

COUNTERACTION OF SALINITY STRESS ON WHEAT PLANTS BY PRE-SOWING SEED TREATMENTS

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Effects of pre-sowing seed treatments (hydropriming, matricconditioning, chilling at -19 ± 2 °C for 24 or 48 h, osmopriming and hardening) on wheat (*Triticum aestivum* cv. Auqab-2000) emergence and seedling growth under non-saline (4 dS m⁻¹) or saline (15 dS m⁻¹) conditions were studied to determine their usefulness in increasing relative salt-tolerance. Hydropriming and 24 h chilling treatments enhanced final emergence percentage under saline conditions. Mean emergence time was un-affected by all the priming treatments, however, root and shoot length, root and shoot ratio, fresh weight of seedlings were significantly increased by hydropriming followed by 24 h chilling treatments during salinity stress. Although shoot dry weight of seedling was improved with the application of these seed treatments but root dry weight of seedling was unaffected. All pre-sowing seed treatments decreased the electrolyte leakage of steep water as compared to that of non-primed seeds even after 12 h of soaking. Hydropriming induced maximum decrease in electrolyte leakage while an increase in electrolyte leakage was observed by chilling for 48 h. It is concluded that hydropriming and chilling were the most effective in alleviating the adverse effects of salinity in wheat whereas matricpriming, osmopriming and hardening were proved to be the least effective under present experimental material and conditions.

Keywords: Pre-sowing seed treatments, salinity tolerance, seedling vigor, wheat seed

Abbreviations: Mean emergence time = MET, Electrical conductivity= EC

INTRODUCTION

Studies on salt stress in germinating seeds have shown that during this stage seeds are particularly sensitive to saline environments (Bewley and Black, 1982; Mayer and Poljakoff-Mayber, 1982). 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 seedling establishment (Rogers *et al.*, 1995). The need to develop crops with higher salt tolerance has increased strongly due to increased salinity problems. Therefore, physiological treatments to improve seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades (Bradford, 1986). 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. Most of these involve a period of controlled hydration of the seeds to a point close to, but before, the emergence of the radicle after which the seeds are dried back to their initial moisture before sowing (Khan, 1992). Such

treatments include hydropriming (Nagar *et al.*, 1998), osmoconditioning with polyethylene glycol 6000 (PEG) (Dell'Aquila & Tritto, 1990), solid matrix priming (Afzal *et al.*, 2002), chilling (Equiza *et al.*, 2001), hardening (Lee and Kim, 2000) especially under stressful condition like salinity.

Priming is one of the physiological methods, which improves seed performance and provides faster and synchronized germination (Sivritepe and Dourado, 1995). A wide range of species show beneficial responses to controlled hydration treatments in aerated and diluted salt solutions (Rehman *et al.*, 1998) or in aerated polyethylene glycol (PEG) solutions (Fu *et al.*, 1988) prior to sowing. These primed seeds in general give earlier, more uniform and sometimes greater germination and seedling emergence (Bradford, 1986). 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. Idris and Aslam (1975) found that osmopriming of wheat seeds hastened germination under varying sodium chloride (NaCl) regimes. Recently, Ashraf *et al.* (2003) found that chilling and PEG were effective pre-sowing seed treatments in alleviating adverse effects of salt stress

in pearl millet cultivars during germination. Similarly, soaking in distilled water enhanced germination under saline conditions as much as any seed pretreatment used on cotton (Shannon and Francois, 1977).

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 various invigoration techniques upon the germination of wheat cv. Auqab-2000 and its 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. Before the start of experiment, seeds were surface sterilized in 1% sodium hypochlorite (NaOCl) solution for 3 min followed by rinsing with sterilized water and air-drying (Afzal *et al.*, 2002).

Seed treatments

Hydropriming. Seeds were soaked in distilled water for 12 h. 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).

Osmotic Priming. Low osmotica solution was prepared by dissolving 450 g of Polyethylene glycol (ave. mol. wt. 6,000) per 1L of distilled water. An electro-magnetic stirrer (Magnetic Stirrer/Hot Plate, Model CJJ-1, Japan) was used to dissolve the solution completely. Then, seeds were primed with aerated low water potential PEG solution (Bennet and Waters, 1987).

Solid Matrix Priming. Solid matrix priming was carried out with a solid matrix carrier "press mud" a cheap local source as compared to commercial products i.e., *Calcined Clay* and *Micro-Cell-E*. Press mud was sterilized in an autoclave for 24 h to avoid any fungal infection before conditioning.

First of all seeds were mixed with 1 kg sterilized press mud separately and 350 mL of distilled water in closed plastic containers. The containers were placed under shade at room temperature for 24 h. The partially hydrated seeds then were sieved from press mud (Afzal *et al.*, 2002).

Hardening. Seeds were soaked in tap water at 25 °C for 12 h followed by redrying to initial moisture under shade with forced air. The cycle was repeated twice (Lee *et al.*, 1998).

Chilling. A weighed quantity of wheat seeds was sealed in polythene bags and placed in refrigerator (Model National NR 245 TES, Japan) at -19 ± 2 °C for 24 h and 48 h.

Seedling Vigor Evaluation

Before the start of the experiment, salinity was developed in each plastic pot by giving the first irrigation of 15 dS m⁻¹ saline water with NaCl salt (USDA Salinity Lab. Staff, 1954) and a control group of plants supplied with water having EC 4 dS m⁻¹ was maintained. Control and treated seeds were sown at the depth of 3 cm in plastic pots having moist sand and were placed in a growth chamber (Type 8194, VINDON) 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). During this, mean emergence time (MET) was calculated according to the equation of Ellis & Roberts (1981):

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

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 emergence.

The reduction percentage of germination (RPG) or (RPE) was calculated according to the following formula (Madidi *et al.* 2004);

$$RPG = (1 - Nx / Nc) \times 100$$

"Nx" is the number of germinated seedlings under salt treatments and "Nc" is the number of germinated seedlings under control.

The data regarding the 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)

Membrane Permeability Test

After washing in distilled water, 5 g of nonprimed and treated 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, Japan) and expressed as $\mu\text{S}/\text{cm}$ (Basra *et al.*, 2002).

Statistical analysis

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

RESULTS

Pre-sowing seed treatments exerted a significant effect on emergence percentage, mean emergence time and reduction percentage of germination under both normal and saline conditions (Fig. 1). All the pre-sowing seed treatments failed to improve final emergence percentage under saline conditions but maximum emergence (61%) was recorded in hydroprimed seeds and closely followed by 24 h chilling which was statistically similar to 48 h chilling treatment under saline conditions ((Fig. 1a). Matricconditioning and hardening showed minimum emergence percentage under stress conditions.

Salinity significantly increased mean emergence time (MET) as compared to non-saline conditions (Fig. 1b). During normal conditions, all of the treatments indicated the same MET as that of control but under saline conditions, all the presowing seed treatments took less time to emergence as compared to non-primed seeds except hardening treatment. The seeds treated after chilling for 24 or 48h and hydropriming treatments took minimum time to emerge under saline conditions. The order of RPE was hydropriming > chilling24h > control > chillingfor48h > osmopriming > matirpriming > hardening (Fig. 1c)

Root and shoot lengths of wheat were highly less under saline conditions ((Fig. 2). However, maximal shoot length was recorded in seeds subjected to hydropriming and chilling treatments while remaining treatments failed to improve shoot length as compared to non-treated seeds under normal conditions (Fig. 2a). Minimum shoot length was recorded in osmoprimed and matricconditioned seeds as compared to non-primed seeds. During saline conditions, maximum shoot length was observed in hydroprimed seeds while remaining presowing seed treatments failed to improve shoot length. Matricconditioning and hardening treatments adversely affected shoot length as compared to control. Root length was highly increased by hydropriming followed by chilling for 24 h while

remaining treatments reduced root length as compared to non-primed seeds under both conditions (Fig. 2b). Under saline conditions, lowest root length was recorded in seeds due to hardening which was statistically non-significant to matricconditioning. During salinity stress, maximum root shoot ratio was recorded in seeds due to chilling for 24 h followed by hydropriming while remaining treatments were statistically non-significant to that of control (Fig. 2c).

Various pre-sowing seed treatments had a significant effect on shoot fresh and dry weights under both conditions but overall, salinity highly decreased shoot fresh and dry weights (Fig. 3). Under saline conditions, maximum shoot fresh weight was recorded in plants raised from hydroprimed seeds and 24h chilling which was statistically similar to chilling (48 h) and osmopriming treatments as compared to control whereas minimum shoot fresh weight was recorded in those plants raised from hardened seeds.

Under normal conditions, fresh weight of roots was statistically increased in plants raised from hydroprimed seeds as compared to non-primed seeds (Fig. 3b). However, these hydropriming, osmopriming and chilling treatments significantly improved root fresh weight under saline conditions as compared to non-primed seeds.

Maximum dry weight of shoot was recorded in hydroprimed and non-primed seeds while remaining seed treatments failed to improve shoot dry weight, whereas, hydropriming showed maximum shoot dry weigh followed by chilling for 24h under saline conditions (Fig. 3c). Minimum shoot dry weight was achieved due to hardening which was statistically similar to matricconditioning with press mud.

Dry weight of roots under non-saline conditions was maximally increased due to hydropriming statistically similar to chilling (24 h) as compared to non-primed seeds whereas minimum root dry weight was recorded in matricconditioned seeds, which was statistically non-significant with chilling for 48 h (Fig. 3d). Under saline conditions, all the pre-sowing seed treatments attained statistically same root dry weight as those by untreated seeds except hardening.

Different pre-sowing seed treatments have pronounced effect on solute leakage of wheat seeds (Fig. 4). The seed leachates was decreased by all pre-sowing treatments except 48 h chilling treatment. Hydropriming followed by chilling (24), hardening and osmopriming treatments had minimum electrical conductivity on all measuring periods than control.

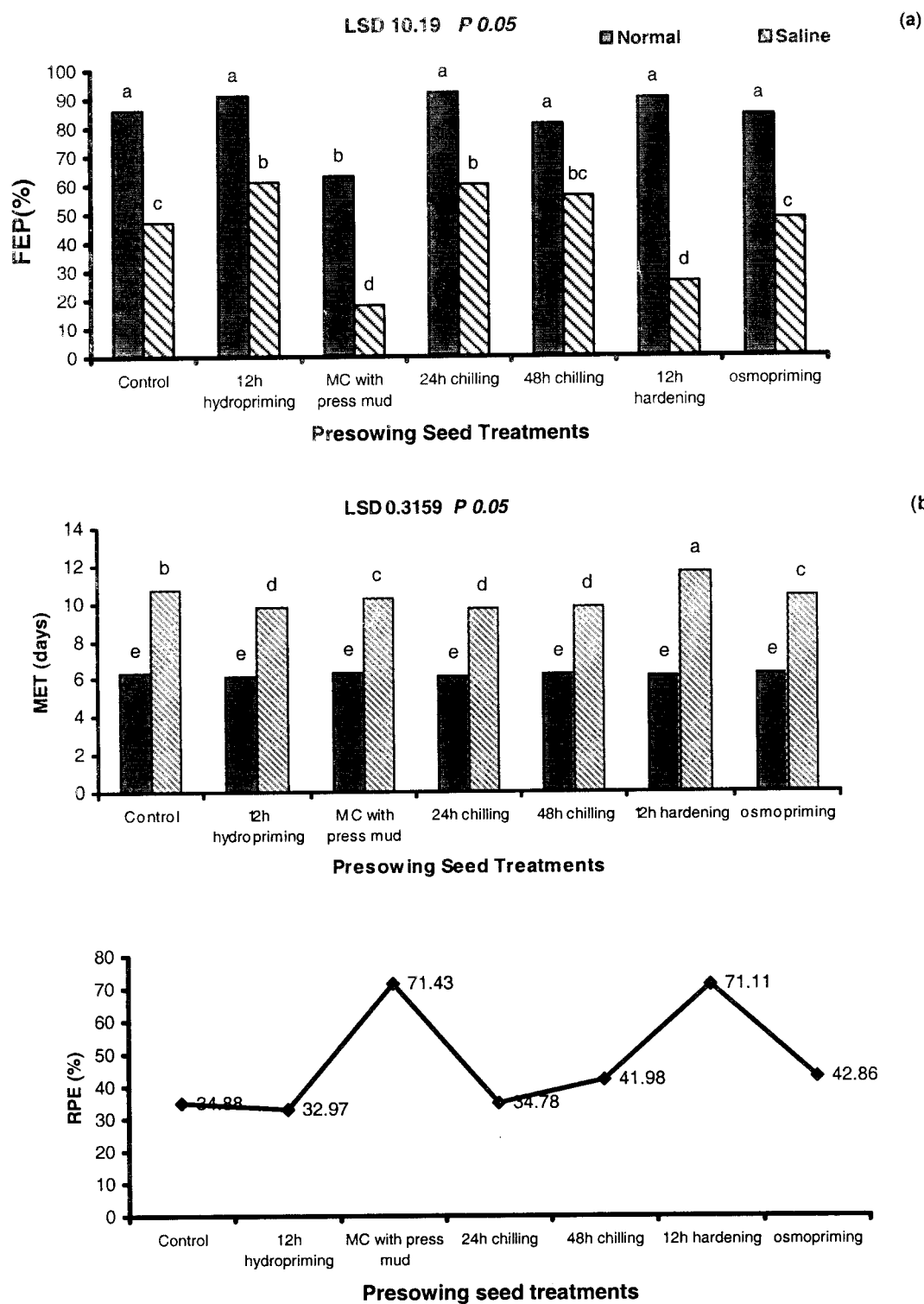


Fig.1. Effect of different priming techniques on (a) final emergence (FEP), (b) mean emergence time (MET) and (c) reduction percentage of emergence (RPE) of wheat cv. Auqab-2000 grown under normal and saline conditions.

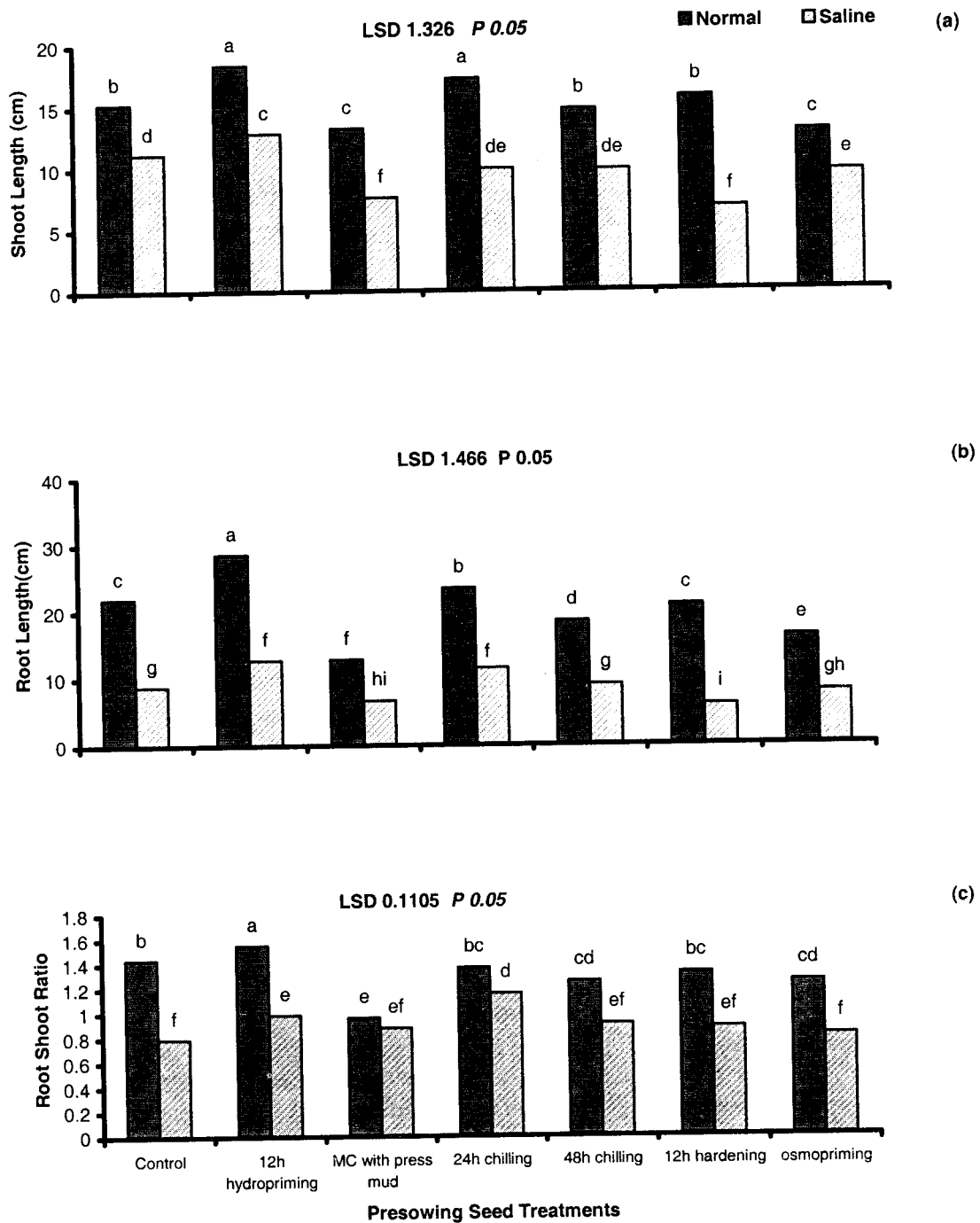


Fig. 2. Effect of different pre-sowing seed treatments on (a) root length, (b) shoot length and (c) root shoot ratio of wheat cv. Auqab-2000 grown under normal and saline conditions.

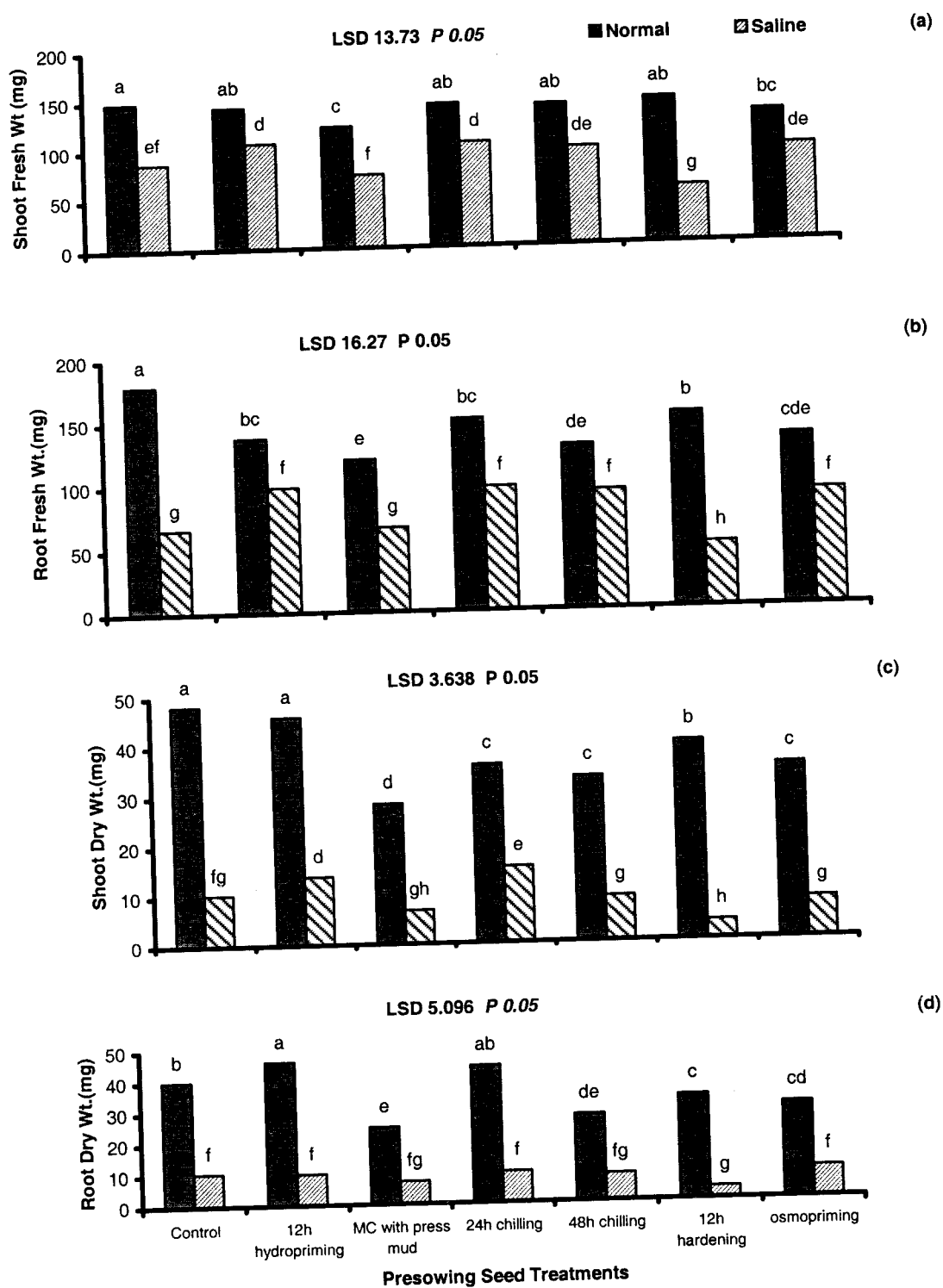


Fig. 3. Effect of different pre-sowing seed treatments on (a) shoot fresh weight, (b) root fresh weight, (c) root dry weight and (d) shoot dry weight of wheat grown under normal and saline condition.

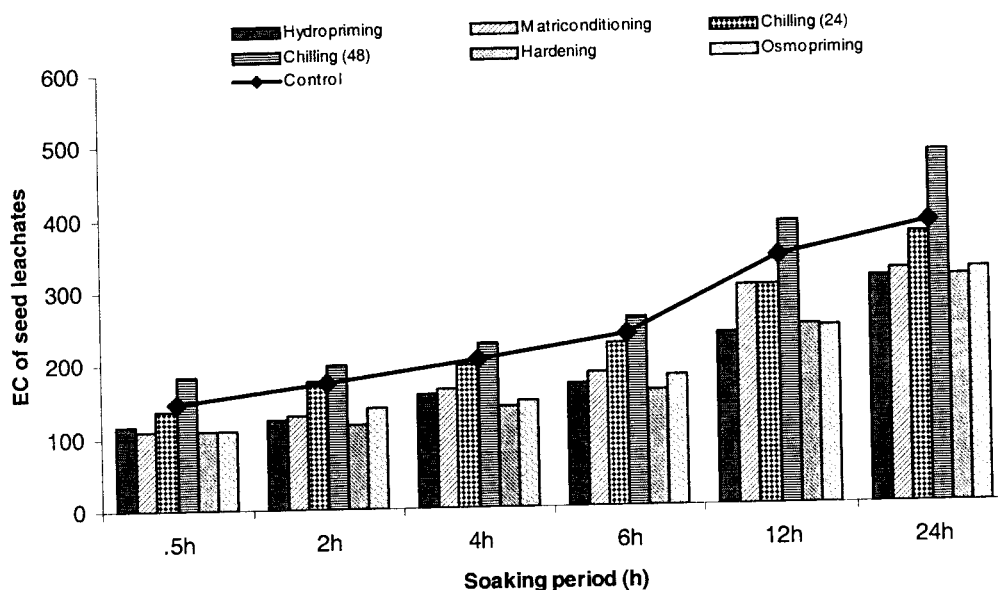


Fig. 4. Effect of different pre-sowing seed treatments on the electrical conductivity (EC) of seed leachates in wheat cv. Auqab-2000.

DISCUSSION

It is evident that the salinity significantly reduced emergence and seedling vigor of wheat (Fig. 1-3). Hydropriming was the most effective one priming agent in inducing salt tolerance in wheat at emergence stage. Among other treatments, the response of 24 h chilling found better than other treatments including control. Similar results were recorded by Al-Ansari (2003) who reported that high concentration of NaCl and KCl salts reduced final germination percentage, germination rate and increased production of abnormal seedlings in wheat. Early emergence (reduced E_{50}) by various priming tools under saline conditions was due to enhanced pre-emergence metabolic activities during priming and resulted in triggering emergence. This earlier synchronized and faster emergence is due to the enhanced synthesis of DNA, RNA and protein during priming (Bray *et al.*, 1989). The findings of the present study are in line with other findings on maize (Afzal *et al.* 2002) and wheat (Basra *et al.* 2002) where various priming agents reduce emergence time. These results are in agreement with the findings of Ashraf *et al.* (1999) who reported that water treatment had no effect on the percent seed emergence in pot experiment in saline condition. Chilling also improved the seed vigor under non-saline condition and this has been found to some extent alleviate the adverse effects of salt stress in pearl millet (Ashraf *et al.*, 2003).

The increased plant biomass might be due to synchronized emergence and early stand establishment in treated seeds (Khan, 1992). These findings are similar with earlier research on wheat (Bose and Mishra, 1992). Likewise, Basra *et al.* (2005b) reported that all priming agents were effective in decreasing the adverse effects of salt stress on wheat at emergence stage while 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_{50} than those treated with other salts or hardening or matriconditioning. Increased root and shoot lengths (Fig. 1 & 2) might be due to early emergence of hydroprimed seeds. While working on chickpea, similar results are reported by Musa *et al.* (1999). They found that hydropriming resulted in early emergence of seedlings and significantly increased plant stand and initial growth vigor. Similarly, Bose and Mishra (1992) and Basra *et al.* (2002) also reported that shoot length was increased in wheat seeds subjected to hydropriming. These results confirm the findings of Basra *et al.* (2005a) who reported that hydropriming increased root and shoot fresh weights that might be due to early emergence and seedling establishment in treated seeds.

All priming treatments were effective in decreasing electrolyte conductivity of seed leachates, which shows membrane stability. Decreased leakage of solute in primed seeds than non-primed seeds was due to better membrane repair during hydration (Burgass and Powell, 1984, Fu *et al.* 1988). Greater membrane integrity in primed seeds are reported by Rudrapal and Naukamura (1988) for eggplant and radish, Afzal *et al.* (2002) for hybrid maize. Our findings are also in accordance with the finding of Basra *et al.* (2003) who reported that hydroprimed seeds had lesser electrical conductivity of seeds leachates than control. However, an increase in electrolyte leakage was observed by chilling (48 h) at all soaking periods (Fig. 4), which was due to the loss of ability to reorganize cellular membranes rapidly and completely (McDonald, 1980). In conclusion, most of the presowing treatments were effective in inducing salt tolerance in wheat during germination and emergence, however, hydropriming and chilling (24 h) were more effective pre-sowing seed treatments for wheat cv. Auqab-2000. These treatments improved emergence and seedling vigor to the greater extent under both normal as well as saline conditions. No added advantage was obtained by osmopriming, matricconditioning and hardening treatments for alleviating salinity stress in wheat.

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