

## COMPARATIVE EFFECT OF NaCl AND SEA SALT ON GERMINATION OF HALOPHYTIC GRASS *PHRAGMITES KARKA* AT DIFFERENT TEMPERATURE REGIMES

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### Abstract

Seed germination of salt tolerant grasses, which could serve as non-conventional fodder/forage crop for saline land is more sensitive to salinity than dicotyledonous halophytes. *Phragmites karka* (Retz.). Steud., is a perennial halophytic grass, distributed in marshy places around Karachi. To determine tolerance limits of germination, we conducted germination experiments in NaCl and sea salt (0, 10, 20, 30, 40 & 50 dS m<sup>-1</sup>) at alternating temperature regimes (10:20, 15:25, 20:30 and 25:35°C) for 12h: 12h light: dark period & 24- h dark photoperiod. Increase in salinity substantially inhibited seed germination both in NaCl and sea salt. Germination in light was higher in comparison to dark. Effect of NaCl in general was not significantly different from sea salt and optimal germination percentages were obtained at 20:30°C. However, NaCl prevented more seeds from germination at 25:35°C in comparison to sea salt. Ungerminated seeds were transferred from salinity to distilled water after 20 days and the highest percentage of recovery was at 50 dS m<sup>-1</sup> both in NaCl (20:30°C) and sea salt (10:20°C & 20:30°C). Increase in salinity significantly inhibited seed viability and lowest viable seeds were obtained at highest salinity.

### Introduction

Salinization due to human interference is rapidly increasing and has reached alarming levels in the arid and semi-arid regions of world. The sub-standard canal irrigation system in arid regions has substantially decreased the productivity of 400 million hectares of agricultural land (Akinel & Simsek, 2004). Utilization of saline lands and poor quality irrigation water in arid regions are major challenges. The use of economically useful salt tolerant plants in rehabilitation programmes for a saline ecosystem would be a useful option.

A major impediment in implementing such an approach is the poor germination of halophytes under saline conditions: germination decreases with an increase in salinity (Khan & Gul, 2006). Salinity levels and their effect on germination vary from species to species (Baskin & Baskin, 1998). Salinity causes osmotic stress (Nandwal *et al.*, 2000; Daniela *et al.*, 2004) or specific ionic effects, which delay, reduce or completely inhibit seed germination (Munns, 2002; Hanselin & Eggen, 2005). The data available on the effect of salinity on the seed germination of salt tolerant perennial grasses showed that most of them could germinate in NaCl concentrations below 300 mM (Lombardi *et al.*, 1997, 1998, Khan & Gul 2006), while some could germinate at 500 mM NaCl (Khan & Gulzar, 2003). Mooring *et al.*, (1971) reported that *Spartina alterniflora* seeds could germinate in up to 1027 mM NaCl.

Most studies focused primarily on the effect of NaCl on seed germination because it is the most abundant salt. The effects of sea water on germination is poorly reported (Joshi & Kumar, 1993; Houle *et al.*, 2001; Dauod *et al.*, 2001; Hanselin & Eggen, 2005) despite the fact that effect of sea water on seed germination of coastal halophytes may be different from NaCl (Zia & Khan, 2002; Joshi *et al.*, 2005).

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Temperature is probably also an important factor influencing seed germination of halophytes (Masuda *et al.*, 1999). The maintenance of viability, dormancy regulation, ability to germinate, and speed of germination are affected by temperature to various degrees (Al-Khateeb, 2006; Godoi & Takaki, 2004). Some halophytes, such as *Halostachys capsica*, *Kalidium foliatum*, *Halocnemum strobilaceum*, *Panicum turgidum* and *Prosopis juliflora* are sensitive to change in temperature regime (Al-Khateeb, 2006; El-Keblawy & Al-Rawai, 2005; Song *et al.*, 2004) while *Arthrocnemum macrostachyum*, *Caragana koshinskii* and *Suaeda fruticosa* showed only a small response to changes in temperature (Khan & Ungar, 1996, 1998; Khan & Gul, 1998; Zheng *et al.*, 2004).

Light appears to play a major role in seed germination of a number of halophytes (Guterman, 1993; Baskin & Baskin, 1998; Redondo *et al.*, 2004). Germination of seeds of various halophytes occurs during that time of the year when a combination of day length, temperature, and salinity are optimal (Ungar, 1995). Studies on the interaction between salinity and light on germination indicated a high degree of variability of the effect of light on seed germination under saline and non-saline conditions (Guterman *et al.*, 1995; Lorenzen *et al.*, 2000; Godoi & Takaki, 2004; Redondo *et al.*, 2004; Zheng *et al.*, 2004, 2005; El-Keblawy & Al-Rawai, 2005; Kambizi *et al.*, 2006).

*Phragmites karka* (Retz.) Steud is distributed along streams, wet grassland and swamps in Africa, Asia, New Guinea, Northern Australia, and is also widely distributed in Pakistan. *Phragmites karka* usually form a pure community, however, it has been found in association with *Sporobolus ioclados*, *Dicanthium annulatum*, *Suaeda fruticosa* and *Haloxylon stocksii* in saline areas of Karachi University campus. *Phragmites karka* is used as decoration, weaving material and for making musical instruments. It is also grazed in some regions of New Guinea and can withstand heavy floods and is an excellent stabilizer of eroding river banks (Rosa-Innes, 1977).

The objectives of this research were to evaluate the following: 1) The salt tolerance of seeds of *Phragmites karka* during germination. 2) Compare the inhibitory effect of sea water on seed germination compared to NaCl. 3) The role of light on seed germination under saline conditions, 4) The role of thermoperiods in controlling seed germination under saline conditions. 5) Dormancy induction under highly saline conditions.

## Materials and Methods

Seeds of *Phragmites karka* collected in December 2004 from salt flats situated on the Karachi University campus were separated from the inflorescences, surface sterilized with 0.85% Sodium hypochlorite solution (clorox) for 1 minute and then thoroughly washed with distilled water. The germination experiments were carried out in 50 x 9 mm (Gelman No. 7232) air tight plastic Petri dishes. Four replicates of 25 seeds were placed in each Petri dish and imbibed in 5 ml of test solution. The salinity concentrations used were 0, 10, 20, 30, 40 and 50 dS m<sup>-1</sup> NaCl and sea salt. Dishes were placed in germinators maintained at temperature regimes 10-20, 15-25, 20-30 and 25-35°C with 12 h photoperiods (Sylvania cool white fluorescent lamps, 25 µmol.m<sup>-2</sup>.s<sup>-1</sup>, 400-750 nm). Seed germination was recorded on every alternate day for 20 days. Seeds were also germinated in complete darkness by using photographic envelopes at above mentioned temperature regimes and germination was recorded once after 20 days. Seeds were considered to be germinated once the radicle had emerged. The rate of germination was estimated by using modified Timson Index of germination velocity,  $\Sigma G/t$ , where  $G$  is the percentage of seed germinated at 2-day intervals and  $t$  is the total germination period (Khan & Ungar, 1984). The maximum value possible for our data using this index was 50 (i.e., 1000/20). The higher the value, more rapid the germination.

After incubation while imbibed in saline treatments for 20 days, seeds that did not germinate were transferred to distilled water, in order to study the recovery of the ability to germinate. Recovery was determined using the index  $(a-b)/(c-b) \times 100$ , where  $a$  is the total number of seeds germinated after being transferred to distilled water,  $b$  is the total number of seeds germinated in saline solution and  $c$  is the total number of seeds. Imbibing seeds in a 1% tetrazolium solution determined viability of remaining ungerminated seeds for 24 hours (Copeland & McDonald, 1995). Embryos that turned red were counted as viable.

Germination and recovery data was transformed (arcsine) prior to statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS version for windows release 9.0 (Anony., 1999). The effect of salts, their concentrations and temperature on percent germination, recovery and viability were examined using a three-way analysis of variance (ANOVA). A Bonferroni test was used ( $P < 0.05$ ) to determine significant differences between germination means in light and dark conditions in NaCl and sea salt, at various levels of salinity and temperatures. Linear regression analysis was used to determine the relationship between rate of germination and rate of recovery in salinity at different temperatures and in different salts.

## Results

A two-way ANOVA indicated significant ( $P < 0.05$ ) individual effects of salinity, temperature and their interactions on seed germination, recovery and viability of *P. karka* (Table 1). Higher number of seeds germinated in distilled water, compared to those imbibed in salts, at all temperature regimes (Fig. 1). At 25-35°C, NaCl inhibited germination of comparatively more seeds than sea salt (Fig. 1). However, seeds were still able to germinate at highest salinity concentration (50 dS m<sup>-1</sup>) at moderate temperatures (15-25 and 20-30°C).

Seed germination in the dark, compared to those germinated in 12 h photoperiods was substantially reduced both in the control and saline treatments, with reduction greatest at warmer temperatures where only a few seeds germinated (Fig. 2). A linear regression showed decrease in rate of germination with increasing salinity at different temperature regimes with  $R^2$  values from 0.96-0.558 in NaCl and from 0.94-0.77 in sea salt. The rate was highest in non-saline control and decreased significantly with an increase in salinity (Fig. 3). Temperature also influenced the rate of germination and at higher temperature seeds showed low rate of germination in both salts in comparison to 15-25°C and 20-30°C temperature regimes (Fig. 3).

After 20 days under various salinity, temperature and light treatments with subsequent transfer to distilled water, seeds germinated more readily and in higher numbers than in salinity treatments (Fig. 4). Cooler temperatures prevented recovery due to induced dormancy, more so in NaCl treatments than in sea salt.

Linear regression demonstrated the promotion in rate of recovery with the increase in salinity with  $R^2$  value from 0.41-0.85 in NaCl and from 0.40-0.91 in sea salt at different temperature regimes (Fig. 5). When imbibed in NaCl, recovery was low at both higher and lower temperatures, whereas in sea salt, recovery was not affected by temperature (Fig. 5).

Seed viability remained largely unaffected with both NaCl and sea salt treatments (Fig. 6). At cooler and warmer thermoperiods and at higher salinity concentrations few seeds lost their viability. However, secondary dormancy was induced at extreme temperature regimes and it increased with the increase in NaCl concentrations (Fig. 6).

**Table 1. A two-way ANOVA of the effect of salinity (S), temperature (T) and their interactions in NaCl on seeds of *Phragmites karka* (p< 0.05).**

Dependent variables	S	T	SXT
Germination (%)	5.903*	55.436***	2.509*
Recovery (%)	4.194*	4.856*	5.442**
Viability (%)	5.397*	11.42**	5.169**

**Table 2. A two-way ANOVA of the effect of salinity (S), temperature (T) and their interactions in sea salt on seeds of *Phragmites karka* (p< 0.05).**

Dependent variables	S	T	SXT
Germination (%)	8.199**	16.75***	9.175***
Recovery (%)	1.371 <sup>ns</sup>	12.268***	6.834***
Viability (%)	1.731 <sup>ns</sup>	0.343 <sup>ns</sup>	6.79***

## Discussion

Successful establishment of perennial halophytic grasses in coastal and inland salt marshes depends on their ability to germinate as the environmental conditions—water temperature and light become favorable during the smallest window of opportunity that may open only occasionally (Khan & Gulzar, 2003; Ungar, 1995; Baskin & Baskin, 1998; Song *et al.*, 2004; Khan & Ungar, 1997). Species distributed around the coastal areas have to contend with sea water which contains a variety of salts dominated by NaCl. There are a number of reports that the effect of NaCl on germination is usually different from that of sea water and that this difference could vary with species (Joshi *et al.*, 2005).

Seeds germinated best in non-saline conditions and germination decreased with an increase in both NaCl and sea salt concentrations, as expected. The seeds of perennial grasses are reported to be moderately salt tolerant at germination (Khan & Gul, 2006) with the exception of *Spartina alterniflora*, which is reported to germinate at 1.03 M NaCl (Mooring *et al.*, 1971). The seeds of *Aeluropus lagopoides*, *Sporobolus ioclados* and *Urochondra setulosa* showed some germination at 500 mM NaCl and this high salt tolerance was attributed to their zone of distribution along the coast where they are regularly inundated by sea water or exposed to underground brackish or sea water regularly (Khan & Gulzar, 2003). *Halopyrum mucronatum*, on the other hand, always occupies high ground on the sand dunes and is occasionally exposed to high tides (Khan & Ungar, 2001) and germinate only up to 350 mM NaCl. Similar reports are available for other grasses (Myers & Morgan, 1989; Lombardi *et al.*, 1998; Al-Khateeb, 2006), whereas, seeds of *P. karka* tolerated 50 dSm<sup>-1</sup> (500 mM) of NaCl and sea salt at germination stage.

Similar response has been altered for seed germination of *Aeluropus lagopoides*, *Limonium stocksii* and *Sporobolus madraspatanus* (Joshi *et al.*, 2005; Zia & Khan, 2002). Salt damage to seed germination is attributed to the reduction in water availability, toxicity of specific ions and nutritional imbalance caused by such ions (Murillo - Amador *et al.*, 2002). In the present study, germination was inhibited more by NaCl than sea salt. The salts present in sea water possess different properties and could potentially affect germination differently from NaCl (Ungar, 1978). NaCl may also affect germination by facilitating the intake of toxic ions, which may change certain enzymatic or hormonal activities of the seed (Smith & Comb, 1991). These physiochemical effects upon the seed seem to result in slower and/or lower germination or emergence.

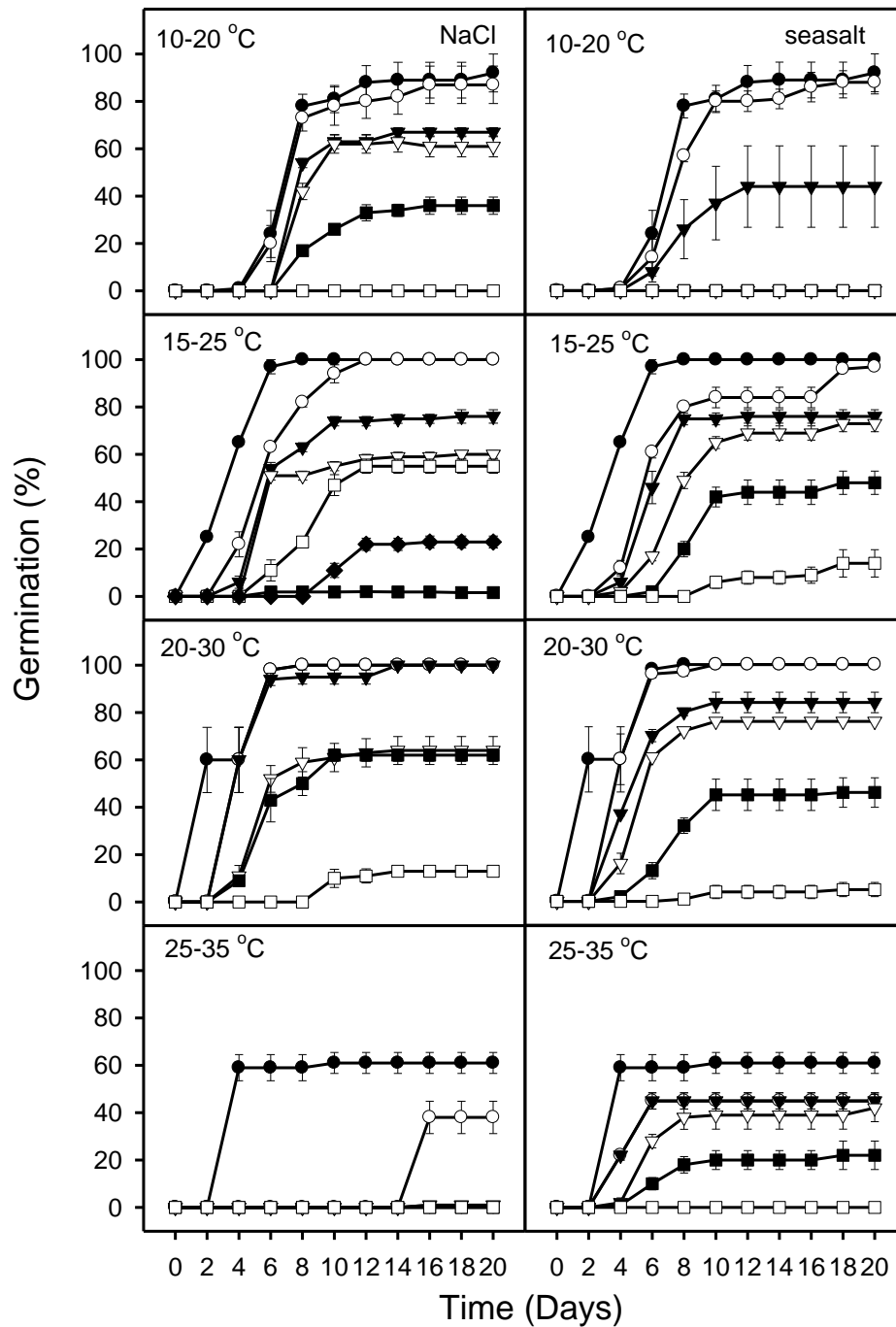


Fig. 1. Effect of NaCl and sea salt (— 0, — 10, — 20, — 30, — 40 and — 50 dSm<sup>-1</sup>) on seed germination of *Phragmites karka* at different temperatures. Different symbols represents mean  $\pm$  SE.

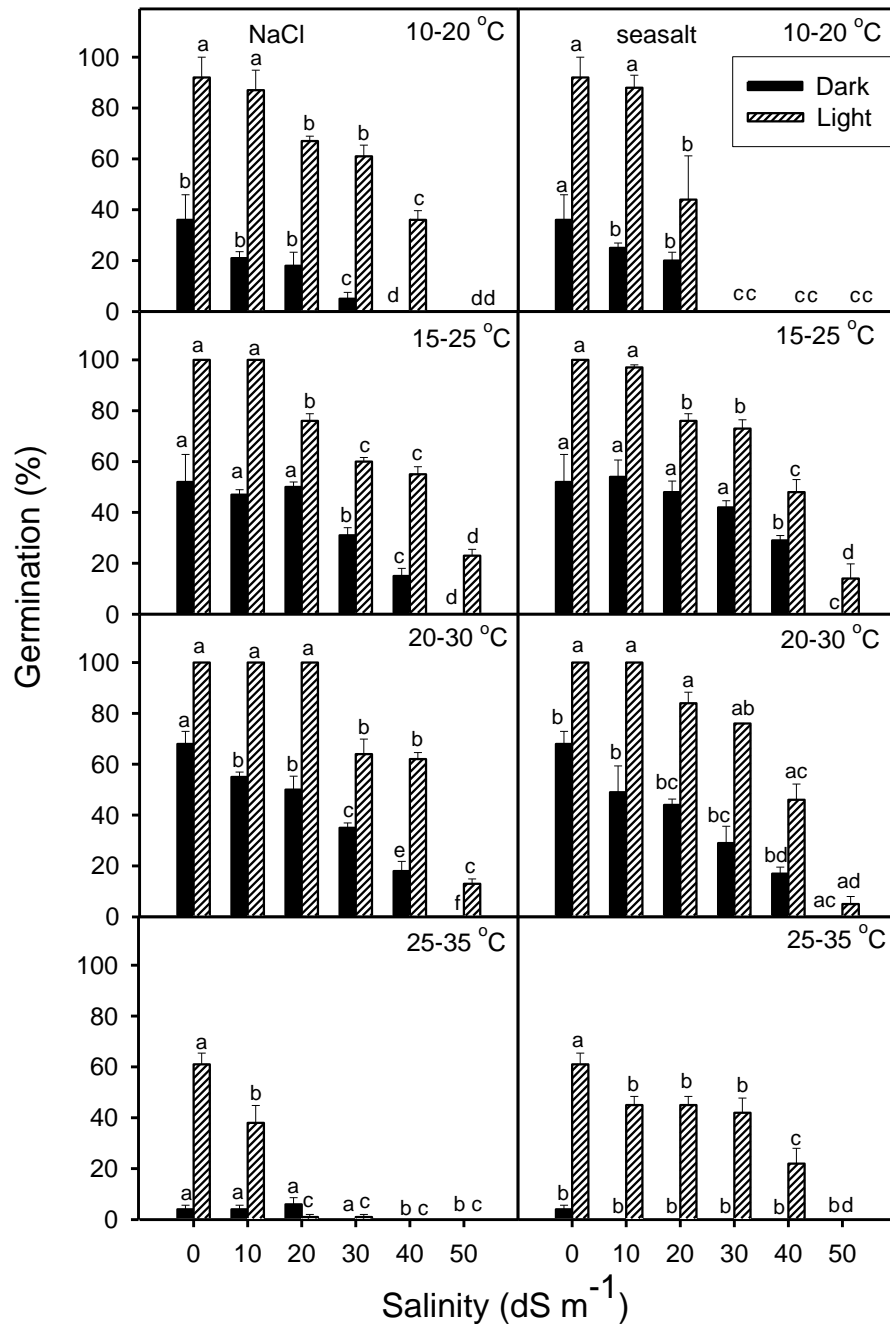


Fig. 2. Percent seed germination of *Phragmites karka* in light and dark. Bars having the same letters within light or dark treatment are not significantly different ( $p < 0.05$ ) from each other (Bonferroni test). Bars represents means  $\pm$  SE.

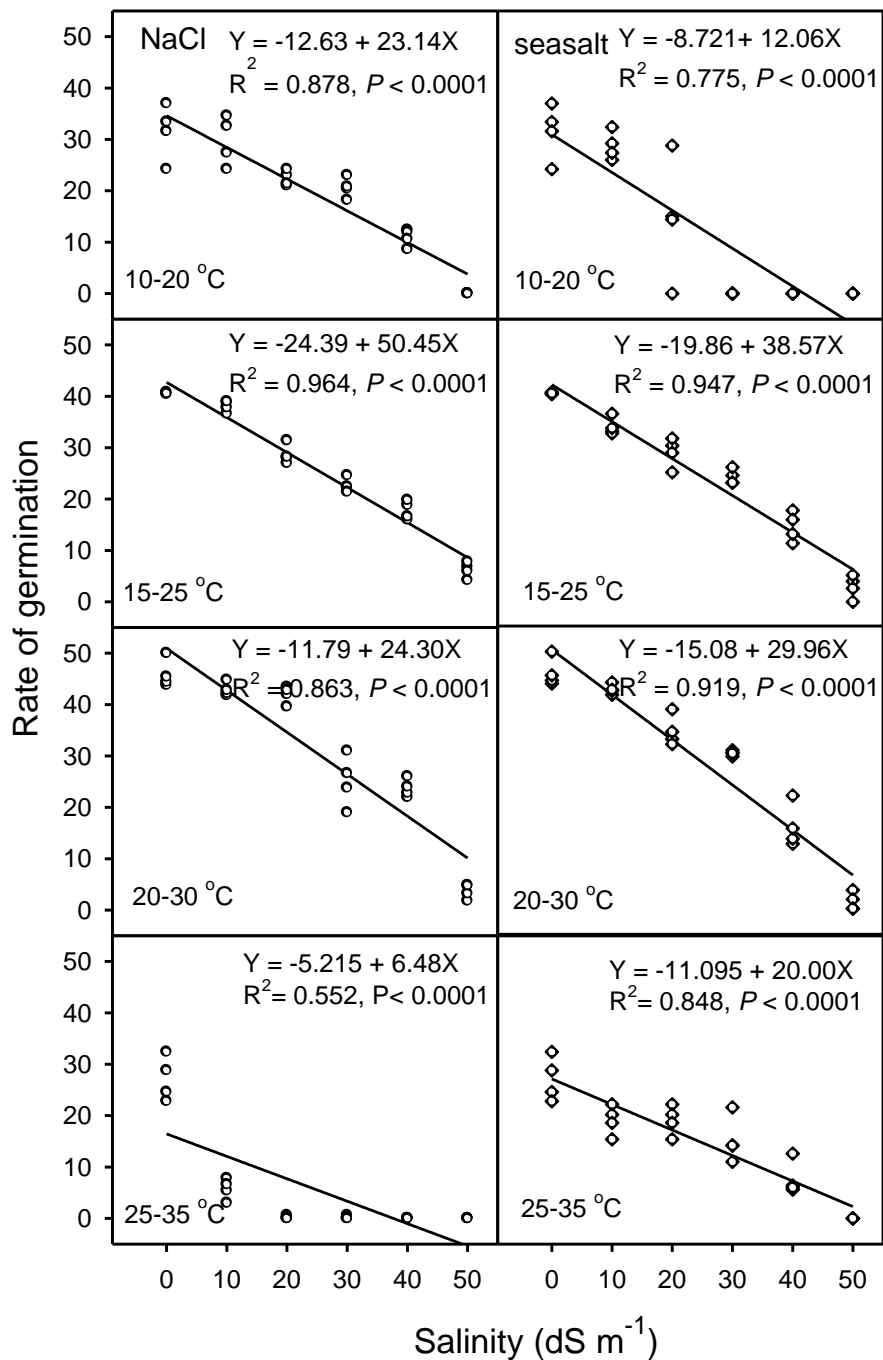


Fig. 3. Regression plots for percent germination of *Phragmites karka* seeds in various NaCl and sea salt concentrations at different temperature regimes.

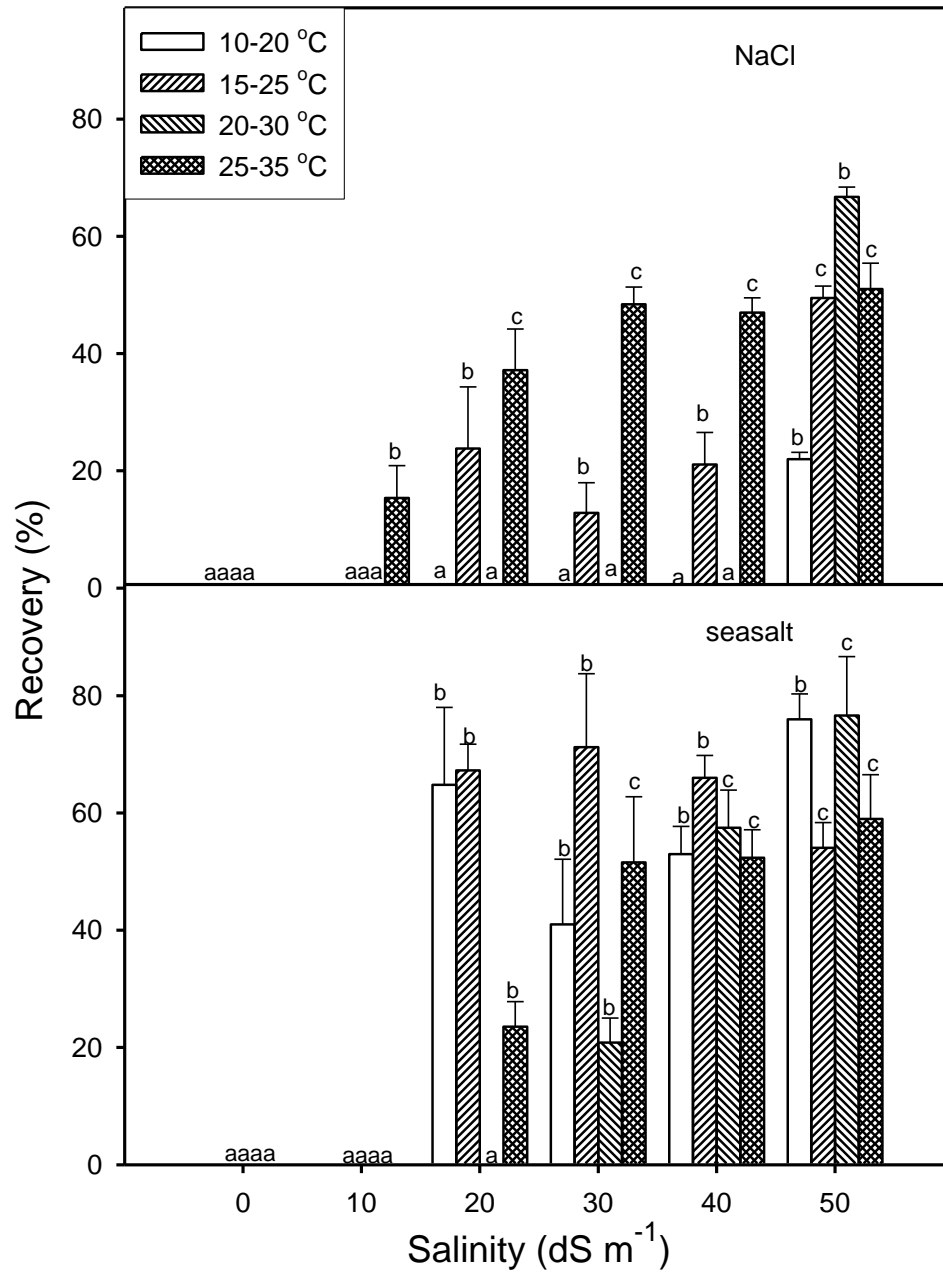


Fig. 4. Percent recovery (mean  $\pm$  SE) germination of *Phragmites karka* in various salinity treatment in NaCl and sea salt. Bars having the same letter within each temperature regimes are not significantly different ( $p < 0.05$ ) from the control (Bonferroni test).



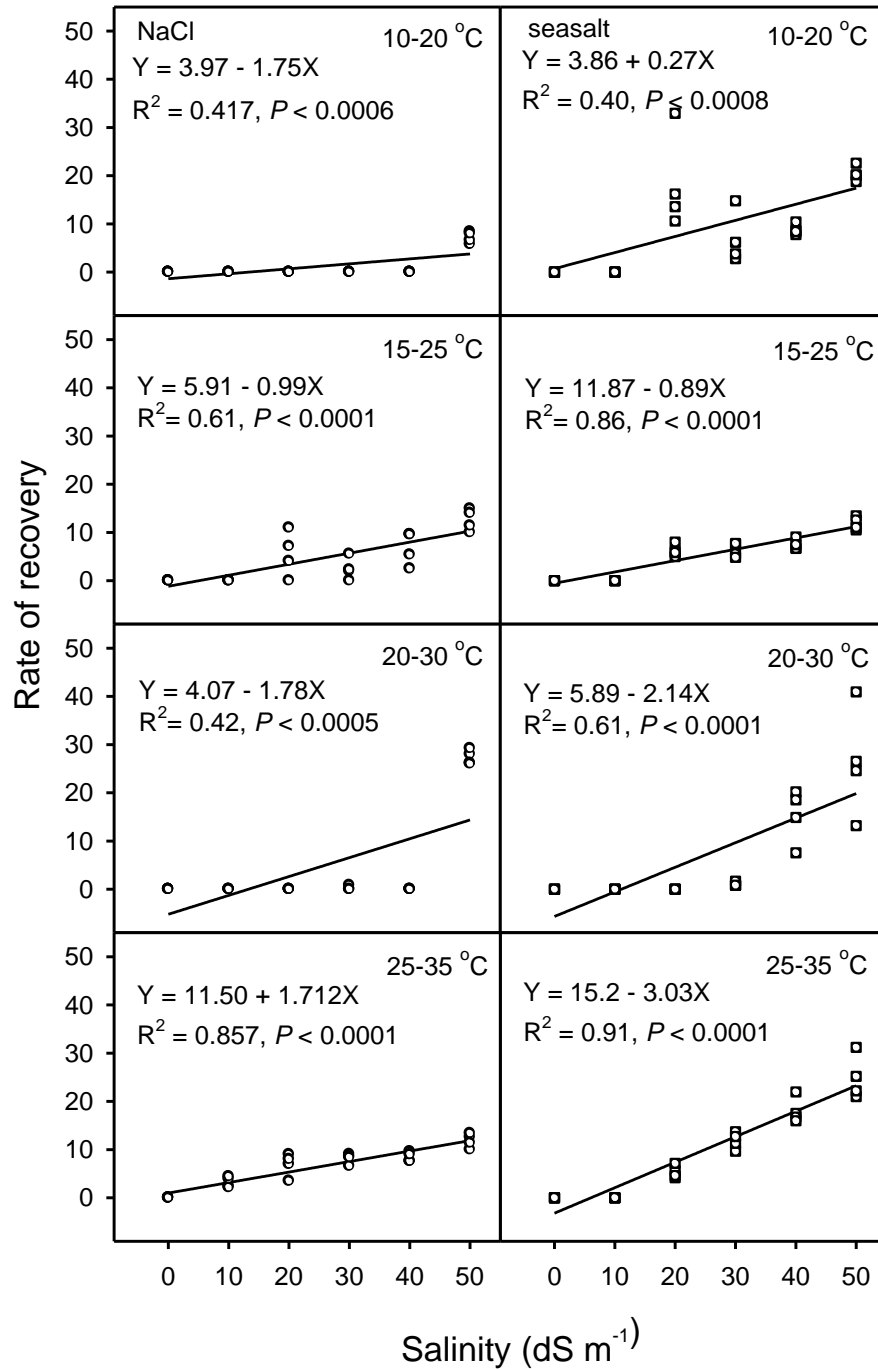


Fig. 5. Regression plots for the rate of recovery germination of *Phragmites karka* seeds in NaCl and sea salt at different temperature regimes.

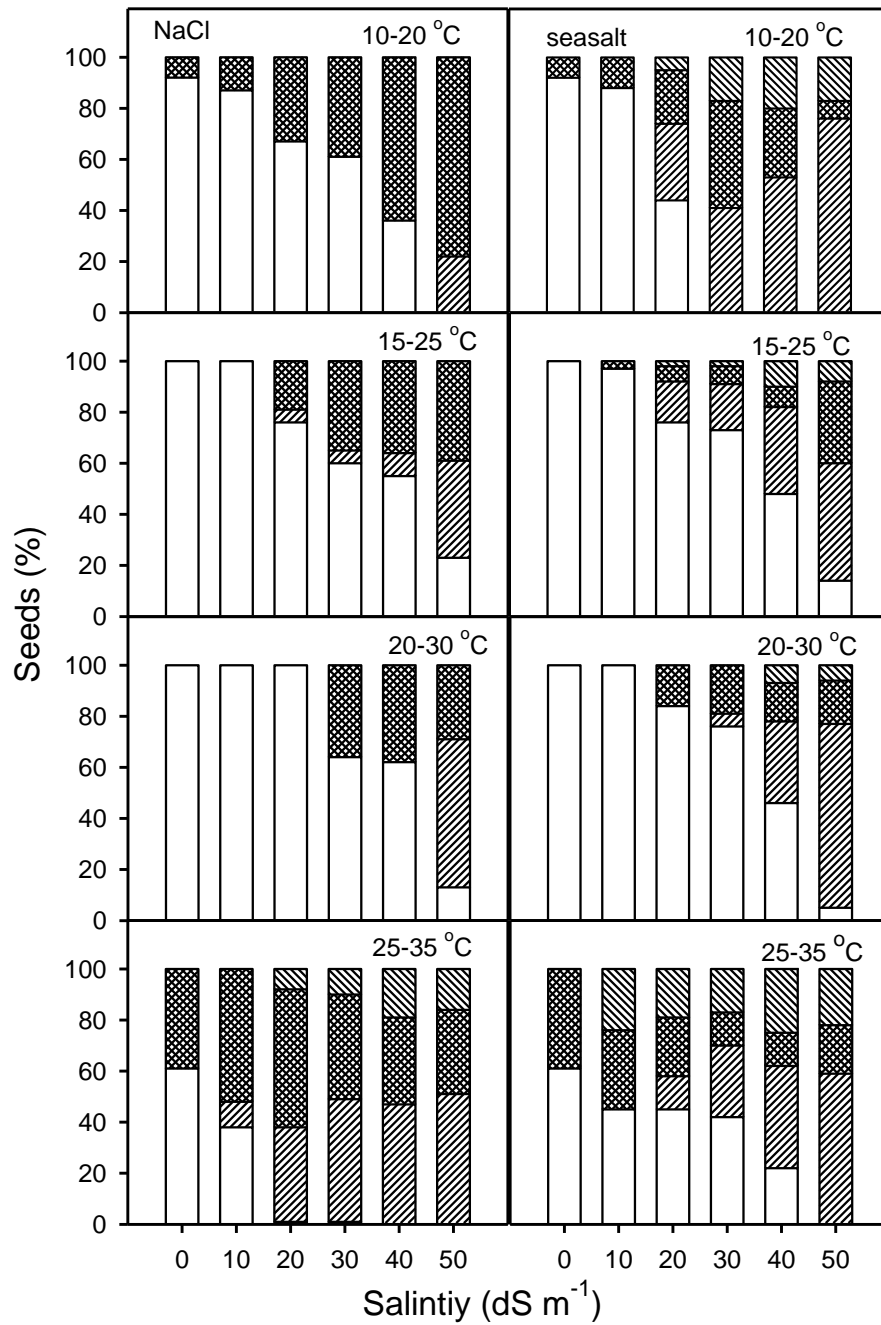


Fig. 6. Effect of NaCl and sea salt on percent seed viability of *Phragmites karka* at different temperatures. (□ germinated, ▨ recoverd, ▩ viable, ▀ dead seeds)

Grasses found in this region have the ability to germinate in warm (20 – 30 °C) conditions (Gulzar *et al.*, 2001; Khan & Ungar, 2001) and any change in optimal temperature will result in a decrease in germination (Khan & Gulzar, 2003). Seed of *Phragmites karka* were germinated in similar temperature regimes reported for the other grasses of the region. In a number of halophytic grass species viz., *Hordeum jubatum* (Badger & Ungar, 1989), *Iva annua* (Ungar & Hogan, 1970), *Puccinellia festucaeformis* (Onnis, 1981), *Diplachne fusca* (Myers & Morgan, 1989) and *Briza maxima* (Lombardi *et al.*, 1998), seed germination increased at the optimal temperature. It seems that halophytic grass species of our region are well adapted to germinate under a seasonal temperature regime when availability of moisture and soil salinity levels is favourable for germination for instance. Seeds after monsoon rains germinated at higher temperatures in high NaCl or sea salt concentrations caused a delay, or substantial inhibition in comparison optimum temperature regime in salinity. Presumably such high salt concentrations result in malfunctioning of enzymatic systems at sub optimal temperature limiting seed germination (Al- Khateeb *et al.*, 2006).

Germination of *P. karka* seeds was better in light as also reported earlier for twenty three halophytic species (Baskin & Baskin, 1998). However, some halophytes have an obligate requirement of light for germination, while in others the presence of light enhances seed germination to various degrees and still others do not require light for germination (DeVilliers *et al.*, 1994; Andrews, 1997).

Seeds of *P. karka* showed highest recovery at 20–30°C and 15–25°C. Similar results were reported by Gulzar & Khan (2001), where in *Aeluropus lagopoides* showed 80% recovery at 20:30°C. Recovery was also inhibited at low and high temperature regimes in *Urochondra setulosa* and best recovery of seeds was obtained at moderate temperature (20:30° C) regime (Gulzar *et al.*, 2001). Halophytic grasses have the ability to maintain their viability even in higher salinities and at high temperatures by adapting dormancy mechanism and they germinate quickly as the conditions become favorable. That is also an adaptive strategy to survive in salt stress conditions.

Seed viability was generally maintained in the presence of salinity at optimal temperatures, however, at cooler and warmer temperature regimes about 20% seeds lost their viability while most of them entered into salt and temperature induced seed dormancy. Redondo *et al.*, (2004) reported that higher salinity and temperatures caused the loss of viability in *Sarcocornia* spp. High temperature accelerates evaporation from the soil surface thus increasing soil salinity and causing anoxia (absence of O<sub>2</sub>) which affects respiration of seeds and inhibits the process of germination.

In conclusion, seeds of *Phragmites karka* are highly salt tolerant during germination with a significant difference between sea water and NaCl -later having more harmful effect. Seeds also become dormant at high salinity and lose viability if temperature and salinity stress increased. Our study indicates that seeds of *Phragmites karka* could germinate under higher salinity concentrations approaching sea water under moderate thermoperiod and they also germinate quickly when high salt and temperature stresses are removed for instance after monsoon rainfall. *Phragmites karka* could germinate equally well in coastal and inland salt marshes. Seed burial in mud may be harmful because it prevents seed from germination and it may also provide protection from the combination of high salinity and temperature, which may cause death of seed. During the monsoon, heavy rainfall not only brings down the salinity and temperature but also helps seed buried under mud to expose to the light providing ideal conditions for seed germination and recruitment.

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