IMPROVING GERMINATION AND SEEDLING VIGOUR IN WHEAT BY HALOPRIMING UNDER SALINE CONDITIONS

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The effects of halopriming (10, 25 or 50 mM NaCl and $CaCl_2.2H_2O$) were investigated in wheat (*Triticum aestivum* cv. Auqab-2000) under normal (4 dS m^{-1}) and saline (15 dS m^{-1}) conditions. Most of priming agents were not effective in improving germination and seedling vigour of wheat under saline conditions. Final germination count was unaffected by all priming tools while seeds subjected to halopriming with 25 and 50 mM $CaCl_2.2H_2O$ significantly reduced the germination time under both normal and saline conditions. Seeds subjected to halopriming with 50 mM $CaCl_2.2H_2O$ had significantly higher shoot length, fresh and dry weight of seedlings than those treated with other salts or control. During emergence test, emergence percentage, mean emergence time (MET) and dry weight of seedlings were un-affected by all the priming treatments, however, root and shoot length and shoot fresh weight were significantly increased by 50 mM $CaCl_2.2H_2O$ under both normal and saline conditions. All pre-sowing seed treatments caused a decrease in electrolyte leakage as compared to that in untreated seeds even after 12 hours of soaking period. Halopriming with 50 mM $CaCl_2.2H_2O$ induced maximum decrease in electrolyte leakage while an increase in electrolyte leakage was observed by treating seeds with increasing concentration of NaCl salt. It is concluded that priming of seed with 50 mM $CaCl_2.2H_2O$ induces physiological changes in the seed against salt stress conditions and can be used to induce salinity tolerance in wheat.
Keywords: Halopriming, salinity tolerance, seedling vigour, wheat seed
Abbreviations: Final emergence percentage = FEP, Mean emergence time = MET, Electrical conductivity = EC

INTRODUCTION

Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu, 2001). In Pakistan, salt affected lands are estimated to be about 6.67 Mha (Khan, 1998). Salinity adversely affects almost all stages of growth and development such as germination, seedling growth and vigour, vegetative growth, flowering and fruit set, ultimately causing diminished economic yield and also quality of produce. There are also reports suggesting that salt may affect the germination rate to a greater extent than the germination percentage. Therefore seeds with more rapid germination under salt stress may be expected to achieve a high final germination percentage and rapid and vigorous seedling establishment (Rogers *et al.,* 1995). The need to develop crops with higher salt tolerance has increased strongly due to increased salinity problems but the process for development of salt tolerance varieties in very slow due to our incomplete knowledge of salt tolerance mechanism and complex nature of salt tolerance of various crop species. In general plants do not develop salt tolerance unless they are grown in 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 (Bradford, 1986). A number of workers used different types of salts in pre-sowing soaking treatment to the seeds of various crops to get either better establishment of seedling or better plant development/yield (Raul, 1992; Bose and Mishra, 1999). 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 as to synchronize emergence, which leads to uniform stand and improved yield.
Halopriming is a pre-sowing soaking of seeds in salt solutions an alternative to priming, which enhances germination and seedling emergence uniformity under adverse environmental conditions (Cantliffe, 1997). It has been reported that salt tolerance in wheat can be increased by treatment of seeds with NaCl solutions prior to sowing (Ashraf *et al.*, 1999). The use of salt as an osmoticum can lead to an increase in fresh weight of vegetable seeds (Cantliffe, 1997). It has been shown that NaCl priming could be used as an adaptation method to improve the salt tolerance of seeds and it is important to understand the physiological effect of this technique on plants, which mediate their responses to

salinity. Cayuela *et al.* (1996) reported that the higher salt tolerance of plants from primed seeds is the result of a 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.

So an understanding of the physiological basis of seed germination under saline conditions is important since research is in progress to ameliorate the adverse effects of salinity on germination by employing certain chemical and biochemical agents. The present study is therefore, conceived with to investigate the effects of presoaking of wheat seeds in varying concentration of salts upon their germination and subsequent growth under saline conditions.

MATERIALS AND METHODS

Seed materials

Seeds of wheat (*Triticum aestivum* L.) cv. Uqab-2000 were obtained from Punjab seed corporation, Faisalabad. 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 solution of 10, 25 and 50 mM $CaCl_2.2H_2O$ or NaCl for 12 h and redried near to original weight with forced air under shade. The treated seeds were packed in polythene bags and stored in a refrigerator at 7±2 °C for further studies.

Germination Test

Germination potential of the wheat seeds was estimated 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 at 25 °C in growth chamber. Saline solution with NaCl of 15 dS m^{-1} was applied in each petri dish to impose salinity stress while distilled water with EC 4 dS m^{-1} was applied for normal conditions. A seed was scored germinated when coleoptile and root 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. During this, 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.

Seedling Vigour Evaluation

Salinity of 15 dS m^{-1} was developed in each plastic pot by giving the first irrigation of 15 dS m^{-1} saline water (USDA Salinity Lab. Staff, 1954). Control and treated seeds were sown in plastic pots having moist sand and were placed in a net house. After sowing the seed in sand at the depth of 3 cm, the pots were placed in growth chamber at temperature of 25 ± 2 °C. Half strength Hoagland solution was applied when the sand began to dry out, but there was no excess water visible. Emergence was recorded daily according to the Seedling Evaluation Handbook of Association of Official Seed Analysts (1990). The experiment was proceded for three weeks. The data regarding the final emergence percentage (%), mean emergence time (MET) (days), shoot length (cm), root length (cm), root/ shoot ratio and fresh and dry weight of seedling (g) were recorded according to Basra *et al.* (2002)

Electrical conductivity of seed leachates

After washing in distilled water, 5 g treated or non treated seeds were soaked in 50 mL distilled water at 25ºC. Electrical conductivity of steep water was measured 0.5,1.0,1.5,2.0,6.0,12.0 and 24.0 h after soaking using conductivity meter (Model Twin Cod B-173) and expressed as µS/cm (Basra et *al.*, 2002).

The data collected was analyzed using the Fisher's analysis of variance technique under completely randomized block design (CRD) and the treatment means were compared by Least Significant Difference (LSD) test at 0.05 probability level (Steel and Torrie, 1984).

RESULTS

Germination Test

Halopriming treatments had a significant effect on germination percentage, mean germination time and root length of wheat under both normal and saline conditions (Fig. 1). Germination percentage was significantly reduced under saline conditions, however maximum germination (85%) was observed in seeds primed with 50 mM $CaCl_2.2H_2O$ which was statistically similar to control. All the remaining treatments failed to improve germination under saline conditions (Fig. 1a).

Most of the halopriming treatments along with control took longer time to germinate i.e., due to higher MGT under normal conditions. Lowest MGT was recorded in seeds primed with 25 or 50 mM $CaCl_2.2H_2O$ while highest MGT was recorded in seeds treated with 10 and 25 mM $CaCl_2.2H_2O$. Under saline conditions, halopriming with 25 or 50 mM $CaCl_2.2H_2O$ were found successful in reducing time for germination as compared to control (Fig. 1b).

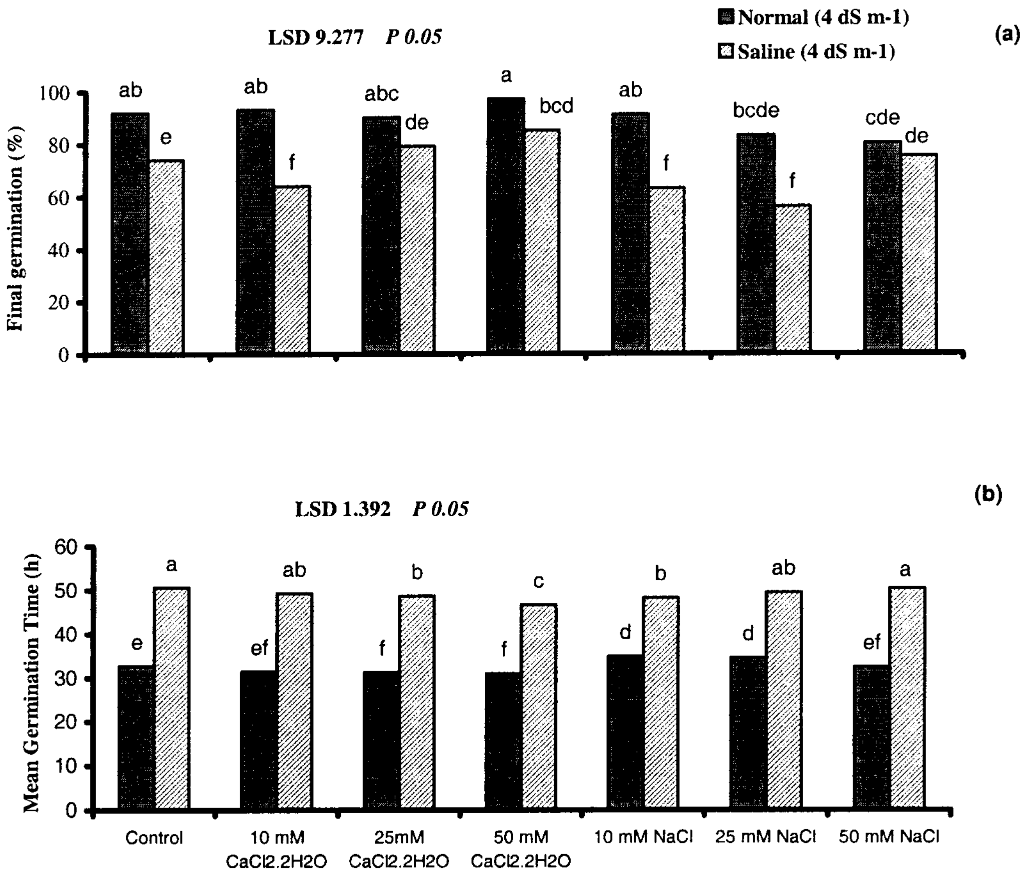

Fig. 1. Effect of different halopriming treatments on (a) final germination and (b) MGT of wheat cv. Auqab-2000 growing under normal and saline conditions during germination test. The vertical bars with different alphabets are statistically different indicating interactive effect of halopriming treatments and salinity.

All the halopriming treatments showed significant effect on root, shoot lengths and but root shoot ratio was non-significantly affected under both normal and saline conditions (Fig. 2). Maximum shoot length was obtained in those plants raised from seeds primed with 50 mM $CaCl_2.2H_2O$ under non-saline or saline conditions (Fig. 2a). Only $CaCl_2.2H_2O$ (50 mM) significantly improved root length under non-saline and saline conditions (Fig. 2b).

Priming treatments affected significantly fresh and dry weight of seedlings in normal and saline conditions (Fig. 3). Under normal conditions, all priming treatments except 10 or 50 mM NaCl proved better in improving root and shoot fresh weight as compared to untreated seeds under normal conditions. Root fresh weight was significantly decreased by salinity. Seeds treated with 50 mM $CaCl_2.2H_2O$ performed better as compared to control but statistically similar to all the treatments except 10 mM NaCl (Fig. 3a and 3b).

Maximum dry weight of root was attained in seeds treated with 50 mM $CaCl_2.2H_2O$ and 25 mM NaCl while minimum was recorded in 25 mM $CaCl_2.2H_2O$ and 10mM NaCl treated seeds under normal conditions. Under saline conditions, maximum dry weight (3.26mg) was achieved in seeds treated with 50 mM $CaCl_2.2H_2O$ which was statistically similar to all the treatments except halopriming with10 mM NaCl and control (Fig. 3c). Under normal conditions, maximum shoot dry weight was achieved in seeds treated with 10 or 50 mM $CaCl_2.2H_2O$ while minimum as recorded in non-primed and 50 mM NaCl treated seeds. Under saline conditions, maximum shoot dry weight was achieved in seeds treated with 10 and 50 mM $CaCl_2.2H_2O$ while remaining treatments failed to improve shoot dry weight as compared to control (Fig. 3d).

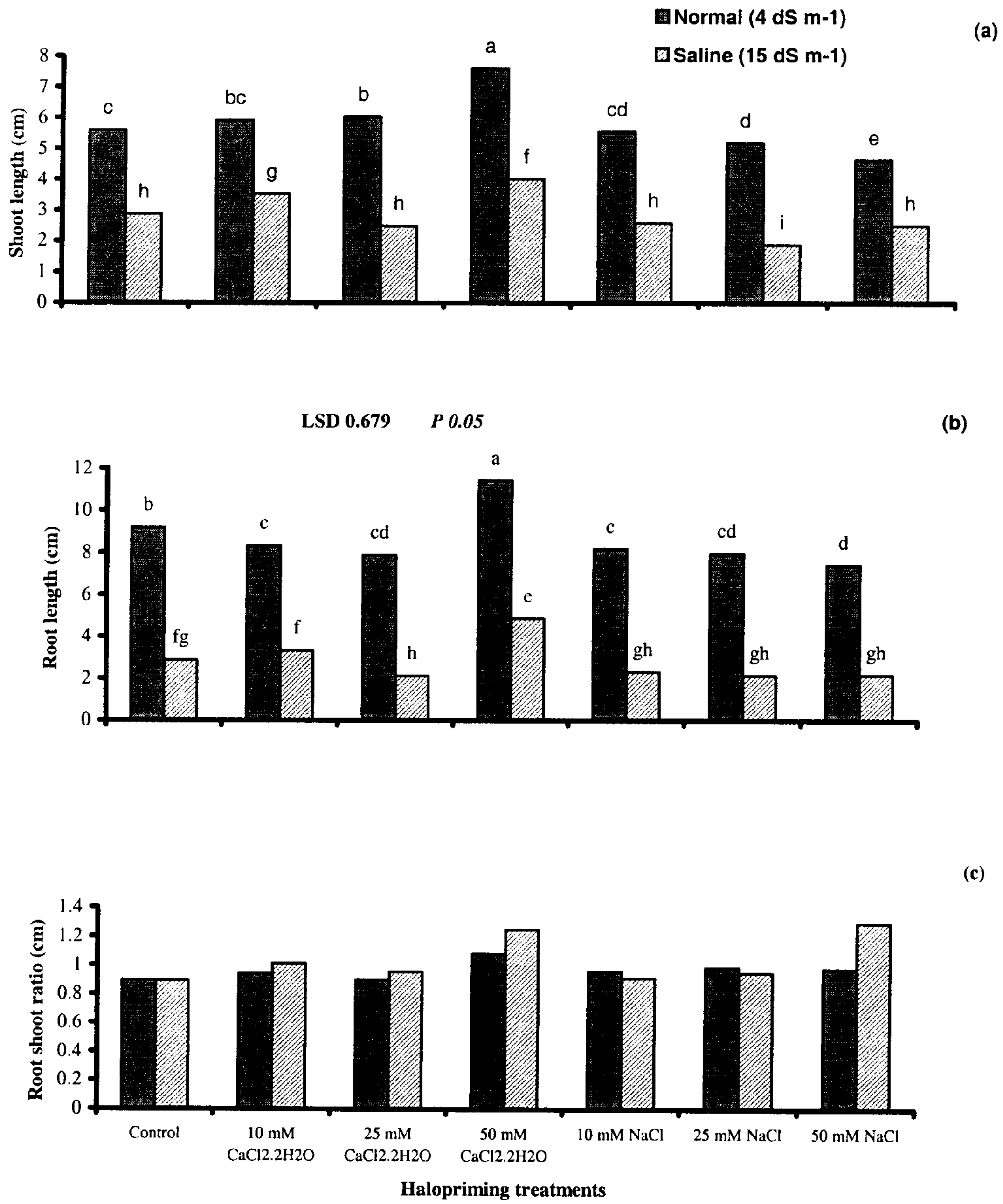

Fig. 2. Effect of different halopriming treatments on (a) shoot length (b) root length and (c) root shoot ratio of wheat cv. Auqab-2000 under normal and saline conditions during germination test. The vertical bars with different alphabets are statistically different indicating interactive effect of halopriming treatments and salinity.

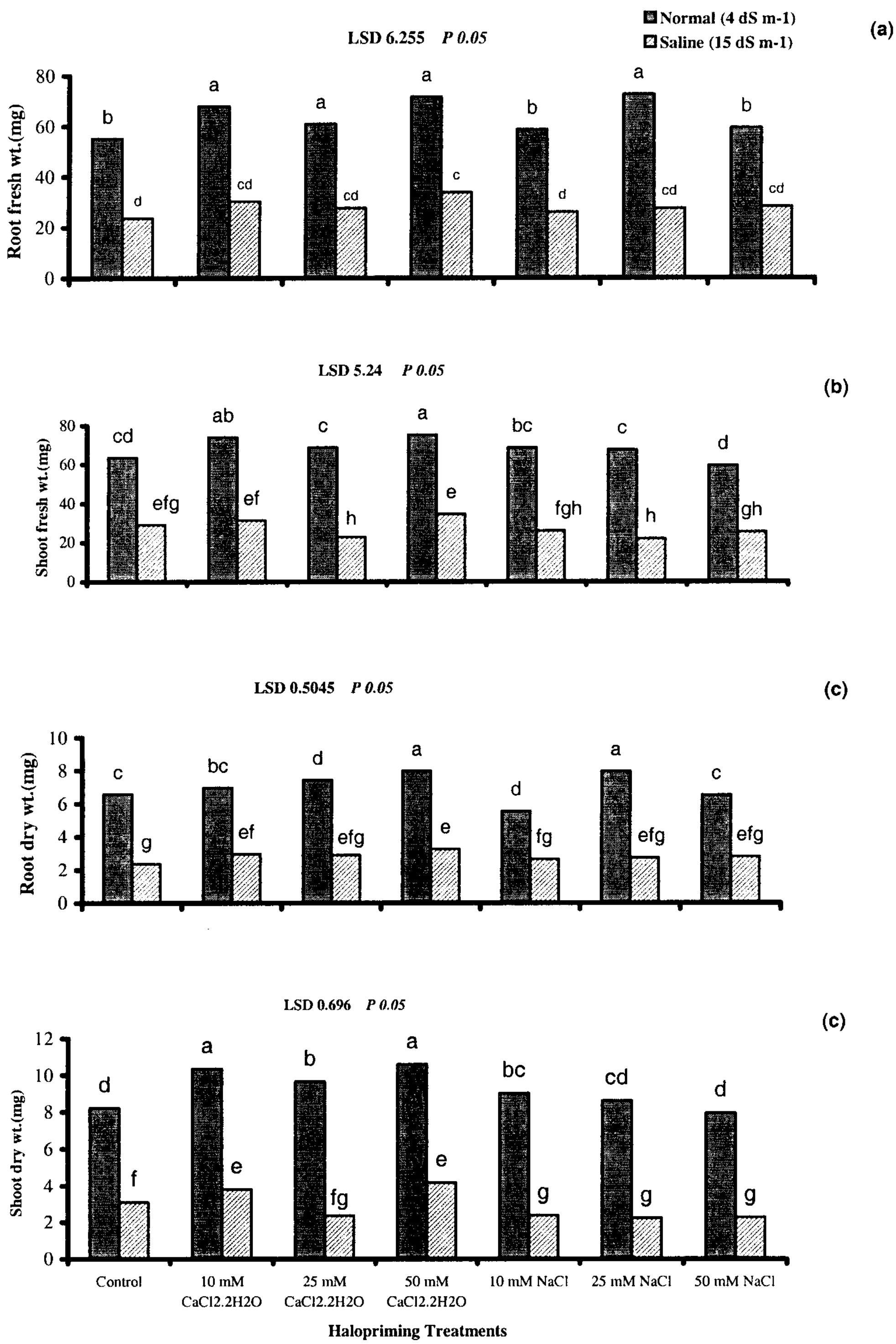

Fig. 3. Effect of different halopriming treatments on (a) root fresh weight (b) shoot fresh weight (c) root dry weight and (d) shoot dry weight of wheat cv. Auqab-2000 growing under normal and saline condition during germination test. The vertical bars with different alphabets are statistically different indicating interactive effect of halopriming treatments and salinity.

Emergence Test

Halopriming treatments had a significant effect on emergence percentage, mean emergence time and shoot length of wheat plants under both normal and saline conditions (Table 1). Under non-saline environment, all the treatments failed to improve emergence while NaCl 10 mM priming has suppressive effect. Halopriming of seeds with 50 mM $CaCl_2.2H_2O$ improved emergence maximally as compared to control under salinity stress. Whereas halopriming with 10 or 25mM $CaCl_2.2H_2O$ and 25 or 50 mM NaCl adversely effected the emergence. All the halopriming treatments along with control took longer time to emerge i.e., due to higher MET under normal conditions. Lowest MET was recorded in seeds primed with 50 mM $CaCl_2.2H_2O$ as compared to remaining treatments including control. However under saline conditions, MET was unaffected by all the treatments.

Root and shoot lengths were maximally improved by priming with 50 mM $CaCl_2.2H_2O$ but these treatments had a non-significant effect on root shoot ratio under both normal and saline conditions (Table 1).

Salt treatments significantly affected the fresh and dry weight of shoot and root under normal and saline conditions (Table 1). Maximum shoot fresh weight was recorded in seeds haloprimed with 50 mM NaCl, which was statistically similar to 50 mM $CaCl_2$ under normal conditions. In seeds treated with 10mM NaCl, decrease in shoot fresh weight was recovered to maximum limit. Under saline conditions, shoot fresh weight was increased in seeds treated with 50 mM $CaCl_2.2H_2O$ and was statistically similar to priming with 25 mM NaCl. Under normal conditions, maximum shoot dry weight was observed in seeds treated with 50 mM $CaCl_2.2H_2O$ which was statistically non-significant with that of control and 50 mM NaCl. All the pre-sowing treatments did not improve shoot dry weight under saline conditions except 50 mM $CaCl_2.2H_2O$. However, all the treatment failed to improve root fresh and dry weight under both normal and saline conditions (Table 1).

Electrical Conductivity

All pre-sowing seed treatments were observed effective in decreasing electrolyte conductivity of seed leachates (Fig. 4). After a longer period of imbibition ranging from 1h to 24h all the priming treatments lowered down the electrolyte leakage except control. Maximum decrease in electrolyte leakage was induced by 10mM $CaCl_2$ on all measuring periods. Halopriming with 25 and 50 mM $CaCl_2$ followed by 10 mM $CaCl_2$ were successful in decreasing electrolyte leakage. An increase in electrolyte leakage was observed by 10, 25 and 50 mM NaCl at all soaking periods.

DISCUSSION

Studies on salt stress in germinating seeds showed that during this stage the seeds are particularly sensitive to the saline environments (Bewely and Black, 1982; Mayer and Poljakoff-Mayber, 1982). The present findings clearly indicate that the salinity significantly reduced germination percentage both by causing delay in germination and by lowering the final population of germinated seeds thus confirming the results of Sarin and Narayanan (1968) in wheat. Kocacaliskan (1990) also found the final percent germination decreased as salinity increased and the seed germination failed at high NaCl concentration in maize. Salt stressed seeds are dessication sensitive, which caused physiological injuries in seeds resulting in reductions in seed germinability (Wiebe and Tiesses, 1979). In view of some earlier studies it is now evident that pre-soaking or priming of seed of different crops causes improvement in germination, seedling establishment and in some cases enhances crop yield (Ahmad *et al.,* 1998; Harris *et al.,* 1999). In present studies, all the priming treatments did not improve final germination percentage but priming with 25 or 50 mM $CaCl_2$ increased germination speed as compared to control under saline conditions. These results can be related to the earlier findings by Chaudhuri and Wiebe (1968) in wheat, Kader and Jutzi (2004) in sorghum and Shannon and Francois (1977) in cotton. Generally, faster germination is due to the synthesis of DNA, RNA and protein during priming (Bray *et al.,* 1989). There more rapid germination in primed seeds may be due to enhanced activities of seed β-amylase and shoot catalase in rice (ChangZheng *et al.*, 2002). NaCl priming treatments failed to improve germination as compared to 50 mM $CaCl_2$ seed treatment under both non-saline and saline conditions. It might be that NaCl treated seeds had taken up more Na^+ and/or Cl^- from the salt solution, hence leading to the toxic effect as suggested by Bradford (1995) and Ungar (1995). While seed pretreated with 50 mM $CaCl_2$ had an advantage in maintaining germination under saline conditions due to the influence of calcium on membranes (Shannon and Francois, 1977). Similarly, Chaudhri and Wiebe (1968) found no germination in NaCl when wheat seeds were primed only with water but a considerable improvement in germination when $CaCl_2$ was used as a priming agent. The results regarding root and shoot fresh an dry weight are in agreement with those of Ashraf and Rauf (2001) who reported that fresh and dry weights of wheat seeds treated with $CaCl_2.2H_2O$ were significantly higher as compared to other salt treatments and control.

Table 1. Effect of halopriming treatments on emergence and seedling vigour of wheat cv. Auqab-2000 under saline and non-saline conditions.

Treatments		FEP (%)	MET (hours)	Shoot length (cm)	Root length (cm)	Root shoot ratio (cm)	Shoot fresh wt. (mg)	Root fresh wt. (mg)	Shoot dry wt. (mg)	Root dry wt. (mg)
Normal (4 dS m^{-1})	Control	85.75 a	6.38 d	14.95 c	21.42 e	1.428	150.1 bc	178.2 a	47.42 a	45.2 a
	10 mM $CaCl_2.2H_2O$	81 a	6.085 d	17.31 d	25.5 b	1.475	143.1 c	117.9 c	39.24 b	22.46 e
	25 mM $CaCl_2.2H_2O$	91 a	6.12 d	17.48 b	25.38 bc	1.455	147.6 bc	160.3 ab	39.99 b	32.32 bc
	50mM $CaCl_2.2H_2O$	92 a	6.075 d	20.72 a	28.77 a	1.39	163.2 ab	157.4 b	48.19 a	36.79 b
	10 mM NaCl	44.5 c	6.262 d	16.65 b	22.94 d	1.38	110.9 d	112.3 cb	25.92 c	23.88 de
	25 mM NaCl	86 a	6.145 d	17.33 b	24.96 bc	1.442	149.6 bc	149.9 b	41.22 b	29.45 cd
	50 mM NaCl	88.25 a	6.153 d	17.77 b	23.99 cd	1.345	175.3 a	163.9 ab	48.18 a	37.28 b
Saline (15 dS m^{-1})	Control	56 b	10.65 b	9.15 e	8.7 g	0.793	87.43 ef	89.87 efg	7.72 e	10.53 f
	10 mM $CaCl_2.2H_2O$	34 c	10.48 b	7.2 fg	6.225 i	0.865	82.1 ef	77.7 fg	10.41 de	3.637 g
	25 mM $CaCl_2.2H_2O$	34 c	10.8 ab	11 d	7.7 gh	0.84	54.2 g	48 h	3.49 f	3.717 g
	50 mM $CaCl_2.2H_2O$	85 a	8.818 c	12.17 d	10.78 f	0.885	111 d	103.9 cde	11.86 d	5.73 fg
	10 mM NaCl	58 b	10.48 b	7.35 f	6.5 hi	0.885	78.9 f	73.6 g	7.62 e	6.557 fg
	25 mM NaCl	44 c	10.73 b	8.625 e	7.75 gh	0.897	98.1 de	93.8 def	10.06 de	9.3 fg
	50 mM NaCl	33 c	11.15 a	6.15 g	5.32 i	0.875	78.1 f	71.5 g	8.13 de	7.637 fg
	LSD	**11.12**	**0.385**	**1.179**	**1.35**	**NS**	**16.2**	**6.25**	**11.12**	**5.69**

Note: Within a column, means sharing the same letters are nonsignificantly different ($P>0.05$) according to the LSD test.

The increase in emergence percentage in 50 mM $CaCl_2.2H_2O$ pretreatment under saline conditions may be due to enhanced oxygen uptake, increased α-amylase activity and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis as reported by Karthiresan *et al.* (1984) in sunflower. The findings of the present study are not in line with other findings on pea (Gurrier and Pinel, 1989), pepper (Smith and Cobb, 1991) and tomato (Cano *et al.*, 1991). NaCl pretreatment could have two effects: a stimulative effect related to salt acclimation, and a toxic effect due to salt stress. At the high NaCl pretreatment level (50 mM), the toxic effect would be increased and the stimulative effect would be nullified. This conclusion is further supported by the data in Table 1. Salt priming and other organic molecules might pose toxicity problems as ions accumulate in tissues as reported in various vegetable species (Brocklehurst and Dearman, 1984). That's why reduction in emergence percentage of seeds subjected to NaCl was due to accumulation of salts in tissue, which cause toxicity (Smith and Cobb, 1991). Similar results were recorded by Al-Ansari (2003) who reported that high concentrations of NaCl and KCl salts reduced final germination percentage, germination rate and increased the production of abnormal seedlings in wheat.

Salt tolerance was increased in seeds subjected to priming (50 mM $CaCl_2.2H_2O$) compared with other treatments including control as indicated by shoot and root length, shoot fresh and dry weight. An increase in root length was recorded in $CaCl_2$ treatment as compared control and remaining priming treatments, which might be the result of higher embryo cell wall extensibility. Demir and Oztokat (2003) also found that root and shoot lengths were increased in water melon seeds due to salt priming as compared to non-primed seeds. Similarly, Kamboh *et al.* (2000) reported that Ca salt seed treatments significantly improved shoot growth during the early seedling establishment stage in wheat under saline conditions.

Seed leachate electrical conductivity is considered as an effective indicator of seed germination (Waters and Blanchette, 1983). All priming treatments were effective in decreasing electrolyte conductivity of seed leachates, which shows membrane stability. An increase in electrolyte leakage was observed by 10, 25 and 50 mM NaCl at all soaking periods which shows the toxic behaviours and/or penetration of salts in the seed tissues and was probably due to the loss of ability to reorganize cellular membranes rapidly and completely (McDonald, 1980). The membrane damage and enhanced permeability may be affected by the displacement of Ca^{2+} by Na^{+} from the binding sites of

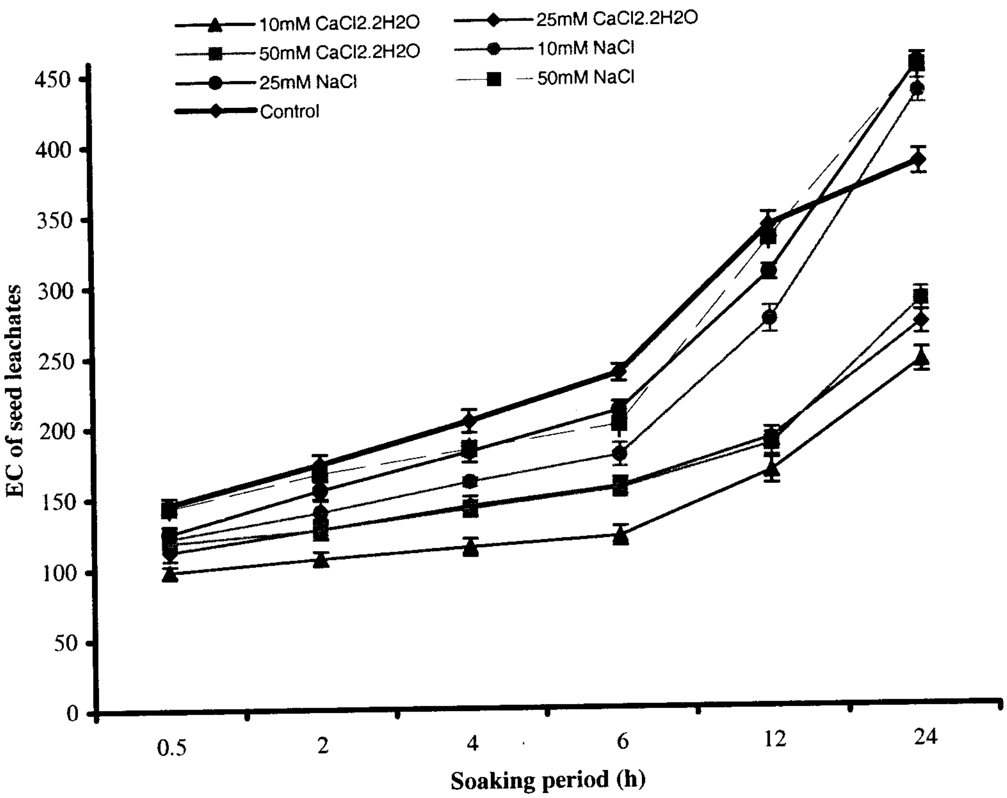

Fig. 4. Effect of different halopriming treatments on the electrical conductivity of seed leachates (μS cm^{-1} g^{-1}) in wheat cv. Auqab-2000. Vertical bars indicate the SE

phospholipids of membranes (Zhao and Mingliang, 1988). Similarly, Leopold and Willing (1984) concluded that salt toxicity results in the production of lesions on the membranes, generally the plasmalemma membrane, resulting in the leakage of solutes from the cell. These results are in agreement with the work done by Ashraf *et al.* (1999) who reported that higher EC values were observed in seeds treated with NaCl than in the non-primed seeds. Decreased leakage of solute in $CaCl_2$ treatment than control may be because of better membrane repair during hydration as it was proved by previous workers that calcium has a positive influence on membranes (Shannon and Francois, 1977). Greater membrane integrity in primed seeds was reported by Rudrapal and Naukamura (1998) for eggplant and radish and Afzal *et al.* (2002) for hybrid maize. However, our results are not in line with the findings of Agerich and Bradford (1988) for tomato seeds and Basra *et al.* (2004) for wheat seeds.

In conclusion, halopriming with 50 mM $CaCl_2.2H_2O$ was effective priming treatment in alleviating adverse effect of salt stress in wheat during germination and emergence. Priming with higher concentration of NaCl salt exacerbated the adverse effect of salt stress on wheat plant. Further research is needed to optimize the effectiveness of halopriming treatments with different salts on number of cultivars of wheat.

ACKNOWLEDGEMENTS

This work was supported by a scholarship awarded to Mr. Irfan Afzal by Higher Education Commission, Ministry of Science and Technology, Government of Pakistan.

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