***Pak. J. Bot.*, 45(3): 743-754, 2013.**

COMPARATIVE EFFECTS OF NACL AND SEA SALT ON SEED GERMINATION OF TWO HALOPHYTIC GRASSES UNDER VARIOUS LIGHT AND TEMPERATURE REGIMES

FAIZA SHAIKH[1], BILQUEES GUL[1], RAZIUDDIN ANSARI[1], ABDUL REHMAN A. ALATAR[2,]
AHMED. K. HEGAZY[2+] AND M. AJMAL KHAN[1,2,3*]

[1]Institute of Sustainable Halophyte Utilization (ISHU), University of Karachi, Karachi-75270, Pakistan
[2]Department of Botany & Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia,
[+]Permanent address of A. K. Hegazy is Botany Department, Faculty of Science, Cairo University, Giza, Egypt
[3]Qatar Shell Professorial Chair for Sustainable Development, ***Department of International Affairs,***
College of Arts and Sciences, Qatar University, P O Box 2713, Doha, Qatar.
[*]Corresponding author's e-mail: ajmal.khan@qu.edu.qa

Abstract

Effect of several concentrations of NaCl or sea salt was studied on seeds germination of two halophytic grasses- an annual (*Eragrostis ciliaris*) and a perennial (*Dichanthium annulatum*) in controlled growth chambers maintained at 10:20, 15:25, 20:30 and 25:35^{o}C (12 h dark: light) and 24 h dark photoperiods. Sea salt and NaCl both inhibited seed germination but NaCl prevented more seed from germination. Optimal germination was obtained at temperature regime of 25:35^{o}C for both grasses. Seeds of *D. annulatum* had similar germination both in light and dark at cooler temperatures in distilled water while addition of salinity in dark inhibited more seed germination compared to light treated seeds. No seeds of *E. ciliaris* germinated in dark and this positive photoblastic response was associated with temperature irrespective of salinity treatment. All seeds recovered from salts stress when returned to distilled water. Most of the un-germinated seeds were dormant and viable at the end of experiment indicating that they could form a viable seed bank to ensure the continuity of the population in saline habitat.

Introduction

Seeds of halophytes can tolerate high salinity and temperature stress when stored in the soil and germinate when conditions are conducive for their germination (Khan & Ungar, 1984; Al-Khateeb, 2006; Khan & Gul, 2006). However, during germination seeds are more sensitive to salinity and germinated better under non-saline conditions (Khan & Gul, 2006). Seeds of all halophytes germinate better under non-saline conditions but halophytes have the ability to tolerate salinity more than other groups and this tolerance may go up to 1.5 M NaCl (Chapman, 1960). Seed germination response of annuals and perennials is different because there are under different ecological and evolutionary compulsions to either have a dormancy control to produce new genetic individual when conditions are appropriate or little control at seed level because of utilization of clonal growth to perpetuate the populations. In this report we have made comparison of annual and perennial salt tolerant grasses at germination stage expecting the annual to exercise more control at germination in comparison to perennials.

Sea salt is a combination of various salts with more contribution of NaCl. Therefore sea salt supposed is to be different from NaCl solution alone because ionic and osmotic effects vary with kind and quantity of salt present in the solution (Pujol *et al*., 2000; Saeed *et al*., 2011). In addition, effects of both cation and anion also vary substantially. Most studies predominantly reported so far are NaCl based because it is the most common salt of saline soils and a dominant component of seawater which often influences coastal plants either by direct inundation or through lateral seepage (Khan & Gul, 2006). Effect on NaCl and sea salt have quite different effects on seed germination when used at iso-osmotic conditions (Zia & Khan, 2002; Hanselin & Eggen, 2005; Joshi *et al*., 2005; Zehra & Khan, 2007). However, nature and quantity of salts are not the only constraints but other environmental factors, individually or collectively may also have a significant influence. *Suaeda salsa* and *Atriplex centralasiatica* are highly salt tolerant halophyte species where *S. salsa* is more salt tolerant than *Atriplex centralasiatica*. Seeds of both species could germinate at seawater level but more germination was recorded in the seeds of *S. salsa* (Liu *et al*., 2006).

Light is an important environmental signal and the effects may vary from none to an obligate requirement (Thanos *et al*., 1991; Garcia *et al*., 1995; Andrew *et al*., 1997; Khan & Ungar, 1999; Khan & Gulzar, 2003). Seed during germination may also respond differently due to the variation in other environmental factors (Flores *et al*., 2011) Seeds of various halophytes generally germinate during the season when a combination of day length, temperature, and salinity are appropriate. Most species are sensitive to the change in temperature and the diurnal variation in temperatures, however, their sensitivity varies with species (Ungar, 1995) and any deviation from the optimum conditions, may delay, decrease or completely inhibit seed germination (Khan, 2002). For instance, local grasses of this region like *Urochondra setulosa, Aeluropus lagopoides, Halopyrum mucronatum* and *Sporobolus ioclados* germinated best at moderate temperatures of 20:30^{o}C and ample moisture (Khan & Gulzar, 2003). Mortality of some halophyte seeds increased when exposed to high salinity and showed a further increase with the increase in temperature (Khan & Gul, 2006). Seeds of species like *Zygophyllum simplex*, *A. lagopoides*, *Atriplex stocksii* showed a considerable recovery of germination when salt stress was removed while those of grasses like *S. ioclados, U. setulosa,* and *H. mucronatum* did not recover (Khan & Gul, 2006). Zia & Khan (2002) showed that seed germination in *Limonium stocksii* decreased with increase in salinity and few seeds germinated above 30 dS m^{-1} in both NaCl solution and seawater. Absence of light had little effect under non-saline condition however, addition of salinity synergistically inhibited seed germination. Seawater inhibited seed germination of *L. stocksii* more than NaCl solutions under both light and dark conditions. All un-germinated seeds when transferred to distilled water after 20 days of salinity treatments readily germinated. Zehra & Khan (2007) showed that seed germination of *P. karka* 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. Un-germinated seeds were transferred from salinity to distilled water after 20 days and the highest percentage of recovery was at 50 dS m^{-1} both in NaCl (20:30 °C) and sea salt (10:20°C & 20:30°C).

Halophytic grasses like (*Dichanthium annulatum* (Forssk.) Stapf and *Eragrostis ciliaris* (L.) R.Br. are widely distributed in a range of salt affected lands and have potential to be used as fodder crop. The former is a perennial of up to 100 cm height occurring in arid/semi-arid regions of the world and throughout the inland and coastal areas of Pakistan while the latter is a good grazing grass, 5-60 cm high distributed widely in tropical America, tropical and South Africa, extending through Arabia and Pakistan to India (Cope, 1982). We propose the hypothesis that their relative tolerance or response to saline water during seed germination stage is more dependent on factors like light, salinity and temperature acting in concert rather than individually. Results obtained from this study may provide clues to the relative tolerance of these species to sea salt or NaCl during germination and to their interaction with light and temperature regimes. This will help in understanding their distribution pattern, optimizing the plant population in the field where conditions vary considerably and in using these species as cash crop.

Materials and Methods

Seeds of *D. annulatum* and *E. ciliaris* were collected from Karachi University campus during September 2008, separated from the inflorescence, cleaned and stored at room temperature (25:35ºC) in opaque plastic bottles. During October, these seeds were surface sterilized for 1 minute with 1% sodium hypochlorite solution before experiment. Four replicates of 25 seeds each were used for each treatment. Germination was carried out using tight-fitting plastic Petri plates (5 cm diameter) with 7 ml of test solution. Each dish was placed in a larger 10 cm diameter plastic Petri plate to avoid evaporation. Sea salt and NaCl were used at similar concentrations (0, 10, 20, 30, 40 and 50 dS m^{-1} NaCl or sea salt for *D. annulatum* and 0, 2.5, 7.5, 10.0, 12.5 and 15.0 dS m^{-1} NaCl or sea salt for *E. ciliaris*). These concentrations were determined in preliminary tests on their salt tolerance. Germination was tested at 10:20, 15:25, 20:30 and 25:35ºC (night: day) temperature regimes with a 12 h photoperiod at 25 μmol m^{-2} s^{-1}, 400-700 nm Sylvania cool white fluorescent lamps or in complete darkness. The above temperatures cover the conditions experienced by germinating seeds during the year. Seed germination (emergence of radicle) was noted every alternate day for 20 days in 12 h photoperiod and only once after 20 days in seeds kept in dark. All un-germinated seeds from dark were subsequently allowed to germinate in distilled water under their respective temperature regimes and 'dark-light' treatments as above for another 20 days and germination noted thereafter. Tetrazolium chloride was used to test the viability of un-germinated seeds.

Rate of germination were calculated with the help of a modified Timson's germination velocity index = ΣG/t, where G = percentage of seed germinated after 20 days, and t = total time of germination (Khan & Ungar, 1999). Percentage recovery of germination was calculated by the formula: (a-b)/(c-b)*100 where, a = number of seeds germinated after transfer to distilled water, b = number of seeds germinated in saline solution and c = total number of seeds. Data were arcsine transformed before statistical analysis to ensure homogeneity of variance using SPSS 9.0 (Anonymous, 1999). The effect of salinity and temperature on the rate of germination and recovery were examined using two-way analysis of variance (ANOVA). Post-hoc Bonferroni analysis (Multiple range test) was carried out to delineate differences between individual treatment means.

Results

A two-way ANOVA indicated significant individual effect of salinity, temperature and their interaction on germination, rate of germination, recovery, and rate of recovery of seeds of both species in NaCl and sea salt (Table 1). Germination was higher in distilled water control than in any of the saline treatments while salinity decreased as well as delayed germination progressively with increasing concentration irrespective of the source-NaCl or sea salt. However, compared to sea salt, germination was generally lower in NaCl (Figs. 1-4). In light/dark condition, seeds germinated rapidly in non-saline controls at all temperatures tested and reached final germination in less than 4 days in *D. annulatum* (Fig. 1) while in case of *E. ciliaris* (Fig. 2), it took 4-6 days. Maximum seeds germinated at 25:35 ºC followed by 20:30ºC and 15:25ºC in both the species. Seeds germinated better under light in comparison to dark (Figs. 3 and 4). Absence of light almost completely inhibited germination in *E. ciliaris* seeds under various treatments used (Figs. 3 and 4).

Seed germination was generally optimal for both species at 25:35°C and decreased with a decrease in temperature. Seeds under NaCl and sea salt regimes when transferred to distilled water after 20 days exposure to salinity, germinated readily and their recovery as well as the rate of recovery were related with salinity concentrations and temperature regimes i.e., rate of germination increased with increase in temperature and decreased with increasing salinity (Tables 2 and 3) while rate of recovery increased with increasing salinity and decreased with increasing temperature (Tables 4 and 5). Most of the un-germinated seeds remained viable however; mortality increased with decrease in optimum temperature and the number of dead seed was generally higher in *E. ciliaris* compared to *D. annulatum* (Tables 6 and 7).

Table 1. Two-way ANOVA of the effect of salt (S), temperature (T) and their interaction (SxT) on various germination parameters of *Dichanthium annulatum* and *Eragrostis ciliaris*.

	Source of variation	*Dichanthium annulatum*		*Eragrostis ciliaris*	
		NaCl	**Sea salt**	**NaCl**	**Sea salt**
Germination (%)	S	393.65	604.75	13.36	48.89
	T	16.83	52.99	70.67	118.32
	SxT	4.82	17.66	5.60	16.28
Rate of germination	S	524.52	996.80	14.09	52.07
	T	25.13	85.07	75.54	121.87
	SxT	4.98	23.79	5.28	18.06
Recovery (%)	S	53.80	163.80	6.87	7.04
	T	11.09	19.31	17.49	30.94
	SxT	1.21	10.20	5.06	4.50
Rate of recovery	S	66.01	263.10	5.23	5.37
	T	10.26	21.43	19.34	25.83
	SxT	1.76	9.59	3.84	3.55

Data represent *F*-values showing significant differences at $p<0.0001$

Table 2. Effect of NaCl salt and temperature regimes on the rate of seed germination of + *Dichanthium annulatum* at 12 h photoperiod.

NaCl (dS m^{-1})	Temperature regime			
	10:20°C	**15:25°C**	**20:30°C**	**25:35°C**
0	49.5 ± 0.3	50.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0
10	25.2 ± 2.5	37.2 ± 0.7	39.0 ± 4.0	44.0 ± 1.0
20	18.6 ± 0.9	22.8 ± 1.1	27.4 ± 2.2	31.9 ± 4.8
30	1.9 ± 0.2	4.0 ± 0.8	17.9 ± 3.4	9.4 ± 0.6
40	0.4 ± 0.2	2.6 ± 0.5	4.9 ± 2.0	3.6 ± 0.9
50	0.0 ± 0.0	0.5 ± 023	2.2 ± 0.8	0.0 ± 0.0

Table 3. Effect of NaCl salt and temperature regimes on the rate of seed germination of *Eragrostis ciliaris* at 12 h photoperiod.

NaCl (dS m^{-1})	Temperature regime			
	10:20°C	**15:25°C**	**20:30°C**	**25:35°C**
0	49.5 ± 0.3	50.0 ± 0.0	50.0 ± 0.0	50.0 ± 0.0
2.5	42.5 ± 2.4	38.6 ± 1.6	37.4 ± 3.9	49.7 ± 0.2
7.5	10.9 ± 1.0	26.6 ± 0.6	25.9 ± 0.6	43.7 ± 0.9
10.0	1.6 ± 0.5	6.9 ± 1.7	19.8 ± 1.1	24.2 ± 1.7
12.5	1.7 ± 0.5	4.7 ± 1.0	6.6 ± 0.6	6.7 ± 1.8
15.0	0.0 ± 0.0	1.1 ± 0.3	1.8 ± 0.5	0.3 ± 0.2

Table 4. Effect of NaCl salt and temperature regime on the rate of recovery of seed germination of *Dichanthium annulatum* at 12 h photoperiod.

NaCl (dS m^{-1})	Temperature regime			
	10:20°C	**15:25°C**	**20:30°C**	**25:35°C**
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10	2.7 ± 0.2	0.0 ± 0.0	2.3 ± 1.7	0.0 ± 1.0
20	6.3 ± 0.7	7.1 ± 1.0	9.1 ± 0.5	4.3 ± 0.1
30	12.0 ± 1.3	13.9 ± 0.7	15.5 ± 0.9	7.6 ± 1.2
40	20.2 ± 1.4	23.6 ± 1.4	23.3 ± 1.7	17.5 ± 1.8
50	28.7 ± 1.2	30.8 ± 0.6	36.7 ± 0.5	25.8 ± 1.1

Table 5. Effect of NaCl salt and temperature regime on the rate of recovery of seed germination of *Eragrostis ciliaris* at 12 h photoperiod.

NaCl (dS m^{-1})	Temperature regime			
	10:20°C	**15:25°C**	**20:30°C**	**25:35°C**
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2.5	0.0 ± 0.0	1.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7.5	6.2 ± 0.2	4.1 ± 0.2	4.5 ± 0.6	0.0 ± 0.0
10.0	11.0 ± 0.6	7.9 ± 0.5	9.2 ± 1.7	5.4 ± 0.3
12.5	22.9 ± 0.9	20.1 ± 1.8	30.2 ± 1.1	13.6 ± 1.3
15.0	33.7 ± 1.2	27.6 ± 1.9	25.3 ± 3.1	21.5 ± 1.4

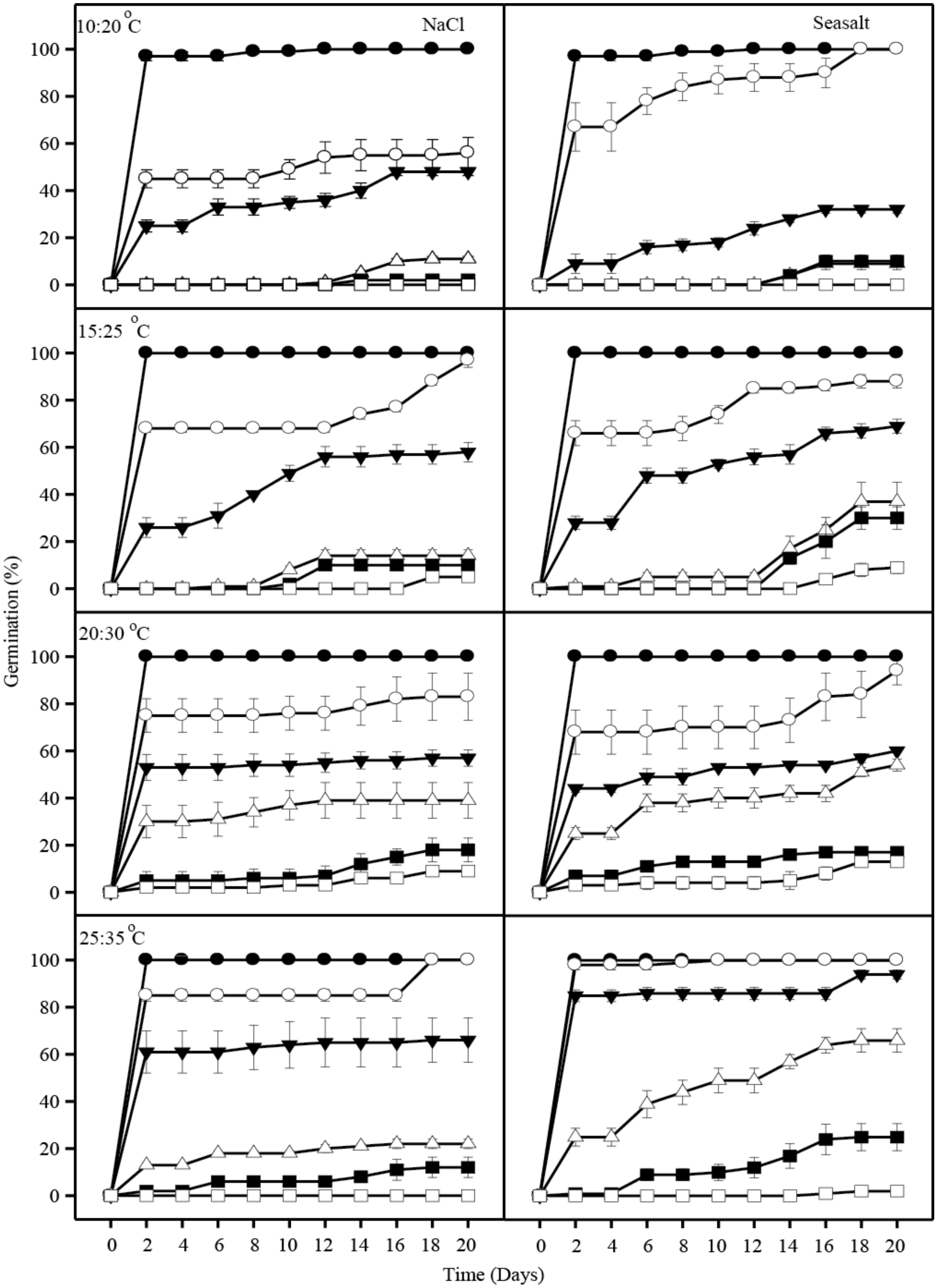

Fig. 1. Effect of salt (NaCl and sea salt), their concentration in dS m^{-1} (black circles: 0; white circles: 10; black triangles: 20; white triangles: 30; black squares: 40; white squares: 50) and temperature (10:20, 15:25, 20:30 and 25:35°C) on seed germination of *Dichanthium annulatum*.

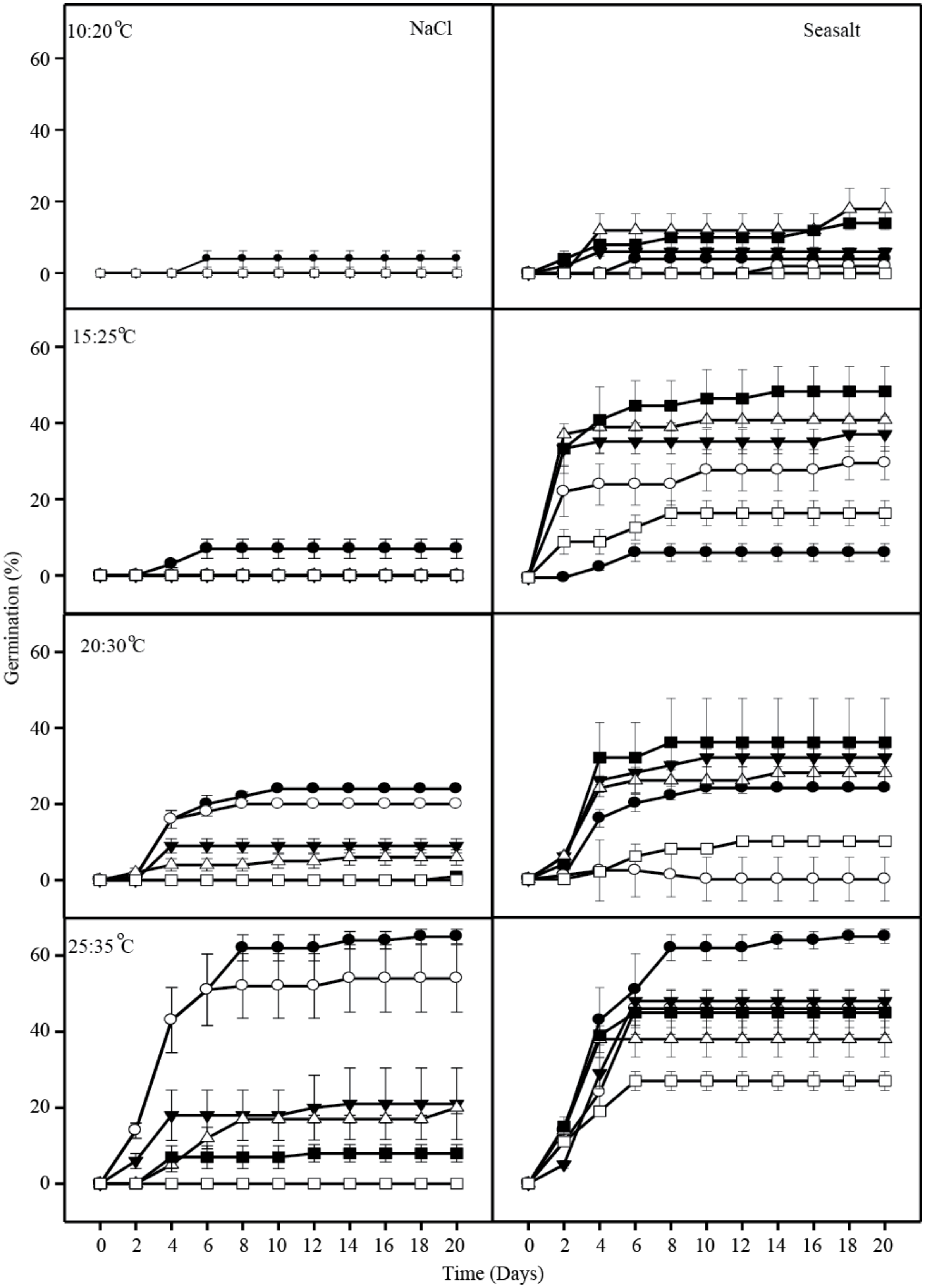

Fig. 2. Effect of salt (NaCl and sea salt), their concentration in dS m-1 (black circles: 0; white circles: 2.5; black triangles: 7.5; white triangles: 10.0; black squares: 12.5; white squares: 15.0) and temperature (10:20, 15:25, 20:30 and 25:35°C) on seed germination of *Eragrostis ciliaris*.

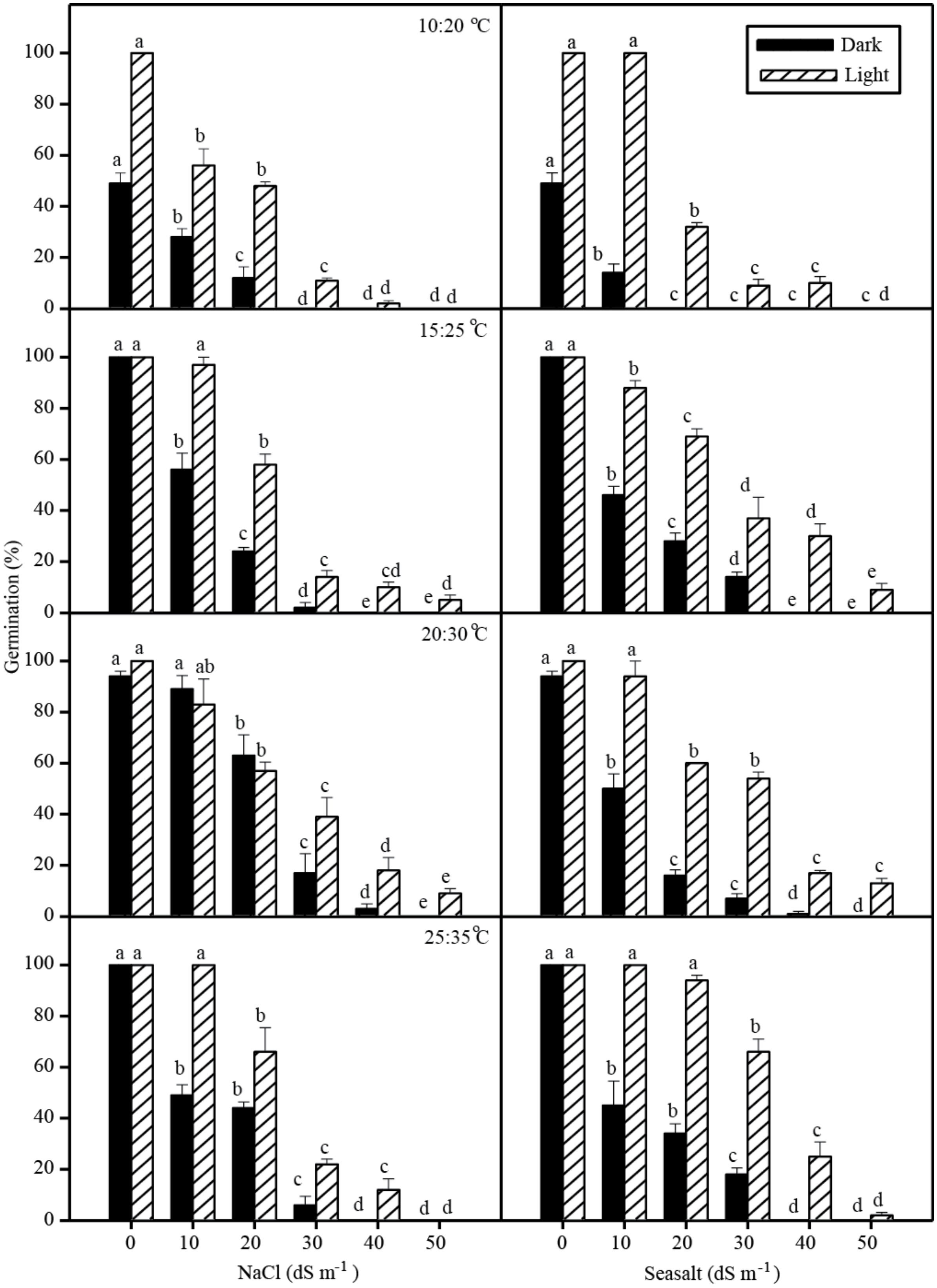

Fig. 3. Effect of salt (NaCl and sea salt), temperature regimes and light (complete darkness and 12 h light/dark photoperiod) on final seed germination of *Dichanthium annulatum*. Similar letters over means (±S.E. n = 4) within each light treatment are not significantly ($p<0.05$) different from each other (t-test).

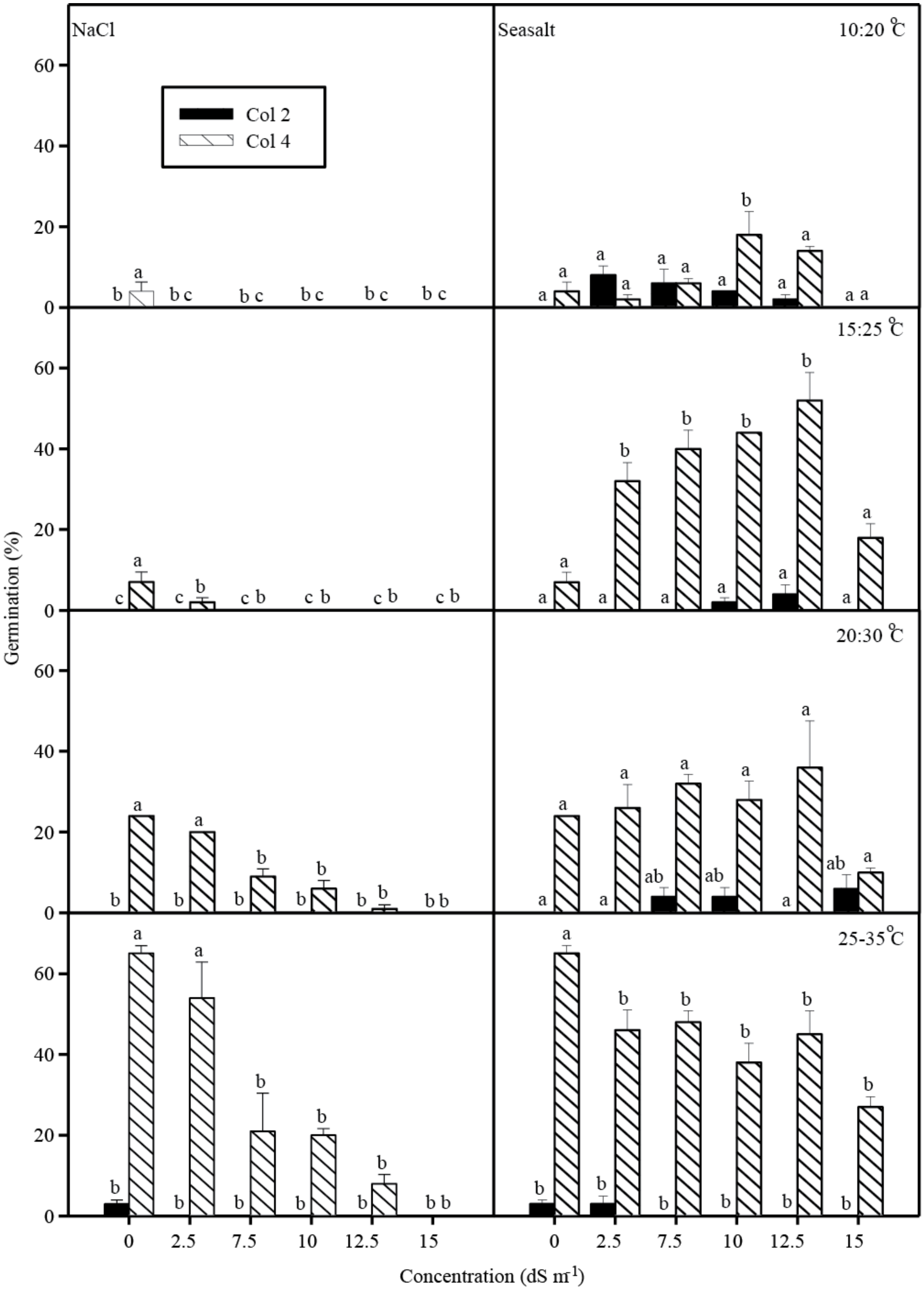

Fig. 4. Effect of salt (NaCl and sea salt), temperature regimes and light (complete darkness and 12 h light/dark photoperiod) on final seed germination of *Eragrostis ciliaris*. Similar letters over means (± S.E. n = 4) within each light treatment are not significantly ($p<0.05$) different from each other (t-test).

Table 6. Effect of NaCl, sea salt and temperature regime on the seed germination, recovery, dormancy induction and mortality of *Dichnathium annulatum* at 12 h photoperiod.

Conductivity dS m^{-1}		NaCl				Sea salt			
		10:20	15:25	20:30	25:35	10:20	15:25	20:30	25:35
Germination (%)	0	100	100	100	100	100	100	100	100
	10	56	97	83	100	100	88	94	100
	20	48	58	57	66	32	69	60	94
	30	11	14	39	22	9	37	54	66
	40	2	10	18	12	10	30	17	25
	50	0	5	9	0	0	9	12	2
Recovery (%)	0	0	0	0	0	0	0	0	0
	10	14	0	10	0	0	7	0	0
	20	29	28	33	25	25	17	25	0
	30	49	61	46	33	40	29	37	21
	40	82	83	70	72	74	58	83	54
	50	100	95	91	88	95	86	88	83
Dormancy (%)	0	0	0	0	0	0	0	0	0
	10	30	3	7	0	0	5	6	0
	20	21	13	10	8	41	13	14	6
	30	35	21	13	36	50	33	9	12
	40	12	6	10	12	15	11	0	18
	50	0	0	0	10	4	4	0	10
Mortality (%)	0	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0
	20	2	1	0	1	2	1	1	0
	30	5	4	2	9	1	1	0	1
	40	4	1	2	4	1	1	0	3
	50	0	0	0	2	1	1	0	5

Table 7. Effect of NaCl, sea salt and temperature regime on the seed germination, recovery, dormancy induction and mortality of *Eragrostis cilliaris* at 12 h photoperiod.

Conductivity dS m^{-1}		NaCl				Sea salt			
		10:20	15:25	20:30	25:35	10:20	15:25	20:30	25:35
Germination (%)	0	4	7	24	65	4	7	24	65
	2.5	0	2	20	54	2	32	26	46
	7.5	0	0	9	21	6	40	32	48
	10.0	0	0	6	20	18	44	28	38
	12.5	0	0	1	8	14	52	36	45
	15.0	0	0	0	0	0	18	10	27
Recovery (%)	0	2	0	0	0	2	0	0	0
	2.5	0	1	1	2	10	8	0	1
	7.5	0	0	8	14	16	8	2	11
	10.0	7	9	6	17	10	4	0	15
	12.5	0	0	4	33	10	0	2	30
	15.0	0	0	8	25	12	6	12	20
Dormancy (%)	0	89	85	72	30	89	85	72	30
	2.5	95	92	73	40	76	53	72	52
	7.5	90	90	77	60	57	43	62	39
	10.0	83	85	82	58	54	49	68	42
	12.5	95	88	86	55	47	40	59	24
	15.0	93	87	83	69	43	54	63	48
Mortality (%)	0	5	8	4	5	5	8	4	5
	2.5	5	5	6	4	12	7	2	1
	7.5	10	10	6	5	21	9	4	2
	10.0	10	6	6	5	18	3	4	5
	12.0	5	12	9	4	29	8	3	1
	15.0	7	13	9	6	45	22	15	5

Discussion

Perennial grasses are more ubiquitous among 150 halophytes distributed along the Arabian Sea coast of Pakistan. During germination and growth these species are subjected to various degree of temperature, moisture and salinity stress due to the unpredictable monsoon rains leading to adaption of different strategies to maximize their fitness (Kigel, 1995; Khan, 1999; Saeed *et al*., 2011). In the present study, seeds of both *E. ciliaris* and *D. annulatum* showed 100% germination in non-saline control at all temperature regimes at 12 h photoperiod. However germination was reduced in dark in *D. annulatum* and almost no seed germinated in *E. ciliaris* in any treatment (Fig. 4). This indicates that both temperature and light played a significant role in seed germination.

Seed germination was delayed and inhibited by application of both NaCl and sea salt; similar responses were reported over a wide range of halophytes (Joshi *et al.,* 2005; Khan *et al*., 2006). At the onset of germination, passive water uptake leads to activation of metabolic processes as the dormancy of the seed is broken following imbibition (Bewley & Black, 1994). Elevated NaCl and sea salt concentrations reduce water uptake by seeds, thereby partially or completely inhibiting the germination process. Laboratory studies on various halophytes from Karachi coast indicated that most of them attain optimal germination in distilled water and their salinity tolerance varied between 300-1000 mM NaCl (Khan & Gul, 2006).

Sodium chloride inhibited seed germination more in comparison to sea salt. Limited information is available on the effect of sea water on the germination of halophytes (Joshi & Khairatkar, 1995; Arun *et al*., 2001; Houle *et al*., 2001; Naidoo & Kift, 2006), and on the relative tolerance of sea water and NaCl solutions during germination (Tirmizi *et al*., 1993; Mehrun-Nisa, 2001; Zia & Khan, 2004, Joshi *et al*., 2005, Liu *et al*., 2006, Zehra & Khan, 2007). Seed germination of *A. lagopoides* and *Sporobolous madraspatanus* was inhibited more by NaCl than sea water (Joshi *et al*., 2005). However seed germination of *Salvadora persica* (Joshi & Khairatkar, 1995) and *Salicornia brachiata* (Joshi & Iyengar, 1982) was inhibited more by sea water than different individual chlorides of Na^+, K^+ and Mg^{++}. Atayat (2001) compared the germination of various halophyte seeds in both sea water and NaCl and found the effect to be species-specific i.e. promoting effect of seawater was found in *Salsola imbricata* and inhibitory in *Suaeda fruticosa* at all thermoperiods. Sea water inhibited seed germination of *Arthrocnemum macrostachyum* at all thermoperiods except at 25:35°C, where sea water proved to be less inhibitory than NaCl (Atayat, 2001).

Halophyte seeds vary in their response to light during seed germination which ranges from no effect of light to little germination without light (Baskin & Baskin, 1998). In both species of this study, light was required for better germination in saline as well as non-saline conditions, more so in *E. ciliaris* whose seeds failed to germinate in dark. This inhibition of seed germination could be due to the inactivity of Pfr in darkness which regulates several genes coding both for enzymes and structural proteins (Ni *et al*., 1999; Martinez-Garcia *et al*., 2000; Orozco-Segovia *et al*., 2000; Hennig *et al*., 2002) while ecologically, the light dependence of seeds may be a mechanism evolved to prevent germination when seeds are buried in the soil, leaf litter or are under a canopy (Eshetu & Kari, 2002; Teel & Buck, 2002).

Light could also control the timing of seed germination, a crucial factor in the seedling survival and completions of its life cycle with high fecundity. Germination in 23 species showed substantial variation in response to light (Baskin & Baskin, 1998). In an earlier study we observed that light increased seed germination in local halophytic grasses such as *U. setulosa, H. mucronatum, S. ioclados* and *A. lagopoides* (Khan & Gulzar, 2003). Germination was also influenced by light and salinity in the present study where it was improved in presence of light, more so in *E. ciliaris* while salinity decreased germination at all temperature regimes in both species (Figs. 3 and 4). Interactive effects of light with salinity (NaCl and seawater) and temperature have also been reported in other halophytes (Neo & Zedler, 2000; Huang *et al*., 2003; Redondo *et al*., 2004; Zia & Khan, 2004; Zehra & Khan, 2007).

Temperature is reported to be another important factor in modulating seed germination responses under saline conditions (Al-Khateeb, 2006; Mauromicale & Licandro, 2002) but the response may be species-specific e.g. *S. ioclados* is more sensitive to change in temperature in comparison to *A. lagopoides*, *H. mucronatum*, *U. setulosa*, *A. sphaerocephala*, *A. glabra* and *P. aquatica* (Khan & Gulzar, 2003; Mata & Moreno-Casasola, 2005; Zheng *et al*., 2005). Diurnal fluctuation in temperature has also been reported to stimulate seed germination of *Phragmites australis* and *Typha latifolia* (Ekstam & Forseby, 1999; Ekstam *et al*., 1999). Although salinity reduces germination and growth but the detrimental effect is generally lower at optimum temperature for a particular species (Aiazzi *et al*., 2002) and any deviation is harmful (Khan & Gulzar, 2003). At high temperature perhaps the salt concentration increases due to higher evaporation (Khan & Ungar, 1996) while at low temperature the physiological mechanisms to tolerate salts may be impaired. This is also evident in Tables 6 and 7 where low temperature (10/20°C) resulted in high mortality more so in *E. ciliaris*. However, in some species, salt tolerance has been reported to be not dependent on temperature (Khan & Gul, 2002). Halophytes from this region like *L. stocksii* and *P. karka* interacted when germinated either in NaCl or sea salt showed similar effect in change in temperature (Zia & Khan, 2004, Zehra & Khan, 2007). The grasses under test here are widely distributed in coastal and inland areas of Karachi where average ambient diurnal temperatures in summer generally corresponds to the optimum temperature for germination observed in the present study i.e. 35:25°C with sea breeze. Monsoon rain brings down mid-summer higher temperatures as well as causes reduction in soil salinity due to leaching and apparently provides suitable conditions for recruitment.

Seeds of halophytes have been found to germinate even after lying dormant for prolonged periods under hyper-saline conditions; however, they differ in their ability to recover from this stress (Keiffer & Ungar, 1997; Khan & Ungar, 1997). Sub-tropical halophytes generally do not maintain a persistent seed bank and suffer higher mortality during storage under high temperature and salinity stress (Khan & Gul, 2006). In the present study, seed of *D. annulatum* germinated readily when transferred to distilled water after 20 days of salinity treatment (Table 6). However, most of the un-germinated seeds were found to be dormant (Tables 6 and 7). Recovery of seed germination from salt stress was not affected by salt type. Seeds exposed to higher concentrations (40 and 50 dS m^{-1}) of NaCl and sea salt showed higher recovery, which was higher than the final germination percentage at these salinity levels. Seeds of *U. setulosa* are reported to recover best (>80% of un-germinated seeds) at 200 mM NaCl under optimal temperature of 20-30°C (Gulzar & Khan, 2001). Few halophyte seeds maintain viability after 21 d at sea water salinity higher than 400 mM NaCl with rapid and high germination after transfer to distilled water (Martin & Trine, 2005). Salinity seems to have a priming effect on these seeds by promoting germination in comparison to seeds which are not exposed to saline conditions. Zhang *et al*., (2010) suggested that barley seeds exposed to NaCl salinity could take up sodium which helps in generating additional osmotic potential, absorbing more water and thus resulting in rapid germination in environment of low water potential.

Seeds of *E. ciliaris* exposed to NaCl and sea salt for 20 days responded differently when transferred to distilled water under various temperature regimes. Our data indicate low recovery response in NaCl in comparison to sea salt. Similar result was reported for *Zygophyllum simplex* seeds, which had little recovery from all NaCl concentrations at all thermo-periods tested (Khan & Ungar, 1997). However, various species of *Limonium* and *Carpobrotus* recovered substantially when transferred to distilled water from sea water (Woodell, 1985; Weber & D' Antonio, 1999). Most of the seeds of *E. ciliaris* remained viable but were dormant; however, low temperature did affect their viability especially in sea salt (Table 7). Our results confirm the conclusion that halophyte seeds, unlike glycophytes, could remain viable for long periods under extremely high ionic / osmotic stress and germinate at a time when the salinity stress is reduced (Katembe *et al*., 1998; Pujol *et al*., 2000; Martin & Trine, 2005).

It is concluded that: Species differ in their response to abiotic factors including salinity, temperature and light. In the present study, best germination was observed at relatively high temperatures of 25:35°C which is generally prevalent in this region after monsoon rains and when most of the recruitment takes place. This could be considered as an adaptation to germinate in summer months when ambient temperatures are favorable for seeds to germinate.

Most of the seeds exposed to salinity remained viable and germinated readily after removal of salinity stress. The halophytic grasses generally produce a large number of seeds but for which the soil salinity prevalent at the time of seed dispersal may be too high for germination. A suitable strategy would be to remain dormant and germinate after monsoon when the substrate salinity has been diluted. This appears to be a strategy which helps in sustaining continuity of the population under unfavorable conditions by skipping the harsher period. It is especially necessary for the annuals to have viable seeds ready to germinate whenever conditions are favorable whereas there is no such compulsion for the perennials. In the present study more seeds of the annual grass (*E. ciliaris*) went into dormancy in comparison to the perennial *D. annulatum*. These dormant seeds could germinate at suitable time to maintain the progeny when the older plants died after seed production.

NaCl prevented more seeds from germination in comparison to sea salt solutions and this inhibition markedly increased in darkness, especially in *E. ciliaris*. The greater inhibition in NaCl may be due to specific ion toxicity interfering with metabolic processes of seeds while the presence of multiple ions in sea salt may have an antagonistic effect that reduced toxicity of hazardous ions. This indicates the need to use sea water in such studies for a more practical approach and a better perception of what happens to seeds in their natural habitat near the seashore. The light-dependent germination appears an ecological adaptation to restrict germination while the seed is under a thick canopy, buried in debris or deep in saline soil; seedlings emerging under such conditions may have difficulty in surviving.

The highest inhibition of germination was observed at the lowest thermo-period, which explains the failure of their establishment during winter despite the readiness of seeds to germinate and availability of moisture from the winter rains. There is also a need to exploit the potential of these and other species as cash crop fodder/forage for coastal and nearby inland saline areas.

Acknowledgments

This work was supported by the National Plan for Science and Technology (NPST) project number 11-ENV1756-02.

References

Aiazzi, M.T., P.D. Carpane and J.A. Rienzo. 2002. Effect of salinity and temperature on the germination and early seedling growth of *Atriplex cordobensis* Gandoger et Stuckert (Chenopodiaceae). *Seed Science & Technology,* 30: 329-338.

Al Khateeb, S.A. 2006. Effect of salinity and temperature on germination, growth and ion relations of *Panicum turgidum* Forssk. *Bioresource Technology,* 97: 292-298.

Andrew, T.S., C.E. Jones and R.D.B. Whalley. 1997. Factors affecting the germination of Giant Parramatta grass. *Australian Journal of Experimental Agriculture*, 37: 439-446.

Anonymous. 1999. SPSS 9.0 for Windows Update. SPSS, USA

Arun, A.B., N.S. Raviraja and K.R. Sridhar. 2001. Effect of temperature, salinity and burial on seed germination and seedling emergence of five coastal sand dune legumes. *International Journal of Ecology & Environmental Science*, 27: 23-29.

Atayat, A. 2001. Comparative effects of NaCl and seawater on the germination of halophytes. M.Sc. Thesis, Department of Botany, University of Karachi, Pakistan.

Baskin, C.C. and J.M. Baskin. 1998. Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, USA.

Bewley, J.D. and M. Black. 1994. Seeds: physiology of development and germination. Plenum Press, London.

Chapman, V.J. 1960. Salt marshes and salt deserts of the world. New York, New York: Interscience Publishers.

Cope, T.A. 1982. Poaceae. In: Flora of Pakistan, (Eds.): E. Nasir and S.I. Ali. University of Karachi, Pakistan.

Ekstam, B. and A. Forseby. 1999. Germination response of *Phragmites australis* and *Typha latifolia* to diurnal fluctuations in temperature. *Seed Science Research,* 9: 157-163.

Ekstam, B., R. Johannesson and P. Milberg. 1999. The effect of light and number of diurnal temperature fluctuations on germination of *Phragmites australis. Seed Science Research*, 9: 165-170.

Eshetu, Y. and I. Kari. 2002. Seed germination responses of four montane tree species to red/far-red ratio and temperature. *Forest Ecology and Management*, 168: 53-61.

Flores, J., E. Jurado, L. Chapa-Vargas, A. Ceroni-Stuva, P. Dávila-Aranda, G. Galíndez, D. Gurvich, P. León-Lobos, C. Ordó˜nez, P. Ortega-Baes, N. Ramírez-Bullón, A. Sandoval, C.E. Seal, T. Ullian and H.W. Pritchard. 2011. Seeds photoblastism and its relationship with some plant traits in 136 cacti taxa. *Environmental and Experimental Botany*, 71: 79-88.

Garcia, F.P., J.M. Pita, M.E.G. Benito and J.M. Iriondo. 1995. Effect of light, temperature and seed priming on germination of celery seeds (*Apium graveolens* L.). *Seed Science & Technology*, 23: 377-383.

Gulzar, S. and M.A. Khan. 2001. Seed germination of a halophytic grass *Aeluropus lagopoides*. *Annals of Botany*, 87: 319-324.

Hanselin, M.H. and T. Eggen. 2005. Salinity tolerance during germination of seashore halophytes and salt tolerant grass cultivars. *Seed Science Research*, 15: 43-50.

Hennig, L., W.M. Stoddart, M. Dieterle, G.C. Whiteman and E. Schafer. 2002. Phytochrome E controls light-induced germination of *Arabidopsis*. *Plant Physiology*, 128: 194-200.

Houle, G., L. Moral, C.E. Reynolds and J. Siegel. 2001. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). *American Journal of Botany*, 88: 621-67.

Huang, Z., X. Zhang, G. Zheng and Y. Gutterman. 2003. Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron. Journal of Arid Environment,* 55: 453-464.

Joshi, A.J. and E.R.R. Iyengar. 1982. Effect of salinity on the germination of *Salicornia brachiata* Roxb. *Indian Journal of Plant Physiology*, 25: 390-392.

Joshi, A.J. and P.P. Khairatkar. 1995. Seed germination, amino acids and sugars in seedlings of *Juncus maritimus* and *J. acutus* under salt stress. *Journal of the Indian Botanical Society*, 74: 15-17.

Joshi, A.J., B.S. Mali and H. Hinglajia. 2005. Salt tolerance at germination and early growth of two forage grasses growing in marshy habitats. *Environmental & Experimental Botany*, 54: 267-274.

Katembe, W.J. and I.A. Ungar and J.P. Mitchell. 1998. Effect of salinity on germination and seedling growth of two *Atriplex* species (Chenopodiaceae). *Annals of Botany*, 82: 167-175.

Keiffer, C.W. and I.A. Ungar. 1997. The effect of extended exposure to hyper-saline conditions on the germination of five inland species. *American Journal of Botany*, 84: 104-111.

Khan, M.A. 1999. Comparative influence of salinity and temperature on the germination of subtropical halophytes. Halophyte uses in different climates I: Ecological and ecophysiological studies. In: Progress in biometeorology, (Eds.): H. Leith, M. Moschenko, M. Lohman, H.W. Koyro and A. Hamdy. Vol. 13. Backhuys Publishers, The Netherlands. pp. 77-88.

Khan, M.A. 2002. An ecological overview of halophytes from Pakistan. In: Cash crop halophytes: potentials, pilot projects, basic and applied research on halophytes and saline irrigation, (Eds.): H. Leith and M. Moschenko. 10 years after Al-Ain meeting**.** Kluwer Academic Press, The Netherlands.

Khan, M.A. and B. Gul. 2002. Salt tolerant plants of coastal sabkhas of Pakistan. In: Sabkha ecosystem Vol. I, (Eds.): H. Barth and B. Boer. Kluwer Academic Press, The Netherlands.

Khan, M.A. and B. Gul. 2006. Halophyte seed germination, In: Ecophysiology of high salinity tolerant plants, (Eds.): M.A. Khan and D.J. Weber. Springer Academic Publishers, The Netherlands.

Khan, M.A. and I.A. Ungar. 1984. Effect of salinity and temperature on the germination and growth of *Atriplex triangularis* Willd. *American Journal of Botany*, 71: 481-489.

Khan, M.A. and I.A. Ungar. 1996. Alleviation of seed dormancy in the desert forb *Zygophyllum simplex* L. from Pakistan. *Annals of Botany*, 80: 395-400.

Khan, M.A. and I.A. Ungar. 1997. Effect of thermpoeriod on recovery of seed germination of halophytes from saline conditions. *American Journal of Botany*, 84: 79-83.

Khan, M.A. and I.A. Ungar. 1999. Effect of salinity on the seed germination of *Triglochin maritima* under various temperature regimes. *Great Basin Naturalist*, 59: 44-150.

Khan, M.A. and S. Gulzar. 2003. Light, salinity and temperature effects on the seed germination of perennial grasses. *American Journal of Botany*, 90: 131-134.

Khan, M.A., R. Ansari, B. Gul and M. Qadir. 2006. Crop diversification through halophyte production on salt prone land resources. CAB reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources No. 48.

Kigel, J. 1995. Seed germination in arid and semi-arid regons. In: Seed development and germination, (Eds.): J. Kigel and G. Galili. Marcel and Dekker, New York. pp. 645-700.

Liu, X., H. Qiao, W. Li, T. Tadano and M.A. Khan, S. Yamaguchi and Y. Kamiya. 2006. Comparative effect of NaCl and seawater on seed germination of *Suaeda salsa* and *Atriplex centralasiatica*. In: Biosaline agriculture and salinity tolerance in plants, (Eds.): M. Ozturk, Y. Waisel, M.A. Khan and G. Gork.. pp. 45-54. Birkhauser, Berlin.

Martin, H.H. and E. Trine. 2005. Salinity tolerance during germination of seashore halophytes and salt-tolerant grass cultivars. *Seed Science Research*, 15: 43-50.

Martinez-Garcia, J.F., E. Huq and P.H. Quail. 2000. Direct targeting of light signals to a promoter element-bound transcription factor. *Science*, 288: 859-863.

Mata, D.I. and P. Moreno-Casasola. 2005. Effect of *in situ* storage, light, and moisture on the germination of two wetland tropical trees. *Aquatic Bot.*, 83: 206-218.

Mauromicale, G. and P. Licandro. 2002. Salinity and temperature effects on germination, emergence and seedling growth of globe artichoke. *Agronomie*, 22: 443-450.

Mehrun-Nisa. 2001. Effect of NaCl and seawater on seed germination of *Salsola imbricata* under light and various temperature regimes. M.Sc. Thesis, Department of Botany, University of Karachi, Pakistan.

Naidoo, G. and J. Kift. 2006. Responses of the salt marsh rush *Juncus krausii* to salinity and water logging. *Aquatic Botany*, 84: 217-225.

Neo, G.B. and J.B. Zedler. 2000. Differential effects of four abiotic factors on the germination of salt marsh annuals. *American Journal of Botany*, 87: 1679-1692.

Ni, M., J.M. Tepperman and P.H. Quail. 1999. Binding of phytochrome B to its nuclear signaling partner PIF3 is reversibly induced by light. *Nature*, 400: 781-784.

Orozco-Segovia, A., A.E. Brechú-Franco, L. Zambrano-Polanco, R. Osuna-Fernández, G. Laguna-Hernández and M.E. Sánchez-Corondo. 2000. Effects of maternal light environment on germination and morphological characteristics of *Sicyos deppei* seeds. *Weed Research*, 40: 495-506.

Pujol, J.A., J.F. Calvo and L.R. Diaz. 2000. Recovery of germination from different osmotic conditions by four halophytes from Southeastern Spain. *Annals of Botany*, 85: 279-286.

Redondo, S., A.E. Rubio-Casal, J.M. Castillo, C.J. Luque, A.A. Alvarez, T. Luque and M.E. Figueroa. 2004. Influence of salinity and light on germination of three taxa with contrasted habitats. *Aquatic Botany*, 78: 225-264.

Saeed, S., B. Gul and M.A. Khan. 2011. Comparative effects of NaCl and sea salt on seed germination of *Arthrocnemum indicum*. *Pakistan Journal of Botany*, 43: 1091-1103.

Teel, W.S. and L.E. Buck. 2002. Between wild crafting and monocultures: agroforestry options. In: Nontimber forest products in the United States, (Eds.): E.T. Jones, R.J. Mclain and J. Weigand. University of Kansas Press, Lawrence, KS, pp. 199-222.

Thanos, C.A., K. Georghious, D.J. Douma and C.J. Marangaki. 1991. Photoinhibition of seed germination in Mediterranean maritime plants. *Annals of Botany*, 68: 469-475.

Tirmizi, S.A.S., K.M. Khan and S.I. Qadir. 1993. Study on salt tolerance of *Hippophae rhamnoides* L. during germination. *Pakistan Journal of Science & Industrial Research*, 36: 252-257.

Ungar, I.A. 1995. Seed germination and seed bank ecology in halophytes. In: Seed development and seed germination, (Eds.): J. Kiegel and G. Galili. Marcel and Dekker, New York. pp. 599-628.

Weber, E. and C.M. D'Antonio. 1999. Germination and growth responses of hybridizing *Carpobrotus* species (Aizoaceae) from coastal California to soil salinity. *American Journal of Botany*, 86: 1257-1263.

Woodell, S.R.J. 1985. Salinity and seed germination patterns in coastal plants. *Vegetation*, 61: 223-230.

Zehra, A. and M.A. Khan. 2007. Comparative effect of NaCl and sea salt on germination of halophytic grass *Phragmites karka* at different temperature regimes. *Pakistan Journal of Botany*, 39: 1681-1694.

Zhang, H., I.J. Irving, C. McGill, C. Matthews, D. Zhou and P. Kemp. 2010. The effect of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. *Annals of Botany,* 106: 1027-1035.

Zheng, Y., Z. Xie, Y. Gao, L. Jiang, X. Xing, H. Shimizu and G.N. Rimmington. 2005. Effects of light, temperature and water stress on germination of *Artemisia sphaerocephala*. *Annals of Applied Biology*, 146: 327-335.

Zia, S. and M.A. Khan. 2002. Comparative effect of NaCl and seawater on seed germination of *Limonium stocksii*. *Pakistan Journal of Botany*, 34: 345-350.

Zia, S. and M.A. Khan. 2004. Effect of light, salinity, and temperature on seed germination of *Limonium stocksii*. *Canadian Journal of Botany*, 82: 151-157.

(Received for publication 8 March 2012)