

## INDUCING SALT TOLERANCE IN WHEAT BY SEED VIGOR ENHANCEMENT TECHNIQUES

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### ABSTRACT

The study was conducted to determine whether salt tolerance could be induced in wheat at emergence stage by seed priming. Different seed priming techniques used were, soaking of seeds for 24h in distilled water, hardening for 12h (one cycle), matricconditioning with pressmud for 24h and halopriming with 100 mol m<sup>-3</sup> CaCl<sub>2</sub>, 50 mol m<sup>-3</sup> NaCl, 25 mol m<sup>-3</sup> Ca(NO<sub>3</sub>)<sub>2</sub> for 24h. Both primed and non-primed seeds were subjected to 15dS/cm salinity under controlled conditions. Although all priming agents were effective in decreasing the adverse effects of salt stress on wheat at emergence stage. Hydropriming and halopriming with NaCl treatments proved to be more effective since the seed primed with these treatments had significantly lower mean emergence time, higher shoot and root length, dry weight of seedlings and E<sub>50</sub> than those treated with other salts or hardening or matricconditioning. Except halopriming (CaCl<sub>2</sub>) all pre-sowing seed treatments cause a decrease in electrolyte leakage as compared to that in non-primed seeds even after 24 hours of soaking period. Hardening induced maximum decrease in electrolyte leakage while an increase in electrolyte leakage was observed by 50 mol m<sup>-3</sup> CaCl<sub>2</sub> treatment. It is concluded that priming of seed with distilled water or NaCl induces physiological changes in the seed against salt stress conditions and can be used to induce salinity tolerance in wheat.

**Key Words:** Halopriming, hydropriming, matricconditioning, hydropriming, wheat seed

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### INTRODUCTION

Salinity is amongst the most important problems affecting irrigated agriculture in the world. It has been estimated that salinity limits crop production on 20 million hectare of the world's irrigated land (Greenway and Munns, 1980). Salinity changes nutrient and water availability, lowers the quality of arable lands, and alters the structure of ecological communities. Salinity induced osmotic stress, the physiological equivalent of drought stress, typically reduces growth and photosynthesis in plants (Pasternak and Pietro, 1985). In general, growth reduction due to salinity is attributed to ion toxicity, nutrient imbalance and osmotic effect. Higher concentration of soluble salts in soil causes reduction in the germination percentage and delay in germination of seeds of many plant species (Greenway and Munns, 1980; Khan, 1992). 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. Seed invigoration treatments have therefore, been developed to improve seed performance during germination and emergence under various stress conditions. "Seed invigoration" is a term used in the scientific literature to describe beneficial treatments applied on the seeds after harvest, but prior to sowing in order to improve the germination or seedling growth (Taylor *et al.*, 1998) especially under stressful condition. Many seed invigoration treatments are being used to improve the rate and speed of germination (Lee and Kim, 2000). Pre-sowing treatments with different salts (Idris and Aslam, 1975) and water (Twitchell, 1955) stimulate the germination process and earlier germination in treated than in untreated seed of wheat under salt stress. However, Henckel and Strongonov (1961) advocated that salinity tolerance of plants could be improved by treating seeds with solutions of different salts before sowing, as plants from such treated seeds show more adaptation to saline conditions than the untreated seeds.

The present investigation therefore, was designed to test the response of wheat seeds to priming with distilled water or salts and to develop suitable invigoration techniques for wheat seeds under salt stress conditions.

### MATERIALS AND METHODS

#### Seed materials

The experiment was conducted to study the influence of different seed priming techniques on growth and vigor of wheat under saline conditions. Seeds of wheat (*Triticum aestivum* L.) cv. Uqab-2000 were obtained from Punjab seed corporation, Faisalabad. Before the start of experiment, seeds were surface sterilized in 10% sodium hypochlorite solution for 10 minutes, then rinsed with sterilized water and air-dried.

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**Table 1. Different pre-sowing seed treatments used in this study.**

No.	Treatment
T <sub>1</sub>	Control
T <sub>2</sub>	Hydropriming for 24 h
T <sub>3</sub>	Hardening for 12 h (One cycle)
T <sub>4</sub>	Matricconditioning with Pressmud for 24 h
T <sub>5</sub>	Halopriming with 100 mol m <sup>-3</sup> CaCl <sub>2</sub> for 24 h
T <sub>6</sub>	Halopriming with 50 mol m <sup>-3</sup> NaCl for 24 h
T <sub>7</sub>	Halopriming with 25 mol m <sup>-3</sup> Ca(NO <sub>3</sub> ) <sub>2</sub> for 24 h

### Seed Hydration Treatments

**Hydropriming:** A weighed quantity (250 g) of wheat seeds were soaked in distilled water for 24 hours. After soaking seeds were redried to original weight with forced air under shade. The seeds were sealed in airtight container and placed in refrigerator at 8°C temperature till further use (Bennett and Waters, 1987).

**Hardening:** Seeds were soaked in tap water at 27 °C for 12 h followed by redrying to initial moisture under shade with forced air. The cycle was repeated twice (Lee *et al.*, 1998).

**Halopriming:** Seeds were soaked in aerated solution of 100mM CaCl<sub>2</sub>, 50mM NaCl and 25mM Ca (NO<sub>3</sub>)<sub>2</sub> separately for 24 h.

**Matricconditioning:** matricconditioning was carried out with different solid matrix carriers (compost, press mud and gunny bag) that are cheaper as compared to calcined clay and Micro Cell E. First of all seeds were mixed with 1 kg sterilized press mud separately and 350 mL of distilled water in closed plastic container. The container was placed under shade at room temperature for 24 hours. The partially hydrated seeds were then screened from the press mud (Afzal *et al.*, 2002).

**Post priming operations:** After matricconditioning, seeds were given 3 surface washings with distilled water (Khan *et al.*, 1992) and redried to original weight with forced air under shade. At the end, all the treated seeds were packed in polythene bags and stored in a refrigerator at 7±2 °C till further studies.

**Seedling Vigour Evaluation:** Before the start of the experiment, salinity of 15 dS/cm was developed in each plastic pot by giving the first irrigation of 15dS/cm saline water (USDA Salinity Lab. Staff, 1954). Control and treated seeds were sown in plastic tubs 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 preceded for three weeks. The data regarding the final emergence percentage (%), days to 50% emergence (E<sub>50</sub>) (days), 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 seeds were soaked in 50 mL distilled water at 25°C. 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 (Ashraf *et al.*, 1999). 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).

**Abbreviations:** Final emergence Percentage = FEP, Mean emergence time = MET, Time taken for 50 % emergence = T<sub>50</sub>, Electrical conductivity= EC

### RESULTS

Comparison of treatment means (Fig. 1) indicates that emergence, mean emergence time, E<sub>50</sub>, and fresh weight of seedlings were significantly affected by different priming tools under saline conditions. Although all the seed

treatments resulted in increased invigoration under saline conditions but highest emergence (95.5%) was recorded in hydroprimed seeds and was statistically at par with hardening treatment (Fig. 1a). After control, lowest final emergence percentage (86.53) was recorded in seeds haloprimed with  $\text{Ca}(\text{NO}_3)_2$  followed by matricconditioning. All the priming treatments decrease time taken to 50% emergence as compared to non-primed seeds under saline conditions (Fig. 1b). Statistically lowest  $E_{50}$  was recorded in wheat seeds subjected to hydropriming and halopriming (NaCl) while maximum  $E_{50}$  i.e., delayed emergence was noted in control that reveals that all the seed treatments resulted in earlier emergence i.e., lower  $E_{50}$  with that of control.

For mean emergence time, all the priming treatments significantly reduce emergence time as compared to control (Fig. 1c). Seedling emergence was significantly enhanced by halopriming with NaCl treatment followed by hydropriming treatment. A significant negative effect on seedling emergence was observed by matricconditioning, which resulted in delayed emergence. Seedling fresh weight was significantly increased by all the priming treatments except matricconditioning (Fig. 1d). Maximum increase in fresh weight of seedlings was observed as a result of hardening followed by hydropriming. Seedling fresh weight was not significantly influenced by matricconditioning.

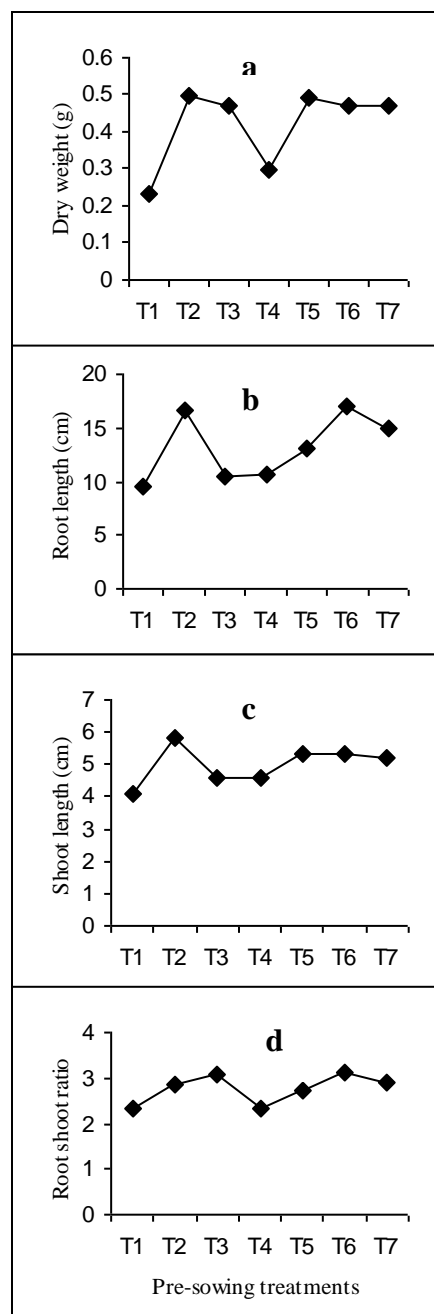
Data presents that different seed priming treatments significantly affected the different parameters of study like root and shoot lengths, root shoot ratio and dry weight of seedlings (Fig. 2). Halopriming with NaCl, and  $\text{Ca}(\text{NO}_3)_2$  and hydro priming of seeds significantly increased the root length as compared to that in control while the affect of other treatments was nonsignificant (Fig. 2b). Maximum root length was achieved in seedlings primed with NaCl. A second maximum root length was observed in seedlings by hydropriming, which was statistically non-significantly different from root length in NaCl primed seedlings. Minimum root length was observed in seedlings after hardening treatment indicating a non-significant affect on root length.

For shoot length, hydropriming, halopriming with NaCl, and  $\text{Ca}(\text{NO}_3)_2$  and  $\text{CaCl}_2$  significantly increase was observed as compared to that in control. The affect of other treatments was non-significant (Fig. 2c). Maximum shoot length was recorded in hydroprimed seeds followed by halopriming with NaCl. Hardening and matricconditioning produced non-significant effects on shoot length of seedlings and the values were minimum for these treatments. Significantly increased root shoot ratio was recorded from all the priming treatments except matricconditioning as compared to that in control (Fig. 2d). Maximum root shoot ratio was observed in seeds haloprimed with NaCl followed by hardening, halopriming with  $\text{Ca}(\text{NO}_3)_2$  and hydropriming. Minimum root shoot ratio was observed in matricconditioned seeds, which was statistically nonsignificantly different from that of control and halopriming with  $\text{CaCl}_2$ .

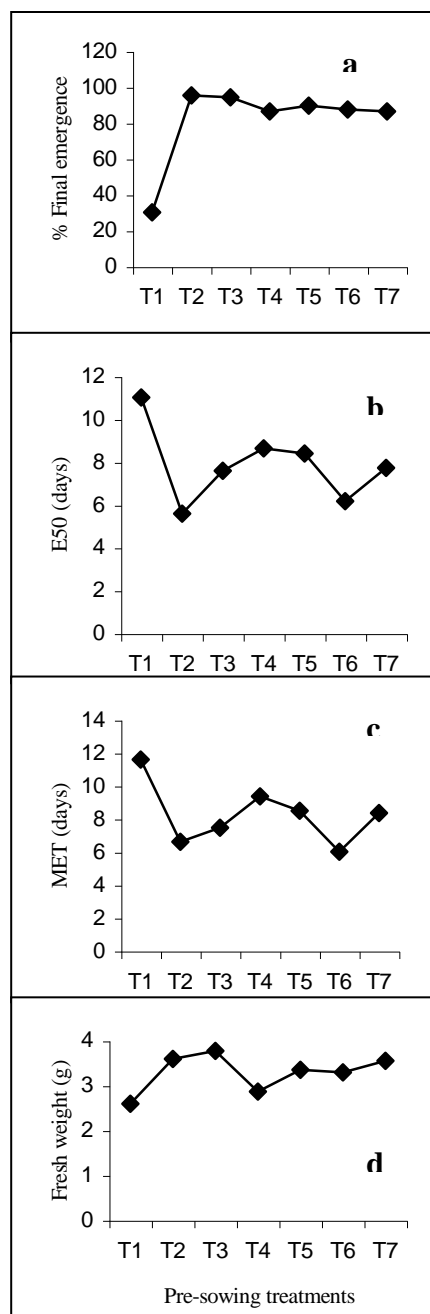
Electrolyte conductivity of seed leachates was decreased by most of pre-sowing treatments (Fig. 3). Except halopriming ( $\text{CaCl}_2$ ) all pre-sowing seed treatments cause a decrease in electrolyte leakage as compared to that in non-primed seeds even after 24 hours soaking period. Generally the electrolyte leakage was increased with increasing imbibition period including all treatments and control. After half an hour of imbibition time, only hydropriming, hardening and matricconditioning resulted in a decreased electrolyte leakage as compared to that in control and other priming treatments. However, after a longer period of imbibition ranging from 1h to 24h all the priming treatments lowered down the electrolyte leakage except halopriming with  $\text{CaCl}_2$ . Maximum decrease in electrolyte leakage was induced by hardening on all measuring periods, indicating an increase in membrane stability. Up to 6h, hydropriming and halopriming (NaCl) followed the hardening in decreasing electrolyte leakage. An increase in electrolyte leakage was observed by 50 mol  $\text{m}^{-3}$   $\text{CaCl}_2$  treatment even higher than the control at all soaking periods.

## DISCUSSION

The increase in emergence percentage in hydropriming may be due to enhanced oxygen uptake, increased  $\alpha$ -amylase activity and the efficiency of mobilizing nutrients from the cotyledons to the embryonic axis (Karthiyesan *et al.*, 1984). The findings of the present experiment are in agreement with earlier research on maize (Kulkarni and Eshanna, 1988) and in wheat (Rao and Singh, 1992) who reported that emergence percentage is increased by hydropriming under saline condition. These findings are also in agreement with the results of Gurrier and Pinel (1989), who reported that presowing seeds treatment with  $\text{CaCl}_2$  improve the emergence percentage in saline media. Early emergence (reduced  $E_{50}$ ) by various priming tools might be due to enhance pre-emergence metabolic activities during priming and resulted in triggering emergence. The findings of the present study are in line with other findings on maize (Guerrier and Pinel, 1989), pepper (Smith and Cobb, 1991), tomato (Cano *et al.*, 1991) and Pancy seeds (Yaan *et al.*, 1997) where time of emergence was increased by  $\text{CaCl}_2$  treatment.



**Fig.2.** Effect of different priming techniques (as listed in table. 1) on dry weight of seedling (a), root length (b), shoot length(c) and root shoot ratio (d) of wheat growing under saline condition



**Fig.1.** Effect of different priming techniques (as listed in table. 1) on MET (a), final emergence (b), E<sub>50</sub> (c) and fresh weight (d) of wheat seedlings growing under saline condition.

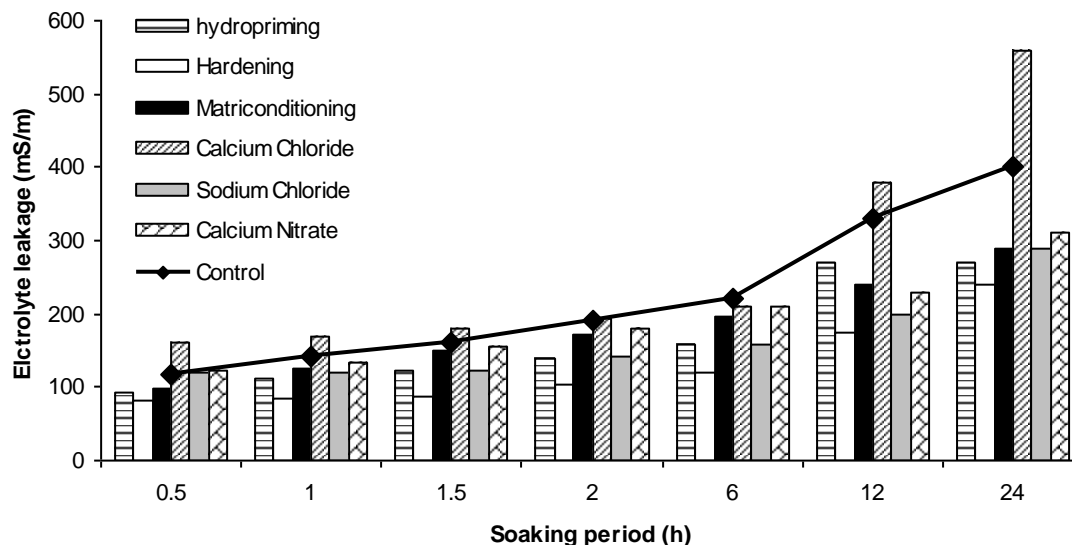


Fig. 3. Effect of various pre-sowing seed treatments on solute leakage in wheat cv. Uqab-2000.

The increased plant biomass might be due to synchronized germination and early stand establishment in treated seeds (Khan, 1992). These findings are similar with earlier research on asparagus (Evan and Pill, 1989) and on wheat (Bose and Mishra, 1992). These results are also in line with the results of Basra *et al.* (2002) who reported that shoot dry weight was increased by hydropriming treatment in wheat.

Shoot length was increased in hydroprimed and NaCl treatments as compared to hardening and non-primed seeds. An increase in root length was recorded in NaCl and hydroprimed treatments as compared to matricconditioning and control treatments, which might be the result of higher embryo cell wall extensibility. These results are in line with the work done by Argerich and Bradford (1989) for tomato seeds and Demir and Van de Venter (1999) for watermelon seeds. The increase in root to shoot ratio by priming may be due to the fact that, priming induced nuclear replication in roots tip of fresh seeds. These observations are in conformity with earlier work on pepper seeds (Stofella *et al.*, 1992).

Seed leachate electrical conductivity is considered as an effective indicator of seed germination (Waters and Blanchette, 1983). Seeds treated with 50 mol m<sup>-3</sup> CaCl<sub>2</sub> had greater EC, which shows the toxic behaviors 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). Decreased leakage of solute in hydropriming treatment than control may be because of better membrane repair during hydration. Greater membrane integrity in primed seeds are 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 Argerich and Bradford (1988) for tomato seeds and Basra *et al.* (2002) for wheat seeds.

## CONCLUSION

It shows that priming (hydropriming and NaCl) can improve the performance of low vigor seeds and induce early, synchronized and healthier crop stand. Matricconditioning and haloprimer with CaCl<sub>2</sub>, and Ca(NO<sub>3</sub>)<sub>2</sub> could invigorate the seeds under saline condition rather these reduced the vigor and increased the emergence time which means that these treatments are unable to induce tolerance against salinity.

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