



Effect of calcium and light on the germination of *Urochondra setulosa* under different salts^{*}

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Abstract: *Urochondra setulosa* (Trin.) C.E. Hubbard is a coastal halophytic grass thriving on the coastal dunes along the Pakistani seashore. This grass could be useful in coastal sand dune stabilization using seawater irrigation. The purpose of this investigation was to test the hypothesis that Ca²⁺ (0.0, 2.5, 5.0, 10.0 and 50.0 mmol/L) alleviates the adverse effects of KCl, MgSO₄, NaCl and Na₂SO₄ at 0, 200, 400, 600, 800 and 1000 mmol/L on the germination of *Urochondra setulosa*. Seed germination was inhibited with increase in salt concentration with few seeds germinated at and above 400 mmol/L concentration. No seed germinated in any of the KCl treatments. Inclusion of CaCl₂ substantially alleviated the inhibitory effects of all salts. Germination was higher under photoperiod in comparison to those seeds germinated under complete darkness. Among the CaCl₂ concentrations used, 10 mmol/L was most effective in alleviating salinity effects and allowing few seeds to germinate at 1000 mmol/L KCl, MgSO₄, NaCl and Na₂SO₄ solution.

Key words: Calcium, Germination, KCl, MgSO₄, NaCl, Na₂SO₄, *Urochondra setulosa*

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INTRODUCTION

Seed germination and seedling emergence are critical to the survival of plants in salt-affected areas (Khan, 2002). Seeds of halophytes often germinate best under non-saline conditions with their germination decreasing with increasing salinity (Ungar, 1995; Khan and Gul, 1998; Khan, 2002). Halophytic grasses that dominate the region have shown variable response to NaCl tolerance during germination (Khan and Gulzar, 2003). *Halopyrum mucronatum* failed to germinate at or above 300 mmol/L NaCl (Khan and Ungar, 2001) 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 mmol/L NaCl ap-

proaching seawater salinity (Khan and Gulzar, 2003).

Seed germination was almost completely inhibited in the absence of light for *Sporobolus indicus* (L.) R. Br. (Andrews, 1997) and *Triglochin maritima* L. (Khan and Ungar, 1999) and partially inhibited germination in *Apium graveolens* L. (Garcia et al., 1995), *Allium stacticiforme* Sibth. & Sm., *Brassica tournefortii* Gouan, *Cakile maritima* Scop., *Onanthus maritimus* (L.) Hoffmanns & Link (Thanos et al., 1991) and *Limonium stocksii* (Zia and Khan, 2004) while germination of seeds of *Atriplex stocksii* Boiss. (Khan and Rizvi, 1994; Khan and Ungar, 2000) and *Suaeda fruticosa* (Khan and Ungar, 1998) was not inhibited by the absence of light. However, little information is available on the effect of Ca²⁺ in improving seed germination under saline conditions.

Saline soils are formed from the accumulation of various chloride and sulfate salts dominated by NaCl. The main salt components in saline soils are Na⁺,

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Mg²⁺ and Ca²⁺ cations and Cl⁻ and SO₄²⁻ anions (Shainberg, 1975). Only few studies (Hardegree and Emmerich, 1990; Mohammad and Sen, 1990; Egan et al., 1997; Agboola, 1998; Pujol et al., 2000; Tobe et al., 2002; Joshi et al., 2005) have compared the effects of various salts on the germination of plants. Some studies indicated an osmotic effect of various chloride and sulfate salts on the germination but no specific ion effect (Mohammad and Sen, 1990; Egan et al., 1997; Agboola, 1998; Pujol et al., 2000) while others indicated both osmotic and ionic effects (Mohammad and Sen, 1990; Duan et al., 2004; Song et al., 2005).

Calcium alleviates the adverse effects of salinity in some plant species (Rengel, 1992; Marschner, 1995; Ebert et al., 2002; Munns, 2002; Bonilla et al., 2004) and promotes plant growth (LaHaye and Epstein, 1969; Cramer et al., 1986; Kurth et al., 1986; Suhayda et al., 1992; Colmer et al., 1996; Kinraide, 1999) and alleviates the toxic effects of Na⁺ and Mg²⁺ on the germination of *Kalidium caspicum* (Tobe et al., 1999; 2001), pea (Bonilla et al., 2004) and *Hordeum vulgare* (Bliss et al., 1986). Tobe et al. (2002; 2004) showed that Ca²⁺ successfully alleviated the toxicity of various chloride and sulfate salts on the germination or seedling growth of *Kalidium caspicum* and *Haloxylon ammodendron* at relatively low concentration, and reduced K⁺ outflux from seedlings, but caused no appreciable decrease in the influx of Na⁺ or Mg²⁺ into seedlings. The implication of the effects of calcium present in saline soil is far from clear. The establishment of *U. setulosa* in highly salinized areas is probably facilitated by the alleviation of salt toxicity to its radicles by the Ca²⁺ present in the alkaline soils of these regions. This could result in increasing use of Ca²⁺ as soil supplement to improve seed emergence.

The present study was done to test the hypothesis that the application of calcium could successfully alleviate the inhibitory effect of chloride and sulfate salts on the seed germination of *Urochondra setulosa*.

MATERIALS AND METHODS

Seeds of *Urochondra setulosa* were collected in 2002 from a salt flat located at Hawkes Bay near the Karachi coast. Tetrazolium test showed 100% viabil-

ity in seeds. Seeds were surface sterilized with 1% sodium hypochlorite solution with germination experiment being started immediately. Germination was carried out in 50 mm×9 mm (Gelman No. 7232) tight-fitting plastic Petri dishes with 5 ml of test solution (0, 200, 400, 600, 800 and 1000 mmol/L NaCl, Na₂SO₄, MgSO₄ and KCl). Calcium chloride (0.0, 2.5, 5.0, 10.0 and 50.0 mmol/L) was also applied in addition to these salts. Four replicates of 25 seeds each were used for each treatment. Germination experiment was carried out at 20 °C:30 °C temperature regimes with a 12-h dark:light photoperiod (Sylvania cool white light, 110 μmol photons, m⁻²·s⁻¹). Germination was recorded every other day.

Germination was also studied in 24 h darkness by placing the sets of Petri dishes in black bags at 20~30 °C. The germination in darkness was recorded on the 20th day of the experiment. Seeds were considered to be germinated with the emergence of the radical. The rate of germination was estimated by using a modified Timson index of germination velocity= $\sum G/t$, where G is the percent germination after 20 d and t is the total time of germination (Khan and Ungar, 1984). Statistical analysis was carried out using SPSS ver. 9.0 (1999).

RESULTS

A three-way ANOVA indicated significant ($F=309.8$, $P<0.001$) effect of calcium in alleviating germination-inhibiting effects of various salts ($F=46.8$, $P<0.001$), their concentrations ($F=2417.9$, $P<0.001$) and all significant interaction ($P<0.001$) on the germination of *Urochondra setulosa* seeds (Table 1). Maximum seed germination was obtained in non-saline control (Fig.1). Addition of NaCl inhibited seed germination with few seeds germinated at 400 mmol/L NaCl (Fig.1). NaCl and darkness had synergistic effect in inhibiting seed germination. Calcium (CaCl₂) alleviated seed germination when included in the growth medium with NaCl (Fig.1). The CaCl₂ concentration of 10 mmol/L appeared to be optimal while few seeds germinated at 1000 mmol/L NaCl. Germination in darkness was significantly lower in all treatments in comparison to photoperiod (Fig.1).

Effect of sodium sulfate on seed germination and its reversal by CaCl₂ showed a similar response as

that of NaCl (Fig.2). In the case of magnesium sulfate, 50 mmol/L CaCl_2 completely alleviated MgSO_4 effect in up to 600 mmol/L and 10 mmol/L CaCl_2 promoted more than 50% seed germination at 800 mmol/L MgSO_4 (Fig.3). Potassium chloride was very toxic to germination and no seed germinated at 200 mmol/L KCl treatment (Fig.4). Inclusion of CaCl_2 , however, substantially alleviated the adverse KCl

effect on seed germination. Application of CaCl_2 (50 mmol/L) completely alleviated the 600 mmol/L KCl effect on seed germination (Fig.4).

Rate of germination decreases with increase of concentrations in all salts (Tables 2~5). CaCl_2 (10.0 mmol/L) had higher rate of germination in comparison to distilled water control. When seed germinated in NaCl, rate of germination was highest when

Table 1 A three-way ANOVA of characteristics of *Urochondra setulosa* due to CaCl_2 , salts, concentrations and their interaction

Sources	Sum of squares	df	Mean square	F	Significance
Calcium	74603.333	4	18650.833	4.719	0.006
Salts	10222.400	4	2555.600	1.288	0.308
Salinity	549490.466	5	109898.093	26.863	0.000
Calcium×salts	12052.133	12	1004.344	2.003	0.040
Calcium×salinity	68984.267	20	3449.213	6.878	0.000
Salts×salinity	29624.400	20	1481.220	2.954	0.001
Calcium×salts×salinity	30089.867	60	501.498	9.359	0.000

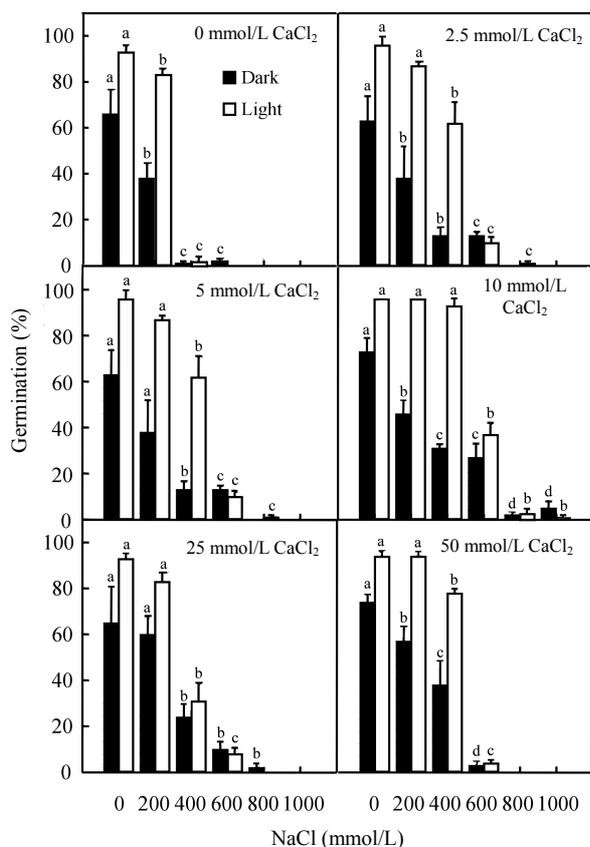


Fig.1 Mean final germination percentage of *U. setulosa* in various NaCl in dark and light conditions
Bar represents mean±SDE. Bonferroni letter represents significant difference ($P < 0.05$) between salt treatments

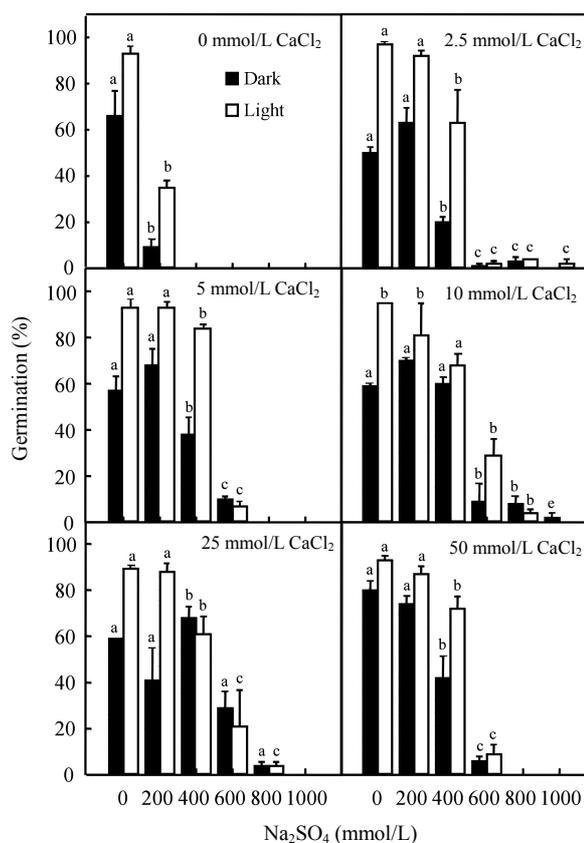


Fig.2 Mean final germination percentage of *U. setulosa* in various Na_2SO_4 in dark and light conditions
Bar represents mean±SDE. Bonferroni letter represents significant difference ($P < 0.05$) between salt treatments

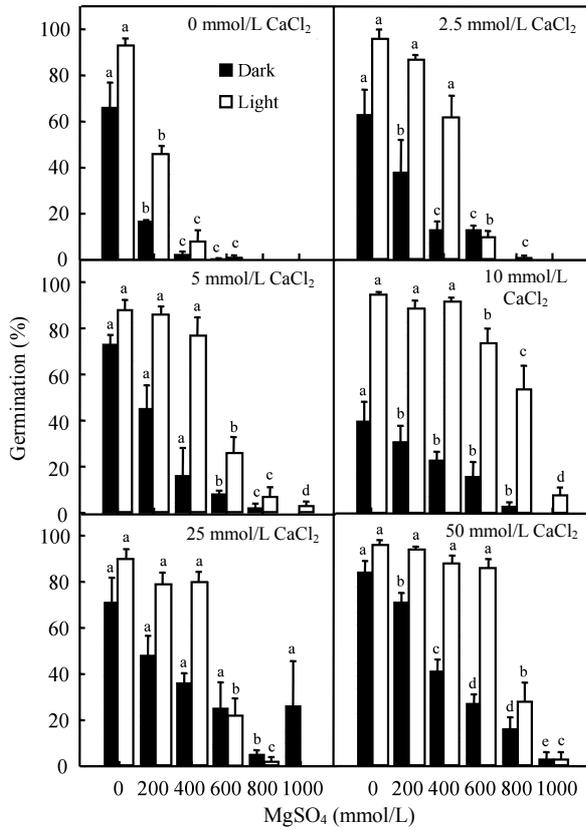


Fig.3 Mean final germination percentage of *U. setulosa* in various $MgSO_4$ in dark and light conditions
Bar represents mean \pm SDE. Bonferroni letter represents significant difference ($P<0.05$) between salt treatments

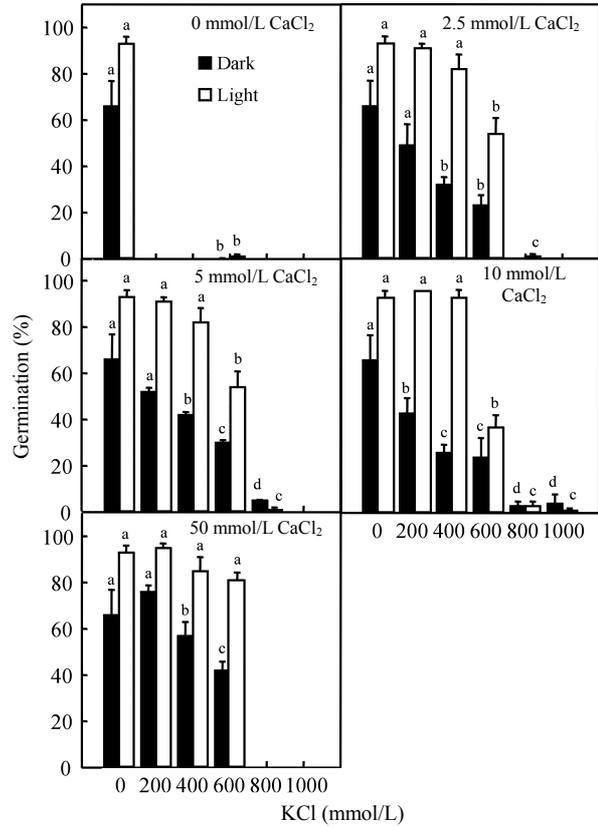


Fig.4 Mean final germination percentage of *U. setulosa* in various KCl in dark and light conditions
Bar represents mean \pm SDE. Bonferroni letter represents significant difference ($P<0.05$) between salt treatments

Table 2 Effect of NaCl with and without $CaCl_2$ on rate of germination of seeds of *Urochondra setulosa*

NaCl (mmol/L)	Rate of germination				
	CaCl ₂ (mmol/L)				
	0	2.5	5	10	50
0	34.3 \pm 1.9	41.0 \pm 1.4	35.2 \pm 1.5	43.2 \pm 0.4	36.4 \pm 0.1
200	27.3 \pm 2.2	36.1 \pm 1.1	40.2 \pm 0.8	41.6 \pm 1.1	36.7 \pm 1.0
400	0.2 \pm 0.2	23.0 \pm 3.1	29.1 \pm 3.2	36.7 \pm 2.0	27.5 \pm 1.1
600	0.0 \pm 0.0	2.4 \pm 0.6	5.5 \pm 0.7	13.7 \pm 2.1	0.7 \pm 0.3
800	0.0 \pm 0.0	0.1 \pm 0.1	0.3 \pm 0.3	1.1 \pm 0.6	0.0 \pm 0.0
1000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.3 \pm 0.2	0.0 \pm 0.0

Table 3 Effect of Na_2SO_4 with and without $CaCl_2$ on rate of germination of seeds of *Urochondra setulosa*

Na_2SO_4 (mmol/L)	Rate of germination				
	CaCl ₂ (mmol/L)				
	0	2.5	5	10	50
0	38.7 \pm 1.2	40.3 \pm 0.7	37.5 \pm 1.3	41.0 \pm 0.4	34.2 \pm 0.5
200	11.5 \pm 1.2	38.4 \pm 1.1	38.2 \pm 1.2	40.6 \pm 0.5	32.6 \pm 0.9
400	0.0 \pm 0.0	22.3 \pm 5.4	27.5 \pm 0.9	30.7 \pm 2.0	23.3 \pm 2.1
600	0.0 \pm 0.0	5.4 \pm 0.2	1.7 \pm 0.8	2.1 \pm 0.7	2.5 \pm 1.1
800	0.0 \pm 0.0	0.9 \pm 0.1	0.0 \pm 0.0	2.1 \pm 0.6	0.0 \pm 0.0
1000	0.0 \pm 0.0	0.4 \pm 0.4	0.0 \pm 0.0	0.2 \pm 0.2	0.0 \pm 0.0

Table 4 Effect of KCl with and without $CaCl_2$ on rate of germination of seeds of *Urochondra setulosa*

KCl (mmol/L)	Rate of germination				
	CaCl ₂ (mmol/L)				
	0	2.5	5	10	50
0	37.9 \pm 1.3	38.2 \pm 1.4	37.7 \pm 1.6	39.5 \pm 0.2	34.4 \pm 0.7
200	0.0 \pm 0.0	37.7 \pm 0.9	36.0 \pm 1.4	39.3 \pm 0.8	36.7 \pm 1.0
400	0.0 \pm 0.0	33.4 \pm 2.4	32.5 \pm 1.8	35.2 \pm 2.0	30.9 \pm 2.5
600	0.2 \pm 0.2	19.2 \pm 2.9	24.0 \pm 3.0	12.4 \pm 2.1	28.2 \pm 1.0
800	0.0 \pm 0.0	0.2 \pm 0.2	14.2 \pm 2.0	1.0 \pm 0.6	0.0 \pm 0.0
1000	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.4	0.0 \pm 0.0

Table 5 Effect of $MgSO_4$ with and without $CaCl_2$ on rate of germination of seeds of *Urochondra setulosa*

$MgSO_4$ (mmol/L)	Rate of germination				
	CaCl ₂ (mmol/L)				
	0	2.5	5	10	50
100	38.3 \pm 1.2	37.7 \pm 1.9	37.4 \pm 1.1	39.6 \pm 0.5	34.6 \pm 0.1
200	16.7 \pm 0.6	36.2 \pm 1.8	35.7 \pm 1.5	33.6 \pm 1.3	34.5 \pm 0.5
400	2.3 \pm 1.4	29.9 \pm 3.5	33.7 \pm 1.3	35.4 \pm 0.8	32.4 \pm 0.5
600	0.4 \pm 0.4	8.6 \pm 1.9	13.4 \pm 1.3	25.6 \pm 2.4	27.2 \pm 1.1
800	0.0 \pm 0.0	2.0 \pm 1.2	2.9 \pm 1.5	16.6 \pm 2.4	7.0 \pm 2.3
1000	0.0 \pm 0.0	0.5 \pm 0.3	0.0 \pm 0.0	3.0 \pm 1.2	0.6 \pm 0.6

10 mmol/L CaCl_2 was included (Table 2). Similar trend was noted with the treatment of sodium sulfate, potassium chloride and magnesium sulfate salts (Tables 3~5). CaCl_2 at higher concentration increased rate of germination more in MgSO_4 , followed by KCl , Na_2SO_4 and NaCl (Tables 2~5).

DISCUSSION

Urochondra setulosa is a dominant species and one of the salt tolerant perennial grasses distributed in the coastal zone of Pakistan and forms hummocks on the farthest side of Pakistan's Manora Creek which rarely gets inundated with seawater with plants probably surviving on oceanic seepage (Gulzar et al., 2001; Khan and Gulzar, 2003). *Urochondra setulosa* has erect and stout culms attaining a height of 15~90 cm, produces a large number of caryopses and also achieves vegetative reproduction by short rhizomes (Gulzar et al., 2001). Other plants growing in association with *U. setulosa* include *Limonium stocksii*, *Cyperus* sp. in the drier zone and *Arthrocnemum macrostachyum* in the relatively wet low-lying zones. The grazing of the *Urochondra setulosa* population at Hawkes Bay by cattle indicates that this species could be used as a forage crop on saline soils (Gulzar et al., 2001). Hawks Bay in Manora Creek also receives domestic and industrial wastes. The analyzed soil samples from the adjacent areas showed high concentrations of various sulfate and chloride ions. The base rock of the region is calcareous in nature with the amount of CaCO_3 in soil of this region being high.

The area's halophytes are reported to tolerate variable concentrations of NaCl during germination. They include *Arthrocnemum macrostachyum* (1000 mmol/L) (Khan and Gul, 1998), *Cressa cretica* (800 mmol/L) (Khan, 1991), *Aeluropus lagopoides*, *Limonium stocksii*, *Sporobolus ioclados* and *Urochondra setulosa* (500 mmol/L) (Khan and Gulzar, 2003; Zia and Khan, 2004), *Halopyrum mucronatum* (300 mmol/L) (Khan and Ungar, 2001). *Urochondra setulosa* showed a similar response to MgCl_2 , KCl , and MgSO_4 , although KCl at lowest concentration (200 mmol/L) prevented any seed from germination. Ungar (1995) reported that salt inhibition to halophyte *Puccinellia festucaeformis* was in the order CaCl_2 , $\text{MgCl}_2 > \text{NaCl}$, NaNO_3 , $\text{KCl} > \text{MgSO}_4$. Several

studies reported the effects of different salts common in the soil (Mohammad and Sen, 1990; Egan et al., 1997; Tobe et al., 2002; Duan et al., 2004). Most studies showed that the effect of salts on the germination is primarily osmotic and few researchers believed that it could be both ionic and osmotic (Mohammad and Sen, 1990; Duan et al., 2004; Joshi et al., 2005; Song et al., 2005). It appears that *U. setulosa* shows both osmotic and ionic effects, for example, KCl appears highly toxic, MgSO_4 less toxic.

Our results for *U. setulosa* showed alleviation of the injurious effects of all salts with the application of CaCl_2 . This alleviating effect was more obvious on the seeds during the photoperiod compared to darkness germinated seeds. Tobe et al. (2002) also showed that calcium alleviated the adverse 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 CaCl_2 inhibition to an osmotic effect, which prevents radicle elongation. Other studies had different results, some showed little ameliorative effects (Leidi et al., 1991) while other showed significant calcium effect in alleviating salinity stress on germination (Bliss et al., 1986; Tobe et al., 2002). The role of calcium in alleviating the adverse effect of NaCl and other salts on plant species (Rengel, 1992; Marschner, 1995; Girija et al., 2002; Munns, 2002; Tobe et al., 2002) is not very well known. Experimental evidence implicates Ca^{2+} function in salt adaptation (Parida and Das, 2005). Externally supplied Ca^{2+} reduces the toxic effects of NaCl , presumably by facilitating higher K^+/Na^+ selectivity (Läuchli and Schubert, 1989; Liu and Zhu, 1998). 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+} increases 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 and Das, 2005). The changes in $[\text{Ca}^{2+}]^{\text{Cyt}}$ 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 $[Ca^{2+}]^{Cyt}$ is an obligate intracellular messenger coordinating the cellular responses to numerous developmental cues and environmental challenges (White and Broadley, 2003; Plieth, 2005). Little information is available on enhancing seed germination in darkness by the application of $CaCl_2$.

The results presented in this paper validated the hypothesis that the application of calcium alleviates the injurious effects of various chloride and sulfate salts on the germination of *U. setulosa*. *Urochondra setulosa* appears to be preferred for grazing by animals, indicating that it would be a potential forage crop that could be grown with seawater irrigation. Presence of Ca^{2+} salt in natural and artificial conditions might increase the emergence and annual productivity of *Urochondra setulosa*, thus providing a rich source of fodder to grazing animals. Further experiments should be done to determine the effect of applied Ca^{2+} in the field on seed emergence.

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