

Role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds

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Abstract

Phragmites karka (Retz.) Trin, ex. steud, a perennial reed with creeping rhizome from the family Poaceae, is distributed as pure population in brackish water swamps. Populations primarily propagate using ramets but also produce numerous seeds which form part of the seed bank after dispersal and are exposed to extremes of temperature, drought, and salinity stress. Seeds were germinated under a range of salinity (0, 100, 200, 300, 400, 500 mM NaCl) and temperature (10/20 °C, 15/25 °C, 20/30 °C, 25/35 °C, night/day) regimes in 12 h light:12 h dark photoperiod or in complete darkness with 0, 5, 10, 25 mM CaCl₂. Salinity, absence of light and high temperature (25/35 °C) reduced germination while calcium generally reversed this effect, more so at cooler temperature regimes. Calcareous soil around Karachi would help alleviate the salinity effect on the germination of *P. karka* and facilitate its survival.

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Keywords: Calcium; Light; NaCl salinity; Photoperiod; *Phragmites*; Seed germination; Temperature

1. Introduction

Seed germination begins with water uptake by the dry seed and is completed with radicle protrusion from embryonic axis (Bewley and Black, 1994; Duval et al., 2002). Under saline conditions, germination is inhibited by either prevention or delay in water absorption or due to ion toxicity (Tobe et al., 2004; Zhu, 2003). Halophytic grasses are usually not as salt tolerant at seed germination as a number of other dicotyledonous halophytes (Khan and Gul, 2006); however, some of them could germinate at about 1000 mM NaCl which is approaching twice the salinity of seawater e.g. *Spartina alterniflora* and *Urochondra setulosa* (Gulzar et al., 2001; Lombardi et al., 1997, 1998; Mooring et al., 1971).

Seeds contain all the essential plant mineral nutrients but their availability is hampered under stresses like cold, drought, salinity (Knight and Knight, 2001). Under such conditions, Ca²⁺ gains significance as it provides stress protection by regulating many

physiological and cellular events (Hirschi, 2004; Tang et al., 2006). Calcium has been reported to inhibit Na⁺ uptake and thereby reduce its adverse effect on seed germination (Bonilla et al., 2004; Marcar, 1986; Nayyar, 2003) as well as increase plant growth (Colmer et al., 1996; Ebert et al., 2002; Kinraide, 1999; Kurth et al., 1986; Marschner, 1995; Munns, 2002; Rengel, 1992; Suhayda et al., 1992; Tobe et al., 2003) possibly by suppressing the entry of Na ion through HKT2 transporters like VI-NSCCs and LCT1 (Kronzucker and Britto, 2011). It has been reported that calcium alleviated the toxic effects of Na⁺ and Mg²⁺ on the germination of *Kalidium capsicum* (Tobe et al., 1999, 2001), *Hordeum vulgare* (Bliss et al., 1986) and *U. setulosa* (Shaikh et al., 2007). Tobe et al. (2002, 2004) showed that Ca²⁺ alleviated the toxicity of various chloride and sulfate salts on the germination or seedling growth of *K. capsicum* and *Haloxylon ammodendron* at low concentration, and reduced K⁺ efflux from seedlings, but caused no appreciable decrease in the influx of Na⁺ or Mg²⁺ into seedling.

Keeping in view, the beneficial effect of calcium on diverse plant species, the current research aims at exploring the possibility of ameliorating the salinity effect on germination of *Phragmites*

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karka seeds and increasing its salt tolerance at this stage through addition of CaCl_2 in the growth medium. *P. karka* is a tall, rhizomatous perennial grass of swamps and moist habitats with diverse usages.

2. Materials and methods

2.1. Experimental details

Seeds of *P. karka* were collected from a population at Karachi University campus, separated from inflorescence and stored in a plastic bottle after surface sterilization with 0.85% Clorox. Seeds: Clorox ratio was 1:10 (weight:volume); the solution was drained after 1 min of soaking and seeds air dried overnight before storage and subsequent use. Experiments were conducted in air-tight plastic Petri plates of 50×9 mm (Gelman No. 7232). Four replicates of 25 seeds each were used for each treatment with 5 ml of test solution. The treatments consisted of six salt concentrations (0, 100, 200, 300, 400, 500 mM NaCl) with CaCl_2 (0, 5, 10, 25 mM). Reagent grade NaCl and CaCl_2 were used. The seeds were immersed in the test solutions directly in contact with the Petri plates which were placed in programmed incubators (Percival, Boone, USA) set at different temperature regimes (10/20 °C, 15/25 °C, 20/30 °C, 25/35 °C, night/day) with 12 h photoperiod (25 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ 400–700 nm Sylvania cool white lamp). Germination (emergence of the radicle) was recorded every alternate day for 20 days. Seeds were also germinated in complete darkness with the above-mentioned treatments and germination was recorded only at the 20th day. The un-germinated seeds were subjected to tetrazolium chloride viability test. The rate of germination velocity was estimated by using modified Timson's Index = $\Sigma G/t$, where G is the percentage of seed germinated at 2-day intervals and t is the total germination period (Gulzar and Khan, 2001). The experiments were repeated twice which showed similar trends.

2.2. Statistical analysis

The effects of salinity, temperature, CaCl_2 were examined using three-way ANOVA using SPSS version 9.0 (1999) and differences between treatment means were determined by Bonferroni test.

3. Results

Seed germination was inhibited with the increase in salinity and in absence of light. More seeds were inhibited from germination at 25/35 °C in comparison to the lower temperatures. Application of calcium alleviated the effect of salinity and dark on the seed germination of *P. karka* particularly at lower temperature regimes.

Application of CaCl_2 at 10/20 °C substantially alleviated salinity effects even at 500 mM NaCl, whereas 10 mM CaCl_2 increased seed germination up to 80% compared to only 2% in non-treated seeds (Fig. 1). A similar seed germination trend was noted at the temperature regimes of 15/25 °C and 20/30 °C. At the optimal temperature regime (20/30 °C), seeds generally showed higher germination under lower salinity even without

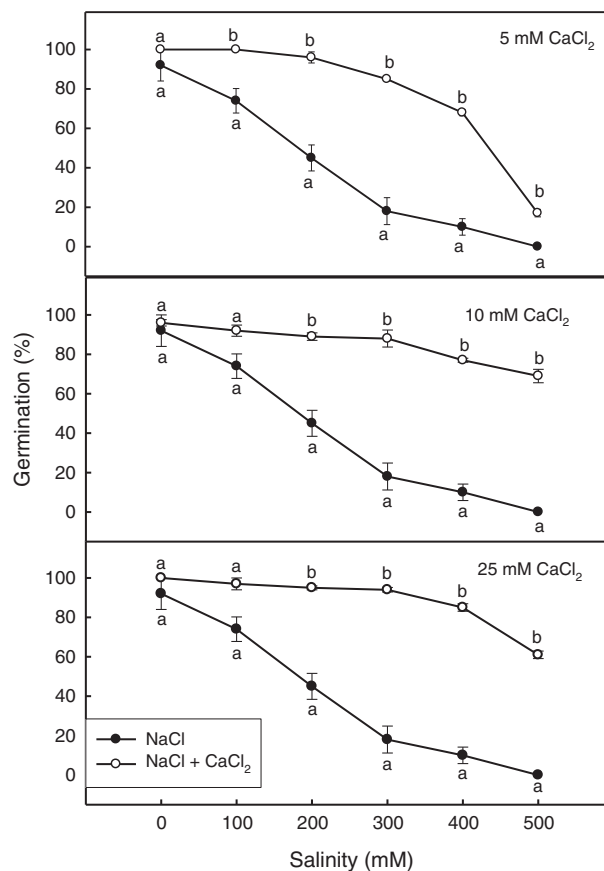


Fig. 1. Percent germination of *Phragmites karka* in NaCl with and without different concentrations of CaCl_2 at 10–20 °C. Symbols having the same letters are not significantly different from each other within each CaCl_2 treated and non-treated seeds at similar salinity levels (Bonferroni test, $P < 0.05$).

calcium and significant differences due to calcium were noted only at the highest salinity (500 mM, Figs. 2 and 3). However calcium application was not helpful in alleviating the salinity effect at the warmer temperature regime (25/35 °C) used in this experiment (Fig. 4).

Seed germination of *P. karka* was inhibited in the dark and the application of CaCl_2 alleviated both salinity and dark effects. At cooler temperature regimes, higher reversal of both salinity and light effects was recorded at 5 and 10 mM CaCl_2 (Fig. 5). High concentration of CaCl_2 (25 mM) also completely reversed salinity and light effects on seed germination at 15/25 °C and 20/30 °C (Figs. 6 and 7). Lower concentrations of CaCl_2 partially alleviated salinity and light effects. Application of CaCl_2 substantially inhibited seed germination in all treatments at the warmer temperature where no seed germinated at 10 and 25 mM CaCl_2 application (Fig. 8). Rate of seed germination of *P. karka* followed a similar response as that of percent germination when different concentrations of calcium chloride were applied at various temperature regimes (Table 1).

A three-way ANOVA showed significant ($P < 0.05$) individual effect of salinity, temperature, CaCl_2 and their interaction on final germination and germination rate (Table 2).

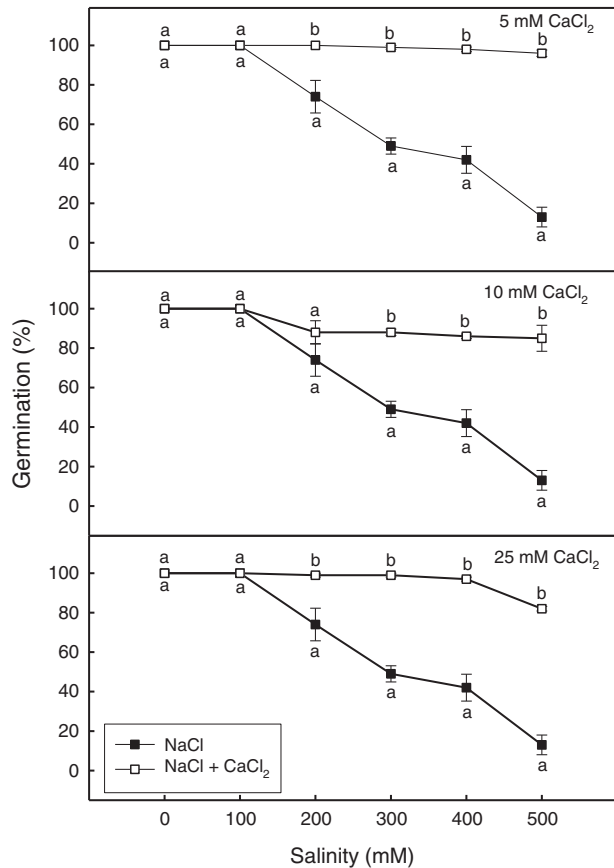


Fig. 2. Percent germination of *Phragmites karka* in NaCl with and without different concentrations of CaCl_2 at 15–25 °C. Symbols having the same letters are not significantly different from each other within each CaCl_2 treated and non-treated seeds at similar salinity levels (Bonferroni test, $P < 0.05$).

4. Discussion

Continuity of the life cycle of the plant populations which occupy salt marshes depends on the success in coping with high and sudden variations in salinity and temperature. This is particularly important at the seed germination stage which is probably the most sensitive part of the life cycle and requires either avoidance (dormancy) or tolerance strategy for recruitment under such conditions. The test species (*P. karka*) appeared to be inherently salt tolerant showing germination in an earlier study (Zehra and Khan, 2007). Seed viability was generally maintained in the presence of salinity at cooler and optimal temperatures; however, at warmer temperature regimes about 20% seeds lost viability while most of them entered into salt and temperature induced dormancy (Zehra and Khan, 2007). In the present study, good germination was observed in up to 400 mM NaCl at the optimum temperature regime (20:30 °C) (Figs. 1–4). Mean temperature ranges between 10 and 30 °C were favorable for germination of *Phragmites australis* and *Typha latifolia* (Ekstam and Forseby, 1999) while Gorai et al. (2006) reported *Phragmites communis* tolerant of 400 mM NaCl at 20 °C. Kushwaha et al. (1990) reported that the combination of 30 °C for 8 h and 10 °C for 16 h resulted in highest germination of *P. karka* seeds.

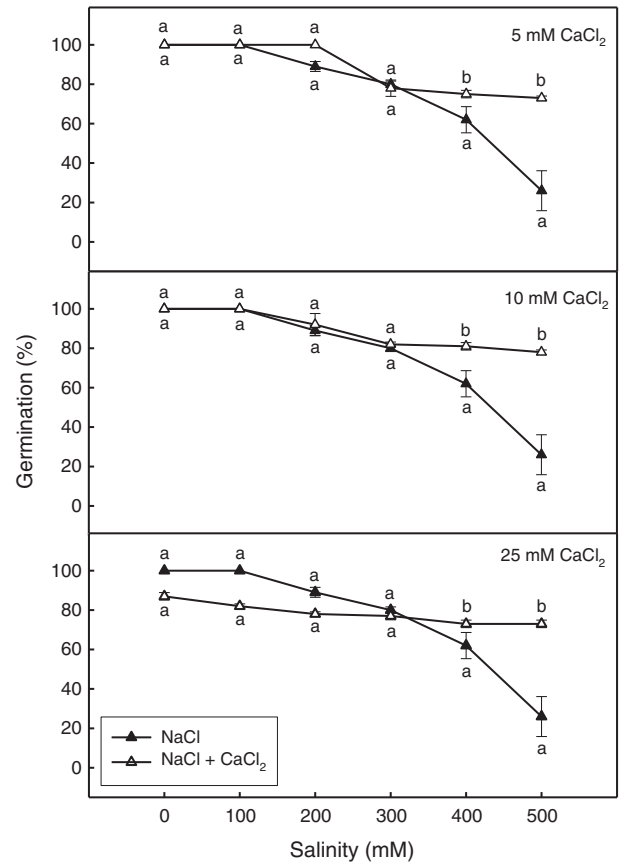


Fig. 3. Percent germination of *Phragmites karka* in NaCl with and without different concentrations of CaCl_2 at 20–30 °C. Symbols having the same letters are not significantly different from each other within each CaCl_2 treated and non-treated seeds at similar salinity levels (Bonferroni test, $P < 0.05$).

Sodium is generally the dominant cation in saline substrates which leads to increase in Na^+ flux that competes with Ca^{2+} at the binding sites. Addition of calcium may counter the adverse consequences by providing protection to the integrity and permeability of plasma membranes against Na^+ toxicity (Tester and Davenport, 2003).

Alleviation of salt toxicity on germination by Ca^{2+} has been studied in several crop plants like peas, wheat, sunflower, tomato (Bonilla et al., 2004; Daowei and Moxin, 2010; Li-Yun and Ming-You, 2010; Turkmen et al., 2004), *H. ammodendron*, *Artemisia ordosica*, *Aristida adscensionis*, *Bassia dasyphylla*, *Pisum sativum*, *Chenopodium album* (Tobe et al., 2003, 2004; Yao et al., 2010) and several other halophytic species of Pakistan coast (Gul and Khan, 2006). In the present study addition of Ca^{2+} to the growth medium also generally improved seed germination, which was more evident at the optimal temperature of 20:30 °C; being conducive for germination (Zehra and Khan, 2007).

It has been argued that high concentration of salts result in a rise of cytoplasmic calcium and extracellular calcium may double this concentration. Another possibility is that extracellular Ca^{2+} first alters Na^+ influx directly, and maintains Na^+ and K^+ homeostasis via SOS (salt overly sensitive) pathway. Increasing cytoplasmic calcium activates a Ca^{2+} sensor protein SOS3, which binds to a ser/thr protein kinase SOS2 (Halfter et al.,

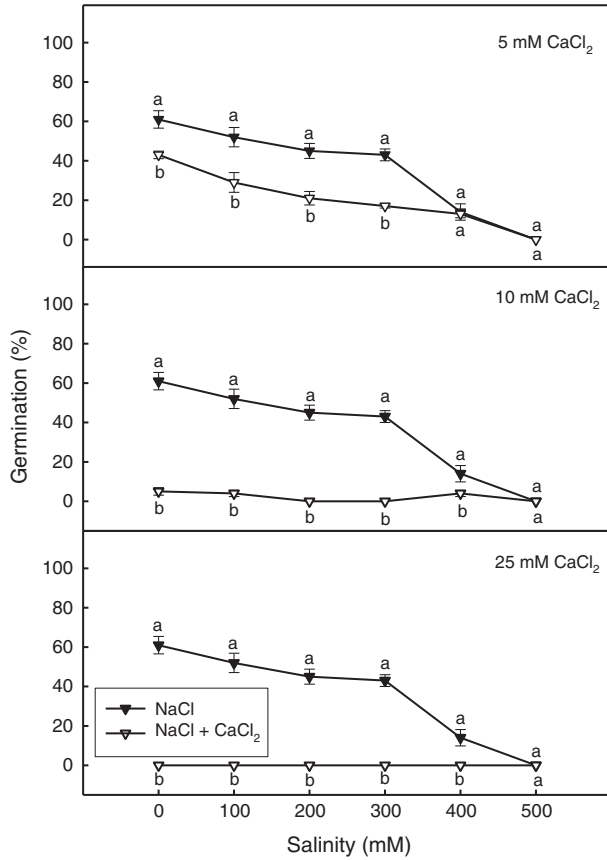


Fig. 4. Percent germination of *Phragmites karka* in NaCl with and without different concentrations of CaCl₂ at 25–35 °C. Symbols having the same letters are not significantly different from each other within each CaCl₂ treated and non-treated seeds at similar salinity levels (Bonferroni test, $P < 0.05$).

2000). SOS2 phosphorylates and increases activity of SOS1, a Na⁺/H⁺ antiporter localized at the plasma membrane which transports Na⁺ out of the cytoplasm (Chinnusamy et al., 2005; Shi et al., 2002).

Germination of *P. karka* seeds was inhibited by solutions with increasing NaCl concentrations, in the absence of light and at cooler and higher temperatures regimes than at the optimum (20/30 °C). Application of calcium partially or completely alleviated the effect of salinity at cooler temperature and compensated adequately for light. However at warmer thermo-period, it synergistically inhibited seed germination with NaCl. Ca²⁺ is required for the activation of many developmental events that are triggered by light. Increase in cytoplasmic calcium in leaf development of *Sorghum bicolor* activates G-proteins, which is involved in signal transduction of phytochromes (Sonan and Sopory, 1998). A direct link between phytohormones and transient rise in cytoplasmic free calcium in chloroplast of wheat as well as in protoplast of dark-grown wheat seedlings was demonstrated (Mehta et al., 1993; Shacklock et al., 1992).

Dark-induced germination inhibition of *P. karka* was partially alleviated by the addition of Ca²⁺ as also reported for *U. setulosa* and *Arthrocnemum macrostachyum* (Saeed, 2002; Shaikh et al., 2007). Perturbation in cytoplasmic calcium activates calcium sensor proteins such as calmodulin (CaM),

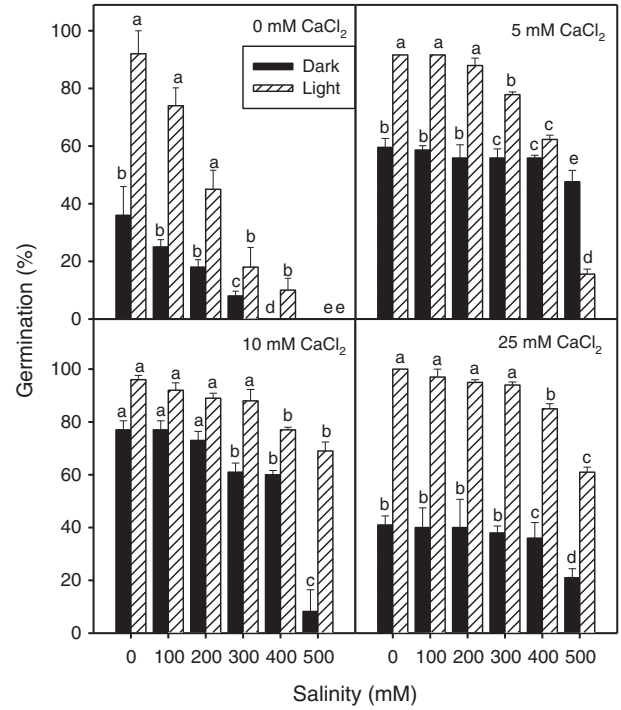


Fig. 5. Percent seed germination of *Phragmites karka* in light and dark treatments with and without different concentrations of CaCl₂ at 10–20 °C. Bars having the same letters within each light and dark treatments are not significantly ($P < 0.05$) different to each other (Bonferroni test). Bars represent mean ± SE.

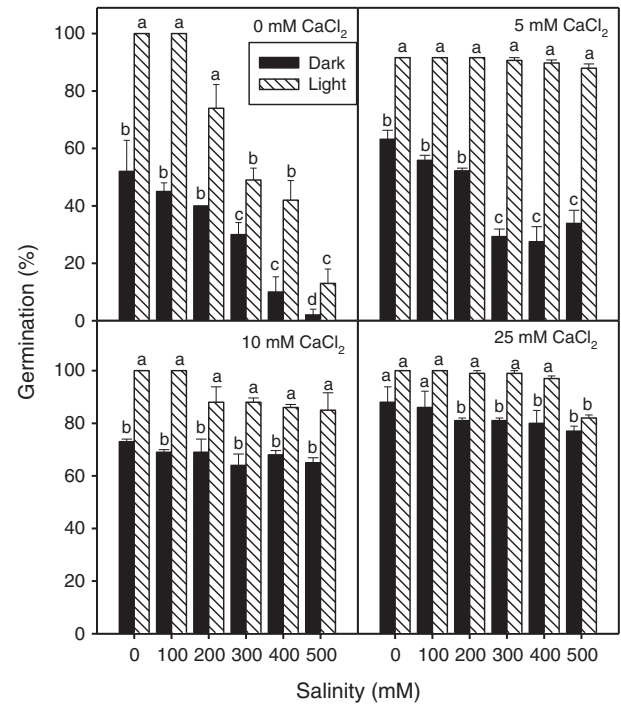


Fig. 6. Percent seed germination of *Phragmites karka* in light and dark treatments with and without different concentrations of CaCl₂ at 15–25 °C. Bars having the same letters within each light and dark treatments are not significantly ($P < 0.05$) different to each other (Bonferroni test). Bars represent mean ± SE.

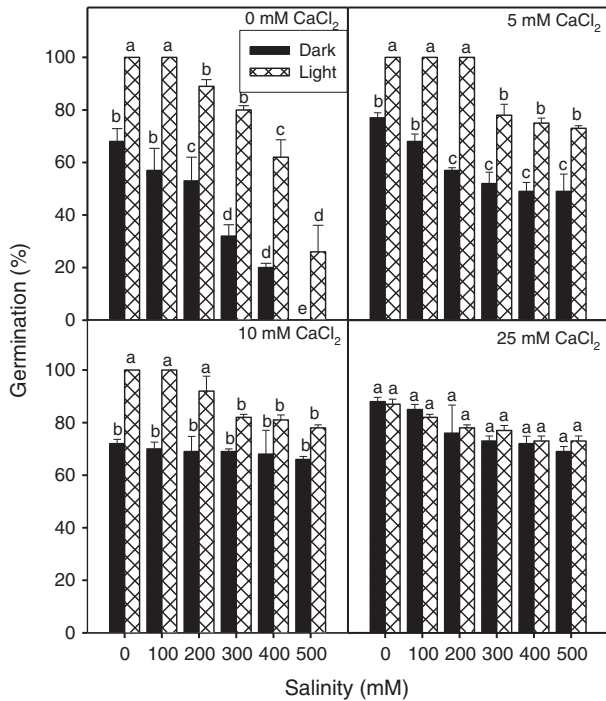


Fig. 7. Percent seed germination of *Phragmites karka* in light and dark treatments with and without different concentrations of CaCl_2 at 20–30 °C. Bars having the same letters within each light and dark treatments are not significantly ($P < 0.05$) different to each other (Bonferroni test). Bars represent mean \pm SE.

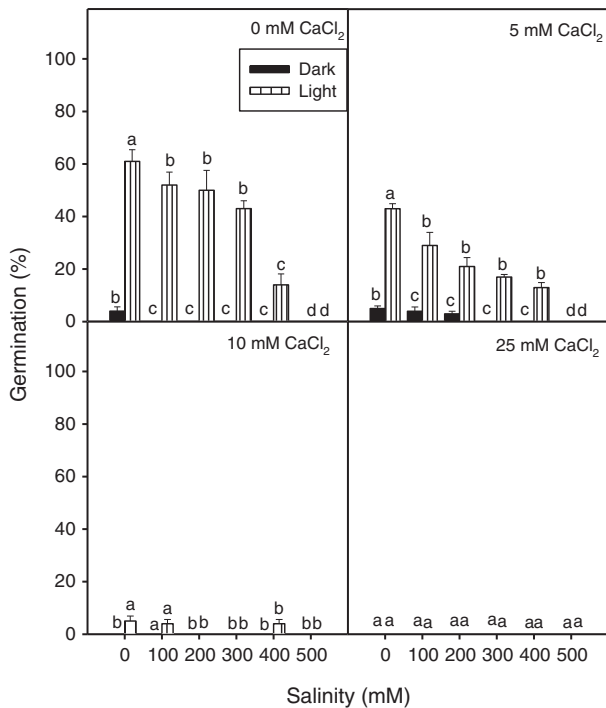


Fig. 8. Percent seed germination of *Phragmites karka* in light and dark treatments with and without different concentrations of CaCl_2 at 25–35 °C. Bars having the same letters within each light and dark treatments are not significantly ($P < 0.05$) different to each other (Bonferroni test). Bars represent mean \pm SE.

Table 1

Rate of germination (mean \pm standard error) of *P. karka* in different salinities with various concentrations of CaCl_2 at different temperature regimes. Different letters in superscript represent significant ($P < 0.05$) differences within each salinity treatment, Bonferroni test.

CaCl_2	NaCl	Temperature regimes			
		10–20 °C	15–25 °C	20–30 °C	25–35 °C
0 mM	0	31.55 \pm 2.69 ^a	40.60 \pm 0.08 ^a	45.90 \pm 1.41 ^a	27.15 \pm 2.15 ^a
	100	23.10 \pm 2.23 ^a	40.10 \pm 0.51 ^a	43.05 \pm 0.83 ^a	20.10 \pm 1.93 ^a
	200	11.00 \pm 1.25 ^a	26.80 \pm 2.30 ^a	35.30 \pm 1.05 ^a	18.55 \pm 1.38 ^a
	300	4.85 \pm 1.73 ^a	16.30 \pm 1.15 ^a	25.55 \pm 0.51 ^a	16.85 \pm 0.98 ^a
	400	1.60 \pm 0.80 ^a	12.50 \pm 1.87 ^a	18.95 \pm 1.33 ^a	4.95 \pm 1.47 ^a
5 mM	0	34.75 \pm 0.64 ^a	42.85 \pm 0.17 ^a	40.10 \pm 0.25 ^a	13.85 \pm 1.02 ^a
	100	33.15 \pm 0.50 ^b	42.65 \pm 0.39 ^a	39.35 \pm 0.59 ^a	10.05 \pm 1.08 ^a
	200	29.80 \pm 1.20 ^b	41.95 \pm 0.61 ^b	36.80 \pm 0.68 ^a	6.95 \pm 0.66 ^a
	300	25.70 \pm 0.58 ^b	37.95 \pm 0.46 ^b	34.00 \pm 1.40 ^b	6.40 \pm 0.82 ^a
	400	19.50 \pm 0.54 ^b	36.80 \pm 0.69 ^b	32.50 \pm 0.53 ^b	4.85 \pm 0.86 ^a
10 mM	0	30.70 \pm 0.59 ^a	41.00 \pm 0.29 ^a	43.05 \pm 0.43 ^a	1.00 \pm 0.38 ^b
	100	29.45 \pm 0.90 ^b	37.95 \pm 0.36 ^a	40.80 \pm 1.34 ^a	0.80 \pm 0.33 ^b
	200	27.75 \pm 0.63 ^b	36.20 \pm 2.07 ^b	38.90 \pm 2.33 ^a	0.00 \pm 0.00 ^b
	300	26.90 \pm 1.25 ^b	34.40 \pm 0.79 ^b	36.05 \pm 0.51 ^b	0.00 \pm 0.00 ^b
	400	18.35 \pm 1.13 ^b	32.50 \pm 0.95 ^b	35.05 \pm 1.12 ^b	0.80 \pm 0.33 ^a
25 mM	0	28.55 \pm 0.50 ^a	41.85 \pm 0.40 ^a	38.80 \pm 0.74 ^b	1.40 \pm 0.57 ^b
	100	27.30 \pm 1.33 ^a	41.05 \pm 0.43 ^a	35.95 \pm 0.41 ^a	0.00 \pm 0.00 ^b
	200	26.35 \pm 0.39 ^b	39.20 \pm 0.36 ^b	34.40 \pm 0.77 ^a	0.00 \pm 0.00 ^b
	300	24.85 \pm 0.46 ^b	37.75 \pm 0.71 ^b	32.95 \pm 0.96 ^b	0.70 \pm 0.40 ^b
	400	18.45 \pm 0.62 ^b	38.10 \pm 0.51 ^b	31.45 \pm 0.97 ^b	0.00 \pm 0.00 ^a
500	12.00 \pm 0.59 ^b	28.95 \pm 0.78 ^b	30.55 \pm 0.90 ^b	0.00 \pm 0.00 ^a	

calcunin B-like proteins (CBLs), and calcium dependent protein kinases (CDPKs), and these proteins are involved in activation of certain metabolic pathways. Among them CDPKs are of particular interest, being involved in embryogenesis, seed development and germination in sandalwood (*Santalum album*) (Anil et al., 2000). CaM proteins in vegetative tissues bind with

Table 2

Results of three-way analysis of variance of characteristics by salinity (S), temperature (T) and calcium (Ca) for germination percentage and rate of germination in seeds of *Phragmites karka*.

Dependent variable	Source	df	Sum of square	F	P
Germination (%)	Salinity (S)	5.00	13032.00	343.70	0.0001
	Temperature (T)	3.00	109101.44	2877.40	0.0001
	Calcium (Ca)	3.00	5374.56	141.75	0.0001
	S \times T	15.00	525.04	13.85	0.0001
	S \times Ca	15.00	2056.16	54.23	0.0001
	T \times Ca	9.00	7034.30	185.52	0.0001
	S \times Ca \times T	45	319.54	8.43	0.0001
Rate of germination	Salinity (S)	5.00	2515.73	575.75	0.0001
	Temperature (T)	3.00	17707.38	4052.51	0.0001
	Calcium (Ca)	3.00	813.04	186.07	0.0001
	S \times T	15.00	75.00	17.16	0.0001
	S \times Ca	15.00	342.15	78.30	0.0001
	T \times Ca	9.00	943.09	215.83	0.0001
	S \times Ca \times T	45	39.60	9.06	0.0001

other proteins implicated in physiological processes including cation transport, cyto-skeletal arrangements (Luan et al., 2002; Reddy et al., 2002). Isoforms of calmodulins (Ca^{2+} sensor, CaM) were found in dry axes, cotyledons of pea seeds and they accumulated during imbibitions prior to radicle protrusion (Duval et al., 2002). These isoforms isolated from pea seeds might then be responsible for the differential abilities to bind various CaM binding peptides and could cause different activation of CaM targets other than NAD kinase. CBLs activate multiple stress genes and enhance tolerance in salt stress (Cheng et al., 2002). This may indicate that Ca^{2+} ions perhaps activate proteins also in the dark similar to light triggering germination.

Seed germination of *P. karka* was partially alleviated at the cooler thermoperiod (10/20 °C) by the application of calcium. Ca^{2+} partially ameliorated the chilling induced (10 °C) oxidative stress from germination of wheat grains (Nayyar and Kaushal, 2002). Calcium ions detoxified the oxidative molecules by increasing the activity of antioxidant enzymes which are involved in the direct processing of active oxygen species and calcium catalyzes the reactions of these enzymes (Rental and Knight, 2004). Ca^{2+} could not alleviate the high temperature (25/35 °C) stress on germination perhaps due to the inactivation of certain enzymatic activities.

The sea coast of Pakistan extending over some 1000 km from Seer Creek in Sindh province to Jiwani in Balochistan is home to about 100 halophyte species, the biology of which is not well understood and their utility as cash crops needs investigation (Khan, unpublished). Seed germination studies on *P. karka* reported here provide an explanation of why so many halophytes survive in the sub-tropical maritime desert of Karachi and its vicinity. The base rock of the entire area being calcareous, it is suggested that the calcium which is not only promoting higher germination but also helping the species to sustain growth and reproduce in saline conditions. The mechanisms which are involved in promoting seed germination under high salinity; dark and low temperature stress need elucidation, focusing on how Ca^{2+} modulates seed germination responses at cellular and molecular levels.

Acknowledgements

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