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Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts: Growth and ion transport at early growth stages

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Abstract

The study was conducted to determine whether salt tolerance could be induced in maize at germination stage by soaking of seeds for 8 h in distilled water or in 200 meq·L^{-1} of NaCl, KCl, $CaCl_2 \cdot 2H_2O$. Both primed and un-primed seeds were subjected for 14 days to 0, 100 or 200 mol·m^{-3} NaCl under controlled conditions. Although all priming agents were effective in alleviating adverse effects of salt stress on maize at germination stage, $CaCl_2 \cdot 2H_2O$ proved to be more effective since the seeds primed with this salt had significantly higher final germination, rate of germination and fresh and dry weights of plumules and radicles than those treated with other salts or distilled water. Concentration of Na^+, K^+ and Ca^{2+} increased significantly in all parts of germinating seeds of maize seeds primed with NaCl, KCl, or $CaCl_2 \cdot 2H_2O$, respectively. In addition, seeds primed with $CaCl_2 \cdot 2H_2O$ were the highest in Cl^- accumulation in all parts of the germinating seeds, followed by seeds treated with NaCl and KCl. Most of the Ca^{2+} was retained in seeds and mesocotyl, because of which, transport of this ion to plumules and radicles was low.

Introduction

High concentrations of soluble salts in soil cause reduction in the germination percentage and delay germination of seeds of many plant species (Greenway and Munns 1980, Khan 1993). However, pre-soaking or priming seed of a number of crops has been shown to improve germination, seedling establishment and, in some cases, to enhance crop yield (Ahmad *et al.* 1998, Harris *et al.* 1999). For instance, pre-sowing treatments with different salts stimulated the germination process and germination started earlier in treated than in untreated seed of wheat in varying NaCl levels (Idris and Aslam 1975). Similarly, Khan (1993) reported that osmo-conditioning with seed hydration treatments, seed hardening and moisturizing on vermiculite improved the performance of sweet corn seed. However, Henckel and Strogonov (1961) advocated that salinity tolerance of plants could be improved by treating seeds with solutions of different salts before sowing inasmuch as plants from such primed seed showed more adaptation to saline conditions than the untreated seeds.

It is known that salinity interacts with plant nutrients. For instance, it has been widely reported that Ca^{2+} and K^+ are decreased in plants under saline conditions (Kent and Läuchli 1985, Lin and Kao 1995, Al-Harbi 1995). There are reports that with increasing the Ca^{2+} and K^+ concentrations in seeds of wheat (Chaudhri and Wiebe 1968, Idris and Aslam 1975) and wimmera ryegrass (Marcar 1986), increased germination resulted in NaCl solutions. In contrast, Gurrier and Pinel (1989) reported that treatments with calcium or potassium salts did not give significantly greater final germination than treatment with distilled water.

The objective to undertake the present study was, firstly, to assess up to what extent pre-sowing seed treatments with different inorganic salts (KCl, $CaCl_2{\cdot}2H_2O$, NaCl) improve the salt tolerance of maize, a crop known for its high salt sensitivity (Ashraf 1994, Fortmeier and Schubert 1995). Secondly, it was determined how far these salts affect the mobilization of different nutrients to different parts of germinated seeds. For instance, it was reported that mesocotyl of corn seedlings retained Na^+ thereby translocating less Na^+ to the plumule (Johanson and Cheeseman 1983). Similarly, a marked decrease in K level in wheat embryo was observed under salt stress (Petruzzelli *et al.* 1991).

Materials and Methods

Seeds of maize cultivar C-17 were obtained from the Maize and Millet Research Station, Yousafwala, District Sahiwal, Pakistan. Before the start of experiments, seeds were surface sterilized in 10 percent sodium hypochlorite solution for 10 minutes, then rinsed with sterilized distilled water and air-dried. The sterilized seeds were soaked for 8 h in 200 $meq{\cdot}L^{-1}$ solutions of NaCl, KCl, or $CaCl_2{\cdot}2H_2O$ or in distilled water. After pre-sowing treatment, all seed samples were rinsed with distilled water and dried in an oven at 40 °C. They were used in experiments within 24 h of soaking treatment.

Salt treated seeds, together with untreated seeds, were germinated at 32±4 °C in 11 cm glass Petri dishes on a layer of filter paper, moistened with 5 ml of distilled water or 100 or 200 $mol{\cdot}m^{-3}$ NaCl. Twenty five seeds were used per Petri dish and each treatment replicated three times.

The experiment was laid out in a completely randomized design with three replicates. The data for seed germination was recorded daily up to day 14 after the start of the experiment. A seed was considered germinated when the radicle and plumule had visibly emerged from the seed. The data for rate of germination was calculated from the untranformed data as days to 50 % germination. Fresh weights of plumules and radicles were recorded and after washing with distilled water they were dried in an oven at 65 °C up to their constant weight.

Determination of Na^+, K^+, Ca^{2+} and Cl^- in different parts of germinating seeds

The dried plant material was ground in a grinder to pass through 2 mm sieve. The dried material (0.1 g for plumule and germinating seed and 0.05 g for radicle and mesocotyl) was digested with the mixture of sulfuric acid – hydrogen peroxide according to the method of Allen *et al.* (1986). Na^+, K^+ and Ca^{2+} were determined with a flame photometer (Sherwood model 410, Japan). For Cl^- determination, 0.05-0.1 g dry ground material of the earlier mentioned plant parts was heated at 80 °C for 4 h, and Cl^- content in the extracts was determined with a chloride meter (Central Kagaku Corp., Japan).

Statistical analysis

The data for all the parameters were statistically analysed using the analysis of variance technique (Steel and Torrie 1980). The mean values were compared with the least significance difference test (LSD).

Results

Saline growth medium had adverse effect on total germination percentage, and fresh and dry weights of plumules and radicles but not on rate of germination. This shows that salt had adverse effect on all the growth attributes except rate of germination (days to 50 % germination). Priming agents also differed significantly in alleviating the adverse effects of salt on different growth attributes. Seeds treated with $CaCl_2{\cdot}2H_2O$ had higher final percent germination than those treated with other salts solutions in both non-saline and saline conditions, but they did not differ from the untreated seeds in final germination (Fig. 1a). However, seeds treated with solutions of different salts did not show a consistent pattern of increase or decrease in rate of germination in either NaCl treatment (Fig. 1b).

Seeds treated with 200 $meq{\cdot}L^{-1}$ of $CaCl_2{\cdot}2H_2O$ had slightly higher fresh weights of plumules and radicles than those treated with other salt solutions

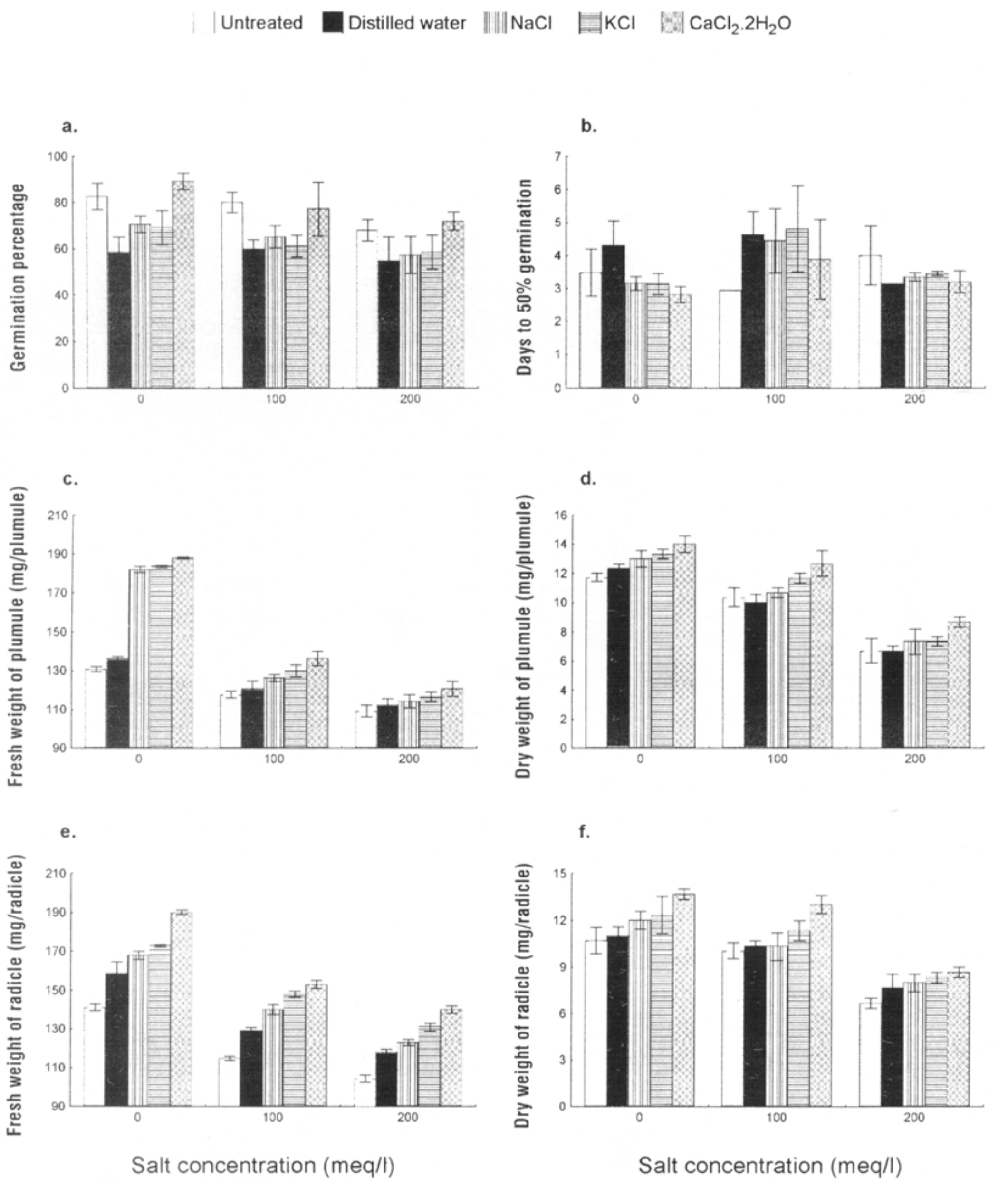

Fig.1: Germination percentage, days to 50% germination, fresh and dry weights of plumule and radicle of maize seedlings primed for 8h with different salts of chloride or distilled water.

or distilled water. In addition, KCl followed by NaCl was also effective in alleviating the adverse effect of NaCl on fresh weights of plumules and radicles compared with the untreated seeds or seeds treated with distilled water (Fig. 1 c & e).

Dry weights of plumules and radicles were also slightly higher for the seeds pre-soaked with $CaCl_2{\cdot}2H_2O$ in both saline and non-saline conditions. But the dry weights of plumules and radicles of seeds primed with KCl or NaCl were much different than those of untreated seeds or seeds treated with distilled water (Fig. 1 d & f).

Analysis of Na^+ of different parts of the seedlings showed that seeds primed with NaCl had signifi-

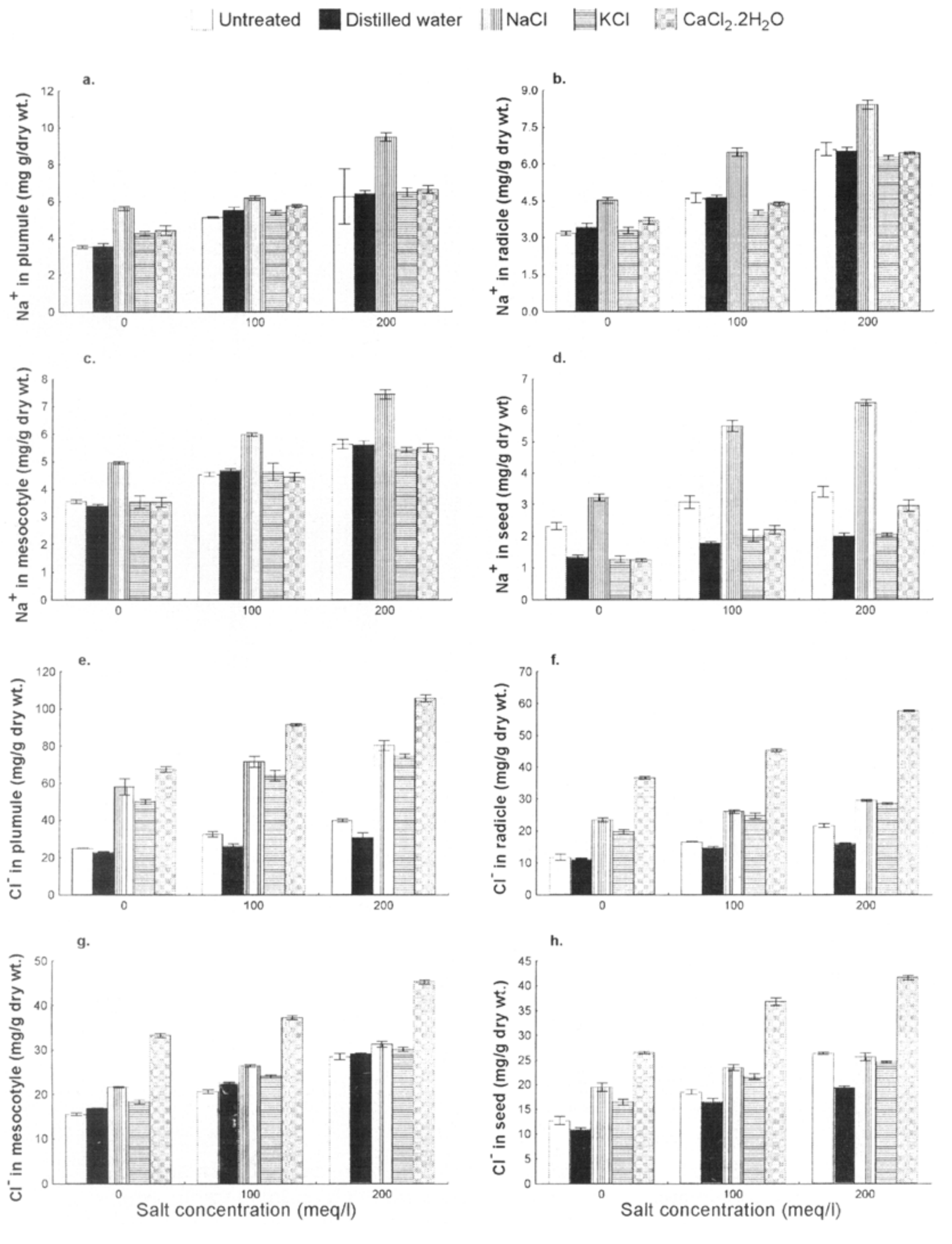

Fig.2: Sodium and Chloride content in different parts of maize seedlings primed for 8h with different salts of chloride or distilled water.

cantly higher Na^+ in plumule, radicle, mesocotyl and seed than those pre-soaked with other salt solutions or distilled water. The other priming agents did not prove in effecting any change in Na^+ concentration of different parts of the seedling (Fig. 2 a-d).

Chloride accumulation in different seedling parts was maximum in seeds treated with $CaCl_2 \cdot 2H_2O$ followed by NaCl and KCl. The lower Cl^- concentration was found in all seedling parts of the untreated seeds or treated with distilled water (Fig. 2 e-h) as compared to that in seeds treated with differ-

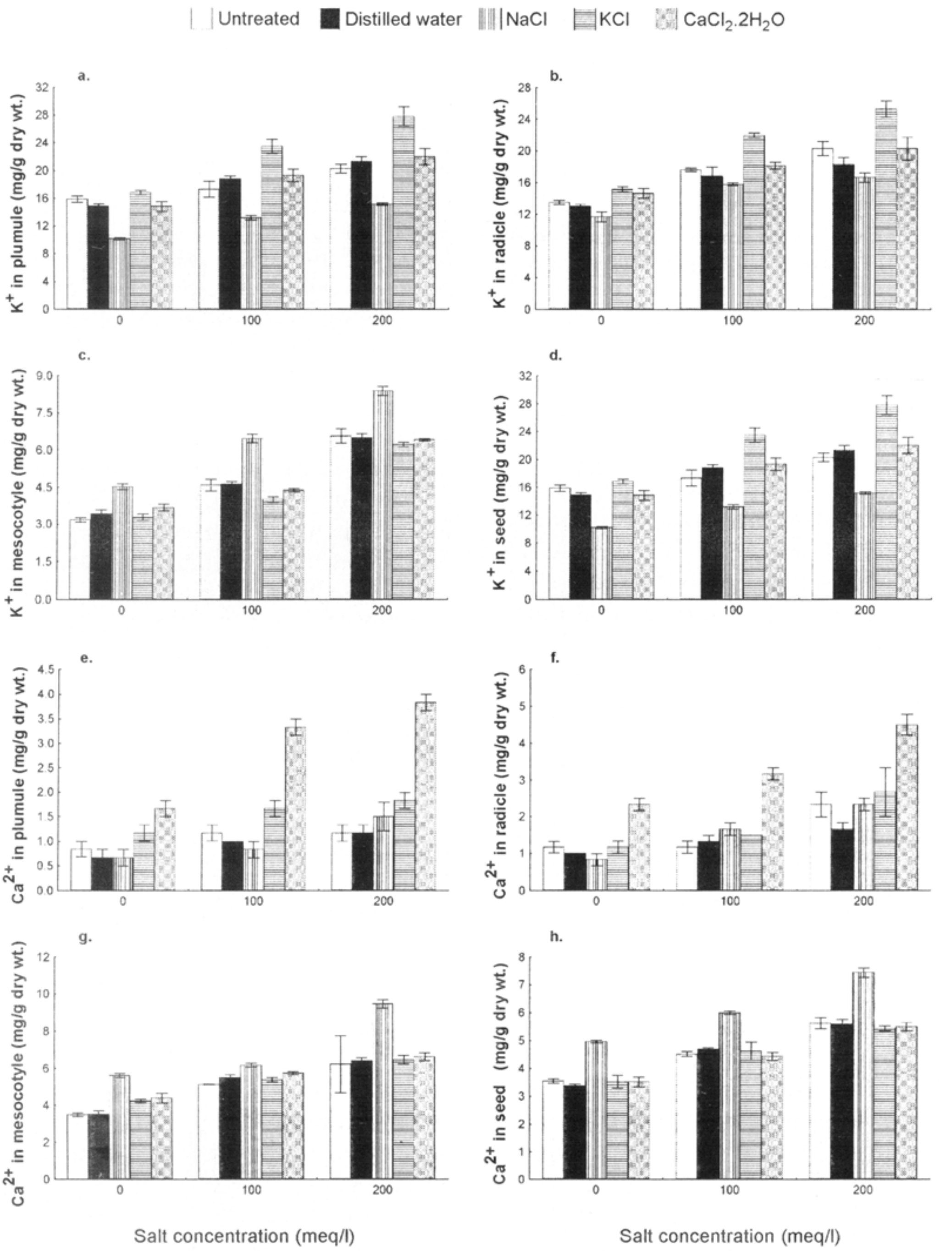

Fig.3: Potassium and Calcium content in different parts of maize seedlings primed for 8h with different salts of chloride or distilled water.

ent Cl^- salts. Although Cl^- accumulation was maximum in mesocotyl of the seeds treated with $CaCl_2{\cdot}2H_2O$, it did not differ among the seeds treated with NaCl, KCl, or distilled water.

Potassium was higher in all four seedling parts of the seeds pre-soaked in KCl and lower in those of the seeds pre-soaked in NaCl as compared to those of untreated or treated with other salts solutions (Fig. 3 a-d).

Calcium concentration was considerably greater in plumules and radicles of the seeds primed with $CaCl_2{\cdot}2H_2O$ than those of the seeds treated with other salts solutions or untreated seeds (Fig. 3 e-h). The Ca^{2+} contents in seedlings of the seeds treated with NaCl were not different.

Discussion

In view of some earlier studies it is now evident that pre-soaking or priming 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). The presoaking of seeds allows the hydration of membranes and proteins, and the initiation of various metabolic systems. These are arrested when the seeds are dried or moisture is withheld, but recommence when the seeds imbibe water for the second time (Bewley and Black 1982). From the results of the present study for final germination percentage, rate of germination (days to 50 percent germination) and fresh and dry weights of 14-day-old plumules and radicles of maize it is evident that pre-sowing treatment of the seeds with distilled water or 200 meq·L^{-1} of NaCl, KCl or $CaCl_2·2H_2O$ proved to be effective in inducing salt tolerance at the germination stage in the maize cultivar. $CaCl_2·2H_2O$ was more effective than the other priming agents. The results for germination percentage can be related to the earlier findings in which Black and El-Hadi (1992) found an improvement in germination of *Acacia senegal* in water, compared with untreated controls, when the seeds were primed with $CaCl_2.2H_2O$. Similarly, Chaudhri and Wiebe (1968) found no germination in NaCl when wheat seeds were primed only with water but a considerable improvement in germination when $CaCl_2·2H_2O$ was used as a priming agent.

The rate of germination of primed seeds of the maize line was also improved due to the solutions of different salts or distilled water as compared to untreated seeds. These results are in agreement with those of Rehman *et al.* (1998) who reported that treatment of seeds of *Acacia tortilis* and *A.coriacea* with distilled water or salts of Ca or K significantly enhanced the rate of germination under saline conditions. Similarly, in wheat, Idris and Aslam (1975) found the stimulatory effects of pre-sowing treatments on germination processes so that rate of germination was higher in treated than in untreated seeds under both non-saline and saline conditions. Working with tomato seeds, Cayuela *et al.* (1996) observed that under salt stress the seedlings emerged earlier from primed seeds with NaCl than from non-primed seeds. Bewley and Black (1982) were of the view that pre-sowing treatments cause initiation of the early metabolic processes and the re-drying of seeds arrested but did not reverse the initial stage of germination so that on the availability of suitable conditions the time taken to germination was reduced.

Sodium, K^+ and Ca^{2+} increased significantly in all the parts of germinating seeds of maize seeds primed with NaCl, KCl, or $CaCl_2·2H_2O$, respectively. It is likely that seeds during soaking may have absorbed considerable amounts of ions of the respective salts in which they were soaked. In addition, seeds primed with $CaCl_2·2H_2O$ were the highest in Cl^- accumulation in all parts of the germinating seeds, followed by the seeds treated with KCl and NaCl. High accumulation of Na^+ and Cl^- in all parts of the germinating seeds of maize including mesocotyl can be related to the greater sensitivity of the crop to salinity compared with other important grain crops (Ashraf and McNeilly 1989), although Johanson and Cheeseman (1983) and Drew and Läuchli (1987) reported the significant role of the mesocotyl in Na^+ exclusion to some extent from the maize shoot at the early seedling stage.

Potassium contents were generally lower in both plumules and radicles of the germinating seeds primed with NaCl than those treated with other salts or distilled water. It is possible that during soaking seeds might have absorbed high amounts of Na^+ which had an antagonistic effect on the transport of K^+ to the growing parts of the germinating seeds. In fact, antagonism of Na^+ with K^+ is one of the common phenomena occurring in plants subjected to saline conditions (Greenway and Munns 1980, Ashraf 1994).

Since Ca is known for its immobility in plants (Marschner 1995), it is evident from the results for Ca^{2+} reported here for different parts of the germinating seeds of maize that most of the Ca^{2+} was retained in the seeds and mesocotyl, because of which, transport of this ion to plumules and radicles was restricted. It is now evident that calcium is one of the important factors that play a significant role in the synthesis of new walls, particularly the middle lamellae that separate newly divided cells (Taiz and Zeiger 1998). The membrane damage and enhanced permeability may be affected by the displacement of Ca^{2+} by Na^+ from the binding sites of phospholipids of membranes (Leopold and Willing 1984). The low Ca^{2+} concentration in the shoot axis and roots of germinating seeds of maize may have been one of the reasons for impairment in initial growth under salt stress.

In conclusion, of the different salts of chloride used for priming maize seeds, $CaCl_2 \cdot 2H_2O$ proved to be the most effective in inducing salt tolerance in maize at the early growth stages. Sodium, K^+ and Ca^{2+} increased significantly in all the parts of germinating seeds of maize seeds primed with NaCl, KCl, or $CaCl_2 \cdot 2H_2O$, respectively. In addition, seeds primed with $CaCl_2 \cdot 2H_2O$ were the highest in Cl^- accumulation in all parts of the germinating seeds, followed by the seeds treated with KCl and NaCl.

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