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ORIGINAL ARTICLE

THE EFFECTS OF SEED SOAKING WITH PLANT GROWTH REGULATORS ON SEEDLING VIGOR OF WHEAT UNDER SALINITY STRESS.

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Abstract— Effects of seed soaking with plant growth regulators (IAA, GA_3, kinetin or prostart) on wheat (*Triticum aestivum* cv. Auqab-2000) emergence and seedling growth under normal (4 dS/cm) and saline (15 dS/cm) conditions were studied to determine their usefulness in increasing relative salt-tolerance. During emergence test, emergence percentage and mean emergence time (MET) were significantly affected by most of priming treatments, however, root and shoot length, fresh and dry weight of seedlings were significantly increased by 25 ppm kinetin followed by 1% prostart for 2 h treatments under both normal and saline conditions. 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. Seed soaking with 25 ppm kinetin induced maximum decrease in electrolyte leakage while an increase in electrolyte leakage was observed by 25, 50 or 100 ppm IAA treatments. It is concluded that priming has reduced the severity of the effect of salinity but the amelioration was better due to 25 ppm kinetin and 1% prostart (2 h) treatments as these showed best results on seedling growth, fresh and dry weights under non-saline and saline conditions whereas seed soaking with IAA and GA_3 were not effective in inducing salt tolerance under present experimental material and conditions.

Key words: seed treatments/plant growth regulators/salinity stress/seedling vigor/wheat seed

INTRODUCTION

The overarching goal of crop establishment is to achieve rapid and uniform germination, followed by rapid and uniform seedling emergence plus autotrophy (Covell *et al.*, 1986). Seeds are particularly vulnerable to stresses encountered between sowing and seedling establishment (Carter and Chesson, 1996). Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results into low agricultural production (Maas, 1990; Garg and Gupta, 1997). 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). The effect of salinity at seedling stage of wheat range from reduction in germination percentage, fresh and dry weight of shoots and roots to the uptake of various nutrient ions.

The use of cultivars with high salt tolerance may also offer an alternative (Wannamaker and Pike, 1987). Pre-sowing seed treatments have been shown to enhance stand establishment in non-saline areas (Khan, 1992) and have potential in saline areas as well (Ashraf and Ruaf, 2001; Basra *et al.,* 2005). Prior to selecting these alternatives, it seems necessary to examine seed vigor enhancement techniques leading to better and synchronized stand establishment under stress conditions. Physiological treatments to improve seed germination and seedling emergence under various stress conditions have been intensively investigated in the past two decades (Bradford, 1986). It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones (Debez *et al.*, 2001)). However, incorporation of plant growth regulators during pre-soaking, priming and other pre-sowing treatments in many vegetables crops have improved seed performance. Typical responses to priming are faster and closer spread of times to emergence over all seedbed environments and wider temperature range of emergence, leading to better crop stands, and hence improved yield and harvest quality, especially under suboptimal and stress condition growing conditions in the field (Halmer, 2004)

Presoaking seeds with optimal concentration of phytohormones has been shown to be beneficial to growth and yield of some crop species growth under saline conditions by increasing nutrient reserves through increased physiological activities and root proliferation (Singh and Dara, 1971). Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators (Datta *et al.,* 1998). Kabar (1987) suggested that endogenous hormone level is

Abbreviations: Final emergence Percentage = FEP, Mean emergence time = MET, Electrical conductivity= EC affected by many environmental stress however, external application of appropriate growth regulators optimize physical metabolic conditions for germination. It is also possible that under high salt concentrations naturally present hormones may be suppressed and that seed soaking with plant growth regulators supplies sufficient hormones for normal growth. Thus the detrimental effects of high salts on the early growth of wheat seedlings may be reduced to some extent by treating seeds with the proper concentration of a suitable hormone (Darra *et al.*, 1973). Changes in IAA and ABA level can be important factor determining plant response to stress conditions (Naqvi, 1999). The importance of IAA was indicated with respect to seed germination process (Gregorio *et al.* 1995) but Gibberellic acid was found to be more effective in improving seed germination than either IBA or IAA (Naidu, 2001).

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 hormones 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. 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.

SEED SOAKING WITH PLANT GROWTH REGULATORS

Hormone solutions of 25, 50 and 100 ppm of IAA, GA_3 and kinetin were prepared separately. 250 gram of seeds were soaked in 500 mL of respective hormonal solution for 12 h and redried to original weight with forced air under shade (Sundstrom *et al.*, 1987). Similarly 250 g of seeds were soaked in aerated solution of 1% prostart for 1 or 2 h and then redried to original weight with forced air under shade.

COMPOSITION OF PROSTART

Ingredients	Concentration (% w/w)
Kinetin	0.009
Gibberellic Acid	0.005
Indole-3-Butyrci Acid	0.005
Magnesium (Mg)	0.2
Humic Acid	0.33
Keylate Zinc (Zn)	7.0
Nitrogen (N)	4.0

Source: Stoller Pakistan (Pvt)Ltd. 296-N, Phase 1, D.H.A., Lahore-54792, Pakistan

SEEDLING VIGOR EVALUATION

Before the start of the experiment, salinity was developed in each plastic pot by giving the first irrigation of 15 dS/cm saline water (USDA Salinity Lab. Staff, 1954). Control and treated seeds were sown at the depth of 3 cm in plastic tubs having moist sand and 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.

During this, mean emergence time (MET) was calculated according to the equation of Ellis and Roberts (1981):

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

Where n is the number of seeds, which were emerged on day D, and D is the number of days counted from the beginning of emergence.

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

RPE = (1- Nx / Nc) x 100

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

Shoot and root lengths at the time of harvest were measured with the help of scale in each replication and averaged to get mean shoot length per replication whereas fresh and dry weights of seedlings were taken with the help of an electric balance at the time of harvest for each replication (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 ºC. 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

EMERGENCE TEST

Seed soaking with plant growth regulators had a.

significant effect on emergence percentage and mean emergence time under both normal and saline conditions (Fig. 1). In normal conditions, except 25 ppm Kinetin all the treatments failed to improve emergence while under saline conditions, 25 or 50 ppm Kinetin and prostart (2h) improved emergence maximally as compared to other treatments including control (Fig. 1a)

MET was unaffected by all the treatments under normal conditions, however, most of the seed soaking treatments took less time to emerge i.e., due to lower MET under saline conditions. Lowest MET was recorded in seeds primed with 25 ppm kinetin and prostart (2 h) as compared to remaining treatments including control (Fig. 1b). However, maximum reduction percentage of emergence was recorded in seeds primed with 100 ppm GA_3 while minimum RPE was observed in kinetin and Prostart treatments (Fig. 1c).

All the seed treatments showed significant effect on shoot and root lengths and root shoot ratio under both normal and saline conditions (Fig. 2). Under both conditions, maximum root and shoot length was recorded in seedlings raised from seeds primed with 25 ppm kinetin and prostart for 2 h. However seeds treated with IAA and GA_3 failed to improve root and shoot lengths under saline conditions (Fig. 2a & b). Regarding root shoot ratio, no treatment was effective under normal conditions but under saline conditions seed soaking with 25, 50 and 100 ppm GA_3 and 25 ppm kinetin significantly increased root shoot ratio as compared to other treatments including control (Fig. 2c).

Seed treatments affected significantly fresh and dry weight of shoot and root under normal and saline conditions (Fig. 3). Maximum shoot fresh and dry weight was recorded in seeds due to 25 ppm kinetin and prostart (2 h) under normal conditions (Fig. 3a & b). Under saline conditions, shoot fresh and dry weight was increased in seeds treated with 1% prostart for 2 h and was statistically similar to seed soaking with 1% prostart (1 h) and 25 or 100 ppm Kinetin as compared to other treatments and non-primed seeds. Under normal conditions, maximum root fresh weight was observed in seeds treated with 25 ppm kinetin than other seed treatments (Fig. 3c). Under saline conditions, except 1% prostart, all the pre-sowing treatments did not improve root fresh weight while 100 ppm GA_3 significantly decreased shoot fresh weight (Fig. 3c). However, all the treatment failed to improve root dry weight under both normal and saline conditions (Fig. 3d).

ELECTRICAL CONDUCTIVITY

All pre-sowing seed treatments were effective in decreasing electrolyte conductivity of seed leachates (Fig. 4). Generally the electrolyte leakage was increased with increasing imbibition period including all treatments and control. After a longer period of imbibition ranging from 1h to 24h all the seed treatments lowered down the electrolyte leakage than control. Maximum decrease in electrolyte leakage was induced by 25 ppm Kinetin on all measuring periods. Seed soaking with Kinetin followed by Prostart was successful in decreasing electrolyte leakage. An increase in electrolyte leakage was observed by IAA at all soaking periods.

DISCUSSION

Salt stressed seeds are desiccation sensitive, which cause 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). Our results show that seedlings raised from seeds primed with various solutions of IAA, GA_3 or kinetin exhibited characteristic morphological responses at only particular concentrations of the plant growth regulators. So salt tolerance can be induced in wheat seeds by seed treatments. Salinity drastically inhibited seedling growth (Fig. 1-3). These results are strongly in accordance with Cicek and Cakirlar (2002) who reported that salinity reduced shoot length, fresh and dry weight of maize seedlings. The increase in emergence percentage in seeds primed with 25 ppm kinetin and prostart (2 h) 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 (Karthiresan *et al.*, 1984). The findings of the present studies are in line with other findings on wheat (Datta *et al.*, 1998; Sastry and Shekhawa, 2001).

Salt tolerance was increased in seeds subjected to 25 ppm kinetin and 1% prostart (2 h) as indicated by shoot and root length, shoot fresh and dry weight. Better performance of seedlings raised from seeds primed with kinetin might be due to increased invertase, α- amylase and starch synthetase in wheat (Sekhon and Singh, 1994). These results are also in accordance, to some extent, with the studies of Patel and Saxena (1994) who reported that fresh and dry weight was increased in seedlings raised from seeds treated with kinetin and GA_3 as compared to seeds treated with NAA and Ethrel. These results further relate with the studies of Angrish *et al.* (2001) who reported that amelioration of salinity was due to enhanced N status and nitrate reductase activity through pre-sowing wheat seeds with plant growth regulators like cytokinins.

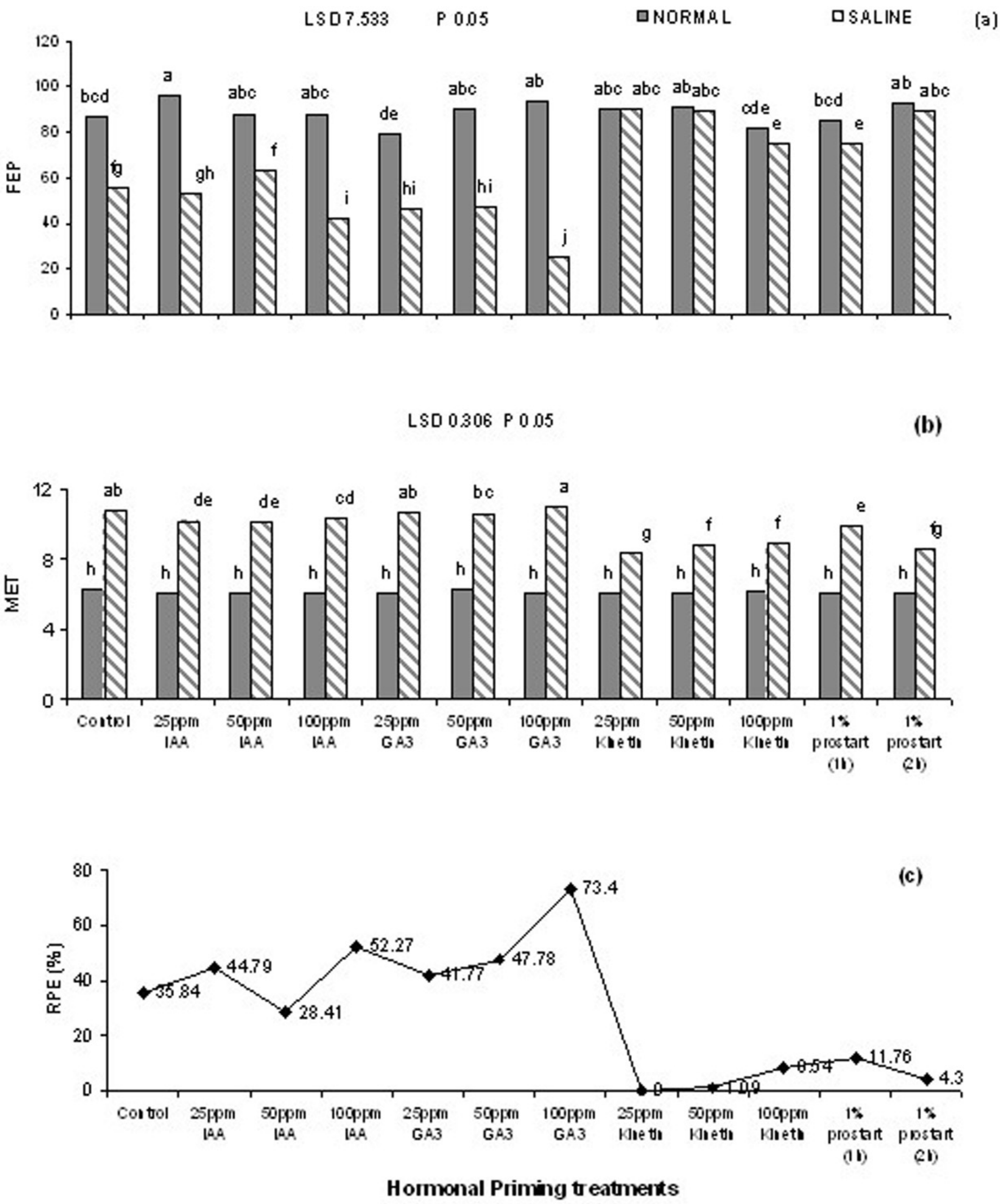

Fig.1. Effects of different pre-sowing seed treatments on final emergence (a), mean emergence time (b) and reduction percentage emergence (c) of wheat cv. Auqab-2000 growing under normal and saline conditions during emergence test.

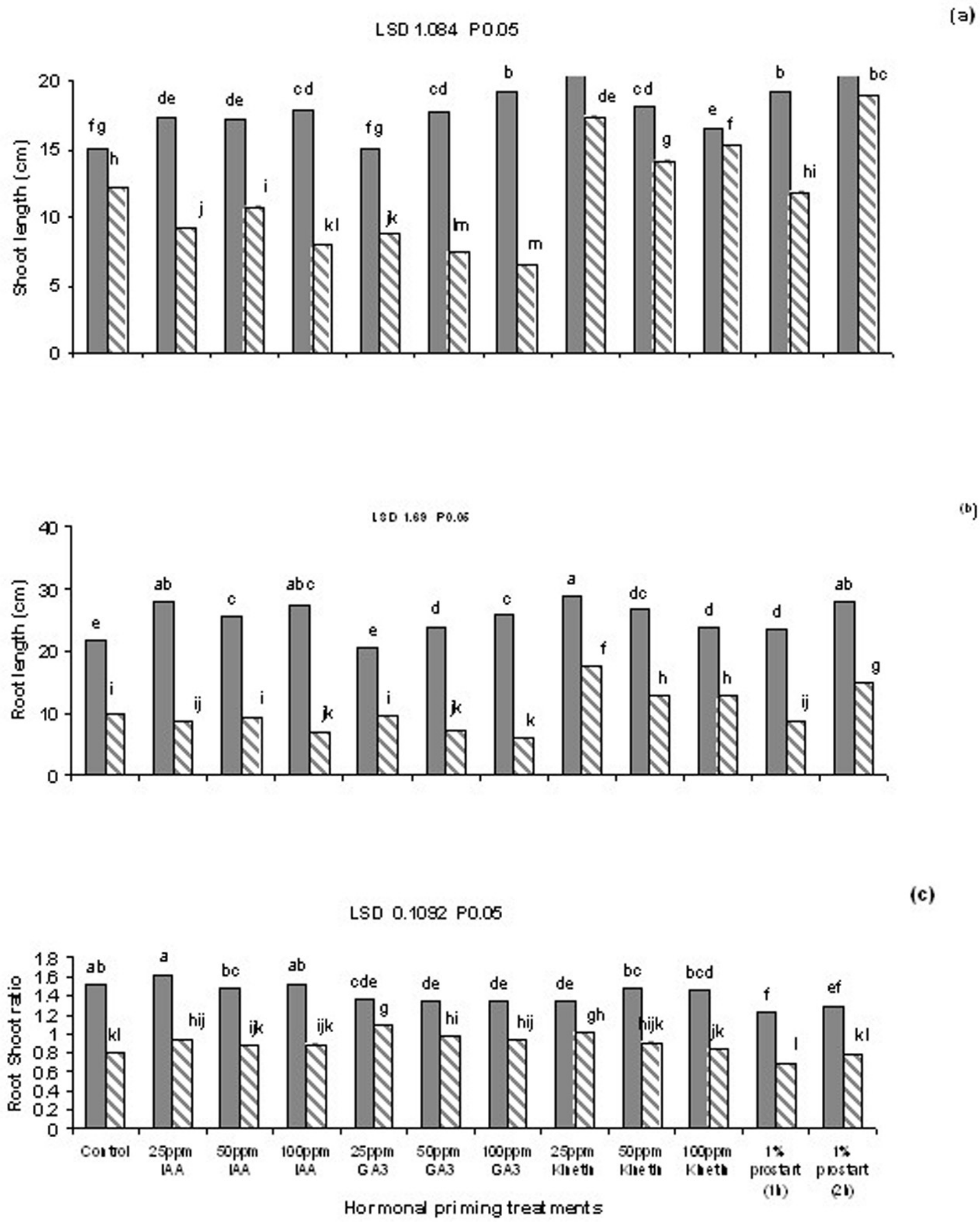

Fig.2. Effect of different pre-sowing seed treatments on root length (a), shoot length (b) and root shoot ratio (c) of wheat cv. Auqab-2000 during emergence test.

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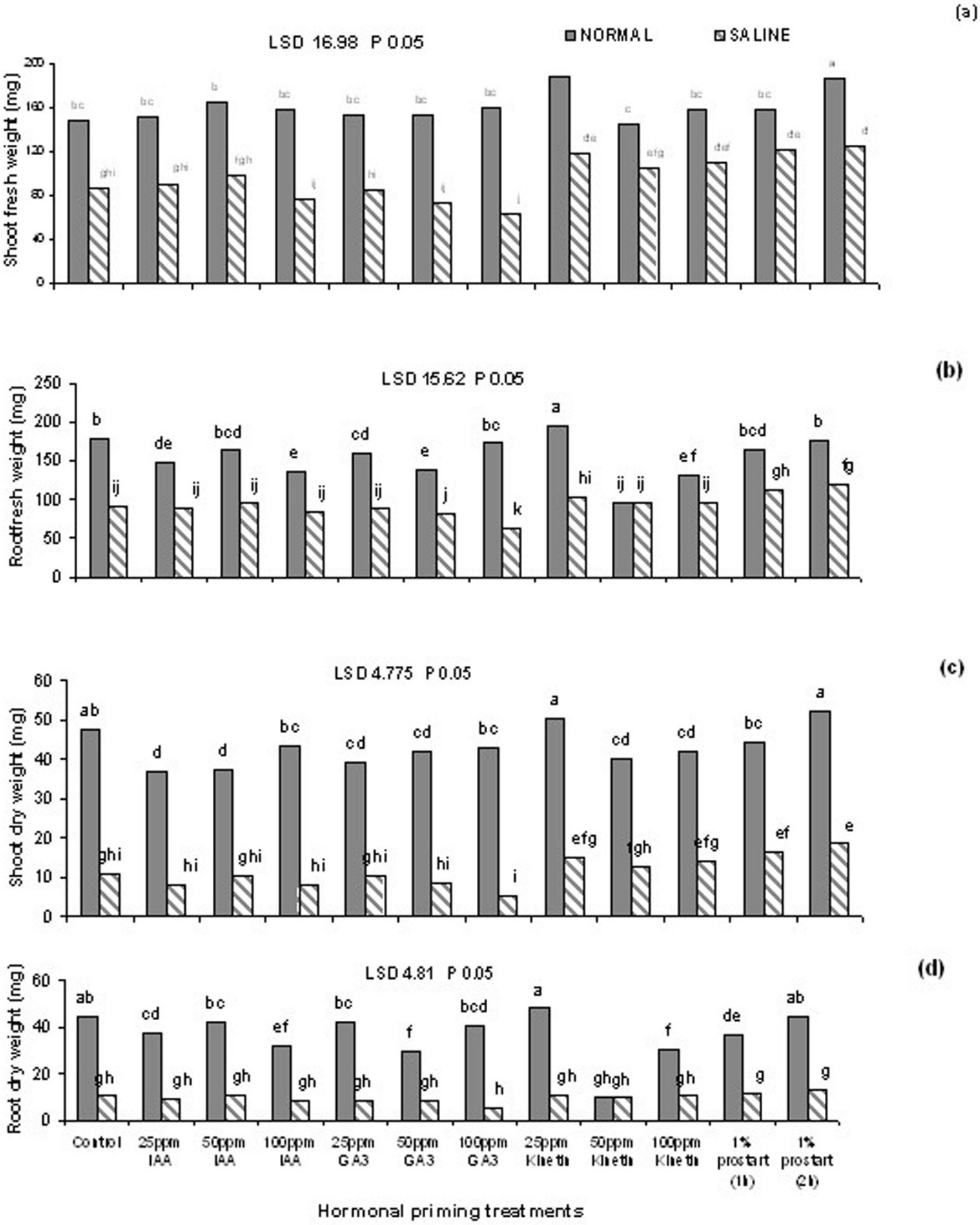

Fig.3. Effect of different pre-sowing seed treatments on Shoot fresh weight (a), root fresh weight (b), root dry weight(c) and shoot dry weight (d) of wheat cv. Auqab-2000 growing under normal and saline condition during emergence test..

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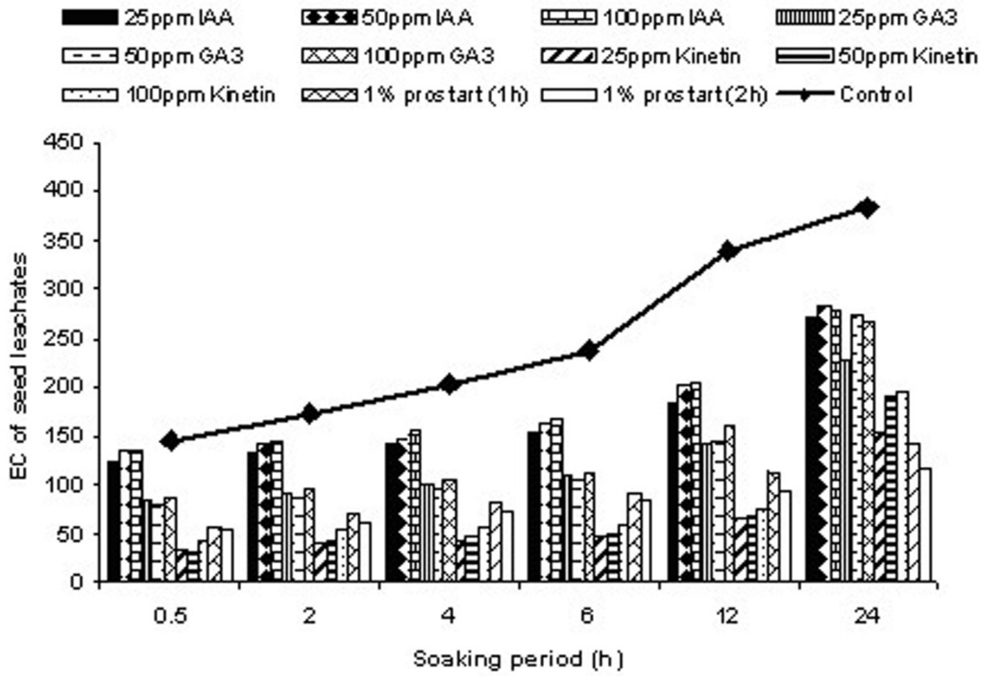

Fig.4: Effect of pre-sowing seed treatments on the EC of seed leachates in wheat cv. Auqab-2000

Seed leachate electrical conductivity is considered as an effective indicator of seed germination (Waters and Blanchette, 1983). All seed soaking treatments were effective in decreasing electrolyte conductivity of seed leachates, which shows membrane stability. An increase in electrolyte leakage was observed by 25, 50 or 100 ppm IAA at all soaking periods which was probably due to the loss of ability to reorganize cellular membranes rapidly and completely (McDonald, 1980). Decreased leakage of solute in kinetin and prostart treatments than control may be because of better membrane repair during hydration (Burgass and Powell, 1984; Fu *et al.,* 1988). 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.

Finally, it can be concluded that seed treatment with 25 ppm kinetin and 1% prostart for 2 h increase the ability of what to grow successfully under saline conditions. The facts mentioned above make it possible to recommend these seed treatments to those plants grown under conditions of soil salinity. Further research is needed to optimize the effectiveness of seed treatments with different plant growth regulators on number of cultivars of wheat.

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