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COMPARATIVE EFFECTS OF NaCl AND SEA SALT ON SEED GERMINATION OF *ARTHROCNEMUM INDICUM*

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Abstract

Arthrocnemum indicum is a stem succulent perennial halophyte from the family Chenopodiaceae. Experiments on seed germination were carried out using NaCl and sea salt (0, 20, 40, 60, 80 & 100 dS m^{-1}) at alternating temperature regimes (10:20ºC, 15:25°C, 20:30°C and 25:35°C) at photoperiod of 12 h dark: 12 h light and in 24 h dark. Seed germination of *A. indicum* was substantially delayed and/or prevented with an increase in NaCl and sea salt concentrations. Only few seeds germinated above 20 dS m^{-1}. Sodium chloride and sea salt differ in their effect on seed germination at all temperature regimes. NaCl prevented more seeds from germination in comparison to sea salt. Optimal germination was obtained in non saline control at lower temperature regime whereas warmer temperatures in our experiment inhibited more seeds from germination. Seed germination was not affected either by photoperiod or dark conditions in non saline control at all temperature regimes. At low NaCl treatments there were significant differences between light and dark germinated seeds at 15:25°C and 20:30°C. Seed germination in sea salt was similar in both light and dark conditions except at 20 dS m^{-1} at 15:25°C. Highest recovery was obtained at cooler temperature regime of 10:20°C while lowest at warmer temperature regime of 25:35°C. Most of the un-germinated seeds were found dead in both salt treatments and their mortality increased with an increase in temperature.

Introduction

The dominance of saline water has lead to the widespread salinisation (over 800 million hectares of land) throughout the world (Flowers & Flowers, 2005; Munns, 2005). Salt tolerant plants (halophytes) which are distributed on these kinds of soils (Flowers & Flowers, 2005) are regarded as a rich source of potential forage or fodder, oilseed etc. (Glenn & Brown, 1999; Aganga *et al*., 2003; Tomar *et al*., 2003; Khan & Ansari, 2008; Reddy *et al*., 2008). Therefore, identifying halophytes that may contribute in recovery of intertidal ecosystems and developing saline agriculture is of importance (Song *et al*., 2008). *Arthrocnemum indicum* is one such halophyte with limited distribution along the Arabian Sea coast at Gadani, Balochistan in association with *Cressa cretica*, *A. macrostachyum*, *Aeluropus lagopoides* and S*uaeda fruticosa*.

The effect of NaCl on seed germination of halophytes is widely reported (Khan & Gul, 2006; Jaleel *et al*., 2008; Wei *et al*., 2008) but little information exists about the effect of other salts on the seed germination (Pujol *et al*., 2000; Tobe *et al*., 2004; Vicente *et al*., 2007). Coastal plants grow under seawater which is a mixture of various salts dominated by NaCl, therefore determining the effect of NaCl alone might not be correlated with the plant responses under natural conditions (Zia & Khan, 2008). The effect of sea salt on seed germination of coastal halophytes may be different from NaCl (Joshi *et al.,* 2004; Zia & Khan, 2008). Some reports have recently appeared on the effect of sea salt on seed germination of halophytes (Joshi & Kumar, 1993; Dauod *et al*., 2001;

Houle *et al*., 2001; Hanselin & Eggen, 2005; Hameed *et al*., 2006; Khan *et al*., 2006; Zehra & Khan, 2007; Zia & Khan, 2008). These reports have indicated that sea salt inhibits more seed germination in comparison to NaCl (De Villiers *et al*., 1994; Houle *et al*., 2001; Zia & Khan, 2008). Similar result was reported for *Aeluropus lagopoides, Desmostachya bipinnata, Suaeda fruticosa* (Hameed *et al*., 2006) and *Limonium stocksii* (Zia & Khan, 2008). However, in *Hipophae rhamnoides* (Tirmizi *et al.,* 1993)*, Crithmum maritimum* (Atia *et al*., 2006), *Dichanthium annulatum* and *Eragrostis ciliaris* (Sheikh, unpublished data) sea salt appeared less inhibitory in comparison to NaCl. In some halophytes like *Haloxylon stocksii* (Hameed *et al*., 2006) and *Phragmites karka* (Zehra & Khan, 2007) seed germination was not significantly different in either salt type.

Temperature can interact with salinity in affecting seed germination in arid saline areas (Gulzar *et al*., 2001; Huang *et al*., 2003; El-Keblawy & Al-Rawai, 2005; Al-Khateeb, 2006; Gorai & Neffati, 2007; Wei *et al*., 2008). Seeds of species like *Haloxylon ammodendron* (Huang *et al*., 2003), *Halostachys capsica*, *Kalidium foliatum*, *Halocnemum strobilaceum* (Song *et al*., 2006) and *Kalidium capsicum* (Tobe *et al*., 2000) showed little germination when exposed to high salinity and temperature stress. Germination was inhibited by either increase or decrease from the optimal temperature (Martinez-Sanchez *et al*., 2006; Tlig *et al*., 2007). Variation in temperature under saline conditions has differential effects on the seed germination of halophytes (Tessier *et al*., 2000; Gulzar & Khan, 2001; Gulzar *et al*., 2001; El-Keblawy & Al-Rawai, 2005; Zehra & Khan, 2007). Temperature also affected the progress of germination under both saline and non saline conditions (Haung *et al*., 2003; Zia & Khan, 2004; Tlig *et al*., 2007).

Light has been recognized as germination controlling factor in some plant species (Baskin & Baskin, 2001; Puppala & Fowlers, 2003). Interaction of light and salinity has been investigated on germination of several species (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; Wei *et al.,* 2008) and varying responses of light under both saline and non-saline conditions were reported. Seed germination of halophytes like *Suaeda fruticosa* (Khan & Ungar, 1998), *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), *Lolium rigidum* (Steadman, 2004), *Carex* species (Kettering *et al*., 2006) and *Eucomis autumnalis* (Kulkarni *et al*., 2006). It is also reported that, the combined effect of dark and salinity greatly reduced germination of halophytes (Zia & Khan, 2004; Khan & Gul, 2006; Zehra & Khan, 2007).

Halophytes are known to maintain seed viability for extended periods during exposure to high salinity. When salinity stress is reduced, partial to complete germination recovery has been observed (Khan, 2003; Song *et al*., 2006; Wai *et al*., 2008; Tlig *et al*., 2007). Recovery of seeds from hyper-saline condition greatly reduced under high temperatures (Khan & Gul, 2006; Zehra & Khan, 2007) and usually high temperatures bring irreversible damage to the seeds (Gorai *et al*., 2006).

In the present study following hypothesis were tested: *A. indicum* seeds tolerate high salinity at germination levels. Seed germination in sea salt is higher in comparison to NaCl. Light affects the seed germination under saline conditions. Temperature affects the time and progress of germination. Seeds of *A. indicum* maintain viability under extended hyper saline conditions.

Materials and Methods

Mature inflorescences of *A. indicum* were collected during July 2002 from a pure population at Gadani, Pakistan. Seeds were separated from their inflorescence and were surface sterilized using 0.85% Sodium hypochlorite solution for 1 minute, followed by thorough rinsing with distilled water and air-drying. Sterilized seeds were stored at room temperature.

Germination was carried out in 50 x 9 mm (Gelman No. 7232) tight fitting plastic petri plates with 5 ml of test solution. Seeds were germinated in distilled water and iso-osmotic solutions (0, 20, 40, 60, 80 and 100 dS m^{-1}) of NaCl and sea salt. Four replicates of 25 seeds per Petri plate were used for each treatment. Petri plates were placed in a germination chamber (25 µmol m^{-2} s^{-1}, 400-750 nm Sylvania cool - white fluorescent lamps) with a 12 h photoperiod and at alternating night and day thermoperiod of 10:20, 15:25, 20:30 and 25:35°C. Seeds were also incubated in complete darkness under similar temperature and salinity treatments. Seed germination from the Petri plates placed under 12 h photoperiod were recorded at 2 d intervals for 20 d. Seeds were considered to be germinated with the emergence of the radical. After 20 d of incubation rate of germination was estimated by using a modified Timson's index of germination velocity $= \sum G/t$, where G is the percentage of seed germination at 2 d interval and t is the total germination period. The maximum value possible using this index with our data was 50 (i.e., 1000/20). The greater the value, the more rapid is germination.

After 20 d un-germinated seeds were transferred to distilled water to study the recovery of germination. Seed germination was recorded at 2 d interval for another 20 d period. The recovery percentage was determined by formula: Percent recovery = (a-b) / (c-b) *100, where *a* is the total number of seeds germinated after being transferred into distilled water, *b* is the total number of seeds germinated in a particular treatment and *c* is the total number of seeds. The seeds that failed to germinate were further tested for their viability by submerging in a 1% aqueous solution of 2,3,5-triphenyle-tetrazolium chloride. Subsequently seeds were dissected and the color of embryo was observed through a magnifying glass.

Values of the percent germination were arcsine transformed before statistical analysis to ensure homogeneity of variance. The entire data were analyzed using SPSS 9.0 for windows release (2000). A two way ANOVA was used to determine the differences among the means. Differences between individual means were tested by Bonferroni (Multiple range tests) test at $p<0.05$ levels.

Results

A two-way ANOVA indicated significant ($p<0.05$) individual effects of NaCl (Table 1), sea salt (Table 2), temperature, and their interaction on germination, rate of germination, recovery, rate of recovery, viability and mortality of *A. indicum* seeds.

Seeds germinated better in distilled water while the introduction of salinity decreased germination (Fig. 1) and rate of germination (Table 3). This decrease was proportional to the salt concentrations employed. Sea salt appeared more inhibitory to seed germination (Fig. 1) which was substantially inhibited both at cooler and warmer temperature regimes and few seeds germinated at 20 dS m^{-1} sea salt at optimal temperature regime of 15:25°C (Figs. 1 and 2). However, at optimal temperature some seeds germinated at 80 dS m^{-1} NaCl (Fig. 2). Seed germination of *A. indicum* in distilled water and under various concentrations of sea salt was similar in light and dark treatments (Fig. 2). However, NaCl at optimal temperatures in dark inhibited more seed from germination.

Table 1. Two-way analysis of variance of the effect of salinity (S), temperature (T) and their interaction in NaCl on seed germination of *A. indicum*.

Sources of variation	S	T	S x T
Germination (%)	227.909***	71.143***	9.131***
Rate of germination	339.597***	86.061***	12.543***
Recovery (%)	30.519***	19.656***	1.408^{ns}
Rate of recovery	29.134***	19.294***	1.620*
Viability (%)	18.236***	1.356^{ns}	7.844***
Dead seeds (%)	61.986***	73.638***	8.399***

Number represents *F*-values. ns = Not significant, ** = $p<0.01$, *** = $p<0.001$

Table 2. Two-way analysis of variance of the effect of salinity (S), temperature (T) and their interaction in sea salt on seed germination of *A. indicum*.

Sources of variation	S	T	S x T
Germination (%)	449.275***	75.234***	33.717***
Rate of germination	548.100***	93.625***	42.853***
Recovery (%)	17.881***	1.696^{ns}	0.221^{ns}
Rate of recovery	7.220***	2.596*	0.268^{ns}
Viability (%)	26.674***	1.136^{ns}	5.321***
Dead seed (%)	2.277*	7.920***	1.564^{ns}

Number represents *F*-values. ns = Not significant, ** = $p<0.01$, *** = $p<0.001$

Table 3. Rate of germination of *A. indicum* seeds under different NaCl and sea salt concentrations.

Salinity (dS m^{-1})	Rate of germination							
	10:20 ^{o}C		15:25 ^{o}C		20:30 ^{o}C		25:35 ^{o}C	
	NaCl	Sea salt	NaCl	Sea salt	NaCl	Sea salt	NaCl	Sea salt
0	24.7 ±1.8	22.0 ±1.9	24.7 ±0.8	21.6 ±1.0	18.9 ±1.4	16.5 ±1.2	11.9 ±0.4	10.2 ±0.4
20	12.0 ±0.4	4.2 ±0.4	17.5 ±1.0	20.8 ±0.8	10.1 ±0.8	15.6 ±1.2	2.1 ±0.8	1.8 ±0.1
40	10.1 ±0.7	2.7 ±0.1	10.6 ±1.0	2.6 ±0.3	9.2 ±1.0	2.9 ±0.2	1.9 ±0.8	0.9 ±0.5
60	4.1 ±1.1	1.6 ±0.1	5.1 ±1.0	1.7 ±0.1	3.6 ±0.4	1.5 ±0.1	1.7 ±0.6	0.0 ±0.0
80	0.6 ±0.3	1.1 ±0.4	2.3 ±1.1	0.7 ±0.4	1.3 ±0.5	0.7 ±0.4	0.0 ±0.0	0.0 ±0.0
100	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Values are mean ± SE at concentration

Seed when transferred to distilled water after 20 days of incubation in NaCl and sea salt, showed difference in their recovery depending on the salts, their concentrations and the temperature regimes (Fig. 3). More seeds recovered from salt stress with the increase in salinity Maximum recovery from NaCl was obtained at cooler thermoperiods in 100 dS m^{-1} (Fig. 3). At warmer temperature regime mortality at all NaCl concentrations was substantially increased. Sea salt caused high seed mortality both at cooler and warmer temperature regimes and most of the remaining seeds found dormant with lower recovery in comparison to NaCl treated seeds (Fig. 2). NaCl caused more death while sea salt made seeds more dormant.

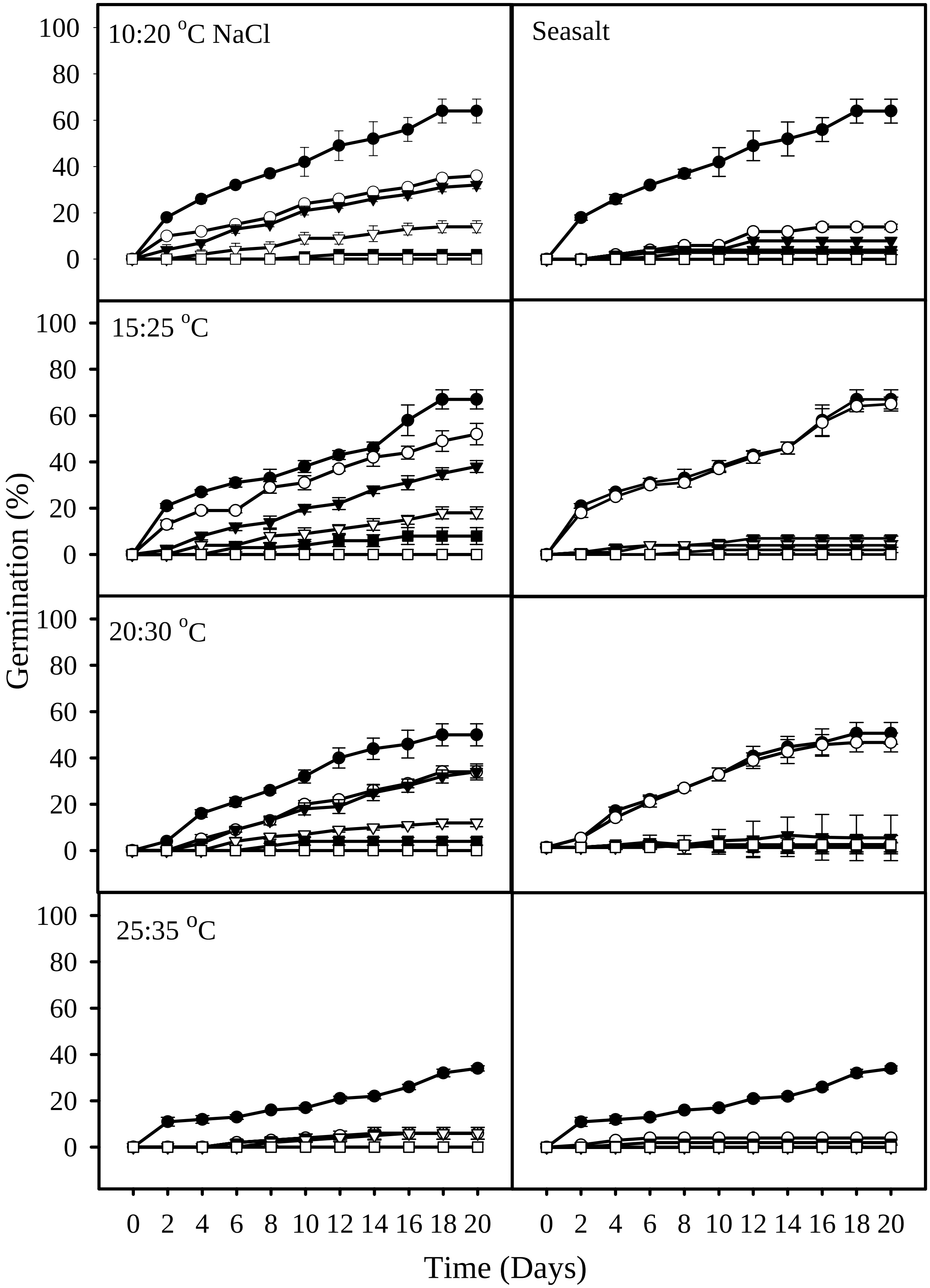

Fig. 1. Effect of NaCl and sea salt on percent germination of *Arthrocnemum indicum* seeds in (—●—0, —○— 20, —▼— 40, —▽— 60, —■— 80 and —□— 100 dS m^{-1} under four different temperature regimes. Each point represents the mean of four replicates.

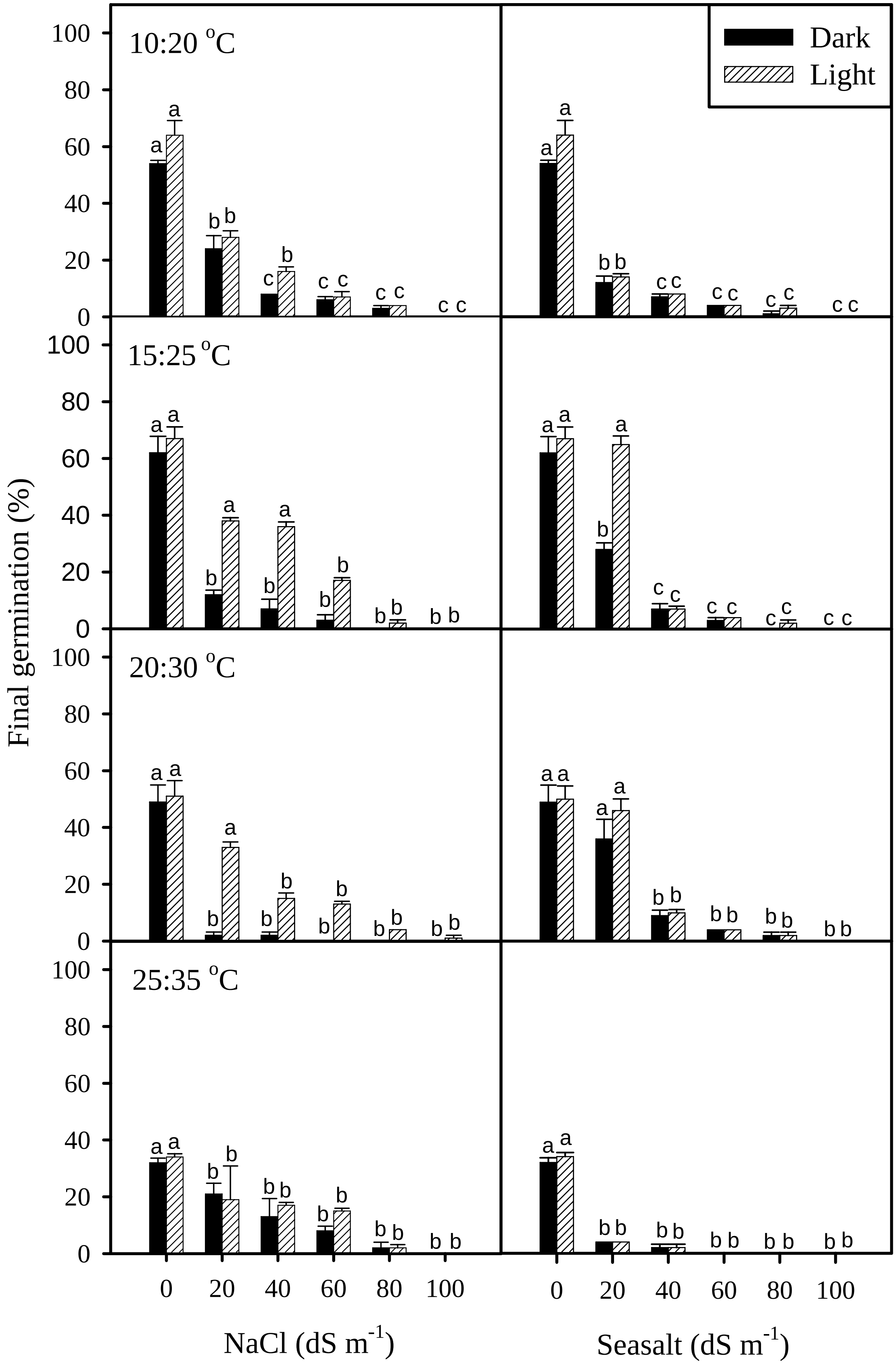

Fig. 2. Effect of NaCl and sea salt on mean final percent germination of *A. indicum* in light & dark. Bars having the same letter within different light and dark treatments are not significantly different (p<0.05) to each other (Bonferroni test). Bars represent mean + SE.

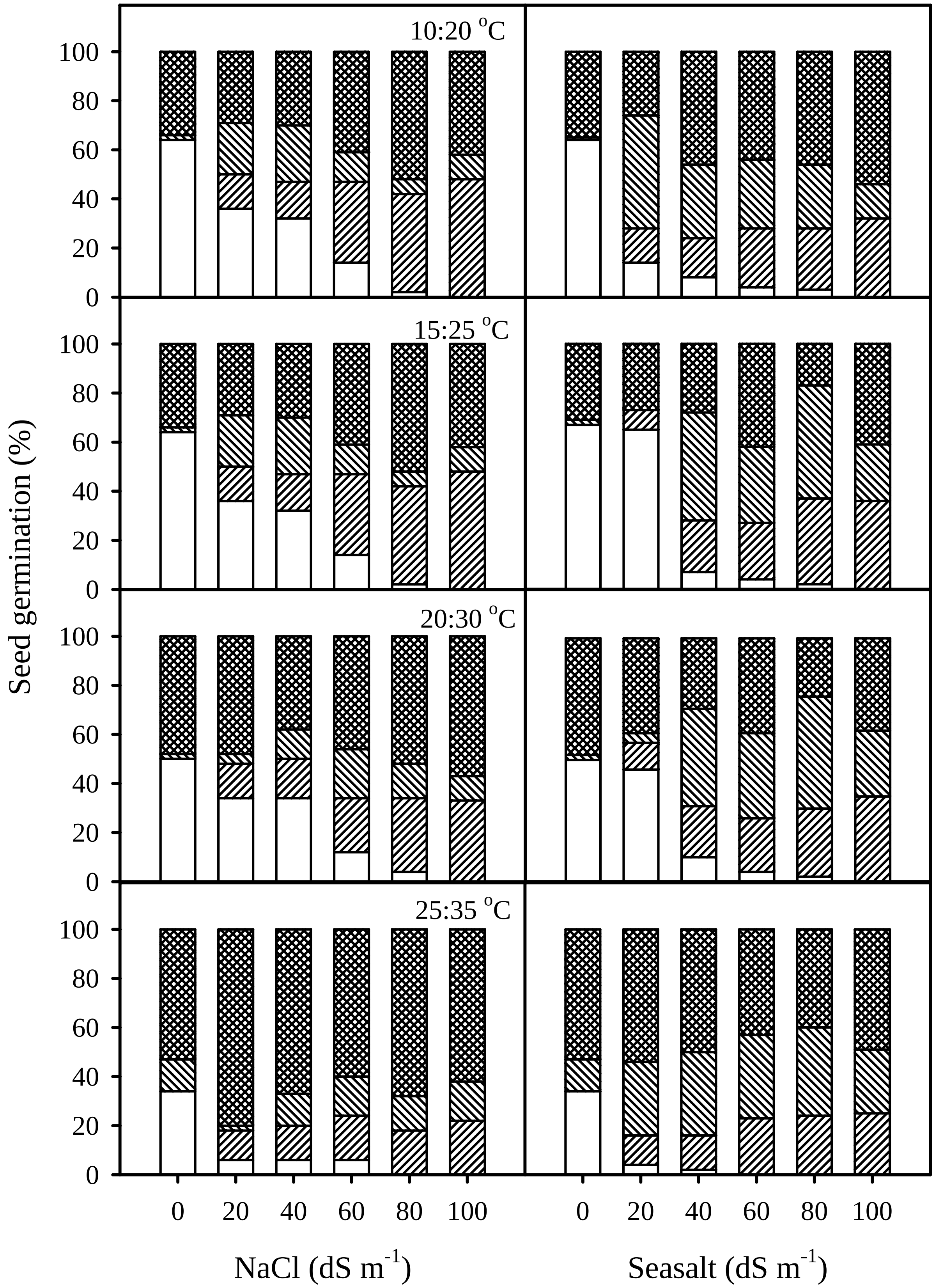

Fig. 3. Effect of NaCl and sea salt on percent germination on (▭), recovery (▨), viability (▧) and mortality (▩) of *A. indicum* seeds.

Table 4. Rate of recovery of *A. indicum* seeds under different NaCl and sea salt concentrations.

Salinity ($dS\ m^{-1}$)	Rate of recovery							
	10:20 °C		15:25 °C		20:30 °C		25:35 °C	
	NaCl	Sea salt	NaCl	Sea salt	NaCl	Sea salt	NaCl	Sea salt
0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
20	5.0 ±0.3	3.6 ±1.0	8.0 ±1.4	5.6 ±0.9	5.3 ±0.7	6.0 ±1.4	3.0 ±0.7	2.9 ±0.6
40	5.0 ±1.0	3.9 ±0.2	8.8 ±2.8	5.4 ±0.5	5.5 ±1.5	6.2 ±1.4	3.4 ±0.4	3.4 ±0.2
60	9.7 ±1.4	5.8 ±0.2	10.3 ±1.8	6.0 ±1.1	6.0 ±0.8	5.5 ±1.3	3.8 ±0.5	4.2 ±1.2
80	10.1 ±0.5	5.2 ±0.2	9.6 ±2.2	8.0 ±2.6	7.7 ±0.9	7.1 ±1.9	3.8 ±0.3	6.0 ±1.8
100	12.0 ±0.2	6.9 ±0.7	11.0 ±0.9	8.3 ±2.5	8.6 ±1.4	8.6 ±2.4	4.6 ±0.3	5.6 ±0.6

Values are mean ± SE at concentration

Discussion

Establishment of the species in salt marsh habitat is related to seed germination under various salinity and temperature regimes (Khan *et al*., 2001). Seeds of the subtropical halophytes show optimal germination at 20:30˚C and in salinities ranging from 0 to 20 $dS\ m^{-1}$ in controlled conditions (Khan & Gul, 2006). However, their recruitment through seed germination is rare in their habitat (Gul & Weber, 2001). They are reported to propagate through ramets despite their massive seed production. *Arthrocnemum indicum* also produces numerous seeds every year, however, seeds have primary dormancy and require an after ripening period of about six to eight months before they are ready to germinate in laboratory condition. Our study indicated that *A. indicum* is moderately salt tolerant at germination when compared with other stem succulent halophytes like *A. macrostachyum* (100 $dS\ m^{-1}$ NaCl; Khan & Gul, 1998) and *Halocnemum strobilaceum* (86 $dS\ m^{-1}$; Pujol *et al*., 2001) and could be categorized as a miohalophyte.

The data reported on comparative effects of sea salt and NaCl on seed germination indicates an upper limit of salt tolerance where no seeds would germinate. This information is an important tool in establishing saline agriculture. Considerable information exist on adverse effects of individual salts on seed germination in halophytes (Khot, 2003), however only few reports are available on relative tolerance of seeds to sea salt and NaCl (Joshi *et al*., 1993, 2005; Hameed *et al*., 2006; Zehra & Khan, 2008; Zia & Khan, 2002; 2008). These reports indicate that the effect of sea salt on seed germination is usually different from that of NaCl and this difference varies with species (Joshi *et al*., 2005; Zia & Khan, 2008). Seed germination of *A. indicum* was also differentially affected by sea salt and NaCl. Sea salt clearly prevented more seeds from germination. Similar results were reported for *Suaeda fruticosa* (Atayat, 2002), *Haloxylon stocksii* (Hameed *et al*., 2006) and *Phargmites karka* (Zehra & Khan, 2007).

Temperature changes may affect a number of processes during seed germination including the membrane permeability, activity of membrane-bound proteins, and cytosolic enzymes leading to germination inhibition and/or seed death (Bewley & Black, 1994). Variation in temperature under saline conditions has differential effect on seed germination of halophytes (Tessier *et al*., 2000; Gulzar & Khan, 2001; Gulzar *et al.,* 2001; Atayat, 2002; El-Keblawy & Al-Rawai, 2005; Zehra & Khan, 2007) and this effect could be an ecological adaptation to survive in their particular habitat. Sub-tropical halophytes predominantly show optimal germination at 20:30^{o}C and any further increase or decrease in temperature affects the seed germination (Zia & Khan, 2002; Khan, 2003; Zia & Khan, 2004; Khan *et al*., 2006). However, certain succulent halophytes such as *Suaeda fruticosa* and *Haloxylon stocksii* are reported to show optimal germination at 15:25^{o}C both in sea salt and NaCl (Atayat, 2002). Optimal germination of *A. indicum* was also recorded at 15:25^{o}C and any increase or decrease in temperature regime inhibited germination percentage in both salts. However sea salt prevented more seeds from germination than NaCl. The optimal temperature and the low germination percentages indicate that *A. indicum* has a narrow germination window. Seed bank studies indicated that seeds are sensitive to salinity and temperature stress and lose viability in the field soon after their dispersal.

Seed germination percentages under non-saline conditions were similar. However, with the induction of salinity there were significant differences in most treatments. At moderate temperature regimes these differences were more obvious in NaCl in comparison to seawater with few exceptions. This light requirement was not observed at extreme temperatures. Interaction of temperature with light is also an important factor in affecting seed germination in many halophytes (Khan & Ungar, 1997a). Seed germination of *A. indicum* in sea salt was light independent at all temperatures except for the optimal thermoperiod. This response could be attributed to the activity and type of phytochrome in the seeds of *A. indicum*. It is reported that phytochrome do not complete its action in seeds at low water potential and probably it has some interaction with temperature. Halophytes respond differently to increasing salinity and temperature particularly at the extremes of their tolerance limits to environmental variables.

Seeds of sub tropical halophytes are usually dispersed after monsoon rains (Khan & Gul, 1998) and are exposed to high salinity and temperature stress if present in salt marshes (Khan, 2003). Halophytes can only maintain the continuity of their lineage if they can survive physicochemical stresses such as drought, salinity, extreme temperature and their interaction while in the seed bank and still maintain their viability (Mehrun-Nisa *et al.,* 2007). However, there are halophytes which do not maintain viability when exposed to hyper-saline conditions (Keiffer & Ungar, 1995) and particularly seeds of sub-tropical species which lose their viability quickly under high salinity and temperature stress (Mehrun-Nisa *et al*., 2007). When seeds of *A. indicum* were exposed to salinity and temperature stress they showed poor recovery response. Recovery was comparatively higher in high concentrations of both salts. However, more seeds recovered from sea salt stress than in NaCl. Low recovery responses were also reported for *Zygophyllum simplex* (Khan & Ungar, 1997b), *A. macrostachyum*, *Salicornia ramosissima* (Rubio-Casal *et al*., 2003) and *Salsola imbricata* (Mehrun-Nisa *et al*., 2007). Our data also showed temperature to influence recovery and a decrease in recovery percentage was observed with an increase in temperature and this effect was more prominent in NaCl treated seeds.

Viability tests showed varying degrees of dormancy and death in the seeds exposed to various temperature and salinity treatments. Seed viability was differentially affected by the type of the salts. Seeds immersed in sea salt exhibited a greater degree of secondary dormancy while the seeds placed in NaCl were more dead than dormant. On the whole poor seed recovery response in *A. indicum* appeared to be the effect of the combination of salinity, temperature and their interaction.

Arthrocnemum indicum is a rare succulent Chenopod confined only in the sabkhat on the Gadani coast, Balochistan. Poor seed germination of *A. indicum* even in low concentrations of salts and high temperature clearly indicates that seeds of *A. indicum* are moderately salt tolerant despite their presence in high saline habitat. Both sea salt and NaCl differentially affected germination, with sea salt causing secondary dormancy while NaCl being lethal for seeds. Seeds do not have obligate light requirement, however, an interaction of darkness and temperature was observed which was negatively affecting the germination. Temperature appeared to be the controlling factor for seed germination of *A. indicum*. Present study helps to understand the response of *A. indicum* seeds under various stress conditions. These observations also explain its ecological response showing limited distribution and rare occurrence of new seedlings in the natural habitats. Furthermore, its vegetative propagation may be an ecological adaptation because of salt sensitive nature of its seeds. This characteristic in turn, would help in establishing saline agriculture by introducing the suitable halophytic species in barren or in saline lands depending on its salinity (Joshi *et al*., 2005). More information is needed to explain variability in NaCl and sea salt effects on the seed germination of *A. indicum*.

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