EFFECT OF SEED PRIMING ON SEED VIGOUR AND SALT TOLERANCE IN HOT PEPPER

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The influence of seed priming using different priming agents (distilled water, NaCl, salicylic acid, acetyl salicylic acid, ascorbic acid, PEG-8000 and KNO_3) on seed vigour of hot pepper cv. Hot Queen was examined. Primed seeds of each treatment were cultured in Petri dishes, kept at 25±2 ºC and 16 hours photoperiod, to evaluate the effect of different priming agents on seed vigour. All priming treatments significantly improved seed performance over the control. KNO_3 primed seeds excelled over all other treatments; decreased time taken to 50% germination, increased root and shoot length, seedling fresh weight and vigour over all other priming agents. Water and NaCl, being cheapest and easily affordable by the farmers were used for further studies on salt tolerance. Seeds were primed in water (hydropriming) and NaCl (1% solution) (halopriming) and sown in pots at different salinity levels [1.17 (control), 3, 5 and 7 dS m^{-1}], along with unprimed seeds. Emergence rate (ER), final emergence percentage (FEP), reduction percentage of emergence (RPE), shoots length, number of secondary roots, seedling fresh weight and vigour were significantly improved by both priming treatments over the control; halopriming was more effective than hydropriming. Number of secondary roots was maximum in haloprimed and unprimed seeds. Seed priming treatment did not significantly affect root length, fresh and dry weight of seedlings. Results indicated that seed priming can be used for improving performance of pepper seeds and seedlings grown under saline conditions.
Keywords: Seed priming, priming agents, seed vigour, salt stress, hot pepper

INTRODUCTION

Pepper (*Capsicum annuum*) is cultivated on large area (38.4 thousand hectares) in Pakistan for its fresh fruit as well as for spice production (Anonymous, 2005-06). It is grown mainly in Sindh and southern Punjab as a summer crop. It is an excellent source of vitamin A, B, C, E and P (Citrin) besides capsaicin. Capsaicin, main constituent causing hotness, helps in digestion; prevents heart diseases and dilates blood vessels. Pepper production in Pakistan not only fulfils domestic demand but also helps in earning foreign exchange (Zia, 2006). Pakistan earned Rs.192.32 million during 2004-05 by exporting red pepper (Govt. of Pakistan, 2004-05) to Middle East, USA and other countries.

Acreage under pepper is increasing, particularly in Punjab due to a shift in production trend from cotton based farming to non-traditional crop production, which in turn is due to a decline in income from cotton crop. But the yield gap is too high; 1 to 2 tones per hectare in Pakistan as compared to 20 tones in other pepper producing countries like China. One of the main problems of this yield gap is high soil salinity in southern Punjab and Sindh; characteristics of arid climate. Pepper is reported as a salt sensitive (Haman, 2000) to moderately salt sensitive (Kanber *et al.*, 1992) crop and prefers soil having salinity less than 1920 ppm (Carter, 1994). Prolonged and non-uniform germination and emergence are the characteristic problem in pepper; duration of both is further lengthened if seeds are sown under stress conditions (Demir and Okcu, 2004) like low temperature or high salinity levels. Soil salinity is a major limiting factor in pepper production limiting crop plant establishment (Al-Karaki, 2001). Yield decreases with increase in salinity; 10% reduction at 2.2 mmhos cm^{-1}, 50% reduction at 5.1 mmhos cm^{-1} and 100% at 8.5 mmhos cm^{-1}.

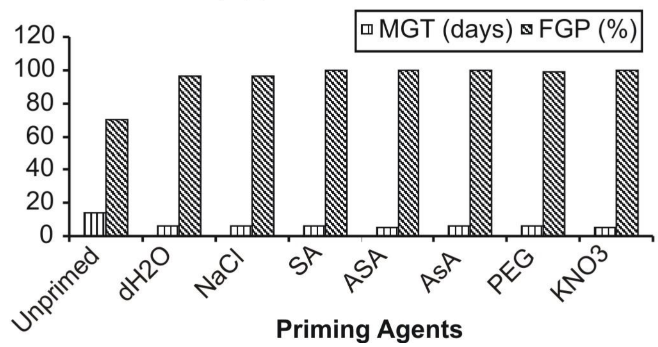
Seed invigoration techniques have been employed to achieve improvements in the rate and uniformity of germination, root growth and seed vigour (Thornton and Powell, 1992) and tolerance to environmental stresses (Heydecker, 1972) in a number of crops.

Positive effects of priming with NaCl have been reported on growth and yield of mature tomato plants when salt treatments were applied with seed sowing (Cano *et al*., 1991). Rivas *et al*. (1984) found increased germination rates in Jalapeno and Tabasco pepper seeds primed in PEG-6000 solution. Fresh seeds of different tomato cultivars primed in PEG-8000, NaCl and KNO_3 performed better than untreated seeds (Farooq *et al*., 2005). Similarly, salicylic acid (SA), acetyl salicylic acid (ASA) and ascorbic acid (AsA) are also known to provide protection against a number of abiotic stresses by triggering the potential to tolerate stress (Senaratna *et al*., 2000). Keeping in view the role of the above mentioned chemicals in improving vigour and increasing salt tolerance in various crops, experiments were designed to evaluate their impact on improvement of seed vigour and salt tolerance in pepper cultivar Hot Queen.

MATERIALS AND METHODS

This study comprised of two experiments; first experiment was to evaluate the effect of different chemicals on seed vigour whereas the second one to assess the effects of selected chemicals on seed and seedling performance under saline conditions.

Fig. I. Efeect of priming agents on germiantion behaviour of pepper cv. Hot Queen



Experiment–1

In the first experiment, seeds of pepper cultivar 'Hot Queen' were surface sterilized by dipping in sodium hypochlorite (5%) solution for five minutes and dried on filter paper. These surface sterilized seeds were primed in distilled water (dH_2O), sodium chloride (NaCl, 1%), salicylic acid (SA, 50 ppm), acetyl salicylic acid (ASA, 50 ppm), ascorbic acid (AsA, 50 ppm), PEG-8000 (PEG, -1.25 MPa) and potassium nitrate (KNO_3, 3%), in darkness for 48 hours. Unprimed seeds (UP) served as control. Seeds were given 2 to 3 washings after priming and surface dried under forced air on filter paper. Primed and unprimed seeds were cultured in Petri dishes on single sheet of Whatman No. 1 filter paper, moistened with 2.5 ml distilled water, at $25\pm2\ ^{o}C$ and kept under 16 hours photoperiod. Data were recorded daily on germination for 14 days and finally on various aspects of seed vigour viz., mean germination time, root length, shoot length and vigour index, following the rules of AOSA (1990).

Experiment–2

Seeds were primed in distilled water (hydroprimed, P_1) and NaCl (1%) solution (haloprimed, P_2) after surface sterilization with sodium hypochlorite. Unprimed (P_o), hydroprimed and haloprimed seeds were sown in pots at four different salinity levels (1.10, 3.0, 5.0 and 7 dS m^{-1}). Salinity was developed in each pot by irrigating the pots with NaCl solution of respective concentration. Pots were kept under lab conditions i.e at $25\pm2\ ^{o}C$ and 16 hours photoperiod. Data were recorded on emergence rate (ER), final emergence percentage (FEP), fresh and dry weight of seedlings and seedling vigour.

Statistical Analysis

Both experiments were arranged according to completely randomized design with four replicates. Data recorded were analyzed statistically using Fisher's analysis of variance technique and Duncan's Multiple Range Test at 5% probability level to compare the differences among treatment means (Steel *et al*., 1997).

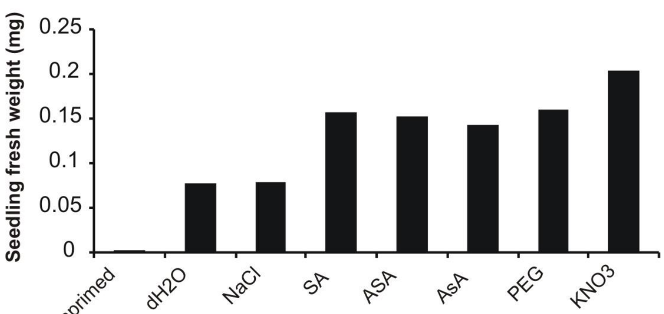
RESULTS

Seed priming decreased mean germination time over the unprimed treatment (Fig. I). Seeds primed in potassium nitrate took minimum time to germinate i.e. 5.63 days, while time for unprimed seeds was 14 days. All the priming treatments were statistically alike. Final germination percentage (FGP) of pepper seeds was significantly improved by different priming treatments over the control (Fig. I). Seeds primed in KNO_3, ascorbic acid, salicylic acid and acetyl salicylic acid showed maximum value of FGP i.e. 100% in each. FGP in hydro- and haloprimed seeds was 96%, while unprimed seeds exhibited 70% germination.

All priming treatments increased root length in primed seeds over unprimed ones (Fig. III); being maximum in KNO_3 (2.87 cm) treated seeds and minimum in seeds primed with NaCl (1.66 cm). Root length was at par in seeds primed in KNO_3 (2.87 cm), acetyl salicylic acid (2.50 cm), PEG-8000 (2.37 cm) and salicylic acid (2.50 cm). Root length of unprimed seeds was mean root length almost negligible (0.5 cm) as most of the seeds did not germinate during the experimental period (14 days).

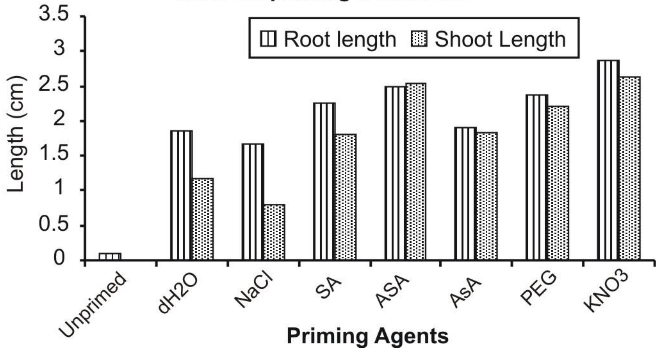
Priming also improve the shoot length; results followed the same pattern for shoot length as in the case of root length is maximum in seeds primed in potassium nitrate (2.63 cm) followed by those primed in acetylic salicylic acid (2.53 cm) and PEG-8000 (2.21 cm), statistically at par with each other (Fig. III). Seeds primed in ascorbic acid and salicylic acid also performed well over the unprimed ones.

Fig. II. Effect of priming on fresh weight of pepper seedlings



Seedling fresh weight was significantly affected by different priming agents. Seeds primed in KNO_3 produced seedling having maximum fresh weight i.e. 0.2038 mg per seedling (Fig. II). Seedlings raised from seeds primed in PEG-8000, salicylic acid and acetyl salicylic acid had fresh weight, statistically at par with each other. Seeds primed in distilled water and NaCl produced seedlings with statistically similar fresh weight.

Fig. III. Root-shoot length of pepper seedlings in response to different priming treatments

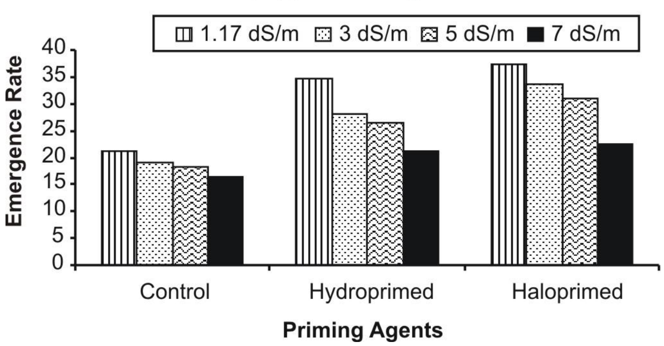


Seeds primed in KNO_3 showed maximum vigour (5.54) followed by acetyl salicylic acid (4.98) and PEG-8000 (4.46), while unprimed seeds were least vigorous (Fig. IV). Vigour index of seeds primed with ascorbic acid, distilled water and NaCl was 3.73, 2.92 and 2.41, respectively; all were statistically at par with each other.

On the basis of results of first experiment, two priming agents (water and NaCl) were selected for

Fig. IV. Effect of priming agents on vigour of pepper seedlings

Fig. V. Effect of priming treatments on emergence rate of pepper seedlings



salinity levels showed statistically significant difference in emergence rate (Fig. V). Seeds primed in NaCl (haloprimed) and distilled water (hydroprimed) exhibited maximum emergence rate in normal growing medium (control) i.e. 37.25 and 34.65, respectively; statistically at par each other, while significantly higher than unprimed seeds (21.30). Emergence rate of haloprimed seeds was maximum at all salinity levels than both hydro- and halo-primed seeds. Emergence rate of haloprimed seeds at 7 dS m^{-1} was statistically at par with emergence rate of unprimed seeds at 1.1 dS m^{-1} (control).

Haloprimed showed more final emergence percentage than unprimed as well as hydroprimed seeds at all salinity levels (Fig. VI). Final emergence percentage of haloprimed seeds at 7 dS m^{-1} (90%) was at par with FEP of unprimed (83.30 %) and hydroprimed (82.50 %) seeds at 3 dS m^{-1}, indicating the supremacy of haloprimed seeds over hydroprimed and unprimed seeds. Performance of both hydroprimed and unprimed seeds was statistically similar at different salinity levels.

Fig. IX. Effect of priming treatments on shoot length of pepper seedlings

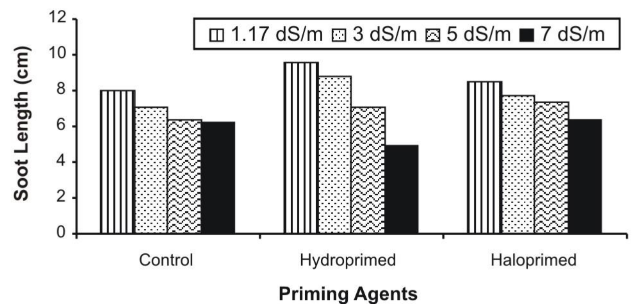
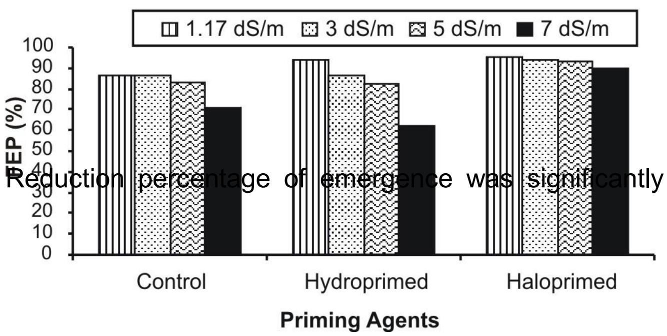
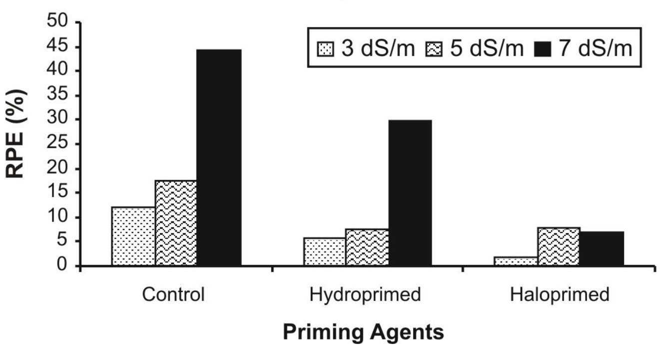


Fig. VI. Effect of priming treatments on final emergence %age



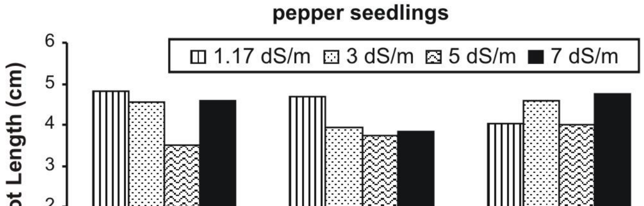
Reduction percentage of emergence was significantly decreased for haloprimed seeds at 3 dS m^{-1}, followed by that for hydroprimed seeds at same salinity level (Fig. VII). RPE value of each treatment increased gradually from lower salinity level (3 dS m^{-1}) to higher salinity level (7 dS m^{-1}) in each priming treatment. Haloprimed seeds had minimum reduction in emergence percentage at all salinity levels, followed by hydroprimed seeds. Unprimed seeds exhibited maximum reduction in emergence percentage.

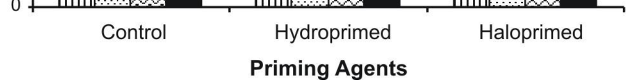
Fig. VII. Effect of priming agents on reduction percentage of emergence



There was no statistical difference among different treatments for root length (Fig. VIII); although root size varied from 3.52 cm to 4.82 cm. Maximum root length was observed in seedlings raised from unprimed seeds at 1.17 dS m^{-1} salinity level followed by 4.76 cm in seedlings raised from haloprimed seeds at 7 dS m^{-1} and hydroprimed seeds (4.70 cm) at 1.17 dS m^{-1}. Minimum root length i.e. 3.52 cm was recorded in unprimed seeds at 5 dS m^{-1}. Priming treatments significantly affected shoot length at different salinity

Fig. VIII. Effect of priming treatments on root length of pepper seedlings





levels (Fig. IX). Shoot size was maximum (9.55 cm) in hydroprimed seedlings at 1.17 dS m^{-1} followed by hydroprimed seeds at 3 dS m^{-1} (8.77 cm). Shoot length of haloprimed seeds at 3 dS m^{-1} (7.72 cm) and 5 dS m^{-1} (7.37 cm) was statistically at par with shoot length of unprimed seeds at 1.17 dS m^{-1}.

Priming significantly affected number of secondary roots (Fig. X). Maximum number of roots per plant (23.25) was observed in seedlings raised from haloprimed seeds at 3 dS m^{-1}, statistically at par with those from hydroprimed seeds (22.65) at 1.17 dS m^{-1}. But, at higher salinity levels performance of both primed and unprimed seeds was variable i.e. at 7 dS m^{-1} unprimed seeds i.e. 19.12 and 14.07 roots per plant, respectively.

Statistically significant results were obtained for fresh weight per plant (Fig. XI). Unprimed seeds at all salinity levels had more fresh weight. Fresh weight of unprimed seeds at 1.17, 3, 5 and 7 dS m^{-1} was at par with that of hydroprimed seeds at 3 and 5 dS m^{-1} and haloprimed seeds at 3 dS m^{-1}. Dry weight was significantly affected different treatments at different salinity levels (Fig. XII). Haloprimed seeds at 3 dS m^{-1} (382 mg) and hydroprimed seeds at 5 dS m^{-1} (380 mg) produced seedlings with dry weights statistically at par with each other. While at higher salinity level (7 dS m^{-1}) performance of different treatments was inconsistent i.e. all treatments behaved alike.

Haloprimed seeds induced maximum seedling vigour at all salinity levels (Fig. XIII). Hydroprimed seeds also improved vigour over the unprimed seeds. Vigour induced by haloprimed seeds at 7 dS m^{-1} was statistically similar to vigour of hydroprimed and unprimed seeds at 1.17 dS m^{-1} i.e. 936, 1248.4 and 1086.55, respectively. Unprimed seeds at 5 and 7 dS m^{-1}, while hydroprimed seeds at 7 dS m^{-} dS m^{-1} produced plants having minimum vigour.

DISCUSSION

Fig. X. Effect of priming treatments on number of secondary roots

Fig. XI. Effect of priming treatments on fresh weight of pepper seedlings

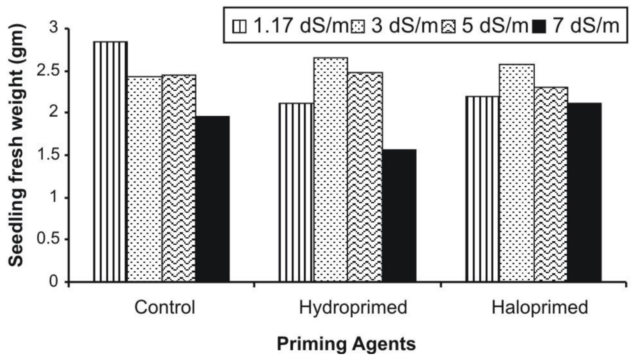
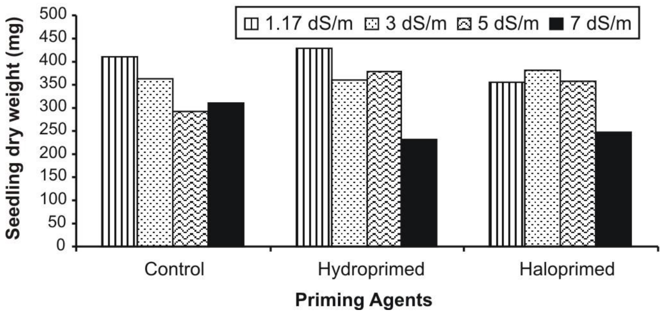


Fig. XII. Effect of priming treatments on dry weight of pepper seedlings



All priming treatments significantly improved MGT, FGP, root length, shoot length, seedling fresh weight and vigour in pepper cv. Hot Queen. Priming with KNO_3, SAS, PEG-8000, significantly improved MGT, root length, shoot length and vigour in pepper cv. Hot Queen (Fig. 1, 2, 3) over other treatments. Earlier and synchronized germination was observed in primed seeds compared with that of unprimed as depicted by lower MGT. These findings are in accordance with the findings of Demir and Van Deventer (1999) who observed improved shoots length in water melon seeds due to seed priming. KNO_3 primed Jalapeno pepper seeds germinated significantly earlier than unprimed seeds as stated by Rivas *et al*. (1984), support our observations. Negligible reduction in emergence percentage of haloprimed seeds over hydroprimed and unprimed seeds indicated that improved vigour of these seeds, which helped the seeds to perform well over the range of salinity levels. Hydropriming was also effective in increasing emergence percentage as reduction percentage of emergence of hydroprimed seeds was significantly lesser than unprimed seeds at all salinity levels (Fig. VI). Significant improvement in root and shoot length may be attributed to earlier germination induced by primed over unprimed seeds (Farooq *et al*., 2005), which resulted in vigorous seedlings with more root and shoot length than the seedlings from unprimed seeds. Our results confirm the findings of Stofella *et al*. (1992), who reported that priming of the pepper seeds significantly improved root length.

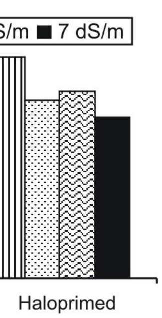
Positive effects of priming with SA and ASA on seed vigour are also confirmed by the observation of Afzal *et al*. (2005). Our findings of maximum vigour in seeds primed with KNO_3 are supported by the findings of Farooq *et al*. (2005). Argerich and Bradford (1989) also observed that priming of tomato seeds with KH_2PO_4+KNO_3 improved vigour over the untreated seeds. Improvement in performance of primed seeds over the unprimed seeds might be due to the repair mechanisms that occur during seed imbibition (Bray, 1995).

Seed germinability is reduced under salt stressed conditions due to physiological injuries under such conditions and these stressed seeds are desiccation sensitive (Wiebe and Tiesses, 1979). Increased emergence of primed seeds over unprimed seeds is in accordance with the findings of Afzal *et al*. (2005). Priming in distilled water enhanced emergence rate in asparagus seeds (de Carvalho Bittencourt *et al*., 2005) support our results of improved performance of hydroprimed seeds over the unprimed ones. Faster emergence rate after priming may be due to increased rate of cell division in the root tips of seedlings from primed seeds as reported in wheat (Bose and Mishra, 1992) and tomato (Farooq *et al*., 2005). Salinity inhibited seedling growth but seed priming induced salt tolerance in pepper seedlings as observed by Afzal *et al*. (2005) in wheat seedlings.

Seedling emergence percentage was markedly affected by salinity. Increased emergence percentage of halo- and hydro-primed seeds under salt stress may be due to reduced uptake of NaCl by the germinating seeds or due to increased uptake of oxygen, and the efficiency of mobilizing nutrients from cotyledons to the embryonic axis (Kathiresan *et al*., 1984).

Seedling growth was suppressed under saline conditions, which is strongly in accordance with Cicek and Cakirlar (2002) who reported that salinity reduced shoot length, fresh and dry weight of maize seedlings.

seedling vigour



higher salinity levels at par with shoot length of unprimed seeds at lowest salinity level (control). However, results signify the supremacy of halopriming over hydropriming. Moreover, under low (3 dS m^{-1}) and moderate (5 dS m^{-1}) saline conditions haloprimed seeds performed comparatively better than hydro- and unprimed seeds. Increased salt tolerance in haloprimed seeds over the unprimed seeds was also observed by Sivritepe *et al.* (2003) who reported increase in seedling dry weight in NaCl primed melons seeds under saline conditions as compared to the unprimed seeds. This enhanced tolerance of seedlings raised from haloprimed seeds may be due to induced resistance by NaCl, as reported in literature for salicylic acid that induce resistance to pathogens (Shirasu *et al*., 1997). Results suggest that priming could ameliorate the destructive effect of salinity on growth.

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