+ in seedlings obtained after priming changed significantly. However, Na^+ was highest in seedlings raised from seeds primed with NaCl whereas the concentration of K^+ was highest in the seedlings primed with $CaSO_4$. Maximum total sugars and reducing sugars were observed when seeds were treated with $CaCl_2$ followed by $CaSO_4$. In addition, SARC-1 overcame Inqlab-91 in all growth parameters of the seedlings. In conclusion, different salts used for priming in wheat seeds improved the salt stress tolerance; however, $CaSO_4$ and $CaCl_2$ proved to be the most effective priming agents in inducing salt tolerance in both wheat cultivars whereas NaCl was a less effective priming agent.

Keywords: priming; ionic homeostasis; salinity tolerance; wheat

Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu 2001). Salinity adversely affects almost all stages of growth and development, flowering and fruit set, ultimately causing low economic yield and poor quality of production (Ashraf and Harris 2004). A need to develop crops with higher salt tolerance has increased strongly due to increasing salinity problems. In general, plants do not develop salt tolerance unless they are grown under saline conditions. This means that they must be hardened to salt stress (Levitt 1980).

Physiological treatments to improve seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades. A number of research workers used different types of salts in pre-sowing soaking treatments to the seeds of various crops to get either better establishment of seedling or better plant development/yield under saline environment (Bose and Mishra 1999, Ashraf et al. 2003, Basra et al. 2005a). The purpose of these treatments is to shorten the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment, so as to synchronize emergence, which leads to uniform stand and improved yield.

Halopriming is a pre-sowing soaking of seeds in salt solutions, which enhances germination and seedling emergence uniformly under adverse environmental conditions. Basra et al. (2005b) reported that salt tolerance in wheat can be increased by treating seeds with various salt solutions prior to sowing; moreover, Cayuela et al. (1996) showed that the higher salt tolerance of plants from primed seeds is the result of higher capacity for osmotic adjustment since plants from primed seeds have more Na^+ and Cl^- in roots and more sugars and organic acids in leaves than plants from non-primed seeds. Therefore, halopriming could be used as a suitable method to improve salt tolerance in crops.

Deeper understanding of the physiological basis of seed germination under saline conditions is thus important because research progresses to ameliorate the adverse effects of salinity on germination by employing certain chemical and biochemical agents. The present study was conducted to investigate the effects of presoaking of wheat seeds in varying concentration of different salts upon their germination and subsequent growth under saline conditions.

MATERIAL AND METHODS

Seed materials. Seeds of wheat (*Triticum aestivum* L.) cvs. Inqlab-91 and SARC-1 were obtained from the Punjab Seed Corporation, Faisalabad, Pakistan. Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and air-dried.

Halopriming. Seeds were soaked in aerated 50 mmol solutions of $CaCl_2$, NaCl, and $CaSO_4$ for 12 h at 20°C in the dark separately and redried up to original weight with forced air under shade following Basra et al. (2005a). The ratio of seed weight to solution volume was 1:5 (g/ml).

Germination test and seedling vigor. Germination potential of the wheat seeds was estimated in accordance with the International Rules for Seed Testing (ISTA 1985). Three replicates of 25 seeds of each cultivar were germinated in 12 cm diameter petri dishes at 25°C and 65% RH in a growth chamber with photosynthetically active photon flux density of 350 mmol/m^2/s and photoperiod of 14/10 h light/dark cycle. Five ml of saline solution of 125 mmol NaCl was applied in each petri dish to impose salinity stress, while deionized water was applied for non-saline conditions. A seed was scored as germinated when coleoptile and radicle lengths reached 2–3 mm. Counts of germinating seeds were made every 6 h, starting on the first day of imbibition, and terminated when maximum germination was achieved. Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

where: n is the number of seeds, which were germinated on day D, and D is the number of days counted from the beginning of germination

The seedlings were collected after seven days and then washed with deionized water. Ten well-washed seedlings from each replication were separated into root and shoot for the determination of seedling fresh and dry weights. Dry weight was determined after oven drying of the samples at 65°C. The remaining seedlings were further used for biochemical analysis.

Determination of Na^+, K^+, Ca^{2+} and Cl^- in different parts of seedlings. Five seedlings from each replication harvested after seven days were used for ionic analysis. The dried plant material of seedlings was grounded. The dried material (0.1 g) was digested in 2 ml of concentrated H_2SO_4. Na^+, K^+ and Ca^+ were determined with a flame photometer (Jenway, PFP7, Essex, UK). For Cl^- determination, 0.1 g dry ground material was heated at 80°C for 4 h, and Cl^- concentration in the extracts was determined with a Sherwood 926 chloride analyzer.

Determination of total, reducing and non-reducing sugars in seeds. Total sugars in all the treated seeds before sowing were estimated in each replication following the method as described by Jayaraman (1981). Reducing sugars were determined by dinitrosalycylic acid method (Miller 1972), while non-reducing sugars, i.e. sucrose, content was calculated following the formula as described by Ranganna (1979).

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

RESULTS AND DISCUSSION

Significant differences ($P < 0.01$) were observed among genotypes, halopriming, and salinity levels for all characters examined. The interaction between cultivar × seed treatment × salinity was found significant for all characters ($P < 0.05$). The time taken for germination of treated and untreated seeds in saline conditions increased significantly (Figure 1a). All seed treatments were able to reduce

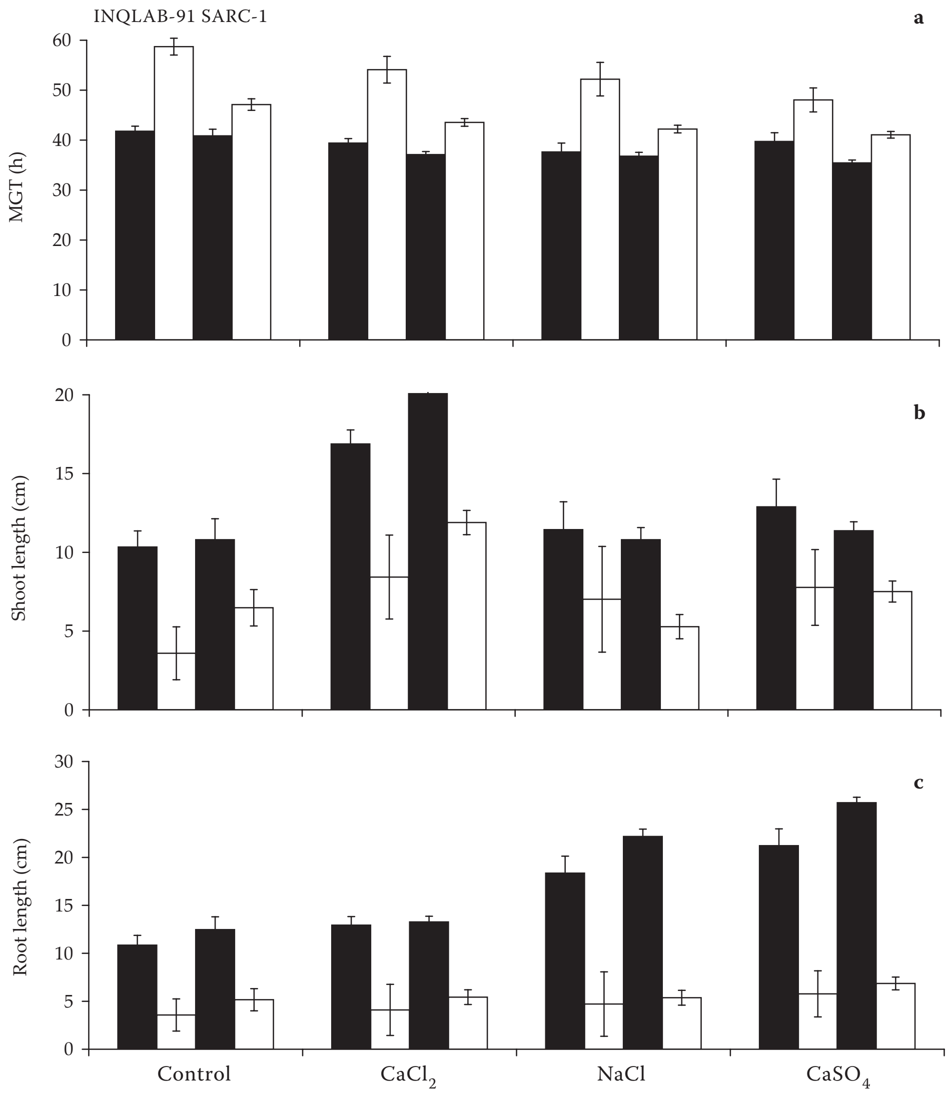

Figure 1. Effect of halopriming on (a) mean germination time (MGT), (b) shoot length and (c) root length of two wheat cultivars grown under normal (■) or saline (□) conditions

the time of germination in both cultivars under saline conditions. Seeds primed with $CaSO_4$ needed minimum time to germinate as compared to other primed or non-primed seeds of both cultivars under saline conditions. The salinity had also considerable effects on root and shoot lengths (Figure 1b and c). Plants raised from seeds primed with $CaCl_2$ maximally improved shoot lengths whereas maximum root length was recorded in plants raised from seeds primed with $CaSO_4$ in both cultivars under both treatments. On the other hand, priming with NaCl failed to improve these parameters in SARC-1 during salinity stress.

SARC-1 produced more biomass under both conditions but it showed a higher decrease in shoot and root weights under saline conditions as compared to Inqlab-91. However, SARC-1 responded well to the seed treatments, as it produced higher shoot and root weights than Inqlab-91, when compared with control under normal or saline conditions. Priming with $CaCl_2$ was the most successful as compared to the other seed treatments in both cultivars under both conditions (Figure 2a and b).

Salinity stress significantly decreased dry weights of roots and shoots in both cultivars. All seed treatments increased dry matter with the exception of SARC-1 treated with NaCl under saline condition. However, $CaSO_4$ treatments were the most successful in both cultivars under normal conditions, while $CaCl_2$ proved successful under saline conditions. Similarly, $CaCl_2$ treatment in both cultivars produced the highest shoots dry matter under both conditions (Figure 2c and d).

Priming of seeds of both cultivars with calcium salts markedly reduced Na^+ accumulation in seed-

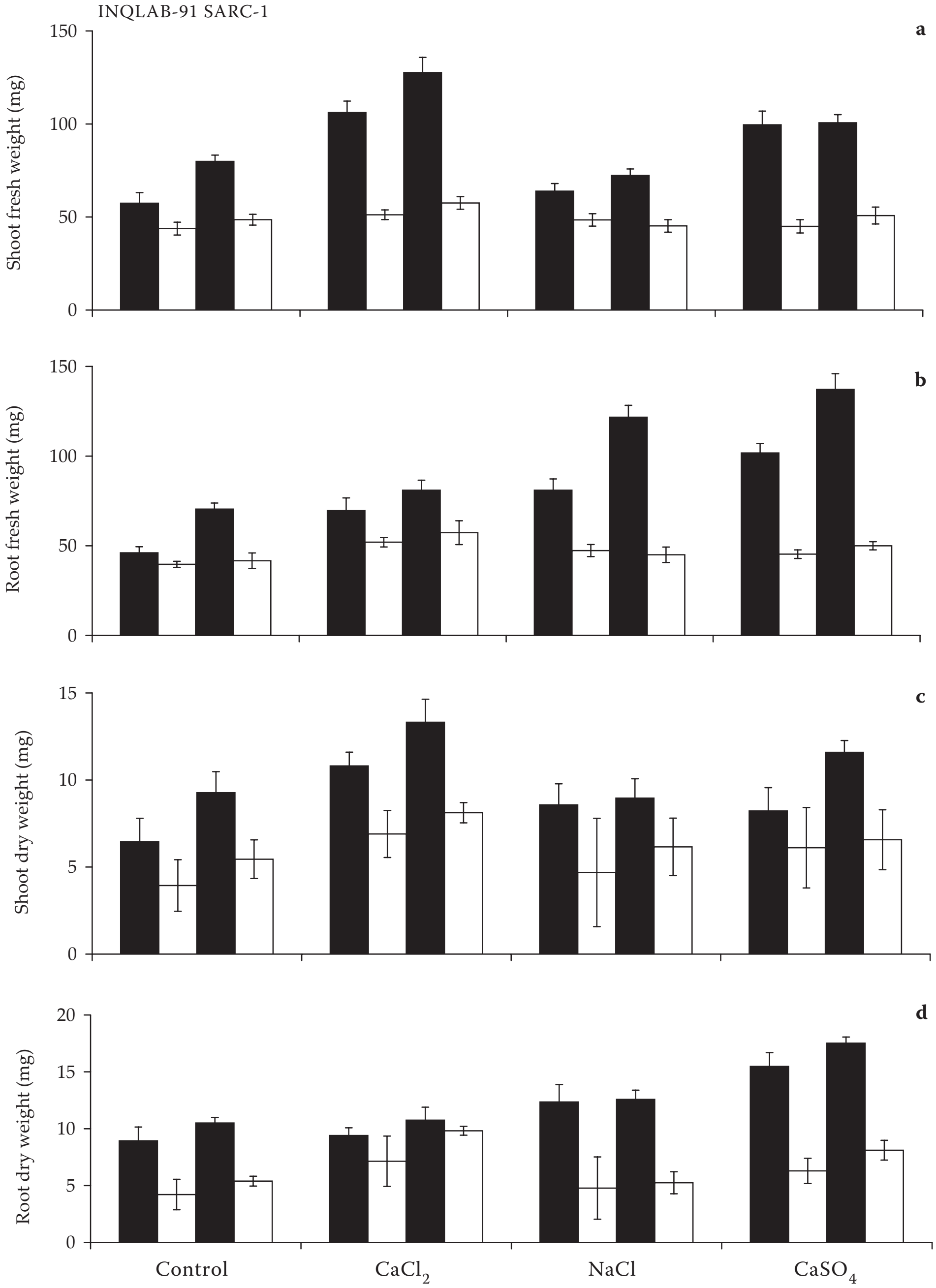

Figure 2. Effect of halopriming on fresh and dry weight of seedlings of two wheat cultivars grown under normal (■) or saline (□) conditions

lings, while priming with NaCl enhanced Na^+ accumulation (Figure 3a). However, the lowest Na^+ accumulation was observed in the plants of both cultivars raised from seeds primed with $CaCl_2$ under saline conditions. The highest Cl^- accumulation was observed in both cultivars due to priming with chloride salts under saline conditions. The lowest accumulation was observed due to priming with $CaSO_4$ in both cultivars under both conditions (Figure 3d). The accumulation of K^+ was the highest when seeds of both cultivars were treated with $CaCl_2$ followed by $CaSO_4$ under saline conditions (Figure 3b). The highest accumulation of Ca^{2+} was observed in plants of both cultivars as a result of priming with $CaSO_4$ followed by $CaCl_2$ under both conditions while the lowest accumulation was recorded in all plants primed with NaCl (Figure 3c).

Total, reducing and non-reducing sugars were also determined to investigate the impact of seed priming on starch metabolism. The highest total sugars and reducing sugars were observed when seeds were primed with $CaCl_2$ followed by $CaSO_4$ (Figure 4a and b). Similarly, the highest amount of

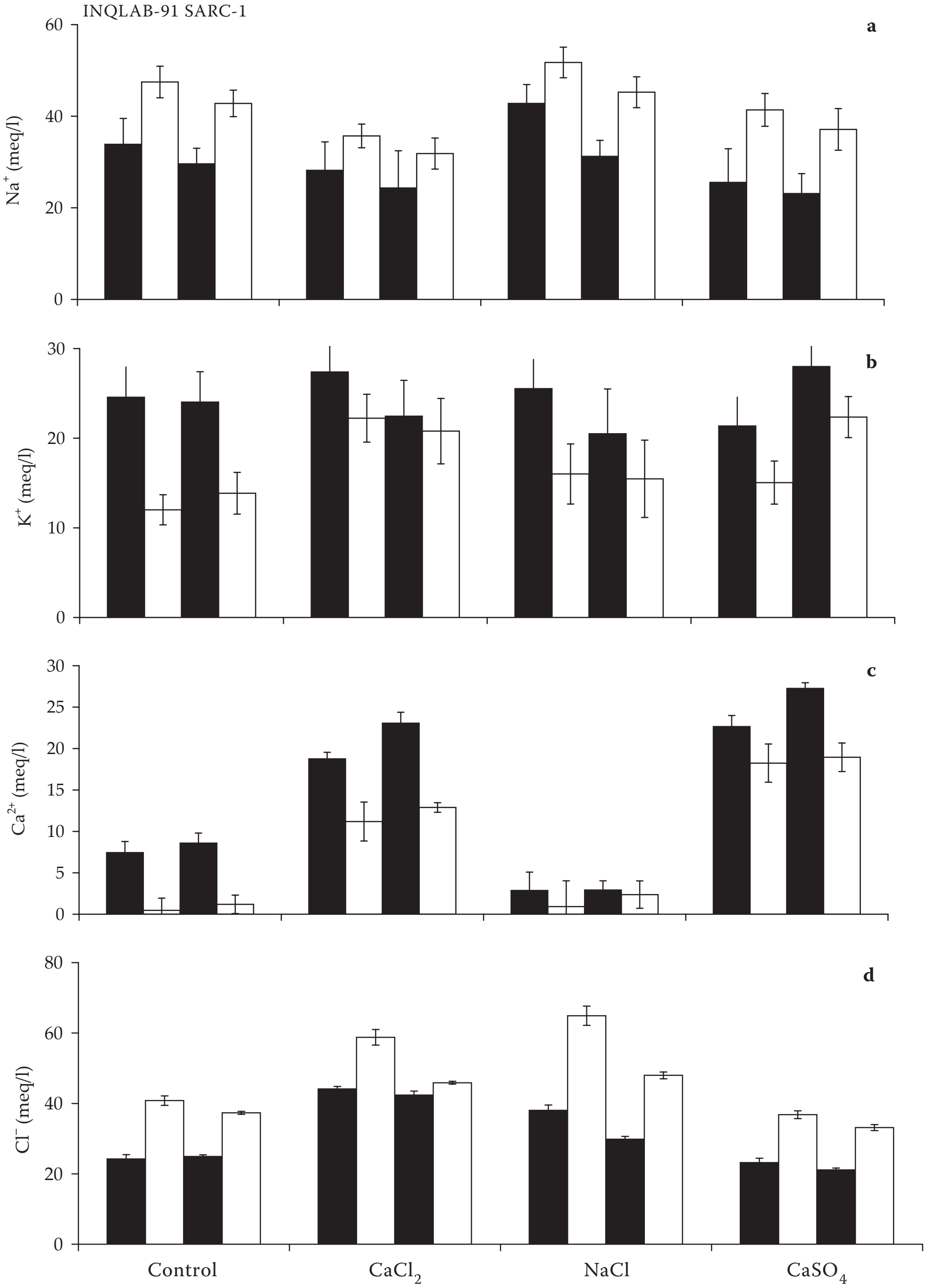

Figure 3. Effect of halopriming on ionic contents of two wheat cultivars grown under normal (■) or saline (□) conditions

non-reducing sugars was observed when seeds were treated with $CaCl_2$ in Inqlab-91, while in SARC-1 highest non-reducing sugars were observed when $CaSO_4$ was used for priming (Figure 4c).

Although salinity significantly reduced germination potential, seedling growth and metabolism, halopriming provided better protection to both wheat cultivars against salinity stress (Figures 1–4). Of all the salts used as priming agents, halopriming with $CaCl_2$ and $CaSO_4$ were found to be the most effective in enhancing germination, seedling vigor, ionic homeostasis and starch metabolism in wheat cultivars both under normal and saline conditions. Seeds primed with calcium salts had an advantage in maintaining germination under saline conditions perhaps due to the influence of calcium on membranes (Shannon and Francois 1977). Calcium thus protects wheat plants from adverse affects of salt stress and improves the growth of plants under saline conditions. NaCl priming failed to improve the growth of seedlings of both wheat cultivars as compared to calcium

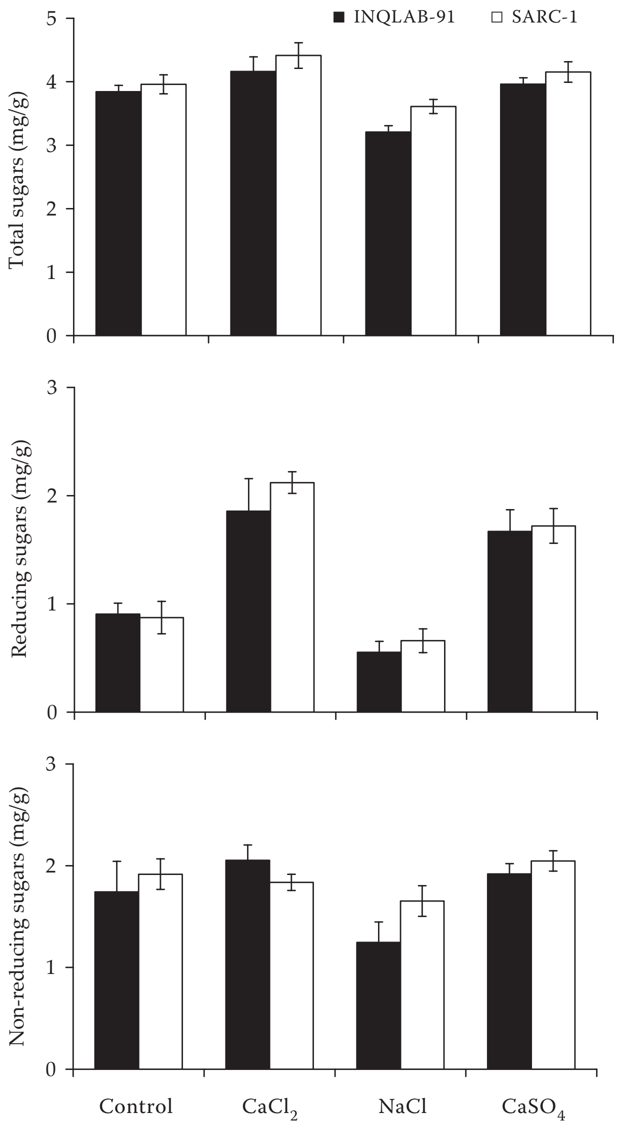

Figure 4. Effect of halopriming on sugar contents of two wheat cultivars

seed treatment under saline condition. It might have been caused by the tendency of NaCl treated seeds to take up more Na^+ and/or Cl^- from the salt solution, thus leading to the toxic effect as suggested by Ungar (1995).

Salt tolerance was increased in seeds of both cultivars subjected to priming (50 mmol $CaCl_2$), compared with other treatments including control, as indicated by shoot length, and root and shoot fresh and dry weights; however, $CaSO_4$ was more effective in both wheat cultivars under control conditions. This improvement might be the result of an increased rate of cell division in the root tips that caused an increase in seedling growth (Bose and Mishra 1999). The higher growth of plants raised from primed seeds of both cultivars exposed to salinity stress seems to result from a higher capacity for osmotic adjustment. The lower uptake of toxic mineral elements and higher uptake of beneficial mineral elements is generally associated with greater salt tolerance of most crop species (Shannon and Grieve 1999). For example, plants raised from SARC-I seeds primed with $CaSO_4$ showed lower Na^+ and Cl^- accumulation under saline and normal conditions. Furthermore, K^+ and Ca^{2+} concentrations were higher in shoots raised from seeds primed with $CaSO_4$ followed by $CaCl_2$ as compared to the seeds treated with NaCl. These results confirm the findings of Ashraf et al. (2003); they reported that pre-sowing seed treatments alleviated the adverse effects of NaCl stress on germination and seedling growth by decreasing Na^+ and slightly increasing K^+ and Ca^{2+} concentrations in the seedlings. In addition, Cl^- concentration was higher in plants raised from seeds primed with $CaCl_2$ or NaCl salts. High accumulation of Na^+ and Cl^- can be related to the greater sensitivity of the crop compared with other important grains (Ashraf and McNeilly 1989). For example, higher accumulation of Na^+ and Cl^- was recorded in seeds of Inqlab-91 as compared to SARC-1 in saline environment.

A clear tendency for increased total soluble sugars, reducing and non-reducing sugars in the seeds of tolerant and intolerant cultivars primed with both Ca^{2+} salts as compared to non-primed or NaCl primed seeds was observed (Figure 4). Halopriming with Ca^{2+} salts can be attributed to proper hydration during imbibition that increased starch hydrolysis, which resulted in increased total soluble sugars. The benefit of increased starch hydrolysis following hydration treatments was not lost during the redrying process, as shown by the better rate of germination. Moreover, Kathiresan et al. (1984) reported that better performance of seeds treated with Ca^{2+} salts under saline conditions may be due to enhanced oxygen uptake, increased α-amylase activity or the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis. It is well reported that increased α-amylase activity results in increased contents of total and reducing sugars subjected to hydration treatments (Lee and Kim 2000). The results of the present studies are also supported by Handa et al. (1983) who found increased concentration of reducing sugars in tomato with the degree of adaptation to the salinity stress.

From the present study, it is concluded that halopriming treatments alleviated the inhibitory effect of salt stress on the seedling growth of both wheat cultivars. Halopriming with $CaCl_2$ or $CaSO_4$ proved to be the most effective in inducing salt tolerance in both wheat cultivars due to improved seedling vigor, increased sugar contents as well as enhanced K^+ and Ca^{2+} accumulation and decreased accumulation of Na^+ in the seedlings.

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Corresponding author:

Dr. Irfan Afzal, University of Agriculture, Department of Crop Physiology, Faisalabad-38040, Pakistan
e-mail: iafzal@plantsciences.ucdavis.edu