Response of maize (Zea mays L.) to seed priming with NaCl and salinity stress

J. Bakht1, M. Shafi2, Y. Jamal2 and H. Sher3

1 Institute of Biotechnology and Genetic Engineering. KPK Agricultural University Peshawar. Pakistan
2 Department of Agronomy. KPK Agricultural University Peshawar. Pakistan
3 Department of Botany and Microbiology. King Saud University. Riyadh. Kingdom of Saudi Arabia

Abstract

Salinity is one of the biggest limitants for agriculture in semi-arid areas of the world. An experiment was conducted to study the effect of seed priming with 6 dS m⁻¹ NaCl on growth and yield responses of two maize cultivars (Azam and Sarhad yellow) exposed to three levels of salinity (0, 6, 8 dS m⁻¹). Statistical analysis of the data revealed that cultivars, seed priming with saline water (6 dS m⁻¹) and subsequent exposure to salinity stress had a significant (p < 0.05) effect on germination, days to emergence, plant height, shoot fresh weight, shoot dry weight, leaf area, shoot Na⁺, K⁺, proline, abscisic acid contents and yield variables. The results suggested that increasing salinity level had a negative effect on the growth and development of both cultivars under study. Analysis of the data also revealed that maize cv Azam performed better than cv. Sarhad yellow when exposed to different levels of salinity. Priming of cv Azam with NaCl resulted in earlier emergence (2 days) and germination rate (31.92%), plant height (12%), shoot proline (950.33 µg g⁻¹ fresh weight) and ABA levels (0.983 and 1.203 µg g⁻¹ fresh weight) and yield (36%) than the non-primed treatment. These results suggest that priming of maize seeds with NaCl before sowing induces physiological and biochemical changes, which resulted in better performance when subsequently exposed to different levels of salinity.

Additional key words: plant growth; proline; salinity tolerance; sodium.

Introduction

Salinity is a major factor limiting crop productivity in the semi-arid areas of the world (Flowers, 2004). High concentration of salts in soils can significantly decrease the value and productivity of agricultural land. Over 800 million hectares of land throughout the world are salt affected, either by salinity (397 million ha) or...
the associated condition of sodicity (434 million ha) (FAO, 2005). Irrigated land is particularly at risk with approximately one-third being significantly affected by salinity. Despite its small area, irrigated land is estimated to produce one-third of the world food (Munn, 2002) so salinization of this resource is particularly critical. Various investigations related to the effects of salt on plant growth have been been undertaken (Shaheen et al., 2005; Mehmood et al., 2009; Akram et al., 2010; Achakzai et al., 2010). Among the most common effects of soil salinity is growth inhibition by Na⁺ and Cl⁻. In some plants, specially woody perennials (such as citrus and grapevines), Na⁺ is retained in the roots and stems and only Cl⁻ accumulate in the shoot which is most damaging to the plants (Mager et al., 2002; Tester and Davenport, 2003). However, for many plants (such as graminaceous crops including maize), Na⁺ is the primary cause of ion-specific damage. Na⁺ specific damage is associated with the accumulation of Na⁺ in the leaf tissues and results in necrosis of older leaves, starting at the tips and the margins, and working back through the leaf. Growth and yield reduction occur as a result of the shortening of life time of individual leaves, thus reducing net productivity and crop yield (Munns, 2005).

Salinity is widely perceived as having adverse effects on farmers and their resource base in Pakistan. It is estimated that approximately 6.8 million hectares of land in Pakistan are affected by salt (Qureshi et al., 2003). Thus production of salinity-tolerant crop plants is very important for agriculture in Pakistan. Unfortunately, the time required for development of more tolerant crops by means of traditional breeding programmes and more recently by biotechnological methods is long. Therefore, it will be very interesting to search new methods which may allow plants to tolerate the effect of salt stress. Binzel et al. (1985) reported the successful adaptation of cell lines to salinity which indicate a genetic potential for salt tolerance present in cells of plants from which these lines were derived and that exposure of the cells to salinity triggers the expression of this information. The induction of salt adaptation has also been observed in whole plants. Thus, Amzallag and Lerner (1990) showed two responses in Sorghum bicolor when pre-treated with NaCl. An adaptive response (i) in which the NaCl tolerance is increased, and a resistance response (ii) in which the plant copes with salinity without modification of its tolerance level. Strogonov (1964) reported that salt tolerance of plants can be increased by priming of seeds with saline water before sowing. The author observed that plants from such primed seeds adapted more easily and quickly to saline condition in the soil than the non-primed seeds. Similarly, Cano et al. (1991) reported that fruit yield was greater in tomato plants derived from NaCl primed seeds than from non-primed ones when grown under saline conditions. The effect of NaCl priming is greater when salt treatment was applied at germination than when applied at seedling stage (Sedghi et al., 2010). The effect of seed priming on growth and yield have rarely been studied in many plants including maize (Foti et al., 2008). The present study investigate the effect of seed priming with NaCl on the growth and yield of two maize cultivars when subsequently exposed to salinity.

Material and methods

The data presented in this paper was obtained from the experiments conducted at KPK Agricultural University Peshawar, Pakistan. The experiment was conducted in completely randomized design (CRD) with six replications having three pots per replication. Before sowing, seeds were imbibed in dark at 25°C in 6 dS m⁻¹ saline water (primed) or distilled water (unprimed) for 24 hours. After priming in saline water, seeds were washed with distilled water for 5 min and then sown in cemented pots (50 × 40 cm) lined with polyethylene sheet containing 20 kg of well dried and meshed (2 mm) soil collected from the surface (0-15 cm) of a normal field. After priming, 15 seeds of each cultivar were sown directly in each pot 15 days after sowing. The N, P and K were applied in the ratio of 100:50:50 kg ha⁻¹ at the time of sowing. The pots were thinned out to four in each pot 15 days after sowing. The N, P and K were applied in the ratio of 100:50:50 kg ha⁻¹ at the time of sowing. The pots were irrigated according to the crop requirement. Data was recorded on germination (%), days to emergence, plant height, shoot fresh weight and dry weight, leaf area, shoot Na⁺, K⁺, proline, ABA concentration and grain yield. All the variables except germination (%) and days to emergence were taken at maturity while endogenous shoot proline and ABA concentrations were measured 4, 6 and 8 weeks after seed priming and salinity stress. Plant height was obtained by measuring three plants in each treatment with a meter rod and then the mean value for each treatment was calculated. Plant harvested from each treatment was immediately weighed for shoot
fresh weight. For recording shoot dry weight, samples were dried at 80°C for 48 hours and again weighed after complete drying for shoot dry weight. Leaf samples were collected and stored in freezer until used for Na⁺ and K⁺ determination. Shoot Na⁺ and K⁺ concentrations were measured by flame photometer (Jenway, PFP 7). Shoot proline content was determined according to the method of Bates et al. (1973) while ABA was measured according to the procedures described by Parry and Horgan (1991).

**Statistical analysis**

The data collected was analyzed using general linear models procedure of SAS (1990). Means were compared between treatments by Duncan Multiple Range Test (DMRT) at the 0.05 confidence level (Steel and Torrie, 1997).

**Results**

**Germination and seedling emergence**

Data regarding germination percentage and day to emergence are presented in Figure 1a and b. Salinity level significantly \((p < 0.05)\) affected germination and days to emergence in both cultivars while the effect of seed priming was non significant. Increasing salinity concentration significantly \((p < 0.05)\) reduced germination of both cultivars. The data suggested that maximum reduction of 60.61% in germination due to salinity exposure was noted in Sarhad yellow when compared with Azam (29.99%). The results also indicated that seed priming did not significantly \((p > 0.05)\) increase germination by 4.49% and 5.58% in Sarhad yellow and 4% and 5% in Azam when exposed to 6 and 8 \(\text{dS m}^{-1}\) salinity respectively when compared with non-primed seeds. Seedlings from Azam emerged 2 days earlier than Sarhad yellow when exposed to different salinity levels. Seed priming had a profound effect on Sarhad yellow with respect to emergence time when exposed to increasing salinity levels compared with Azam. Days to emergence were decreased by 11.64% and 31.92% in Sarhad yellow and 6.3% and 14.99% in Azam when primed seeds were exposed to 6 and 8 \(\text{dS m}^{-1}\) respectively (Fig. 1b).

**Plant growth**

Salinity levels and seed priming had a significant \((p < 0.5)\) effect on plant height, shoot fresh weight, shoot dry weight and leaf area of both cultivars (Fig. 2a, b, c and d). Increasing salinity levels had significantly \((p < 0.05)\) reduced plant height. This reduction was more important (75.76%) in Sarhad yellow when compared with Azam (66.12%) exposed to different salinity levels. Similarly, the effect of seed priming was more profound on Azam than Sarhad yellow at high salinity level (8 \(\text{dS m}^{-1}\)). Seed priming had significantly \((p < 0.05)\) increased plant height of Azam (12%) when exposed to 8 \(\text{dS m}^{-1}\) salinity and compared with non-primed treatment. Increasing salinity levels negatively affected shoot fresh weight. Reduction in shoot fresh due to salinity exposure was less important (65.89%) in Azam when compared with Sarhad yellow (87.89%) and their
respective controls. Seed priming had a non-significant (p > 0.05) effect on shoot fresh weight of Sarhad yellow while the same treatment had significantly (p < 0.05) influenced shoot fresh weight at higher salinity level (8 dS m⁻¹). Shoot fresh weight was 11.36% higher in plants derived from primed seeds of Azam when exposed to 8 dS m⁻¹ salinity when compared with the non-primed treatment. Similarly, shoot dry weight was significantly (p < 0.05) affected by salinity levels and seed priming. Increasing salinity levels had significantly (p < 0.05) reduced shoot dry weight of both cultivars under study (Fig. 2c). This reduction was more profound in Sarhad yellow (75.92%) than Azam (67.81%) compared with their respective controls. Seed priming had significantly (p < 0.05) increased (12.82%) shoot dry weight of the primed Azam at higher salinity level (8 dS m⁻¹) when compared with the non-primed treatment of the same cultivar. On the other hand, seed priming treatment non-significantly (p > 0.05) increased shoot dry weight of Sarhad yellow exposed to increasing salinity levels when compared with non-primed treatment (Fig. 2c). Similar pattern of changes were also observed on leaf area due to salinity and seed priming (Fig. 2d).

Sodium and potassium concentrations

Na⁺ and K⁺ concentrations of shoot were significantly (p < 0.05) affected by salinity levels and seed priming treatments (Fig. 3a and b). Increasing salinity levels increased the accumulation of Na⁺ and decreased K⁺ content of the shoot (Fig. 3a). Sarhad yellow accumulated more Na⁺ and less K⁺ than Azam when exposed to different salinity levels. The effect of seed priming on Na⁺ and K⁺ accumulation in the shoot was also more profound in Azam than Sarhad yellow. Primed seeds of Azam accumulated 10% less Na⁺ and 12.5% more K⁺ at 8 dS m⁻¹ when compared with non-primed treatment of the same cultivar. While on the other hand, Sarhad yellow accumulated non-significantly (p > 0.05) 2.55% less Na⁺ and 4.3% more K⁺ when compared with their non-primed treatment (Fig. 3a and b).

Biochemical variables

Different salinity levels, seed priming and their interactions had a significant (p < 0.05) effect on shoot
ABA levels 4 weeks after treatments. Maximum shoot ABA contents of 0.900 µg g⁻¹ fresh weight were noted in treatments grown with Azam, whereas minimum (0.863 µg g⁻¹ fresh weight) was observed in Sarhad yellow. Shoot ABA contents were increased by 16.51 and 17.25% with increasing salinity stress, i.e. 6 and 8 dS m⁻¹ respectively compared with treatments grown at control (un-primed). Primed treatment enhanced shoot ABA by 7.30 and 7.43% at 6 and 8 dS m⁻¹ salinity respectively (Fig. 4a). Maximum (0.983 µg g⁻¹ fresh weight) shoot ABA contents were noted in Azam when primed with saline water at high salinity level (8 dS m⁻¹), whereas minimum was noted in Sarhad yellow without seed priming at control (Fig. 4 a).

**Figure 3.** Effect of salinity and seed priming on shoot Na⁺ and K⁺ content of two maize cultivars. a) Na⁺ level; b) K⁺ level. Bar shows ±1DMTR at p < 0.05.

**Figure 4.** Effect of salinity and seed priming on shoot ABA contents of two maize cultivars. a, b and c: 4, 6 and 8 weeks respectively after seed priming and salinity stress. Bar shows ±1DMRT at p < 0.05.
Shoot ABA contents were significantly \((p < 0.05)\) affected by different salinity levels, seed priming and their interactions 6 weeks after treatment (Fig. 4b). Azam produced maximum shoot ABA contents \((ca. 1.058 \mu g \text{ g}^{-1} \text{ fresh weight})\) while minimum was observed in Sarhad yellow. Shoot ABA contents increased by 14.25 and 15.84\% with increasing salinity stress, \(i.e.\) 6 and 8 \(dS \text{ m}^{-1}\) respectively compared with treatments grown at control (un-primed). Shoot ABA contents increased by 6.18 and 10.63\% at 6 and 8 \(dS \text{ m}^{-1}\) salinity levels respectively when compared with their untreated plants (un-primed; Fig. 4b). Similarly, maximum \((1.203 \mu g \text{ g}^{-1} \text{ fresh weight})\) shoot ABA contents were noted in Azam when primed with saline water at high salinity level (Fig. 4b). Different salinity levels, seed priming and their interactions had a significant \((p < 0.05)\) effect on shoot ABA levels 8 weeks after treatments (Fig. 4c). Similar pattern of changes were noted in shoot ABA concentration as described for week 4 and 6 treatments.

Different salinity levels, seed priming and their interactions had a significant \((p < 0.05)\) effect on shoot proline levels 4, 6 and 8 weeks after treatments (Fig. 5a, b and c). Azam produced maximum shoot proline contents \((ca. 635.16 \mu g \text{ g}^{-1} \text{ fresh weight})\). Minimum shoot proline contents of 602.78 \(\mu g \text{ g}^{-1} \text{ fresh weight}\) were observed in Sarhad yellow. Shoot proline contents increased by 60.37 and 70\% with increasing salinity stress, \(i.e.\) 6 and 8 \(dS \text{ m}^{-1}\) respectively compared with control shoot derived from un-primed seeds. Shoot proline contents increased by 7.72 and 8.70\% at 6 and 8 \(dS \text{ m}^{-1}\) salinity levels respectively when compared with their untreated plants (un-primed; Fig. 5a). Maximum \((950.33 \mu g \text{ g}^{-1} \text{ fresh weight})\) shoot proline contents were noted in Azam when primed with saline water at high salinity level \((8 \text{ dS m}^{-1})\), whereas minimum in treatments sown with Sarhad yellow with-out seed priming at control (Fig. 5a).

Proline contents of plants exposed to 6 and 8 weeks post treatments were affected in a similar fashion as observed for 4 weeks post-treatments (Fig. 5b and c).

**Grain yield**

Grain yield of both cultivars was significantly \((p < 0.05)\) affected by increasing levels of salinity.
The effect of salinity was more profound on Sarhad yellow than Azam. Salinity reduced grain yield of Azam by 231% when compared with control. The impact of salinity (8 dS m–1) on grain yield was highest in Sarhad yellow when compared with other cultivars and the plants did not reach reproductive stage when exposed to 8 dS m–1 and all the plants were completely barren. Seed priming had significantly ($p < 0.05$) increased grain yield of Azam (8 and 36% at 6 and 8 dS m–1) when compared with the non-primed treatment of the same cultivar at high salinity levels.

Discussion

The present study investigated the effect of salinity and seed priming on the growth and yield of two maize cultivars. The data showed that salinity had significantly affected germination in both maize cultivars under study. The present study also demonstrated that germination recorded from primed seeds were non-significantly different from non-primed treatments of both cultivars when exposed to different salinity levels. Similar results are also reported by Ashraf and Rauf (2001). Emergence time in both cultivars was significantly increased due to different salinity exposure. Cicek and Cakirlar (2002) reported that salinity negatively affect coleoptile and radical development which resulted in delay in emergence time. Maize seedling from primed seeds with NaCl emerged earlier than non-primed seeds as has been shown with other priming treatments, such as polyethylene glycol (PEG), inorganic salts or even ABA (Ashraf and Rauf, 2001).

In the present study salt stress caused a significant reduction in plant height, shoot fresh and dry weight and leaf area. Reduction in plant growth as result of salt stress has been reported in several other species (Cicek and Cakirlar, 2002; Ashraf and Harris, 2004; Bakht et al., 2006; Munns et al., 2006; Ashraf et al., 2008; Mehmoood et al., 2009; Ashraf, 2009; Achakzai et al., 2010; Akram et al., 2010). Salinity has both osmotic and specific ionic effects on plant growth (Dioniso-Sese and Tobita, 2000). Addition of salt keeps changing the osmotic potential of soil solution. This fluctuation in osmotic potential adversely influences the physiological availability of water (Suarez and Lebron, 1993) as a result of which plants could not maintain turgor and thus reduce their growth and development. Similarly, toxic ion accumulation (i.e. Na$^+$ and Cl$^-$) in the aerial part of the plant also negatively affect plant metabolism (Grieve and Fujiyama, 1987). It has also been reported that salinity suppresses the uptake of essential nutrients like P and K (Ali et al., 2006; Nasim et al., 2008), which could adversely affect plant growth. Induction of salinity stress at different phenophases hampered photosynthetic activities of the plant.

The results indicate that seed priming significantly improved plant growth of maize when exposed to different salinity levels. This effect was more profound in Azam than in Sarhad yellow. The higher growth rate found in the primed treatment of Azam indicates that there is an early adoptive response in this cultivar to salt. Passam and Kakouriatis (1994) concluded that positive effect of seed priming on plant growth may be due to the earlier emergence of the seedlings. It has been reported that when the stress level used for priming is below a stress threshold, the plants are unable to increase their salt tolerance levels (Amzallag, 1999; Balibrea et al., 1999). The lack of positive response to priming by Sarhad yellow could be due to the fact that salt level used for priming was not sufficient to induce salt adaptation in this genotype and needs further investigation. A positive correlation of Na$^+$ ion accumulation in the shoot with increasing levels of salinity was observed in the present study. Concentration of Na$^+$ increased in both cultivars but this accumulation was more in Sarhad yellow than Azam when exposed to different salinity levels. The opposite trend was observed for K$^+$ accumulation when both cultivars were exposed to salinity. Most crops suffer when exposed to salt stress and showed decline in growth. The ne-
gative effect of salinity was suggested as to be the result of water loss, ion intoxication or imbalance or combination of all these factors (Kurth et al., 1986). Physiological mechanisms conferring exclusion that operate at the cellular and whole plant level have been described elsewhere in the literature and with particular reference to selectivity for K⁺ over Na⁺ (Jeschke and Hartung, 2000; Tester and Davenport, 2003). There is a strong correlation between salt exclusion and salt tolerance in many species (Munns and James, 2003), i.e. for rice (Lee et al., 2003; Zhu et al., 2004) and wheat (Poustini and Siosemardeh, 2004). Similarly, Cuartero et al. (1992) reported that high uptake of Na⁺ inhibit K⁺ accumulation. Tolerant species accumulate less Na⁺ and more K⁺ than the sensitive species (Tipirdamaz and Cakirlar, 1989). Negative correlation for Na⁺ and positive correlation for K⁺ accumulation was observed due to seed priming. This effect was more noticeable in Azam than in Sarhad yellow. Similar results were also reported by Ashraf and Rauf (2001).

The present study also indicated that seed priming with saline water and subsequent exposure to salinity had significantly affected shoot ABA and proline concentration. ABA and proline content was increased with increasing salinity stress. Similarly, seed priming with saline water increased both ABA and proline content of the shoot when compared with unprimed control. These results agree with those reported by Mutlu and Buzcuk (2007) and Cha-um and Kirdmanee (2008; 2009).

Several hypotheses have been put forward to elucidate the role of ABA in the protection of plants to salinity stress (Davies et al., 2000; Jia and Zhang, 2000). Similarly, many plant species exhibit an increase in endogenous ABA concentration in response to environmental stress including salinity, water and low temperature (Setter and Ammgam, 2001). Exogenous application of proline caused a decrease in shoot Na⁺ and Cl⁻ accumulation and thereby enhanced growth under saline conditions in cultured barley embryos (Lone et al., 1987). But Garcia et al. (1997) concluded that exogenously applied proline exacerbated the deleterious effects of salt on rice. Despite of all these questions, proline concentration in many salt tolerant plants has been found to be higher than that in salt sensitive ones. Petrusa and Wincov (1998) reported that salt tolerant alfalfa plants rapidly doubled their proline content in the roots, whereas in salt sensitive plants the increase was slow. Similar results were also reported by Fougere et al. (1991) in alfalfa. Relatively, salt tolerant plants of Brassica juncea showed higher degree of osmotic adjustment in the leaves and a higher critical point concentration of NaCl, at which the endogenous level of free proline rose sharply, than did the relatively salt sensitive genotypes (Jain et al., 1991). Higher proline accumulation was found in salt tolerant B. juncea plants with better growth than the control (Kirti et al., 1991).

Salinity had a negative effect on yield of both cultivars. Sarhad yellow even did not reach reproductive stage when exposed to high salinity levels (8 dS m⁻¹). Seeds of Azam primed with NaCl before sowing resulted in more grain yield when compared with non-primed treatment of the same cultivar. These results agree with those reported by Cayuela et al. (2001) and Mehmood et al. (2009). Amzallag (1999) reported that different sorghum genotypes exposed to similar adaptation-inducting conditions showed different degrees of adaptation, which indicate a genetic component in the capacity of adaptation. In view of many earlier studies grain yield is the net outcome of the synthesis of assimilates by leaves and translocation of these assimilates to the developing seed where they are utilized to synthesize other organic compounds such as starch, proteins, oil etc. (Eagli, 1999). Thus, these processes differ to a varying extent in different genotypes of wheat under saline environment. It has also been reported that wheat and other grain crops under water deficit during grain filling substantially affects grain weight (Rahman and Yoshida, 1985) due to early plant senescence, cessation of grain filling (Hossian et al., 1990) and shortening of the grain filling period (Royo et al., 2000).

As final conclusions, in this paper, research data from a pot experiment is presented to study the effect of salinity and seed priming treatments on the physiological, biochemical and yield variables of two maize cultivars. Both cultivars showed differential response to salinity and seed priming treatments. Reduction in physiological and yield traits was more profound in Sarhad yellow than Azam when exposed to different levels of salinity. Seed priming with saline water and subsequent exposure to salinity had increased shoot K⁺, ABA and proline content which in-turn had improved the salinity tolerance of maize cultivars under study. The improvement in salinity tolerance was more pronounced in Azam than Sarhad yellow. From these results it can be concluded that Sarhad yellow is more sensitive to salinity than Azam and may not be suitable for cultivation in saline soil. Similarly, seed priming
with saline water can be a beneficial treatment for plant to be sown in saline environment. Due to the presence of genetic component in the adaptive mechanism, it will be interesting to extend this study to other osmotic and plant species showing different growth and yield responses to salinity.

References


Tipladmaaz R.H., Cakirlar H., 1989. Effects of salinity on ion (Na+, K+, Cl-) contents in two different wheat cultivars. Hacettepe Fen ve Muhendislik Bilimleri Dergisi 10, 7-20. [In Turkish].