CHAPTER 6

ROLE OF CALCIUM IN ALLEVIATING SALINITY EFFECTS IN COASTAL HALOPLHYTES

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Abstract: The purpose of the current investigation was to study the effect of Ca^{2+} on the seed germination of coastal species like *Arthrocnemum indicum, A. macrostachyum, Desmostachya bipinnata, Halopyrum mucronatum* and *Urochondra setulosa* under NaCl salinity. Seed germination was inhibited with the increase in NaCl concentration and species differ in their range of NaCl tolerance. Inclusion of $CaCl_2$ partially or completely alleviated NaCl effects on the germination of all species. In the case of *A. macrostachyum* calcium application completely alleviated salinity effects at all concentrations of NaCl, while in the case of *A. indicum* and *D. bipinnata* calcium completely alleviated NaCl effects except at 800 mM NaCl. While in the case of *H. mucronatum* and *U. setulosa* Ca^{2+} completely alleviated the NaCl effects at low concentration and partially alleviated at higher concentrations.

1. INTRODUCTION

The success of bio-saline agriculture is greatly dependent on the germination response of seeds of un-conventional halophytic crops (Khan, 2003). The soils where halophytes normally grow becomes more saline due to rapid evaporation of water particularly during summer, therefore, surface of the soil tend to have higher soil salinity and higher water potential (Khan & Gul, 2002). Seed germination in arid and semi-arid regions usually occurs after the rains, which help in reducing soil surface salinity (Khan, 2003). The germination of halophytes are inhibited by salinity for the following reasons: a) causing a complete inhibition of germination process at salinities beyond the tolerance limit of species, b) delaying the germination of seeds at salinities that cause some stress to seeds but do not prevent germination, c) causing the loss of viability of seeds due to high salinity and temperature and d) upsetting growth regulator balance in the embryo to prevent successful initiation of germination process. There is a great deal of variability in the response of halophytes to increasing salinity, moisture, light, and temperature stresses and their interactions (Khan & Ungar, 2000, 2001). Seeds of halophytes

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often germinate best under non-saline conditions and their germination decreases in salinity (Khan, 2003; Ungar, 1995). Halophytic species that dominate the region have shown variable response to NaCl tolerance during germination (Khan & Gulzar, 2003). *Halopyrum mucronatum* failed to germinate at or above 300 mM NaCl (Khan & Ungar, 1995) while *Aeluropus lagopoides* (Linn.) Trin. Ex Thw., *Sporobolus ioclados* (Nees ex Trin.) Nees and *Urochondra setulosa* (Trin.) C.E. Hubbard could germinate in up to 500 mM NaCl approaching seawater salinity (Khan & Gulzar, 2003). Stem succulent halophyte like *A. macrostachyum* could germinate in up to 800 mM NaCl (Khan & Gul, 1998).

It is well known that Ca^{2+} alleviates the adverse effects of salinity on many plant species (Rengle, 1992; Marschner, 1995; Agboola et al., 1998; Munns, 2002; Ebert et al., 2002). Adding Ca^{2+} to root media with NaCl favors plant growth in both halophytic (Colmer et al., 1996) and non-halophytic species (LaHaye & Epstein, 1969; Cramer et al., 1986; Kurth et al., 1986; Suhayda et al., 1992; Kinrade, 1999). Calcium alleviated the toxic effects of Na^{+} and Mg^{2+} on the germination of *Kalidum caspicum* (Tobe et al., 1999, 2001) and *Hordeum vulgare* (Bliss et al., 1986). Tobe et al. (2002) showed that Ca^{2+} successfully alleviated the toxicity of various chloride and sulfate salts on the germination of *Kalidium capsicum*. Plants occupying the coastal area around Karachi suffer high temperature, drought and salinity stresses. It may be that the establishment of these halophytes is facilitated by the alleviation of salt toxicity to its radicles by the Ca^{2+} present in the alkaline soils of this region.

The present study was done to investigate the effect of NaCl on the seed germination of coastal halophytes and to determine whether $CaCl_2$ application alleviate the salinity effect.

2. MATERIALS AND METHODS

Seeds of *Arthrocnemum indicum, A. macrostachyum, Desmostachya bipinnata, Halopyrum mucronatum* and *Urochondra setulosa* were collected in 2004 from a salt flats located at coastal regions from Karachi to Gadani. Tetrazolium test showed 100% viability in seeds. Seeds were surface sterilized with 1% sodium hypochlorite and germination experiments were started immediately. Germination was carried out in 50 mm by 9 mm (Gelman no.7232) tight-fitting plastic petri dishes with 5 ml of test solution (0 to 1000 mM NaCl depending on the range of tolerance of a given species). Calcium (10.0 mM $CaCl_2$) was also applied in addition to NaCl to study whether it would promote germination under saline conditions. Four replicates of 25 seeds were each used for each treatment. The germination experiments were carried out at a temperature of 20-30ºC. A 24 hr cycle was used; where the higher temperature was 30ºC coincided with the 12-light period (Sylvania cool white light, 110 µmol photons. m^{-2}. s^{-1}) and the lower temperature 20ºC coincided with the 12-dark period. Germination was recorded at every alternate day. The results were analyzed using a three way ANOVA. A

Bonferroni test was carried to determine the significance among individual means (SPSS, 1999).

3. RESULTS

A two-way ANOVA indicated there was a significant (F = 217.8; P < 0.001) effect of calcium in alleviating germination-inhibiting effects of NaCl (F = 46.8; P< 0.01), and all significant interaction (P < 0.001) on the germination of *Arthrocnemum indicum* seeds. *Arthrocnemum indicum* showed less than 20% germination under non-saline conditions and the seed germination decreased with the increase in salinity (Figure 1). Inclusion of calcium significantly alleviated salinity effects at all concentrations (Figure 1).

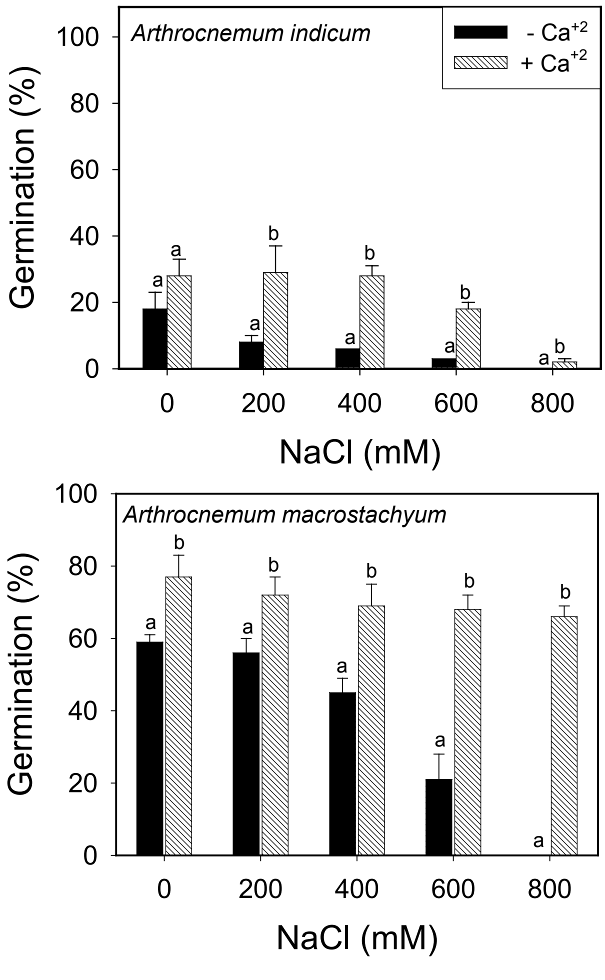

Figure 1. *Effect of calcium chloride (10 mM) on the seed germination of* Arthrocnemum indicum *and* A. macrostachyum *under saline conditions. (Mean with in each having different letters are significantly different from one another (P < 0.05), Bonferroni test)*

Germination in *A. macrostachyum* seeds were about 60% in control and germination was inhibited with the increase in salinity ($F = 50.7$; $P < 0.001$), however, application of calcium ($F = 351.7$; $P < 0.001$) almost completely alleviated salinity effects on germination at all salinity concentrations (Figure 1). A two-way ANOVA indicated significant ($F = 220.8$; $P < 0.001$) effect of calcium in alleviating germination-inhibiting effects of various salts ($F = 56.8$; $P < 0.001$) and all significant interaction ($P < 0.001$) on the germination of *Desmostachya bipinnata* seeds. All seeds of *D. bipinnata* were germinated under controlled conditions and, however, few seeds germinated at 400 mM NaCl. Inclusion of calcium completely alleviated salinity effects in all concentrations except at 800 mM NaCl where 63% seeds germinated in comparison to none in non-treated control (Figure 2). A two-way ANOVA indicated significant ($F = 198.4$; $P < 0.001$) effect of calcium in alleviating germination-inhibiting effects of various salts ($F = 144.6$; $P < 0.001$), and all significant interaction ($P < 0.001$) on the germination of *H. mucronatum* seeds. In *H. mucronatum*, calcium completely alleviated salinity effects at low concentrations and partially alleviated at higher levels (Figure 2). A two-way ANOVA indicated significant ($F = 179.8$; $P < 0.001$) effect of calcium in alleviating germination-inhibiting effects of various salts ($F = 78.8$; $P < 0.001$) and all significant interaction ($P < 0.001$) on the germination of *U. setulosa* seeds. Maximum seed germination was obtained in non-saline control (Figure 3). Addition of NaCl inhibited seed germination and few seeds germinated at 400 mM NaCl (Figure 3) and calcium ($CaCl_2$) alleviated seed germination when it was included in the growth medium with NaCl (Figure 3).

4. DISCUSSION

The diversity of coastal vegetation of Pakistan is very low and the vegetation on the entire coast is dominated by *Avicennia marina, Arthrocenmum macrostachyum, Suaeda fruticosa, Cressa cretica* and *Halopyrum mucronatum* other species are occasional in appearance (Khan & Gul, 2002). The soil samples analyzed from the adjacent areas showed high concentrations of sodium chloride and the base rock of the region is calcareous in nature and the amount of $CaCO_3$ in soil of this region is high.

Halophytes of the area are reported to tolerate variable concentrations of NaCl which includes *Arthrocnemum macrostachyum, A. indicum* (1000 mM, Khan & Gul, 1998; Khan, unpublished), *Salsola imbricata* (800 mM, Mehrunnisa et al., 2005), *Cressa cretica* (800 mM, Khan, 1991), *Limonium stocksii, Aeluropus lagopoides, Sporobolus ioclados* and *Urochondra setulosa* (500 mM, Zia & Khan, 2004; Khan & Gulzar, 2003), *Halopyrum mucronatum* (300 mM, Khan & Ungar, 2001). Our results with *A. indicum, A. macrostachyum, D. bipinnata, H. mucronatum* and *U. setulosa* showed a partial to complete reversal of the injurious effects of sodium chloride with the application of $CaCl_2$.

Tobe et al. (2002) also showed that calcium alleviated the effects of NaCl, Na_2SO_4, $MgCl_2$, and PEG on the germination of *Kalidium capsicum* indicating that the toxicity of different salts to radicles originates from a common mechanism. They attributed this inhibition due to osmotic effect which prevents

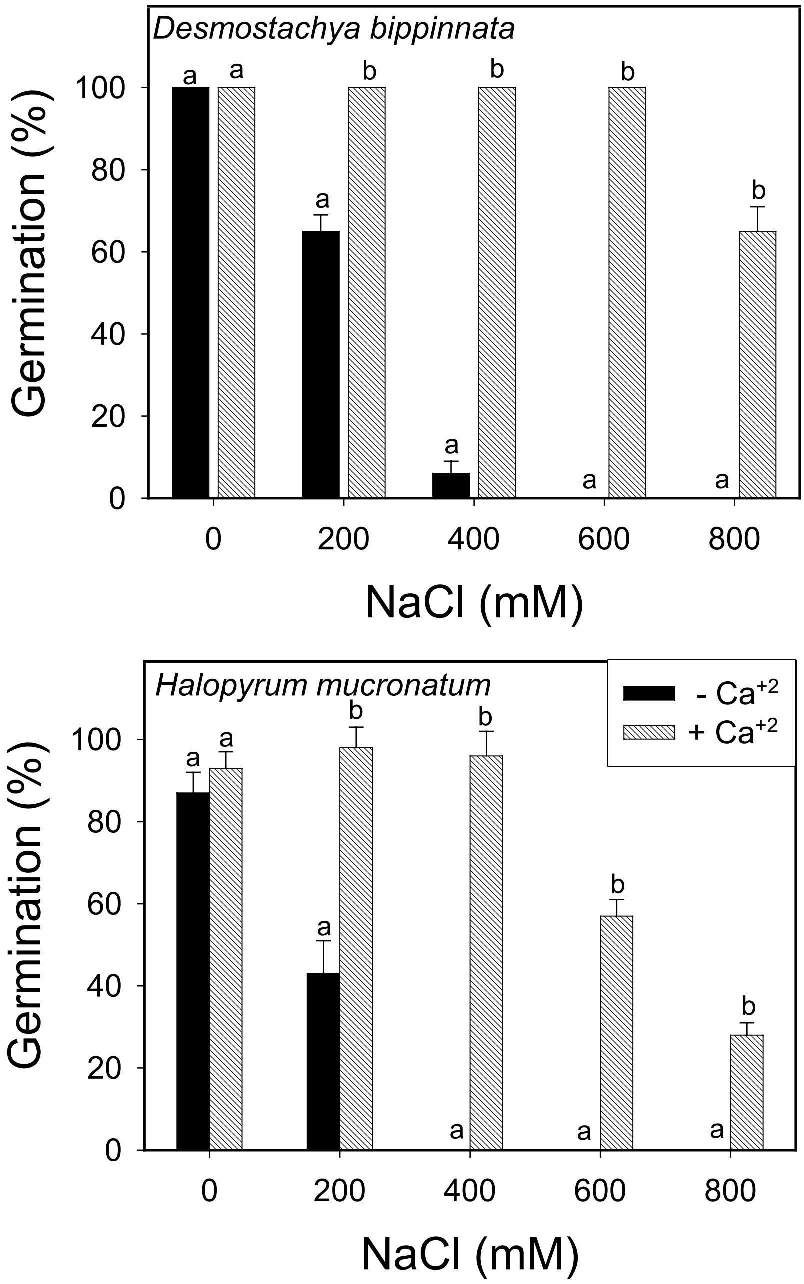

Figure 2. *Effect of calcium chloride (10 mM) on the seed germination of* Desmostachya bippinnata *and* Halopyrum mucronatum *under saline conditions. (Mean with in each having different letters are significantly different from one another ($P < 0.05$), Bonferroni test)*

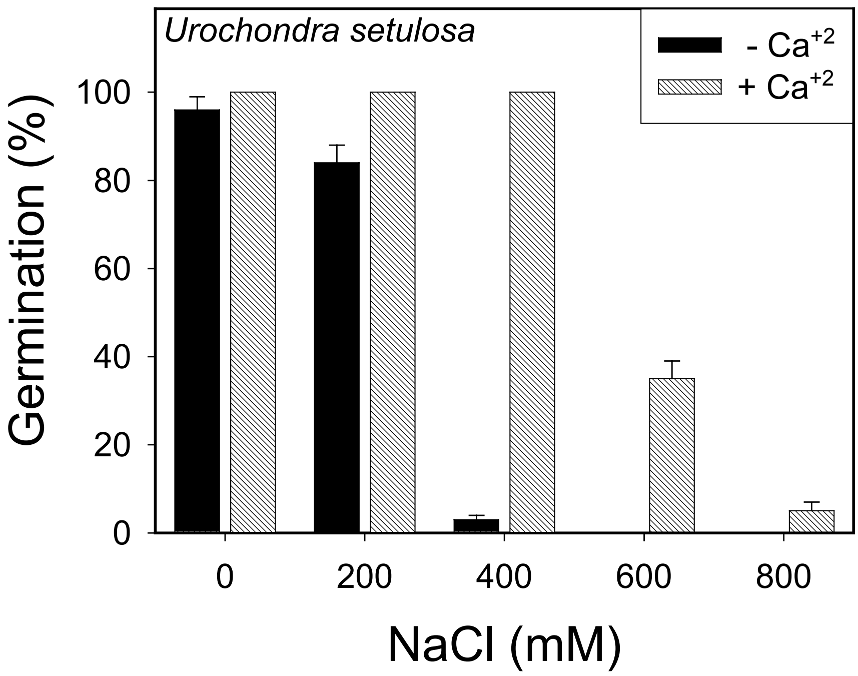

Figure 3. *Effect of calcium chloride (10 mM) on the seed germination of* Urochondra setulosa *under saline conditions. (Mean with in each having different letters are significantly different from one another (P < 0.05), Bonferroni test)*

radicle elongation. Other studies have mixed results some showed little ameliorative effects (Leidi et al*.,* 1991) while other showed a significant calcium effect in alleviating salinity stress on germination (Redmann, 1974; Bliss et al*.,* 1986; Katembe et al., 1998; Rengel, 1992; Tobe et al., 1999, 2001, 2002). The role of calcium to alleviate the adverse effect of NaCl and other salts on plant species (Rengel, 1992; Marshner, 1995; Tobe et al., 2002; Girija et al., 2002; Munns, 2002) is well known. Experimental evidence implicates Ca^{2+} function in salt adaptation (Parida & Das, 2005). Externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating higher K^+/Na^+ selectivity (Liu & Zhu, 1998; Lauchli & Schubert, 1989). High salinity also results in increased cytosolic Ca^{2+} that is transported from the apoplast and intracellular compartments (Knight et al*.,* 1997). The resultant transient Ca^{2+} increase potential stress signal transduction and leads to salt adaptation (Mendoza et al., 1994; Knight et al., 1997). A prolonged elevated Ca^{2+} level may, however, also pose a stress; if so, reestablishment of Ca^{2+} homeostasis is a requisite (Parida & Das, 2005). The changes in $[Ca^{2+}]^{Cyt}$ perturbations following combinations of oxidative stress and hyper-osmotic stress correlated well with the expression of Ca^{2+}-regulated osmotic stress induced genes, and the acquisition of osmotic stress tolerance (Knight et al., 1998). More research on Ca^{2+} in plants has been focused at the cellular level due to realization that $[Ca^{2+}]^{Cyt}$ was an obligate intracellular messenger coordinating the cellular responses to numerous developmental cues and environmental challenges (White & Broadley, 2003).

Arthrocnemum indicum, A. macrostachyum, D. bipinnata, H. mucronatum and *U. setulosa* are among the dominant vegetation along the Pakistani coast. Presence of Ca^{2+} salt in natural and artificial conditions may increase the emergence and the annual productivity of these species that may be a good reason for their survival and dominance in such harsh environmental conditions.

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