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SEED GERMINATION AND RECOVERY RESPONSES OF SUAEDA HETEROPHYLLA TO ABIOTIC STRESSES

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Abstract

Seed germination and recovery responses of an annual C_3 halophyte *Suaeda heterophylla* (Kar. & Kir.) Bunge were investigated under iso-osmotic concentrations (0, -0.46, -0.92, -1.38, -1.84, and -2.30 MPa) of NaCl and PEG-6000. These experiments were conducted at different temperature (15/25, 20/30and 25/35°C) and photoperiod (12h light: 12h dark and 24h dark) regimes. Highest seed germination was observed in distilled water, moderate temperature (20/30°C) and 12h photoperiod. Increasing concentrations of NaCl and PEG-6000 inhibited seed germination at all temperature and photoperiod regimes tested and this inhibition was higher in NaCl. Recovery of germination and seed viability were also lower in NaCl, indicating greater role of ionic toxicity rather than an osmotic effect.

Introduction

Seed germination, being the most important stage in a plant's life cycle, is tightly regulated by a number of environmental factors including soil salinity, moisture, temperature and light (Neo & Zedler, 2000; Koornneef *et al*., 2002; Khan & Gul, 2006; Saeed *et al*., 2011; Gulzar *et al*., 2013). This is particularly true for halophytes, which inhabit saline areas, where they get a brief opportunity to germinate after sufficient rainfall when conditions are suitable for germination (Chapman, 1974, McMahon & Ungar, 1978; Khan & Ungar, 1986). In addition, distribution of halophytes in saline habitats also depends on appropriate seed germination responses to different environmental factors (Tobe *et al*., 2000).

Soil salinity is the major ecological factor which, influences seed germination of halophytes (Elsey-Quirk *et al*., 2009; Khan & Gul, 2006, Al-Khateeb, 2006, Song *et al*., 2008) that generally decreases with the increase in salinity level causing reduced water uptake (osmotic stress) or/and ionic toxicity (Khan & Gul, 2006, Ahmed & Khan, 2010; Saeed *et al*., 2011). Inhibition of germination in seeds of several halophytes like *Atriplex prostrata* (Egan *et al*., 1997), *A. halimus* (Baji *et al*., 2002), *Halocnemum strobilaceum*, *Arthrocnemum macrostachyum* and *Sarcocornia fruticosa* (Pujol *et al*., 2000) was due to osmotic stress imposed by salinity. However, NaCl inhibited germination more than iso-osmotic PEG-6000 in *Aristida adscensionis*, *Artemesia ordosica* (Tobe *et al*., 1999) and *Prosopis strombulifera* (Sosa *et al*., 2005) indicating an ionic effect of salinity. Halophytes also vary in their upper limit to tolerate salinity during germination (Khan, 1999; Hameed *et al*., 2006; Orlovsky *et al*., 2011), with highest tolerance limit observed in *Salicornia herbacea* (1700 mM NaCl; Chapman, 1960). Halophyte seeds may tolerate higher levels of soil salinity and recover their ability to germinate after salinity stress is removed (Woodell, 1985; Hardegree & Emmerich, 1990). For instance, ~80% recovery of seed germination was observed in seeds of *Suaeda fruticosa, Arthrocnemum macrostachyum* and *Salicornia ramosissima* when transferred to water after extended exposure from 500 mM NaCl (Khan & Ungar 1998 and Rubio-Casal *et al*., 2003).

Several studies have reported that variation in temperature also influences seed germination of halophytes (Khan & Ungar, 1998; Khan *et al*., 2001a; Gul & Weber, 1999; Al-Khateeb, 2006; Zheng *et al*., 2005; Benvenuti *et al*., 2004, Zia & Khan, 2008, Tlig *et al*., 2008). In contrary, species like *Suaeda moquinii* (Brown seeds; Khan *et al*., 2001b) and *Sacrobatus vermiculatus* (Khan *et al*., 2001c) are not temperature sensitive. Halophytes inhabiting temperate habitats show optimal germination at a temperature regime of 25-35°C (Khan *et al*., 2000, 2001a, 2002; Gul & Weber, 1999) but species such as *Suaeda moquinii* (Black seeds; Khan *et al*., 2001b), *Triglochin maritima* (Khan & Ungar 1999) and *Sacrobatus vermiculatus* (Khan *et al*., 2001c) germinate optimally at temperature regimes 5-15, 5-25 and 20-30°C respectively. Seed germination at other than optimal temperature regime under saline condition was synergistically inhibited (Khan & Gul, 2006).

Light also influences seed germination of halophytes (Baskin & Baskin, 1998; Ahmed and Khan 2010). Baskin & Baskin (1995) reported that out of 41 halophytic species, germination of 20 species was promoted by light, 10 geminated in dark, and 11 species germinated equally well in light or dark. Light affects germination alone in some species, while in others the light response depends upon salinity and/or temperature (Gul & Weber, 1999; Khan & Ungar, 1997). Seeds of *Sarcocornia fruticosa* germinated more in light under non-saline control, however, seed germination was higher in dark at similar salinity concentrations (Redondo *et al*., 2004).

It is widely reported that effects of salinity on germination of halophytes modulated by other environmental factors (Khan & Gul, 2006). This helps seeds to regulate the timing of seed germination which is optimal. Seeds of halophytes usually germinate in natural habitats when there is reduced soil salinity mainly due to rainfall or melting of snow, coincided with an optimal day length, and adequate temperature regime (Naidoo & Naicker 1992; Gutterman *et al*., 1995).

Suaeda heterophylla (Kar. & Kir.) Bunge is an annual halophyte from Amaranthaceae (Chenopodiaceae). It is a less abundant C_3 herb, found in saline mountainous playas of Upper Hunza, Pakistan. Information about salinity tolerance of this halophyte is not found. Studies on seed germination ecology of this species may give us a new perspective. We determined optimal conditions for seed germination of *Suaeda heterophylla* through controlled experiments, by answering the following questions: a) what are the effects of temperature and light on salt tolerance of *S. heterophylla* seeds? b) whether inhibition of seed germination due to NaCl is because of ionic toxicity or hyper-osmotic stress? and c) how do ionic and osmotic effects, temperature and light collectively influence germination recovery and viability of *S. heterophylla* seeds? Answers to these questions may help in understanding the less frequent occurrence of this species at the study site.

Materials and Methods

Mature inflorescences of *Suaeda heterophylla* were collected from a population located in a saline playa near Borith Lake, Upper Hunza, Pakistan, during September-October, 2005. Geographically, the location is at 2569 m altitude, 36º 25.983′ N, 74º 51.775′ E, with temperate weather conditions, where average temperature ranges from -1.6^{o}C (January) to 36.4^{o}C (July). Area receives low rainfall and main source of freshwater is snow which begins to melt in spring (March to May). Two year (2004-05) meteorological data including temperature (T_{min} and T_{max}), day-length (L_d), rainfall (P_m) and relative humidity (H_r) was obtained from Pakistan Meteorological Department (Table 1).

Table 1. Mean values for two-year meteorological data from Upper Hunza, Pakistan.

Months	T_{min} (^{o}C)	T_{max} (^{o}C)	L_d (H)	P_m (mm)	H_r (%)
Jan	-1.60	12.35	3.22	0.2	48
Feb	0.65	14.55	4.54	20.15	38.5
Mar	6.10	20.50	6.21	12.25	29.5
Apr	9.35	25.05	5.97	34.5	33.5
May	10.40	28.20	7.69	50.25	30.5
Jun	14.20	34.25	9.08	10.85	24
Jul	16.85	36.45	8.73	10.1	33
Aug	17.00	34.50	7.21	9.9	38
Sep	12.15	31.95	6.95	9.95	39
Oct	5.60	25.05	7.28	7.45	39.5
Nov	0.90	19.50	4.74	0.15	43.5
Dec	-0.65	12.50	2.58	20.6	54.5

Seed bearing inflorescences were collected randomly from the plants of uniform size to ensure adequate representation of the genetic diversity among the individuals of population. Collected material was air dried and stored in polyethylene bags and brought to the University of Karachi. Seeds were separated from inflorescence, cleaned and surface sterilized by using 0.82% sodium hypochlorite solution for 1 minute, followed by thorough washing with autoclaved distilled water and air-drying. Germination was carried out in 50-mm diameter tight fitting plastic Petri-plates, with 5 ml of test solution. Seeds were germinated separately in iso-osmotic solutions of NaCl and PEG-6000 (Sigma Chemicals) prepared according to Sosa *et al*., (2005). Six levels of osmotic potential (Ψ_s) i.e., 0, -0.46, -0.92, -1.38, -1.84, and -2.30 MPa were used, on the basis of the results of preliminary tests, which determined the range of tolerance. In this paper, solute concentration refers to the osmotic potential of the solution, as we used iso-osmotic concentrations of NaCl and PEG to determine if the germination inhibition by NaCl is an osmotic or ionic effect. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated with radical emergence (Bewley & Black, 1994).

Seeds were germinated in programmed incubators (Percival Scientific, Boone, Iowa, USA) at three alternating temperature regimes of 15/25, 20/30, and 25/35°C, where higher temperatures (25, 30, and 35°C) coincided with a 12-h light period (25µmol m^{-2} s^{-1}, 400-700 nm Philips cool white florescent lamps) and lower temperatures (15, 20, and 25°C) coincided with a 12-h dark period. Percent germination was recorded after every 48-h for 20 days. After 20 days all un-germinated seeds were transferred to distilled water for further 20 days, to study the recovery of germination. All remaining un-germinated seeds were tested for their viability, with the help of 2, 3, 5-triphenyl tetrazolium chloride (TTC) test after completion of recovery of germination experiment (MacKay, 1972; Bradbeer, 1998).

Rate of germination for light-treated seeds was estimated by using modified Timson's index of germination velocity, germination velocity = ΣG/t, where *G* is the percentage of seed germination at 2-d intervals and *t* is the total germination period (Khan & Ungar 1997). The maximum value possible for our data with this index is 50 (i.e., 1000/20). Higher values for rate of germination indicate faster germination.

Another set of germination experiment was carried out in complete darkness by placing Petri-plates in black plastic bags and then in incubators at the above-mentioned temperature regimes for 20 days. Percent germination was recorded after 20 days. Percent recovery of germination of all un-germinated seeds was also recorded by keeping them in above mentioned temperature regimes coincided with 12-hour photoperiods for further 20 days, and still un-germinated seeds were tested for their viability as described above.

Germination data were arcsine transformed before statistical analyses to ensure homogeneity of variance. These data were analyzed using SPSS Version 10.0 for Windows (Anon., 2001) with the help of ANOVA to demonstrate significance of individual and interactive effects of above-mentioned abiotic factors on different seed germination parameters. A Bonferroni post-hoc test was also used ($p<0.05$) to determine significant differences between means of percent germination and rate among salinity and PEG-6000 treatments under various light and temperature regimes (Anon., 2001).

Results

Final seed germination: A four-way ANOVA showed that the final seed germination (G_F) of *S. heterophylla* was significantly ($p<0.0001$) affected by solute types, solute concentration (osmotic potential) and thermoperiod (Table 2). Maximum G_F (65%) was observed in distilled water (0 MPa) at 12 h photoperiod and decreased subsequently with the increasing osmotic potential of both NaCl and PEG-6000 (Fig. 1A). Optimum temperature regime for G_F was found to be 20/30°C, where about 5% seeds germinated at -2.3 MPa in both NaCl and PEG-6000 (Fig. 1A). Warmer thermoperiods (15/25 and 25/35°C) showed 10 % greater G_F in -1.8 MPa of PEG-6000 than NaCl (Fig. 1A).

Maximum (~50 %, 30% and 20%) seed germination under complete darkness (G_D) was observed in distilled water (0 MPa) at 25/35, 20/30 and 15/25°C respectively (Fig. 2A). Increasing concentrations of both solutes (NaCl and PEG-6000) inhibited the G_D at all temperature regimes. G_D was substantially lower in NaCl than PEG-6000 treatment especially at 25/35°C where seed germinated only in PEG -6000 (Fig. 2A).

Table 2. Germination rate of *Suaeda heterophylla* seeds, in different iso-osmotic solutions of NaCl and PEG-6000 at different temperature regimes and in presence of light (12-hour photoperiod).

Temperature (°C)	Solutes type	Osmotic potential (-MPa)					
		0	0.46	0.92	1.34	1.84	2.3
15/25	NaCl	17 ± 1	14 ± 2	10 ± 1	3 ± 1	0 ± 0	0 ± 0
	PEG	17 ± 1	16 ± 1	12 ± 2	4 ± 1	1 ± 0	0 ± 0
20/30	NaCl	26 ± 1	19 ± 1	15 ± 4	6 ± 2	6 ± 1	1 ± 1
	PEG	26 ± 1	22 ± 1	17 ± 4	8 ± 1	7 ± 1	2 ± 1
25/35	NaCl	27 ± 3	12 ± 4	4 ± 2	1 ± 1	0 ± 0	0 ± 0
	PEG	27 ± 3	14 ± 1	8 ± 1	4 ± 1	1 ± 1	0 ± 1

Germination recovery: Germination recovery from solutes to distilled water (RGS) was significantly ($p<0.0001$) affected by solute types, solute concentration (osmotic potential), thermoperiod and all interactions (Table 3). Un-germinated seeds from osmotic potential of both solute types showed recovery of germination (RGS), when transferred to distilled water, although lower recovery was observed from NaCl than PEG-6000 (Fig. 1B). Partial germination recovery was observed above -0.9 MPa NaCl solute at 15/25 (up to 30%), 20/30 (up to 20%) and 25/35°C (up to 10%). Recovery of seed from PEG-6000 was negatively affected in the following order of thermoperiod 25/35 > 15/25°C whereas 5-20% improvement in seed germination was observed at 20/30°C (Fig. 1B).

Recovery of seed from complete dark to 12 h photoperiod (RGD) was significantly affected by solute types ($p<0.0001$), solute concentration ($p<0.0001$) and thermoperiod ($p<0.001$) (Table 3). RGD was lower in NaCl than PEG-6000 at all iso-osmotic solute concentration and thermoperiod (Fig. 2B). Moreover during recovery seed germination was improved 10% in -0.5 MPa of PEG-6000 at optimal temperature regime (20/30°C) (Fig. 2B).

Seed viability: The four-way ANOVA indicated significant effect of solute types ($p<0.0001$), solute concentration ($p<0.0001$), thermoperiod ($p<0.001$) and photoperiod ($p<0.0001$) on seed viability (Table 2). All seeds were viable in control treatment (0 MPa) of all thermoperiods used at 12 h photoperiod (Fig. 1C). Seed viability was reduced by about 30 to 90% at ≥ 0.9 MPa NaCl irrespective of thermoperiod, while seed viability decreased in the following order of thermoperiod 15/25 > 25/35 > 20/30°C (dead seeds: 80, 30 and 10% respectively) for PEG-6000 solute (Fig. 1C).

Viability of dark-treated seeds was about 90% at 15/25°C and 100% at 20/30 and 25/35°C in distilled water (Fig. 2C). While, seed mortality increased with the increasing concentrations of both NaCl and PEG-6000 solutes. However, mortality in dark-treated seed was greater in NaCl than iso-osmotic PEG-6000 solutions at all thermoperiods (Fig. 2C).

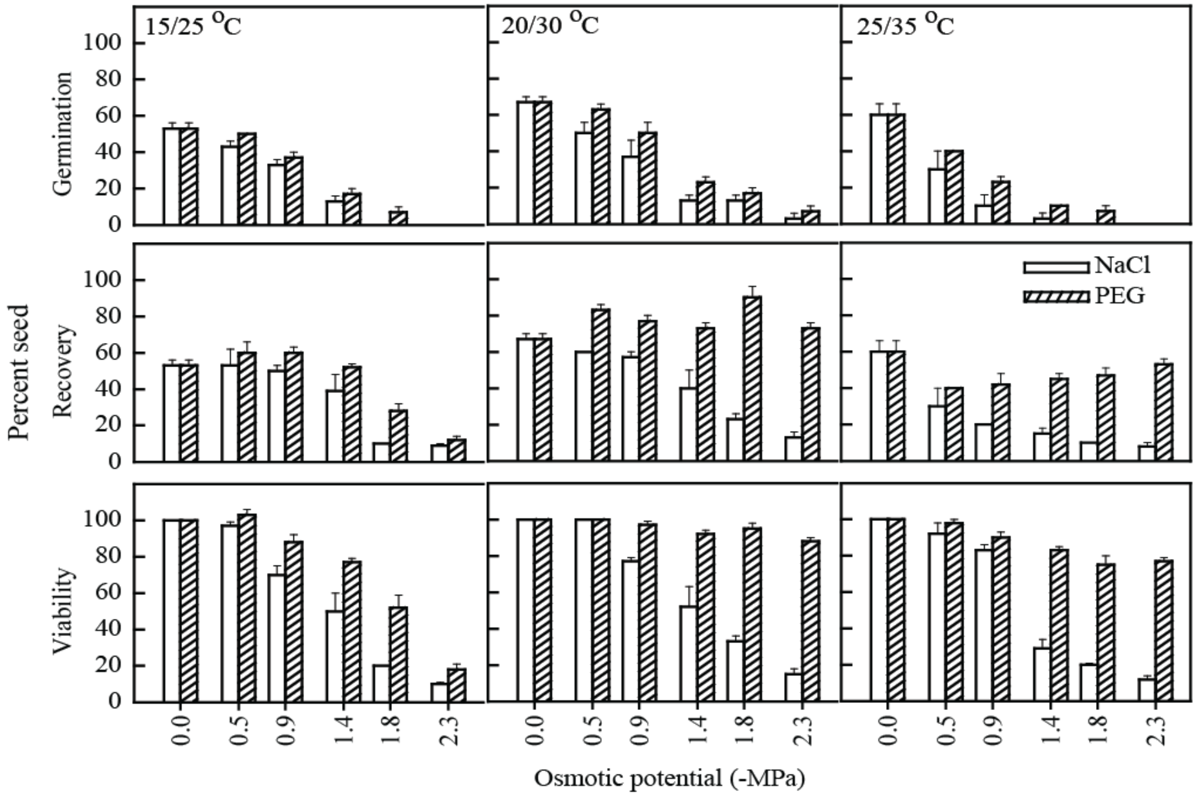

Fig. 1. Germination (A), recovery (B) and viability (C) of *Suaeda heterophylla* seeds in different iso-osmotic solutions of NaCl and PEG-6000 at different temperature regimes and in presence of light (12-hour photoperiod).

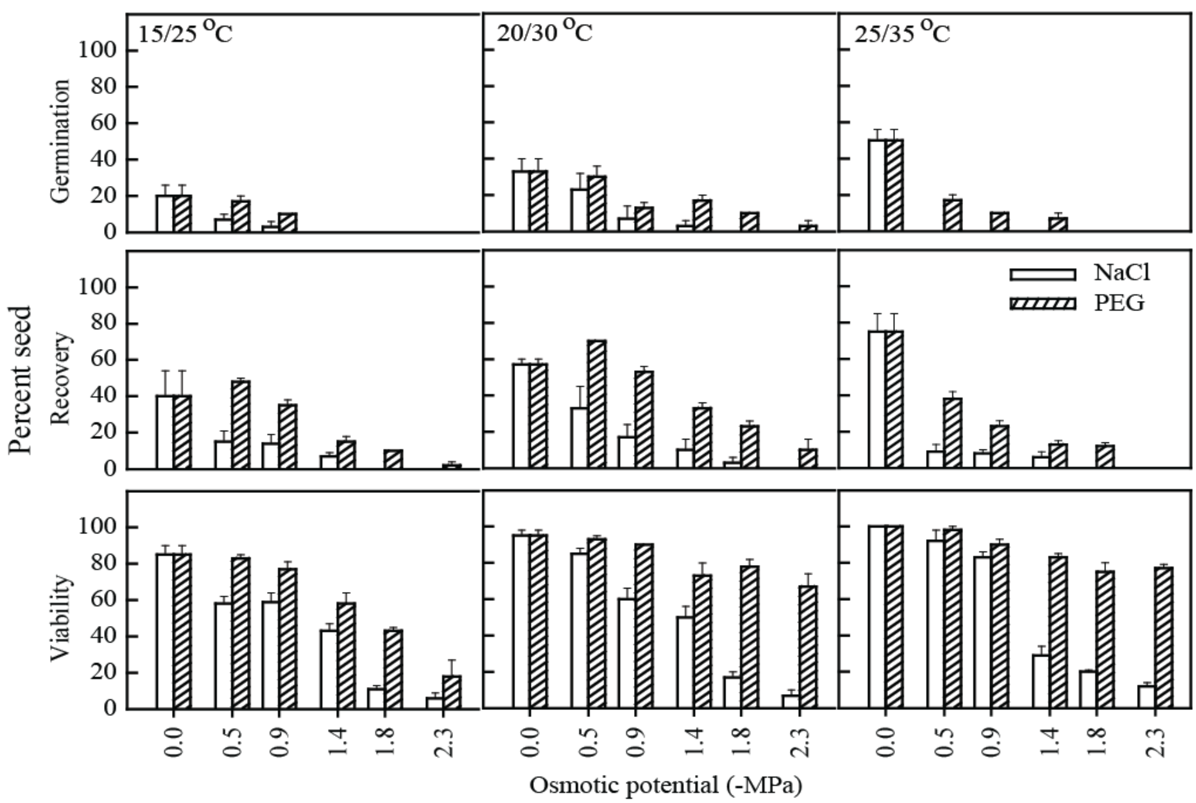

Fig. 2. Germination (A), recovery (B) and viability (C) of *Suaeda heterophylla* seeds in different iso-osmotic solutions of NaCl and PEG-6000 at different temperature regimes and in complete darkness.

Table 3. Analysis of variance for the effects of solute types (S), photoperiod (P), thermoperiod (T) and osmotic potential (OP) on final germination, germination, viability and death of the seeds of *Suaeda heterophylla*.

Factors	Final germination	Viability	Death
S	34.56***	22.72***	570.04***
P	318.39***	34.65***	236.07***
T	39.61***	5.79**	83.50***
OP	259.77***	68.18***	470.41***
S x P	0.09^{ns}	0.80^{ns}	7.25**
S x T	1.66^{ns}	0.29^{ns}	21.19***
P x T	14.59***	44.15***	8.94***
S x OP	3.67**	21.69***	51.78***
P x OP	31.62***	13.37***	3.37**
T x OP	8.91***	10.27***	7.86***
S x P x OP	0.09^{ns}	4.48**	9.12***
S x P x T	0.00ns	4.15*	8.45***
S x T x OP	0.37^{ns}	3.09**	8.97***
P x T x OP	2.32*	1.73^{ns}	3.66***
S x P x T x OP	0.49^{ns}	2.29*	2.15*

Numbers represent *F* values (where * = $p<0.01$, ** = $p<0.001$, $p<0.0001$ and ns = non-significant)

Table 4. Analysis of variance for the effects of solute types (S), thermoperiod (T) and osmotic potential (OP) on germination rate (GR), recovery from solute stress (RGS) and recovery from dark (RGD) of the seeds of *Suaeda heterophylla*

Factors	GR	RGS	RGD
S	5.38*	151.47***	50.65***
T	37.33***	29.09***	5.71**
OP	176.45***	52.06***	26.37***
S x T	0.12^{ns}	19.85***	4.09*
S x OP	0.59^{ns}	21.23***	6.28***
T x OP	5.31***	6.69***	1.09^{ns}
S x T x OP	0.11^{ns}	5.59***	0.86^{ns}

Numbers represent *F* values (where * = $p<0.01$, ** = $p<0.001$, $p<0.0001$ and ns = non-significant)

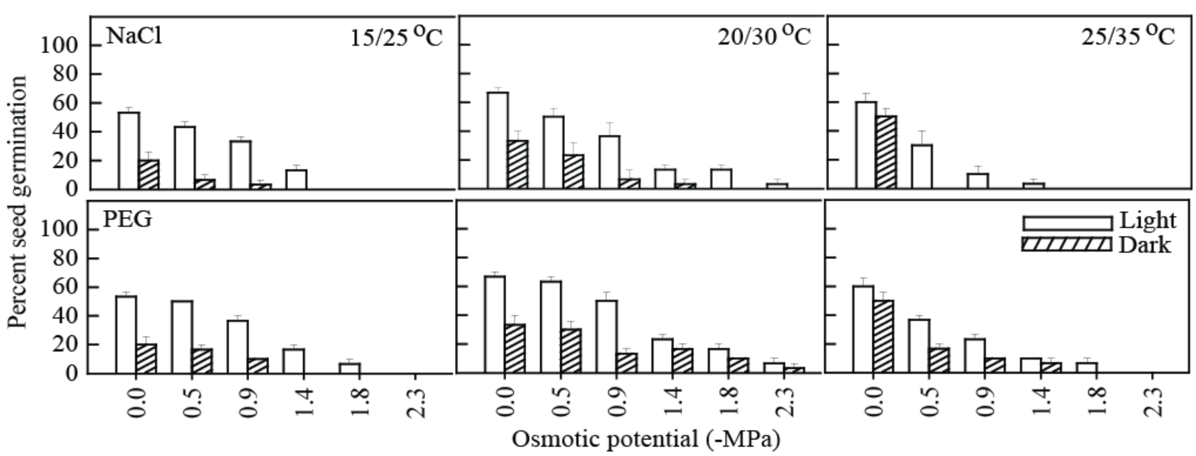

Fig. 3. Seed germination of *Suaeda heterophylla* in different iso-osmotic solutions of NaCl and PEG-6000 at different temperature regimes and in presence of light (12-hour photoperiod) and complete darkness.

Rate of seed germination: Rate of seed germination (G_R) was significantly affected by solute types ($p<0.01$), solute concentration ($p<0.0001$) and thermoperiod ($p<0.0001$) (Table 3). The highest G_R (26) was observed in distilled water control (0 MPa) at 20/30 and 25/35°C and increasing concentration of both solutes (NaCl and PEG-6000) decreased the G_R (Table 4). NaCl treatments were inhibitorier for G_R than PEG-6000. Maximum G_R was obtained at 20/30°C than others (Table 4).

Final seed germination 12 h photoperiod Vs 24 h dark: Final seed germination was significantly ($p<0.0001$) inhibited in 24 h dark than 12 h photoperiod under all experimental conditions except in non-saline control at 25/35°C (Fig. 3).

Discussion

Seed germination of the salt playa halophyte, *Suaeda heterophylla*, decreased with increasing concentrations of both NaCl and PEG-6000 and no seed germination has occurred above -2.3 MPa (=500 mM NaCl), indicating that this species is moderately salt tolerant at the seed germination stage. This is in agreement with results reported for *Chenopodium glaucum* where increasing solute concentration decreased seed germination and no seed germinated above-1.8 MPa in NaCl as well as PEG-6000 (Duan *et al*., 2004). Salinity tolerance during germination for annual halophytes varies considerably, e.g., *Triglochin maritima* (400 mM NaCl, Khan & Ungar, 1999); *Salicornia bigelovii* (856 mM NaCl, Rivers and Weber, 1971); *Salicornia rubra, Ceratoides lanata, Halogeton glomeratus* (1000 mM NaCl, Khan *et al*., 2001a, 2002, 2004) and *Kochia americana* (1712 mM NaCl, Clarke & West, 1969). Seed germination of *S. heterophylla* was inhibited more by NaCl than iso-osmotic PEG-6000, indicating an ionic influence of NaCl, especially under extreme temperature conditions. Tobe *et al*., (1999) and Sosa *et al*., (2005) reported a similar ionic effect of salinity on seed germination of *Aristida adscensionis*, *Artemesia ordosica* and *Prosopis strombulifera*. These findings clearly show that similar to upper limits of salt tolerance during germination, nature of salt effect on seed germination of halophytes is also species specific.

Seeds of *S. heterophylla* appeared to be temperature sensitive during germination and showed higher G_F as well as G_R at a temperature regime 20/30°C under both saline and non-saline conditions. Similar results were also reported for *Kochia scoparia* (Al-Ahmadi & Kafi, 2007) and co-occurring playa halophytes *Halogeton glomeratus*, *Lepidium latifolium* and *Peganum harmala* (Ahmed & Khan, 2010). Zheng *et al*., (2005) also reported similar response to temperature for *Artemisia sphaerocephala* where both G_F and G_R were higher at 20/30°C than at other alternating temperature regimes. In contrast, germination of *Sacrobatus vermiculatus* and brown seeds of *Suaeda moquinii* was not sensitive to temperature (Khan *et al*., 2001b, 2001c). Non-optimal temperatures may enhance deleterious effects of salinity on germination (Khan & Gul, 2006). Temperature-sensitive germination of halophytes is important for their survival in multi-stress natural fields. Test species showed increased germination at 20/30°C. This temperature regime is similar to the average temperature regime of early summers in Upper Hunza, Pakistan, when seeds normally germinated in playa region, as ice melts resulting in lowering the substrate salinity. Seed germination in early summer ensures survival of the seedlings because afterwards more moisture is available due to further ice melting and rainfall.

All un-germinated seeds of *S. heterophylla* from various iso-osmotic solutions of NaCl and PEG-6000 when transferred to distilled water, showed recovery (RGS) of germination. However, response was temperature and solute-type specific. Similar to G_F and G_R, RGS was also higher at moderate temperature regime (20/30°C) than others. In contrary, Khan *et al*., (2001a) reported for a Great Basin halophyte *Halogeton glomeratus* highest RGS at cooler thermoperiod. Similar results were obtained for *S. fruticosa* (Khan & Ungar, 1998) and for black seeds of *S. moquinii* (Khan *et al*., 2001b). This difference in RGS response of our test species and others can be attributed to difference in overall environmental conditions of the habitats to which these species are adapted. Several studies reported that the values of RGS increased with increases in salt stress (Rubio-Casal *et al*., 2003; Huang *et al*., 2003; Gul & Weber, 1999; Khan & Gul, 2006, Song *et al*., 2006). However, Khan *et al*., (2001a) reported inhibition of RGS at higher levels of NaCl for *H. glomeratus*. Similarly un-germinated seeds of annual halophyte *Zygophyllum simplex* showed poor recovery at all NaCl concentrations (Khan & Ungar, 1997). This may be attributed that seeds of both species are not subjected to inundation after dispersal therefore do not usually exposed to high salinity. RGS for *S. heterophylla* varied with solute-type and values of RGS were higher in PEG-6000 than in NaCl. It is worth mentioning that 5-20% improvement in seed germination was observed at 20/30°C by PEG-6000, indicating an osmopriming effect. RGS increased with increasing concentrations of PEG-6000 whereas RGS from NaCl decreased with increases in its concentration. This clearly indicates an ionic influence of NaCl on RGS, like the seed germination response of this species.

Seed germination of *S. heterophylla* was significantly higher in light than in dark at all temperature regimes and in both NaCl and PEG-6000 treatments. In contrary, Huang *et al*., (2003) reported that seed germination of *Haloxylon ammodendron* is not significantly different in light compared to dark. Additionally light induced germination suppression is also reported (Godoi & Takaki, 2004). However, several workers have proposed that light is an essential requirement for seed germination of a large number of plant species, like our test species (El-Keblawy & Al-Rawai, 2005; Zia & Khan, 2004; Khan, 1999; Baskin & Baskin, 1998; Thanos *et al*., 1991; Benvenuti *et al*., 2004). This dark imposed germination reduction could be due to inactivation of Pfr that regulates genes coding for enzymes and/or accessory proteins, essential for germination (Bewley & Black, 1994). Light requirement of the seeds ensures their germination on or near soil surface, so that their seedlings emerge easily, which on other hand may die if seeds germinate in deeper layers of soil, upon reduction in salinity and provision of adequate temperature. Light dependence of seed germination also increases chances of seedlings survival especially in environmental traps, indicating the importance of light as environmental signal.

Un-germinated seeds of *S. heterophylla* from dark also showed recovery of germination (RGD), when exposed to 12-h photoperiod. Seeds from NaCl solutions showed poorer RGD than those from iso-osmotic PEG-6000, a clear indication of ionic toxicity of NaCl on RGD. This also confirms that the seeds of *S. heterophylla* cannot germinate when deeply buried in soil. Various studies have also shown that deep burial in a number of cases inhibits seed germination (Bewley & Black, 1994; Pons, 2000; Ren *et al*., 2002).

Seeds of *S. heterophylla* appeared to be viable in distilled water under all light and temperature treatments. However, seeds begin to lose viability with the introduction of both osmotic and ionic stresses and mortality was higher in case of NaCl treatments particularly in dark. In contrast seeds of co-occurring playa halophytes *Halogeton glomeratus, Lepidium latifolium* and *Peganum hermala* maintained their viability under NaCl at all temperature and light conditions (Ahmed & Khan, 2007). This may explain the low abundance of *S. hetrophylla* in its habitat.

Suaeda heterophylla, is moderately salt tolerant (-2.3 MPa = 500 mM NaCl) for a playa species at seed germination. NaCl appears also to have an ionic influence on seed germination, recovery and viability of this species. Besides, temperature and light have also role to play during seed germination. Reduced salinity, moderate temperature and presence of light are optimal conditions for seed germination of *S. heterophylla*. These conditions are available during early summer in Upper Hunza, Pakistan, when adequate sunlight, moderate temperature and moisture due to rainfall and ice melting reduce the soil salinity. Seed germination in early summer increases the probability of seedling survival, because afterwards water availability increases substantially. Rare occurrence of the species in the habitat can be explained by the ionic toxicity that kills number of seeds during seasons of the year, when there is increased salinity along with non-optimal temperature and inadequate light/snow cover. Similarly light dependence for seed germination plays a crucial role in preventing from environmental heterogeneity traps that may cause species elimination from the habitat.

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