

INTERACTIVE EFFECT OF SALT, LIGHT AND TEMPERATURE ON SEED GERMINATION AND RECOVERY OF A HALOPHYTIC GRASS - *PHRAGMITES KARKA*

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Abstract

The effect of chloride and sulfate salts of Na⁺, K⁺ and Mg²⁺ on seed germination of a halophytic grass *Phragmites karka* was studied under different dark/light conditions and a range of temperature regimes. Seeds germinated better at 20/30°C and their germination decreased with increases in salinity. This reduction in germination could be attributed to specific ion toxicity as well as variable osmotic stress due to the composition of salts. Among cations, K⁺ was generally most toxic followed by Mg²⁺ and Na⁺ but such generalizations could not be made in case of anions. Potassium sulfate was inhibitory than Mg²⁺ salts for seed germination. A better germination recovery from salt stress was found in 10/20°C. More seeds recovered when transferred from NaCl to distilled water at all temperature regimes. Seeds treated with magnesium sulfate showed better recovery at 10/20 °C, while recovery in Na₂SO₄ and MgCl₂ treated seeds was better in moderate temperatures. The salts did not affect viability of seeds which probably entered into dormancy.

Introduction

Saline soils occur worldwide which develop with the accumulation of water soluble salts to a level that inhibits plant growth. Growth inhibition which varies with the nature and properties of salt in all climatic zones is particularly severe in arid and semi-arid regions (Rengasamy, 2006) probably due to the additional stress of high temperature. Chloride and sulfate of sodium generally dominate in such soils, but salts of other ions such as calcium, magnesium, potassium are also found (Gaylord & Egan, 2006). It has been proposed that osmotic shock is usually the primary cause of inhibition in seed germination in saline soils which may be reversed by removal of this stress (Khan & Ungar, 1997); however, specific ion toxicity also plays an important role (Gaylord & Egan, 2006; Mohammad & Sen, 1990; Pujol *et al.*, 2000; Sosa *et al.*, 2005; Tobe *et al.*, 2004; Saeed *et al.*, 2011).

Ungar (1978) reported that in iso-osmotic concentrations, inorganic ions had similar effect as those of mannitol or polyethylene glycol (PEG) in several halophytes. He further stated that some species are tolerant to a combination of wide range of anions such as Cl⁻, SO₄²⁻ and CO₃²⁻ and cations such as Na⁺, K⁺ and Mg²⁺, suggesting that specific ion effects would have less influence on seed germination than the soil water potential. Similar conclusions were also drawn in some other studies on seed germination of halophytes (Duan *et al.*, 2004; Joshi *et al.*, 2005; Katembe *et al.*, 1998; Sosa *et al.*, 2005). Ion toxicity has also been reported to reduce seed germination (Poljakoff-Mayber *et al.*, 1994; Al-Karaki, 2001; Llanes *et al.*, 2005). Egan *et al.*, (1997) reported germination inhibition in *A. prostrata* by using different salts of sodium and potassium which improved after flushing with distilled water. They postulated that if such seeds did not germinate after flushing, the mortality may be due to specific ion effect (Ungar, 1991). In any case, the osmotic and specific ion effects may combine at

high salt concentrations and make it difficult to separate the proportional contribution of two stresses.

Light has been recognized as a germination controlling factor in some plant species (Baskin *et al.*, 2001; El-Keblawy & Al-Rawai, 2005, 2006; El-Keblawy *et al.*, 2011; Huang *et al.*, 2003; Puppala & Fowler, 2003; Wei *et al.*, 2008; Zia and Khan, 2008; Saeed *et al.*, 2011). Seed germination of some halophyte species like *Suaeda fruticosa* (Khan & Ungar, 1998) and *Haloxylon ammodendron* (Huang *et al.*, 2003) was not affected by light however, absence of light almost completely inhibited seed germination of *Triglochin maritima* (Khan & Ungar, 1999), *Carex* species (Kettering *et al.*, 2006) and *Eucomis autumnalis* (Kulkarni *et al.*, 2006), while it partially inhibited seed germination in *Cecropia hololeuca* (Godoi & Takai, 2004), *Juncus acutus* and *Schoenus nigricans* (Martinez-Sanchez *et al.*, 2006). It has been suggested that light may change the membrane permeability of different ions especially K⁺ and Ca²⁺ by changing membrane potential or by regulating the activity of some membrane linked pumps and channels through phosphorylation of protein kinases (Haupt & Häder, 1994; Taiz & Zeiger, 2010).

Another crucial factor in modulating seed germination response of halophytes is the great fluctuations in temperature in their habitats (Badger & Ungar, 1989; Greenwood & DuBow, 2005; Khan, 1999; Ungar, 1995; Zia and Khan, 2008; Saeed *et al.*, 2011). Germination of seeds in response to alternating temperature determines the adaptation to seasonal fluctuation and also indicates the timing of germination in field conditions (Fenner & Thompson, 2005; Kulkarni *et al.*, 2007; Tlig *et al.*, 2008). Alternating day and night temperatures promoted germination in a number of halophytes (Gul & Weber, 1999; Gulzar *et al.*, 2001; Okusanya, 1977; Song *et al.*, 2006). This reflects an adaptation to natural fluctuations of the habitat where it may be associated with germination promoting processes

(Godoi & Takai, 2004; Huang *et al.*, 2003; Kulkarni *et al.*, 2007) and providing opportunity for the recruitment and continuity of the species.

Seeds of annual halophytes usually maintain viability when exposed to high salinity and temperature stress under natural conditions (Llanes *et al.*, 2005; Ungar, 1991). Perennial halophytes generally follow a different adaptive strategy for survival like using ramets to propagate while their seeds lose viability quickly if they do not find suitable conditions for germination under saline conditions (Khan & Gul, 2006; Khan & Ungar, 1997). Recovery of seeds appears to be the function of salinity concentrations when exposed to variable thermo periods at the time of germination (Khan & Gul, 2006). Exposure to high salinity could either cause the priming of seeds before germination or it could result in their death (Gulzar & Khan, 2002; Ungar, 1995). In addition, high salinity and unfavorable photoperiod as well as thermoperiod may prevent seeds from germination however, seeds could germinate when environment becomes favorable (Rubio-Casal *et al.*, 2002; Song *et al.*, 2005). This type of response has been related to the need to take advantage of the periods with suitable conditions for establishment (Neo & Zedler, 2000).

The present study aims at evaluating the effect of different concentrations of sulfate and chloride salts of Na⁺, K⁺ and Mg²⁺ on seed germination of *Phragmites karka* (Retz.) Steud under various temperature regimes and two photoperiods. It is a widely distributed halophytic grass found in Pakistan along streams, wet grasslands, in swamps and is an excellent stabilizer of eroding river banks (Cope, 1982).

Materials and Methods

Seeds of *P. karka* were collected from salt flats at the Karachi University campus; separated from inflorescence and surface sterilized with 0.85% Clorox solution for one minute prior to the commencement of germination

experiments in air tight plastic Petri plates. 25 Seeds/plate with four replicates were germinated in 5 ml distilled water or the test solution. Seed germination (emergence of radicle) was recorded every alternate day for 20 days in dark: light condition whereas it was recorded only at the termination of experiment (20 days) in the set kept in continuous dark. After 20 days, un-germinated seeds from dark were transferred to distilled water to study the recovery of germination under same temperature and light regimes. Viability of un-germinated seeds was checked using tetrazolium chloride test.

Seeds were germinated in different concentrations (0, 100, 200, 300, 400, 500 mM) of NaCl, Na₂SO₄, MgCl₂, MgSO₄, KCl and K₂SO₄. The plates were placed in germinators maintained at different temperature regimes (10/20, 15/25, 20/30 and 25/35°C), lower temperature coinciding with 12 h night and higher with 12 h day. Seeds were illuminated with 25 μmol m⁻² s⁻¹ (400-750 nm Sylvania cool white fluorescent lamps) while another set was enveloped in photographic sheet and placed in complete dark. The osmotic potential of the above solutions was measured with Dew point micro-voltmeter (HR-337 Wescor Inc., Logan, Utah, USA), shown in Table 1.

The rate of germination was estimated by using modified Timson Index of germination velocity, $\Sigma G/t$, where 'G' is the seed germinated at 2-day interval and 't' is the total germination period (Khan & Ungar, 1984). Recovery was calculated by the following formula: (a-b)/(c-b) x100, where 'a' is the number of seeds germinated after transfer to distilled water, 'b' is the number of seeds germinated in saline solution, and 'c' is the total number of seeds. Germination data were arcsine transformed before statistical analysis using SPSS version 9.0 (SPSS Inc., 1999). The effect of treatments on germination and recovery was examined using ANOVA and a Bonferroni test was used to determine significant differences among means.

Table 1. Osmotic potential of different salts at similar mM concentrations.

Concentration (mmol L ⁻¹)	Salt types ψ_s (-MPa)					
	NaCl	KCl	MgCl ₂	Na ₂ SO ₄	K ₂ SO ₄	MgSO ₄
25	0.11	0.11	0.17	0.13	0.14	0.06
75	0.35	0.35	0.53	0.39	0.39	0.19
100	0.45	0.45	0.60	0.57	0.57	0.28
125	0.55	0.55	0.76	0.68	0.69	0.32
150	0.67	0.66	0.96	0.78	0.78	0.39
200	0.95	0.95	1.35	1.05	1.05	0.53
300	1.40	1.40	2.10	1.55	1.55	0.77
400	1.85	1.85	2.85	2.05	2.05	1.00
500	2.30	2.30	3.60	2.55	2.55	1.24

Results

A considerable variation in osmotic potential was found in similar concentration of chloride and sulfate salts on mM basis. For instance, 500 mM NaCl exerts -2.3 MPa of osmotic potential compared to -3.6 in case of magnesium chloride and -1.24 in case of magnesium sulfate (Table 1).

A three-way ANOVA showed significant individual effect of salts, concentrations, temperatures and their interactions on seed germination of *P. karka* (Table 2). Seeds germinated better in the non-saline control and seed germination was inhibited with increase in the concentration of the tested salts (Figs. 1-4). Effect of

various salts was quite variable in different concentrations and their responses were also changed with the change in temperature regimes (Figs. 1-4). A complete germination inhibition was observed in potassium sulfate both in higher and lower temperature regimes and the best germination was obtained at various concentration of magnesium sulfate. Optimum temperature for seed germination was 20/30°C in all salt treatments while in warmer temperature regimes, seeds of *P. karka* germinated only in lower NaCl concentration (Figs. 1-4). The level of toxicity for different salts in decreasing order was as follows: $K_2SO_4 > MgCl_2 > Na_2SO_4 > KCl > NaCl > MgSO_4$.

Table 2. Three-way ANOVA of seed germination, recovery and viability in different salts, concentrations, temperatures and their interactions in *Phragmites karka*.

Independent variables	Dependent variables				
	Germination (%)	Rate of germination	Recovery (%)	Rate of recovery	Viability (%)
Salt types (S)	234***	258***	56***	25***	132***
Concentration (C)	1038***	1728***	121***	127***	132***
Temperature (T)	754***	1253***	49***	67***	295***
S * C	25***	30***	10***	5***	10***
S * T	32***	45***	6***	10***	26***
C * T	31***	50***	8***	15***	3***
S * C * T	6***	7***	3***	3***	3***

Note: Number represents *F*- values: *** $p < 0.0001$

It is evident that better seed germination was found in 12 h photoperiod than complete dark in all salt treatments (Figs. 5-8). Except for NaCl, no seed germinated in dark at 10/20°C when any other salt was present in the medium (Fig. 5). More seeds germinated at 15/25°C and maximum germination in dark was obtained at 20/30°C (Figs. 6-7). Few seeds germinated in dark under any salt treatment at 25/35°C (Fig. 8).

A variable response of germination recovery was observed in various salts and temperature regimes when transferred to distilled water after 20 days of salinity (Fig. 9). At 10/20°C there were some recovery from salt stress in all treatments but it was higher in NaCl and $MgSO_4$. At 15/25°C and 20/30°C considerable recovery was obtained at the highest concentrations of NaCl, Na_2SO_4 and $MgCl_2$ treatments (Fig. 9). However little recovery was noticed in all salts except for NaCl where higher recovery was observed at 25/35°C.

Discussion

Sodium chloride has extensively been used in seed germination studies of plants including halophytes because of its preponderance in saline soils, (Khan & Gul, 2006) but studying the effect of various salts in this context has also gained importance (Katembe *et al.*, 1998; Pujol *et al.*, 2000; Bajji *et al.*, 2002; Joshi *et al.*, 2002, 2005; Tobe *et al.*, 2003, 2004). Following the premise that different cations have different effect on seed germination, the response of above findings may be interpreted as ionic effect. The data in Table 1 shows great variation in OP due

to various salts. For instance, osmotic potential of 500 mM $MgSO_4$ is -1.24 in comparison to -2.55 of K_2SO_4 and Na_2SO_4 at the same concentration whereas 500 mM $MgCl_2$ has the highest OP (-3.60) which is accordingly toxic for seed germination. Hence the role of osmotic potential of the substrate in reducing seed germination attains significance here which implies that the difference in response may be largely due to the difference in osmotic stress caused by different salts of the same concentrations however, the specific ion effects cannot be ignored. Further more in saline soils; salts may act specifically, with effect ranging from toxic to beneficial for germination depending on concentration (Tobe *et al.*, 2004).

Responses of halophytic grasses are similar to other halophytes in terms of optimal seed germination in the absence of salinity (Khan & Gul, 2006) however; they also have the ability to germinate under highly saline conditions (Ungar, 1995). Seeds of this study exhibited germination responses similar to other halophytes of the region which exhibit high germination after monsoon rains when soil salinity is reduced and cooler temperatures prevail. The seeds which are able to attain sufficient hydration have the ability to germinate and the probability of germination decreases substantially with drying of the soil and corresponding increase in salinity and drought stress. In the present study, germination was less affected by $MgSO_4$ followed by sodium salts whereas a greater toxicity of K_2SO_4 was found. Furthermore, K^+ an essential macronutrient for plants inhibited germination more than any other ion, independent of the accompanying anion.

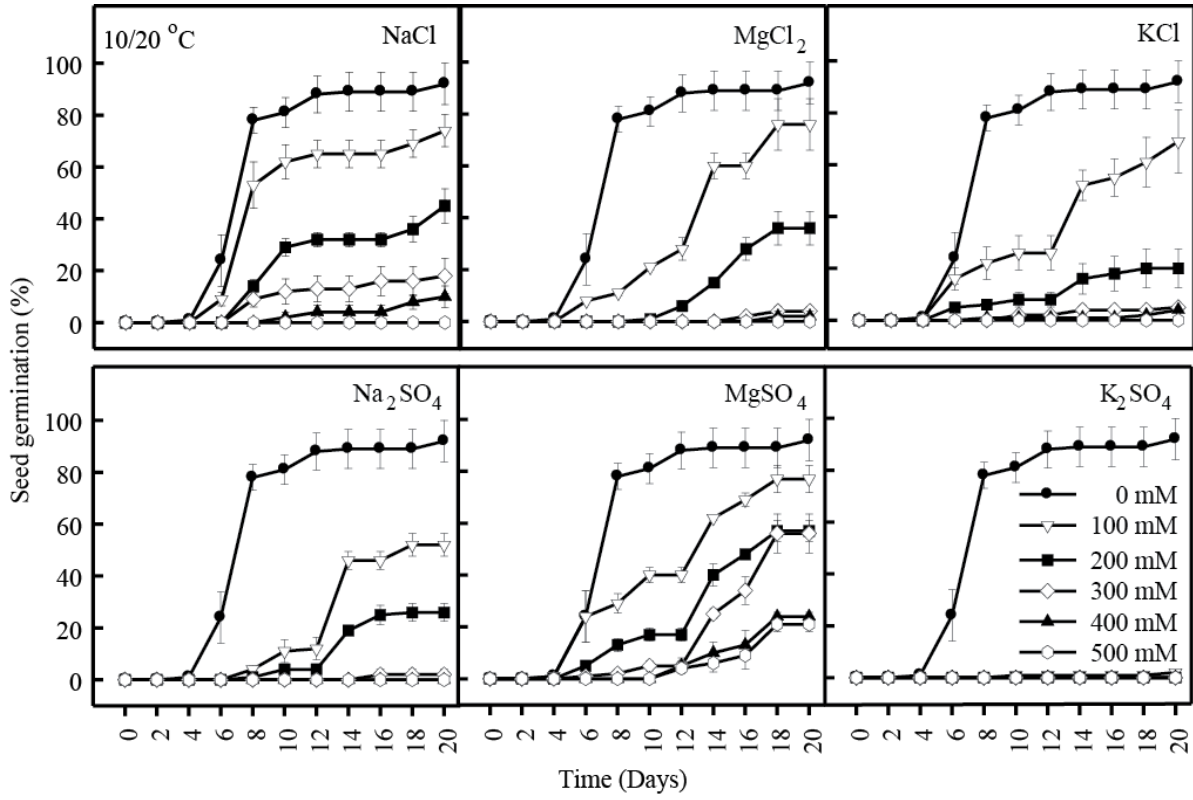


Fig. 1. Final seed germination of *Phragmites karka* in 12 h photoperiod under different salts at 10/20°C. Different symbols represent means +S.E.

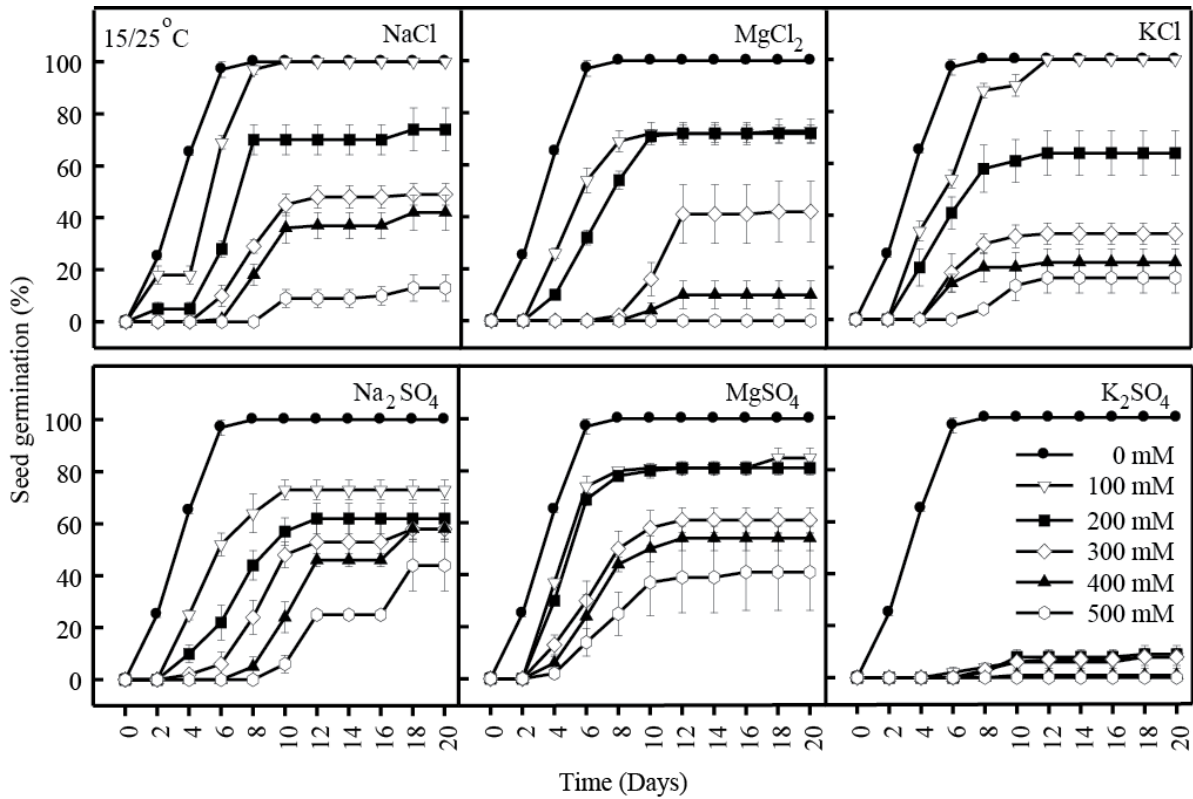


Fig. 2. Final seed germination of *Phragmites karka* in 12 h photoperiod under different salts at 15/25°C. Different symbols represent means +S.E.

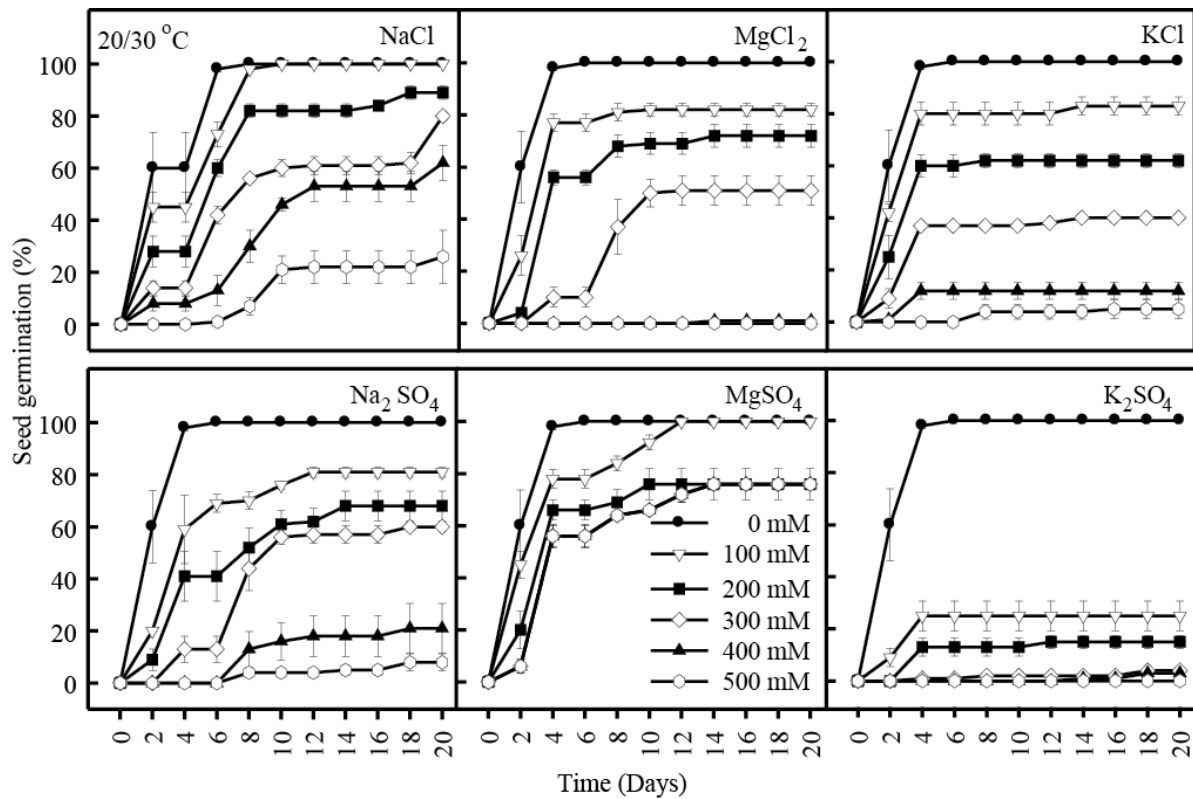


Fig. 3. Final seed germination of *Phragmites karka* in 12 h photoperiod under different salts at 20/30°C. Different symbols represent means + S.E.

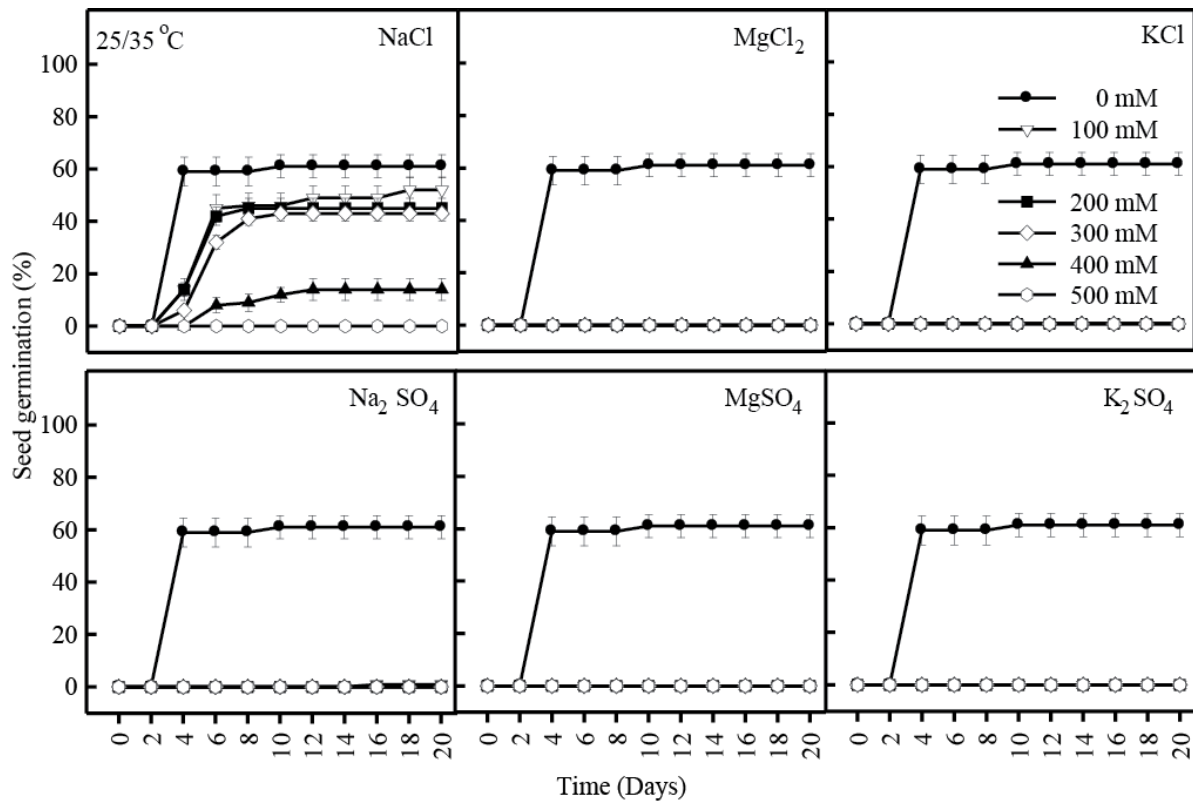


Fig. 4. Final seed germination of *Phragmites karka* in 12 h photoperiod under different salts at 25/35°C. Different symbols represent means + S.E.

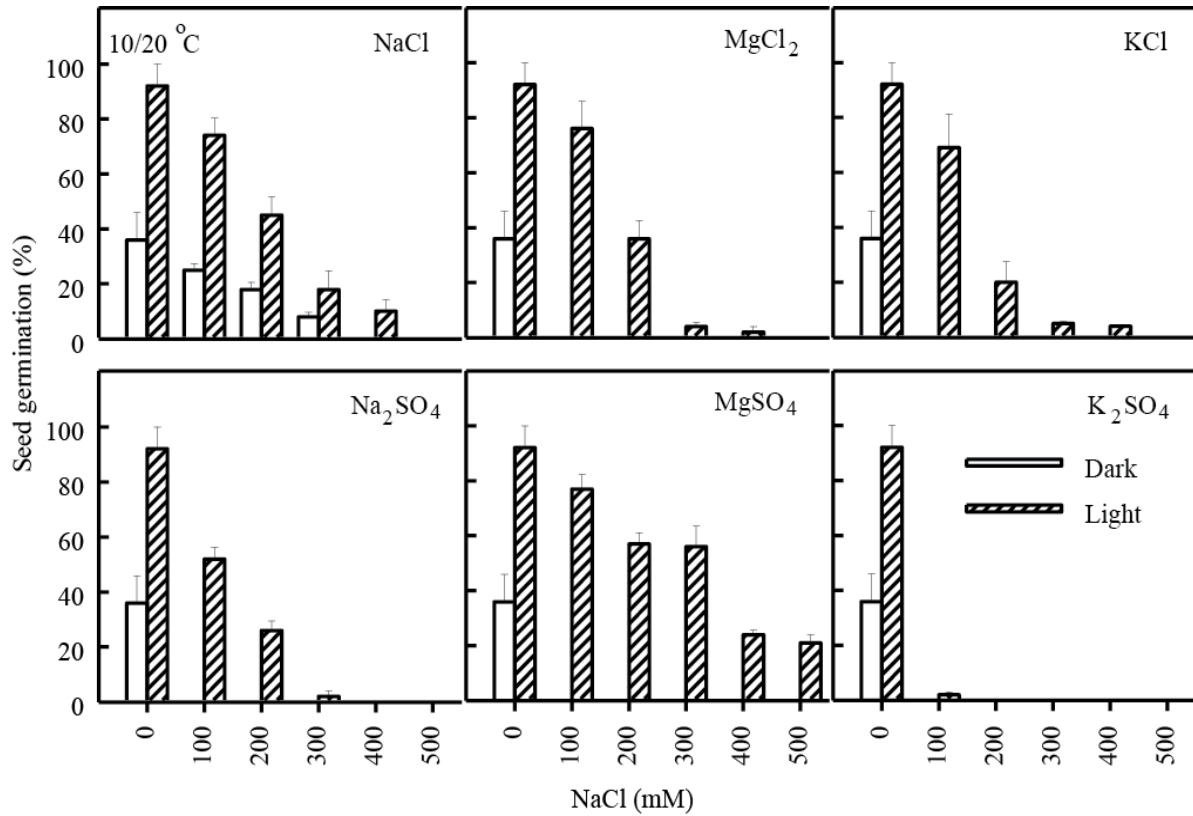


Fig. 5. Seed germination of *Phragmites karka* in 12h photoperiod and 24 h dark at 10/20°C. Bars represents mean + SE.

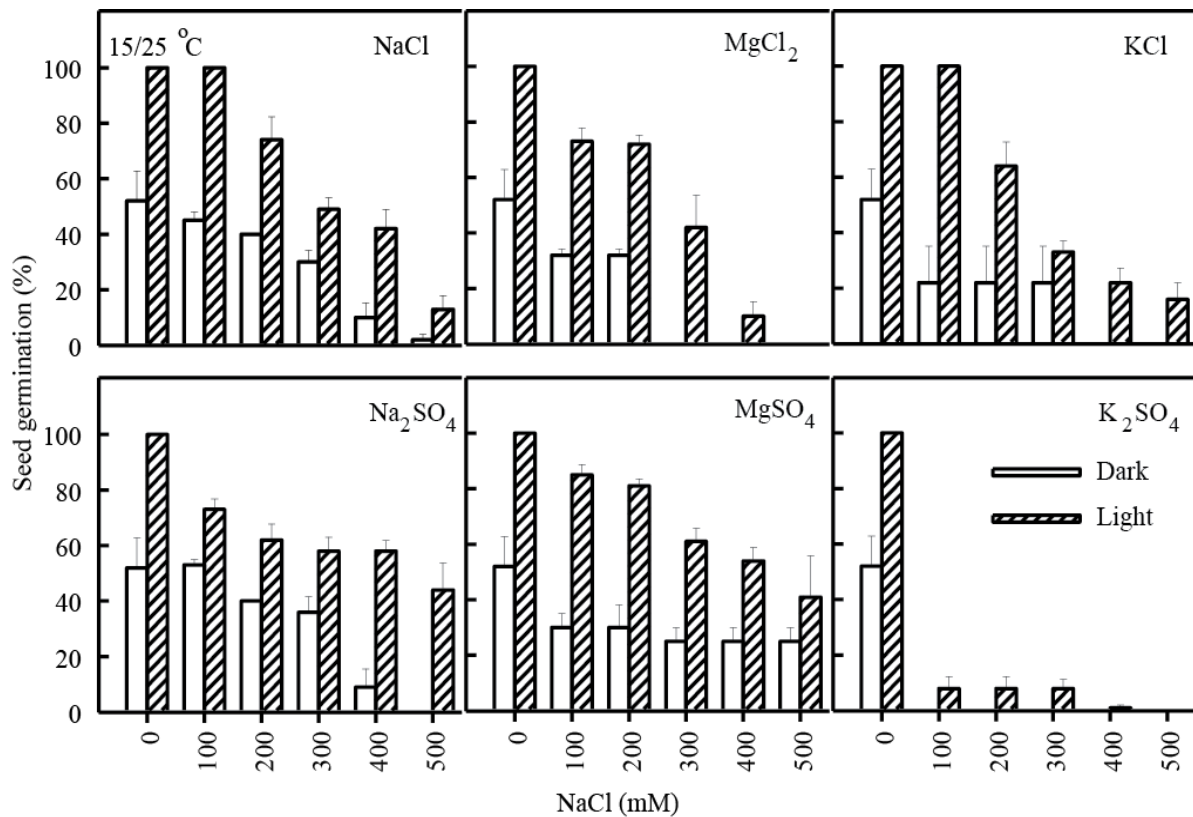


Fig. 6. Seed germination of *Phragmites karka* in 12h photoperiod and 24 h dark at 15/25°C. Bars represents mean + SE.

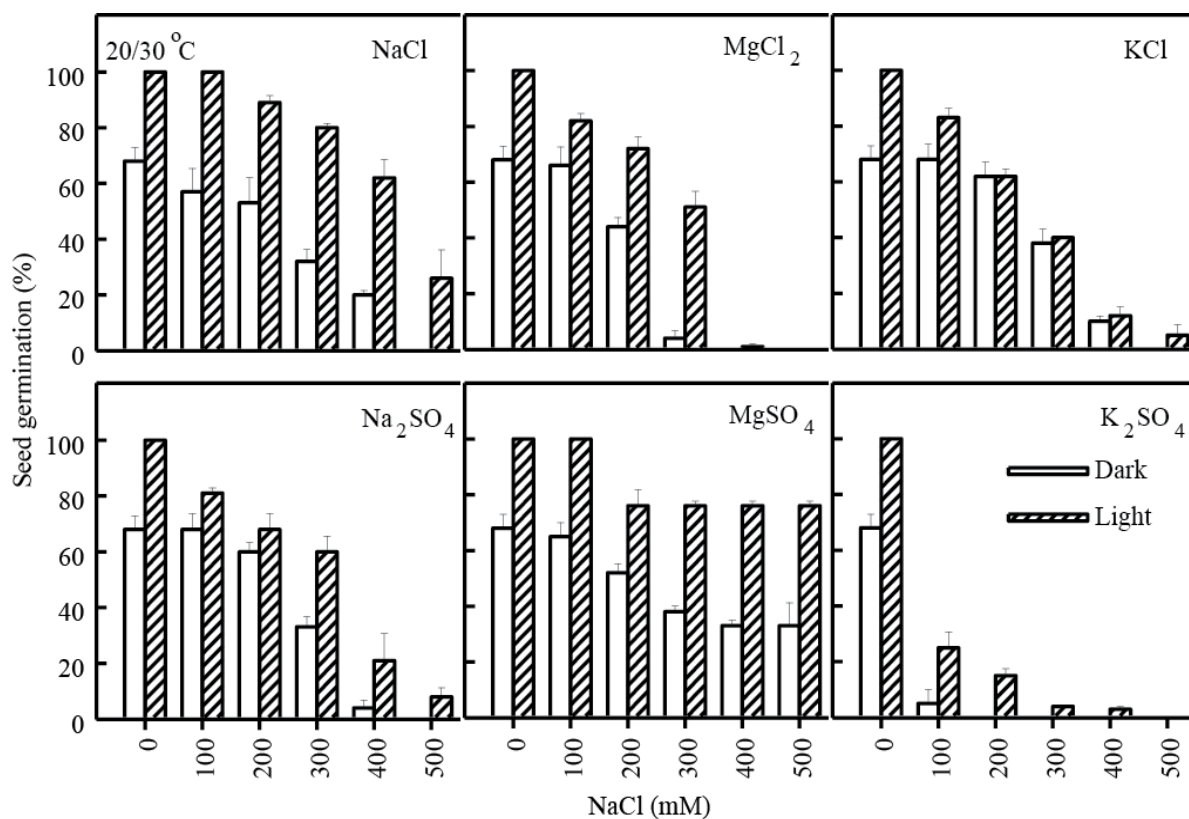


Fig. 7. Seed germination of *Phragmites karka* in 12h photoperiod and 24 h dark at 20/30°C. Bars represents mean + SE.

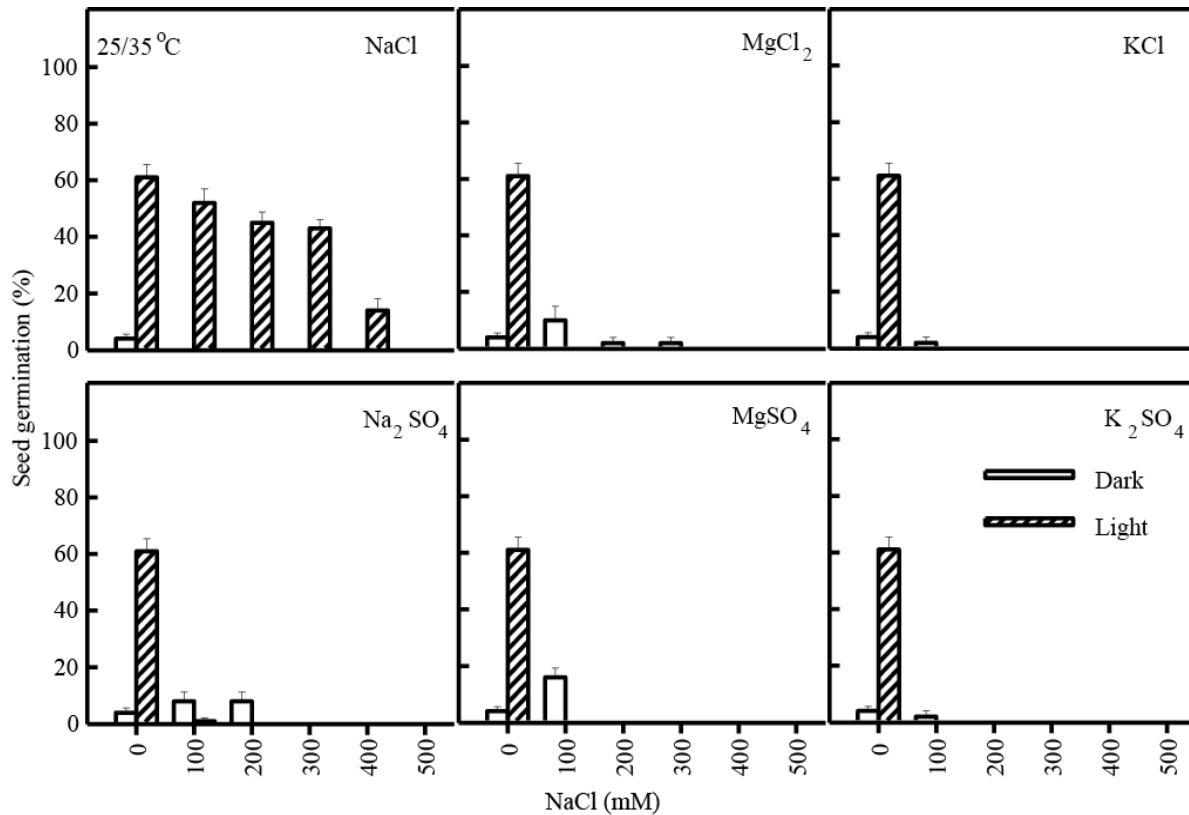


Fig. 8. Seed germination of *Phragmites karka* in 12h photoperiod and 24 h dark at 25/35°C. Bars represents mean + SE.

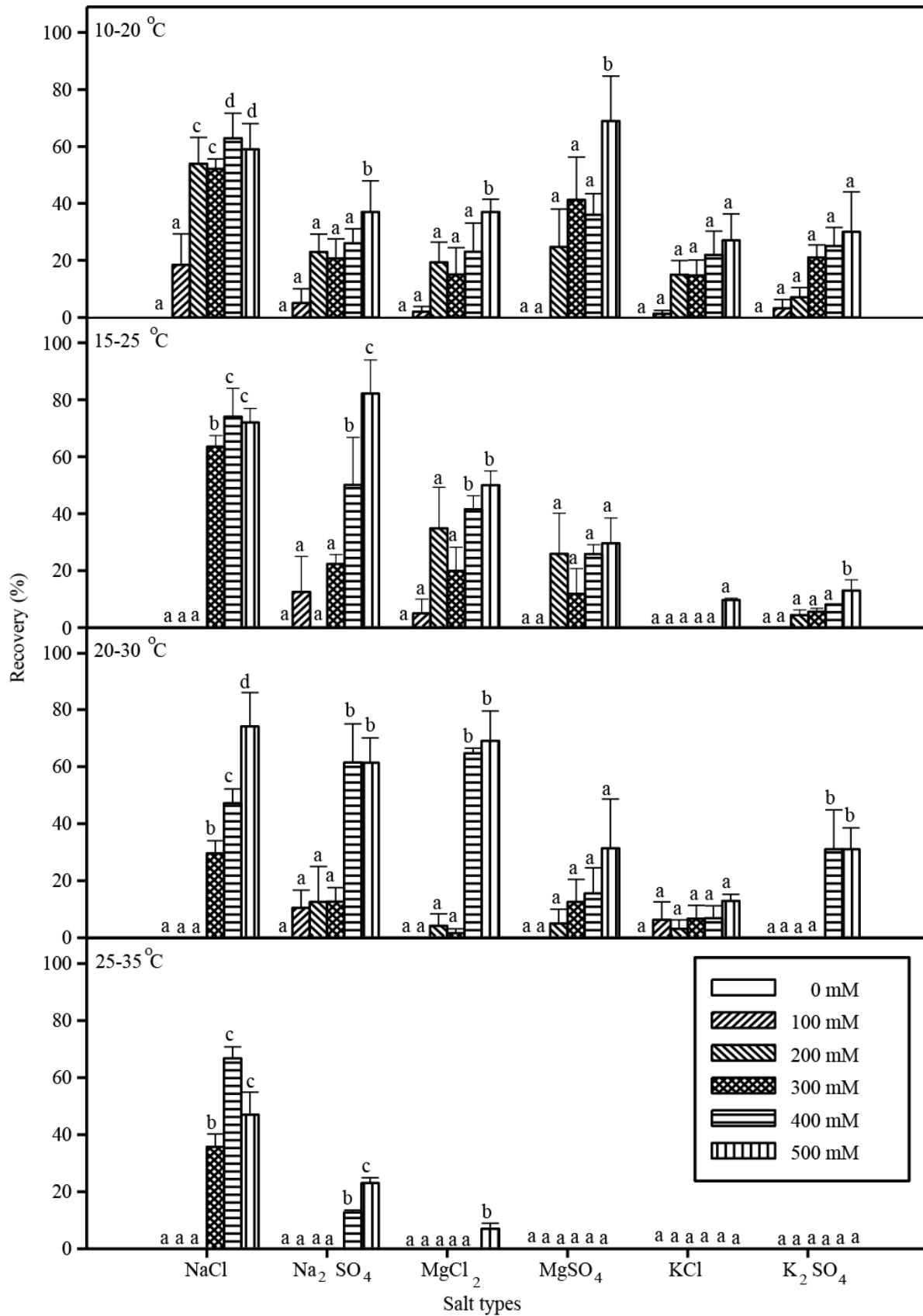


Fig. 9. Final recovery of *Phragmites karka* seed in different concentrations of six salts at various temperature regimes. Bars having same letter within each salt treatment are not significantly different ($p < 0.05$) among means (Bonferroni test). Bars represent mean + SE.

Potassium is known as an important osmotic agent but is also involved in more specific metabolic roles including protein and starch synthesis and enzyme activation (Al-Karaki, 2001). However K^+ toxicity is also reported in halophytes (Egan & Ungar, 1998; Kefu *et al.*, 1995) and glycophytes (El-Haddad & O'Leary, 1994; Kefu *et al.*, 1995). Ungar (1995) reported that salt inhibition to halophyte *Puccinellia festucaeformis* was in the order $CaCl_2, MgCl_2 > NaCl, NaNO_3, KCl > MgSO_4$. Egan *et al.* (2001) hypothesized that K^+ may not be able to transport across membrane, causing specific ion toxicity in the external milieu that inhibit various metabolic functions. Potassium salts also inhibited enzyme activity almost to the same extent as Na^+ salts in some cases (Choukr-Allah *et al.*, 1995).

Variation in light under saline conditions also affected the seed germination of halophytic grasses (Badger & Ungar, 1989; Lorenzen *et al.*, 2000). The fact that light enhances the germination has been previously described for the seeds of species like *Juncus acutus*, *Schoenus nigricans*, (Martinez-Sanchez *et al.*, 2006), *Hypericum avicularifolium* and *Hypericum depilatum* (Cirak *et al.*, 2007). Some other studies in our lab (Sheikh, 2008) have indicated an obligate requirement of light for germination of *E. ciliaris*. Seeds of *P. karka* of this study germinated in dark as well but were better in light.

Germination of those species which are dependent on light, activate certain metabolic processes of seeds during germination under salt stress and in the absence of light these processes are affected in a manner that causes inhibition in germination under unfavorable conditions (Urbano *et al.*, 2005). The light requirement for germination of halophytes has ecological significance also for seeds that have enough reserves for the shoot to reach soil surface (Pons, 2000) and also for a persistent seed bank formation in the dark period (Carter & Ungar, 2004). This has special importance for annuals like *E. ciliaris* for continued progeny maintenance whereas the perennials like *P. karka* generally do not rely that much on seed germination for this purpose. It seems that annual species use seeds as prime mode for the recruitment and usually germinates in condition where plenty of light is available. Therefore it has obligate requirement for light and sensitivity to the salt stress prevents seeds from germination when conditions are not optimal. *P. karka* is a perennial grass and showed little sensitivity to light similar to another halophytic shrub- *Suaeda fruticosa* (Khan & Ungar, 1998) because they are adapted to germinate under the shade.

Temperature is critical in determining successful establishment of plants because it has significant effect on the onset, potential and rate of germination of various species (El-Keblawy & Al-Rawai, 2005; Al-Khateeb, 2006; Gorai & Neffati, 2007; Huang *et al.*, 2003; Kader & Jutzi, 2004; Khan & Gul, 2006). In the present study, the optimum temperature regime for germination of *P. karka* was 20/30°C in which some seeds germinated at the highest concentration of most of the salts used and any deviation was harmful. The highest inhibition in percentage and rate of germination was found at 25/35 °C in all salt treatments. Temperature shifts bring irreversible damage of seeds (Gorai *et al.*, 2006) by affecting a number of processes determining the germination capacity of seeds e.g. membrane permeability, activity of membrane bound proteins and cytosol enzymes, inhibiting hormonal or

enzyme activity (Bewely & Black, 1994; Foment *et al.*, 2002; Zhu, 2001).

Response of halophyte seeds to salinity has been reported to be substantially modulated by variation in temperature regimes in a number of halophytes (Khan & Gul, 2001) but these results suggest that temperature optima may vary with species. For instance, the seeds of *Haloxylon stocksii*, a perennial shrub distributed in deserts of Pakistan, do not germinate at 10-20°C in dark (Khan & Ungar, 1997). Sankary & Barbour (1972) reported that seeds of *H. articulatum*, a desert shrub inhabiting Syria, germinate to higher percentages in light than in darkness. Choi *et al.*, (1976) and Kaul *et al.*, (1990) reported similar response when studying the seed germination of *H. salicornicum*, an Indian desert shrub. Huang *et al.*, (2003) indicated that the seeds of *H. ammodendron* germinate well both in light and dark and the germination percentages do not differ significantly suggesting that the seeds of *H. ammodendron* have different light requirements than several of its congeners.

Seeds of *P. karka*, when transferred to distilled water after 20-days from various concentrations of different salts had varied response of recovery at all temperature regimes. An increase in salinity also increased the recovery germination in all chloride and sulfate salts except for chloride and sulfate of potassium in which seeds showed a poorer recovery. This response may indicate that the germination inhibition of *P. karka* is perhaps due both to osmotic stress and specific ion toxicity. Seeds exposed to higher concentration of different salts at lower thermoperiod (10/20°C) recovered quickly in comparison to warmer thermoperiod (25/35°C) where few seeds germinated. Recovery germination of *Urochondra setulosa* and *Sporobolus ioclados* was also inhibited at warmer temperature regimes and best recovery was obtained at 20/30°C (Gulzar *et al.*, 2001; Gulzar & Khan, 2002). Halophytic grasses recovered quickly when exposed to salinity for short periods and under suitable temperature regimes (Keiffer & Ungar, 1997); a longer exposure and unfavorable temperature conditions prevented their recovery from salinity stress (Gulzar *et al.*, 2001). The annual species has only one chance of the production of seeds and their fitness is linked with the long term viability while perennials get multiple chances for leaving the progeny therefore they generally loose viability soon. However, the tetrazolium test after the recovery showed that the seeds of *P. karka* became dormant but remained viable under the exposure of high salt concentrations and unfavorable temperature regimes.

Conclusion

The present work supports the contention that different salts exhibit both osmotic and ionic effect on germination and single salt effect would not be comparable to saline soil which is a mixture of different salts. It is further evident that the osmotic potential at similar concentration of particular salts was important determinant of germination response in addition to specific ion toxicity. Susceptibility to salt stress indicates that species are able to establish themselves in a wide range of habitats where particular ion composition is favorable for their survival. However, results of germination experiments with a single salt would not

always be applicable to field conditions. Toxicity of a particular salt with various environmental conditions must be assessed in order to determine the specific-ion toxicity of a species in the field. Seeds of this study required light for germination under all salts applications with best germination under optimal temperature regimes and any deviation was inhibitory. The seeds remained viable but were prevented from germination when higher concentrations of different salts were used. During extended exposure to high salinity and temperature stress, seeds are prevented from germination. However, during the monsoon period when temperature becomes relatively cool, monsoon showers dilute the soil salinity, favoring seed germination. This appears to be a good strategy to prevent mortality and provide an opportunity for the induction of new genotypes securing the survival of population both at proximate and ultimate levels.

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