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Tolerance and recovery responses of playa halophytes to light, salinity and temperature stresses during seed germination

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ABSTRACT

Halogeton glomeratus (M. Bieb.) C.A. Mey., *Lepidium latifolium* Linn. and *Peganum harmala* Linn. are distributed in temperate salt playa habitats of Upper Hunza, Pakistan. Seeds were germinated under various salinity (0–500 mM NaCl), light (12 h-light:12 h-dark and 24 h-dark) and temperature (5/15, 10/20, 15/25, 20/30, and 25/35 °C, dark/light) regimes for 20 days to determine the optimal conditions for germination and recovery of seeds from these factors when exposed to less than optimal conditions. Seeds that failed to germinate in dark were transferred successively to 12 h-photoperiod, salinity to distilled water and from various temperature regimes to 20/30 °C, to determine the effect of these stresses and the ability of these seeds to recover respectively. Highest seed germination (*H. glomeratus* and *L. latifolium*: 100%; *P. harmala*: 80%) was obtained in non-saline control at 20/30 °C in 12 h-photoperiod, however, increase in salinity progressively inhibited seed germination. Seed germination of *H. glomeratus* and *P. harmala* was substantially inhibited and that of *L. latifolium* was prevented in dark. Salinity and dark treatments have a synergistic effect in inhibiting seed germination of all species. No seed of any species germinated at 5/15 °C; germination was substantially inhibited at 25/35 °C both for *H. glomeratus* and *P. harmala* while *L. latifolium* failed to germinate at 25/35 °C. Rate of germination also decreased with an increase in salinity at all temperature regimes but this effect was minimal at optimal temperature regime of 20/30 °C. After successive elimination of light, salinity and temperature stresses, final seed germination was identical to respective controls. The results indicate that seeds of these temperate halophytes could endure environmental stresses without losing viability and germinate readily when these stresses are removed. Under the extremely variable conditions of the playa habitat these species are highly opportunistic exploiting the windows of opportunity available during spring or early summer.

Introduction

The temperate moist mountains of northern Pakistan cover about 800,000 km^2 and have more than 149 halophytic species with potential uses as food, fodder, forage, medicine, ornamental, timber, fiber and chemical (Khan and Qaiser, 2006). Halophytes are known for their higher productivity under adverse conditions and may be considered as non-conventional cash-crops because of the ability to deal with saline stress successfully (e.g. Al Sherif, 2009; Nedjimi, 2009). These plants exhibit a variety of life history strategies like seed dormancy to cope with seasonal and diurnal environmental fluctuations to meet specific needs for successful completion of germination and subsequent establishment in their respective habitats (Khan et al., 2001b; Vleeshouwers et al., 1995). Environmental factors like salinity, water, temperature and light regulate the seed dormancy induction and/or release (Baskin and Baskin, 2004; Benech-Arnold et al., 2000; Vleeshouwers et al., 1995) for seedling establishment from a spatial and temporal perspective (Bu et al., 2008; Tlig et al., 2008; Zheng et al., 2005).

Seed germination has long been of interest to plant ecologists because its key role for the establishment of the population (Ungar, 1995). Seeds of most halophytes germinate better and quicker in fresh-water or under low salinity stress (Bu et al., 2008; Guan et al., 2009; Khan et al., 2001a,b,c; Wei et al., 2008). The temperate halophytes have a wide range of salinity tolerance at germination stage, e.g. *Kochia americana* (1712 mM NaCl; Clarke and West, 1969); *Salicornia rubra*, *Suaeda moquinii*, *Halogeton glomeratus* (1000 mM NaCl; Khan et al., 2000, 2001b,c); *Triglochin maritima* (400 mM NaCl; Khan and Ungar, 1999) and *Hordeum jubatum* (320 mM; Badger and Ungar, 1989).

The optimum temperature regime for seed germination of most temperate species is 15/30 °C (Copeland and Mc Donald, 2004) with an average of 21 °C (Baskin and Baskin, 1998; Guan et al., 2009; Khan and Gul, 2006). However, this response is highly variable and

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species specific (Khan and Gul, 2006). During germination, seeds of some species respond to even minor perturbations in temperature regimes (*Allenrolfea occidentalis* and *Salicornia rubra*; Gul et al., 2000; Khan et al., 2000) while other species may have little effect (*Sarcobatus vermiculatus* and *Suaeda moquinii*; Khan et al., 2001b, 2002).

Light plays a crucial role in optimizing the time of seed germination (Baskin and Baskin, 1998; Franklin and Whitelam, 2005). Absence of light inhibits the seed germination either completely (Benvenuti et al., 2004), or partially (Zia and Khan, 2004), or may have no effect (Wei et al., 2008; Zheng et al., 2005). The interaction of light, temperature and soil moisture is also reported previously as a controlling factor for dormancy where seed germination was inhibited at extreme salinity or/and temperature regimes more in the absence of light (Baskin and Baskin, 2004; Benech-Arnold et al., 2000; Vleeshouwers et al., 1995).

Ability of seeds to endure high salinity and temperature stresses under both light and dark conditions and still remain viable would bestow ecological advantage in harsh climatic conditions like the playa habitat (Khan and Gul, 2006). Tolerance and recovery from salinity and temperature stress is also species specific (Khan and Ungar, 1997; Song et al., 2006). For example, seeds of *Limonium stocksii* germinated when they were transferred from 500 mM NaCl to distilled water (Zia and Khan, 2004); a similar trend was also reported for halophytes like *Medicago ruthenica* and *Salsola affinis* (Guan et al., 2009; Wei et al., 2008). Similarly, seeds of *Halostachys capsica* showed a substantial increase in germination when transferred from sub-optimal to optimal temperatures (Song et al., 2006). However, seeds of other halophytes did not recover or showed little recovery response when subjected to high salinity and temperature stress (Khan and Gul, 2006).

Halogeton glomeratus (M. Bieb.) C.A. Mey., *Lepidium latifolium* Linn. and *Peganum harmala* Linn. are among the dominant flora of the temperate northern moist mountainous region of Upper Hunza, Pakistan, which could be grown on marginal lands with brackish water irrigation. Little information however, is available regarding the seed germination strategies of these species in response to major environmental parameters like temperature, salinity and light. Recovery from light, temperature and salinity stress together is not widely reported. The present study was designed to test the following hypotheses (1) seeds of all test species germinate optimally under non-saline condition, (2) all these species germinate quickly under non-saline medium, (3) their maximum seed germination occurs at 20/30 °C, (4) seed germination decreases under (24 h) dark, (5) the combination of unfavorable environmental conditions (salinity, temperature and light) synergistically inhibits seed germination in comparison to factors affecting independently and (6) seeds of *H. glomeratus*, *L. latifolium* and *P. hermala* maintain their viability under harsh environmental conditions.

Materials and methods

Inflorescences of *H. glomeratus* (seed weight: 28.8 ± 0.4 mg/100 seeds; $n = 5$), *L. latifolium* (seed weight: 15.7 ± 0.1 mg/100 seeds; $n = 5$) and *P. harmala* (seed weight: 207.3 ± 0.9 mg/100 seeds; $n = 5$) were collected from the upper Hunza, Pakistan, near Borith (salt) Lake (Elevation: 2569 m, 36°25.983′N and 74°51.775′E). Seeds were collected from individuals of the entire population to get an adequate representation of genetic diversity. Inflorescences were air dried, seeds were separated from spikes and cleaned before surface sterilization with 0.82% sodium hypochlorite for 1 min and after thorough washing and subsequent drying stored in plastic jars at 4 °C.

Germination was carried out using tight-fitting plastic Petri plates (5 cm diameter) with 7 ml of test solution (0, 100, 200, 300, 400, and 500 mM NaCl) by using distilled water. The NaCl concentration was selected after a preliminary test for salt tolerance. Each dish was placed in a 10 cm diameter plastic Petri plate as a precaution against evaporation. Four replicates of 25 seeds each were used for all treatments. Seeds were considered to be germinated at the emergence of the radicle (Bewley and Black, 1994). Seeds were germinated in a programmed incubator (Percival Scientific, Boone, Iowa, USA) for environmental parameters; light (12 h-photoperiod; 25 μmol m^{-2} s^{-1}, 400–700 nm Sylvania cool-white fluorescent lamps [Danvers, Massachusetts, USA]) and temperature (5/15, 10/20, 15/25, 20/30 and 25/35 °C, dark/light). Two experimental designs were used to determine germination response; one set was placed in 12 h-photoperiod (GL), while the second one in complete darkness (24 h; GD) using photographic envelops as light shields. Each experimental design consisted of six salinity and five temperature treatments with four replicates each. Seed germination percentage of GL was recorded on every alternate day for up to 20 days and for GD only after the 20th day. Rate of germination was calculated by using a modified Timson's index (Timson, 1965) of germination velocity $\sum G/t$, where G is the percentage of seed germination at 2-day intervals and t is the total germination period (Khan and Ungar, 1999). The maximum possible value was 50 for our data by using this index (i.e., 1000/20), the higher the value, the more rapid the germination.

Table 1
Summary list of germination tests in experimental sequence with abbreviations, legend pattern and respective formulas.

Germination test	Abbreviation	Formula
Germination in 12 h-photoperiod condition for 20 days	GL	$a/e \times 100$
Keep GL in similar condition for further 10 days	GLL	$a+b/e \times 100$
Transfer GLL from salinity to distilled water for 10 days	GLLW	$a+b+c/e \times 100$
Move GLLW to 20/30 °C for 10 days	GLLWT	$a+b+c+d/e \times 100$
Germination in 24 h-dark condition for 20 days	GD	$a/e \times 100$
Shift GD to 12 h-photoperiod condition for 10 days	GDL	$a+b/e \times 100$
Transfer GDL from salinity to distilled water for 10 days	GDLW	$a+b+c/e \times 100$
Move GDLW to optimum temperature (20/30 °C) for 10 days	GDLWT	$a+b+c+d/e \times 100$

a: the number of seeds germinated during 20 days of GL or GD; *b*: the number of seeds germinated during 10 days of GLL or GDL; *c*: the number of seeds germinated during 10 days of GLLWT or GDLWT; *e*: total number of seeds (25 seeds/Petri plate).

The Petri plates of GL were kept in the same condition for additional 10 days (GLL) after completion of the 20-day incubation period. After this the test solutions of all GLL dishes were changed from saline to distilled water and placed for 10 days in respective temperatures (GLLW). Un-germinated seeds from all GLLW experiments were then transferred to a 20/30 °C temperature regime for 10 days. The Petri plates of GD after completion of 20 days from the day of seed sowing were exposed to light (12 h-photoperiod; GDL) for 10 days. After that the saline solution from all GDL plates was replaced by distilled water (GDLW) and all plates were placed for 10 days in relevant temperature conditions. All Petri plates of GDLW were subsequently transferred from various temperature regimes to 20/30 °C (GDLWT) for 10 days as done above with GLLW.

The final seed germination percentage was determined after each step using formulae as indicated in Table 1. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. The significant effects ($P < 0.05$) of temperature, salinity, light and their interactions on seed germination and rate of germination were determined by analysis of variance (ANOVA) using SPSS version 11.5 for Windows (SPSS Inc., 2002). If the ANOVA indicated a significant effect, than post hoc Bonfer-

Table 2
Results of three-way ANOVA of characteristics by temperature (*T*), salinity (*S*), light (*L*), and their interactions on seed germination parameters; GL-GD [GL: germination in 12 h-photoperiod condition for 20 days under 0–500 mM NaCl at 5 different temperature regimes; GD: germination in 24 h-dark condition for 20 days under 0–500 mM NaCl at 5 different temperature regimes] of three halophytic species (*H. glomeratus*, *L. latifolium* and *P. harmala*).

Independent variables	df	*H. glomeratus*	*L. latifolium*	*P. hermala*
T	4	1008.37***	346.03***	74.59***
S	5	2739.51***	483.84***	197.67***
L	1	6264.47***	1672.07***	183.28***
$T \times S$	20	346.19***	89.28***	18.58***
$T \times L$	4	1064.47***	345.69***	36.39***
$S \times L$	5	916.01***	352.46***	29.00***
$T \times S \times L$	20	173.22***	68.41***	6.130***
Error	60	*MS*: 2.53	*MS*: 25.03	*MS*: 7.47

Superscript *** denotes significant difference at $P < 0.0001$, Data represent *F* values.

roni tests were performed to compare treatment means (SPSS Inc., 2002).

Results

The three-way ANOVA indicated significant ($P < 0.0001$) effects of temperature, salinity, light and their interactions on seed germination of all three test species, *H. glomeratus*, *L. latifolium* and *P. hermala* (Table 2). Rate of germination could not be determined for those seeds that germinated in dark. Thus, only two-way ANOVA were performed, which showed that the temperature, salinity and their interactions had significant ($P < 0.0001$) effects on seed germination velocity of the three species under test (Table 3).

Presence of salinity and absence of light reduced germination. A temperature regime of 20/30 °C led to optimal responses and deviation on either side reduced germination. Elimination of salinity stress while providing light and optimal temperature resulted in almost complete recovery of *H. glomeratus* seed germination. This recovery was however, only partial in *L. latifolium* and *P. hermala*. Temperature seemed to be a dominant factor in improving germination after removal of salinity stress.

Halogeton glomeratus

Higher germination was recorded in the non-saline control (Fig. 1A, GL). Inclusion of NaCl in the medium inhibited ($P < 0.0001$) seed germination and few seeds germinated at 500 mM NaCl (Fig. 1A). In non-saline medium light and dark seed germination were similar except with a slight reduction at 10/20 °C. Presence of salinity inhibited more seeds in dark (*F*: 916.01; $P < 0.0001$) compared to those germinated in light (Fig. 1A). No seed germinated at 5/15 °C, only few seeds germinated at 10/20 °C and germination increased above this temperature, showing optimal values at 20/30 °C and declined at the warmer thermoperiod (25/35 °C; Fig. 1A).

Rate of germination was optimal in distilled water and decreased with an increase in salinity (Table 4). Rate of germination was substantially inhibited both at cooler and at warmer temperature regimes and the highest rate was achieved at 20/30 °C.

Table 3
Results of two-way ANOVA of characteristics by temperature (*T*), salinity (*S*) and their interactions on rate of seed germination [at GL stage: germination in 12 h-photoperiod condition for 20 days under 0–500 mM NaCl at 5 different temperature regimes] of three halophytic species (*H. glomeratus*, *L. latifolium* and *P. harmala*).

Independent variables	df	*H. glomeratus*	*L. latifolium*	*P. hermala*
T	4	1800.63***	560.20***	144.72***
S	5	3116.00***	610.99***	293.82***
$T \times S$	20	340.25***	123.80***	41.60***
Error	30	*MS*: 0.53	*MS*: 1.54	*MS*: 2.62

Superscript *** denotes significant difference at $P < 0.0001$, Data represent *F* values.

Table 4
Rates of seed germination (Modified Timson's index) of all test species in response to variable salinity (0–500 mM NaCl) and all temperature regimes (10/20, 15/25, 20/30 and 25/35 °C) except 5/15 °C (where no seeds germinated). Different letters via Bonferroni test indicate significant differences ($P < 0.05$) between salinity treatments (within one temperature treatment).

Species	NaCl (mM)	10/20 °C	15/25 °C	20/30 °C	25/35 °C
H. glomeratus	0	27^a	43^a	48^a	46^a
	100	3^b	22^b	39^b	24^b
	200	2^b	7^c	33^c	12^c
	300	1^b	2^d	11^d	6^d
	400	0^b	1^d	2^e	2^e
	500	0^b	1^d	0^e	1^e
L. latifolium	0	31^a	38^a	45^a	2^a
	100	9^b	35^a	36^b	0^b
	200	4^c	15^b	27^c	0^b
	300	0^c	1^c	4^d	0^b
	400	0^c	0^c	0^d	0^b
	500	0^c	0^c	0^d	0^b
P. hermala	0	16^a	32^a	32^a	37^a
	100	0^b	21^b	34^a	11^b
	200	0^b	8^c	14^b	4^c
	300	0^b	4^d	6^c	0^c
	400	0^b	0^d	2^d	0^c
	500	0^b	0^d	0^d	0^c

Lepidium latifolium

All seeds were germinated in distilled water in light (12 h-photoperiod, GL) at 20/30 °C temperature regime (Fig. 2A). Seed germination was significantly ($P < 0.0001$) inhibited with an increase in salinity (Tables 2 and 3, Fig. 2A). Few seeds germinated in 300 mM NaCl at the optimal temperature regime. No seeds germinated in dark (GD). Lowest (5/15 °C) and highest (25/35 °C) temperature regimes completely prevented seeds from germinating (Fig. 2A). Maximum seed germination was obtained at 20/30 °C.

Rate of germination was optimal in non-saline control at all temperature regimes however rate was lowest at 25/35 °C (Table 4). Rate decreased with increased salinity. Seeds germinated quickly at optimal temperature but rate decreased and no seeds germinated both at the coolest and warmest temperature regimes studied.

Peganum harmala

More seeds germinated in non-saline control and inclusion of NaCl in the medium substantially inhibited seed germination and few seeds germinated at 400 mM NaCl (Fig. 3A, GL). At similar salinity concentrations seed germination was significantly reduced in dark (GD) in comparison to light (GL). No seeds germinated in dark at salinity concentration of 200 mM and above (Fig. 3A). Seed germination was completely inhibited at 5/15 °C temperature regime and only 42% seeds germinated in water at 10/20 °C. Maximum seed germination was obtained at 20/30 °C while some seeds also germinated at warmer temperature regime (25/35 °C).

Rate of germination was significantly decreased with an increase in salinity and also at temperature regimes higher or lower than optimal (20/30 °C) (Table 4).

GL > GLL > GLLW > GLLWT

There was no seed germination of any of the selected species during an additional 10-day of GLL (Figs. 1B, 2B and 3B). When seeds under GLL were transferred from various saline solutions (100–500 mM of NaCl) to distilled water for 10 days (GLLW), seeds of *H. glomeratus* and *L. latifolium* showed ≥ 90% germination under

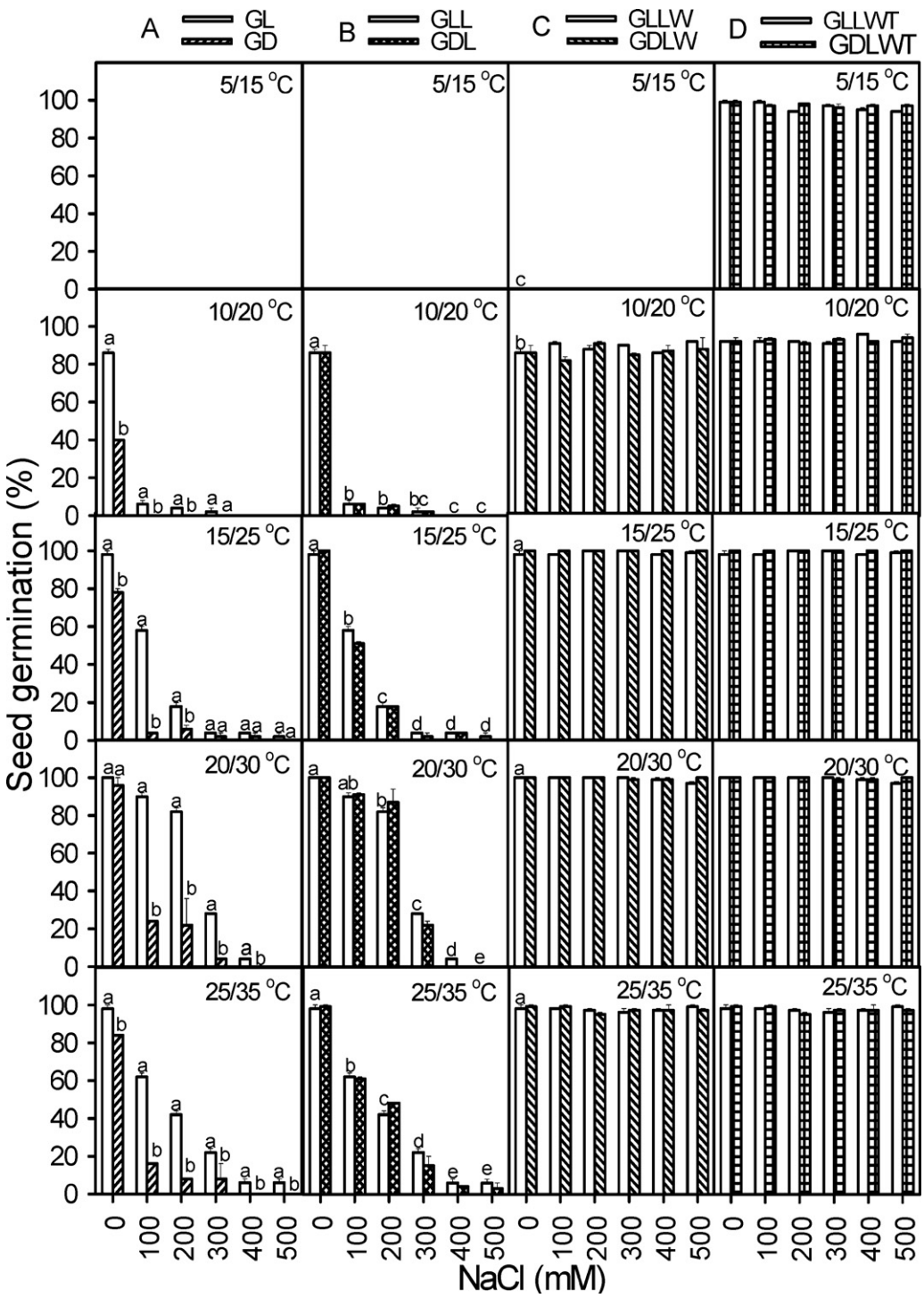

Fig. 1. Seed germination of *H. glomeratus* (for abbreviations and experimental sequence see Table 1). Different letters indicate significant differences ($P < 0.05$) between (A) 12 h-photoperiod and 24 h-darkness (within one salinity and temperature level), (B) various salinity levels (within one temperature level) and (C) among different temperature regimes of non-saline control.

all NaCl concentrations and temperature regimes except for 5/15 °C (Fig. 1C and 2C). Seeds of *P. hermala,* transferred from GLL to GLLW, showed 50% germination at 10/20 °C, 80% at both 15/25 and 20/30 °C, and 70% at 25/35 °C temperature regime (Fig. 3C). Seeds of all species failed to germinate at 5/15 °C, while seed *of L. latifolium* also did not germinate at 25/35 °C (Figs. 1C, 2C and 3C). When GLLW seed of all species were moved from different temperature regimes to 20/30 °C, they showed maximum (*H. glomeratus*: ~100%, *L. latifolium*: ~100%, *P. hermala*: ~80%) germination at all salinity concentrations and temperature regimes, including 5/15 °C and 25/35 °C (Figs. 1D, 2D and 3D).

GD > GDL > GDLW > GDLWT

After moving Petri plates of test species from 24 h-dark (GD) to 12 h-photoperiod for 10 days (GDL), seed germination increased but was not different to GLL at all salinity and temperature conditions (Figs. 1B, 2B and 3B). Although, temperature, salinity and

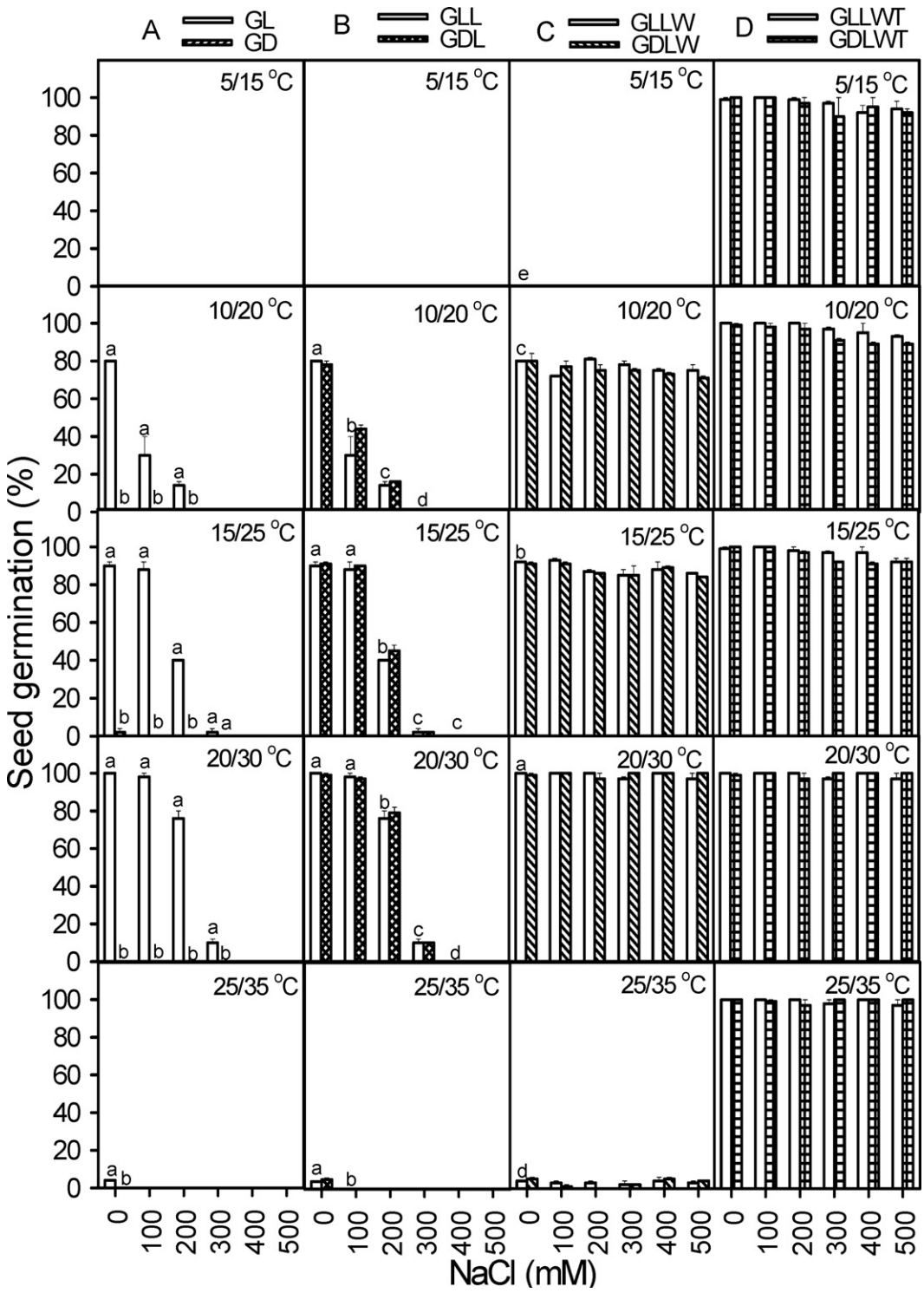

Fig. 2. Seed germination of *L. latifolium* (for abbreviations and experimental sequence see Table 1). Different letters indicate significant differences ($P < 0.05$) between (A) 12 h-photoperiod and 24 h-darkness (within one salinity and temperature level), (B) various salinity levels (within one temperature level) and (C) among different temperature regimes of non-saline control.

their interactions had significant ($P < 0.0001$) affects on seed germination of all test species. When un-germinated seed of GDL were transferred from various salinity levels to distilled water (GDLW), germination was similar to GLLW and this response was identical in all species (Figs. 1C, 2C and 3C), while the temperature effect was significant ($P < 0.0001$). Finally when GDLW moved from different temperature regimes to optimal temperature regime (20/30 °C) for 10 days (GDLWT), seed germination was equal to GLLWT for all three species and maximum seed germination (*H. glomeratus*: ~100%, *L. latifolium*: ~100%, *P. hermala*: ~80%) was recorded at all salinity and temperature regimes (Figs. 1D, 2D and 3D).

Discussion

Playas are regions where water from melting snow and rainfall accumulates in a drained basin. Water evaporates creating highly

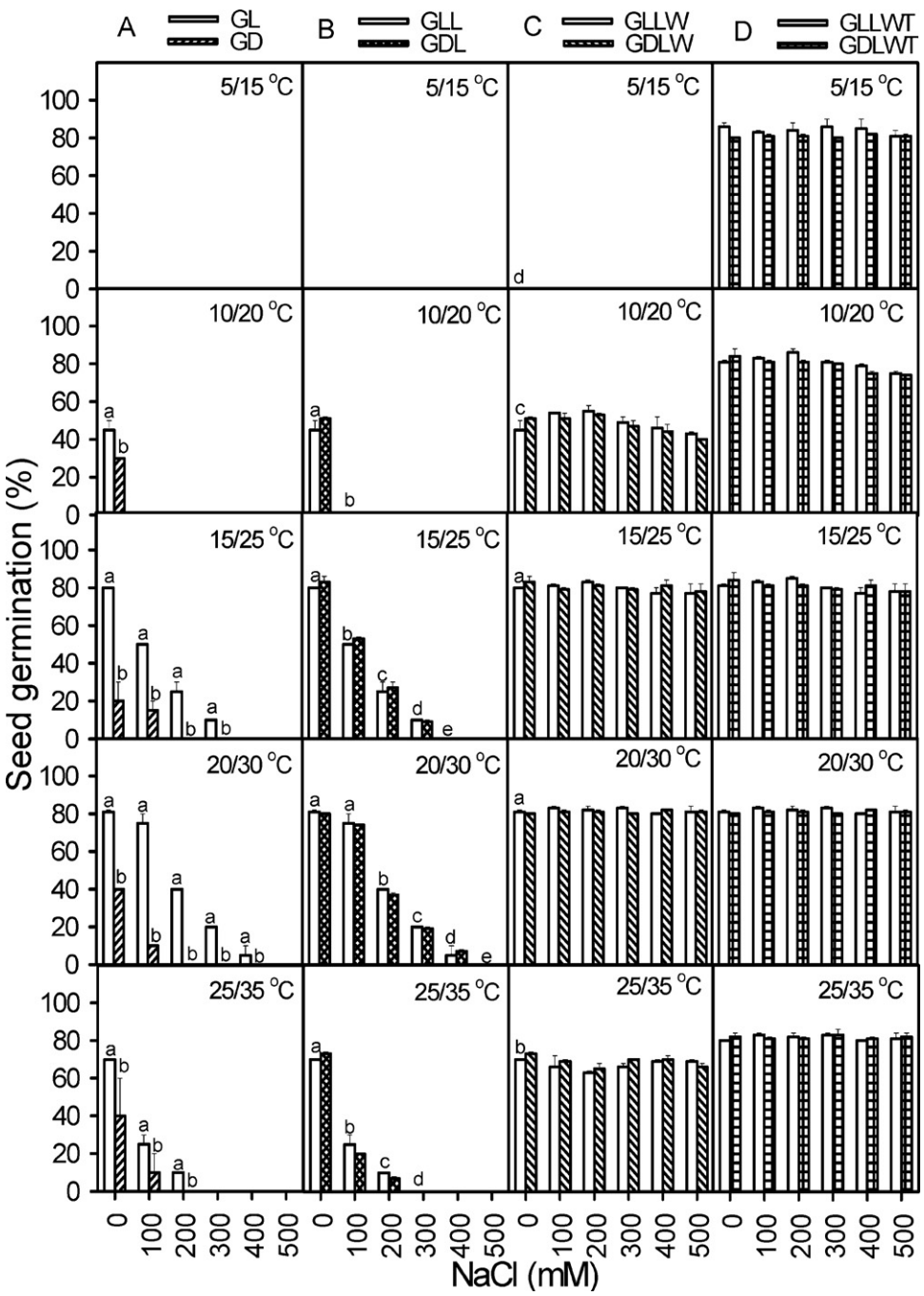

Fig. 3. Seed germination of *P. hermala* (for abbreviations and experimental sequence see Table 1). Different letters indicate significant differences ($P < 0.05$) between (A) 12 h-photoperiod and 24 h-darkness (within one salinity and temperature level), (B) various salinity levels (within one temperature level) and (C) among different temperature regimes of non-saline control.

saline lakes, e.g. Borith lake in upper Hunza where our test species are distributed. The climate of these habitats is harsh with a minimum of −6 °C in winter to a maximum of 36 °C during summer. Average annual rainfall is 149 mm with 70 mm during April to May. The plant populations inhabiting these areas have adapted to these conditions including dependence on snow/glaciers for moisture, both high and low temperature stress, physiological drought and salinity stress by evolving appropriate strategies like recruiting during spring and early summer when suitable temperature and water conditions are available and soil salinity decreases due to availability of moisture from melting snow (Bu et al., 2008; Khan and Gul, 2006). Playa habitats like also in the Great Basin desert, USA, are the home of species which are quite sensitive to variation in temperature and light regimes and are the habitats of most salt tolerant halophytes (Khan and Gul, 2006).

Species from the Borith lake playa, unlike plants from Great Basin playas, are less tolerant to salinity during seed germination (Khan et al., 2001a,b,c). Seeds of *H. glomeratus* collected from Borith lake populations have only few seeds germinated at 500 mM NaCl in comparison to Great Basin populations whose seeds germinated at 800 mM NaCl (Khan et al., 2001c). Other species like *L. latifolium* and *P. harmala* are moderately salt tolerant (300 mM NaCl). Seeds germinated in higher numbers in distilled water similar to that reported before for other temperate halophytes (Bu et al., 2008; Guan et al., 2009; Khan et al., 2001a,b,c; Wei et al., 2008). Some of these temperate halophytes are reported to be very highly

tolerant to NaCl (Khan and Gul, 2006), like *Salicornia europaea* (800 mM NaCl; Grouzis et al., 1976; Langlois, 1966; Ungar, 1995), *Kochia americana* (1700 mM NaCl; Clarke and West, 1969), *Allenrolfea occidentalis* (800 mM NaCl; Gul and Weber, 1999), *Salicornia rubra* (1000 mM NaCl; Khan et al., 2000), *Suaeda moquinii* (1000 mM NaCl; Khan et al., 2001b), *Kochia scoparia* (1000 mM NaCl; Khan et al., 2001a) and *Sarcobatus vermiculatus* (1000 mM NaCl; Khan et al., 2002).

Temperature and the thermoperiod appear to be the critical factors in influencing seed maturity, dormancy and periodicity under temperate climate (Copeland and Mc Donald, 2004; Ungar, 1995). Early seed germination could be subjected to frost leading to heavy mortality of seedlings. It appears that our test species have adapted to germinate when temperature becomes sufficiently warm and do not germinate at cooler (5/15 °C) temperatures. High seedling mortality would also occur during mid-summer due to drought, high temperature and high salinity stress. This may be the reason that species like *L. latifolium* did not germinate at 25/35 °C. All halophytic species studied from the playa of the Great Basin regions progressively modified their seed germination with changes in temperature (Gul and Weber, 1999; Khan et al., 2001a,b). Germination increased with an increase in temperature, and optimal germination was obtained at temperature regime of 25/35 °C (Gul and Weber, 1999; Khan et al., 2000, 2001a,b,c, 2002).

Seed germination in halophytes depends on the range of salt tolerance, which decreases under extreme temperature conditions (Khan et al., 2002; Song et al., 2006). Seeds of our study failed to germinate at cooler (5/15 °C) and warmer (25/35 °C; only for *L. latifolium*) temperatures after salinity and dark stresses were removed. However, seed germination of all species was greater than 80% and comparable to respective controls when shifted into 20/30 °C. Similar results have been observed in *Halostachys caspica* and *Kalidium foliatum* (Song et al., 2006). Seeds of these species show temperature enforced dormancy throughout the winter except for the seeds of *L. latifolium*, which remained dormant during summer. Germination of selected species was affected by temperature in the following order: *L. latifolium* > *P. harmala* > *H. glomeratus*. Additionally, species-specific temperature tolerance limits provide help to survive successfully in the habitat (Bu et al., 2008; Khan et al., 2000; Song et al., 2006).

Light requirement during seed germination varies among halophytes (Baskin and Baskin, 1998), and this is also true for the species studied. *Lepidium latifolium* showed no seed germination in dark both under saline and non-saline conditions while germination of *P. harmala* and *H. glomeratus* was substantially inhibited. Seed germination recorded in those seeds, which were transferred to 12 h-photoperiod was similar to those exposed to photoperiod from the beginning. The difference among species in response to light could be due to the seed reserve in the soil. Seeds of *L. latifolium* accumulate typically in cracks of mountain slopes, where they are exposed to light regularly and hence do not have adaptive mechanisms for the dark environment. Seeds of *P. harmala* and *H. glomeratus*, which are buried under the soil, have evolved adaptive mechanisms in response to dark and to maintain a seed bank which could contribute to the continuity of the population. Baskin and Baskin (1998) reported that 22 out of 27 species respond similarly to both light and dark condition. Light-requiring seeds tend to germinate at a time when other types of stresses are relatively low (Bell et al., 1995; Zheng et al., 2005). Our data suggest that seed germination is inhibited more in dark in comparison to light and high salinity and less optimal temperatures have a synergistic effect in inhibiting seed germination.

The successful establishment and maintenance of a population in temperate regions depends on the ability of the seeds to withstand high temperature and salinity without losing viability and to germinate rapidly when these stresses are reduced (Khan and Gul, 2006; Song et al., 2006). Seeds of selected species were prevented from germination under salinity stress and readily germinated when salinity stress was removed; the germination recovery was almost equal to the non-saline control. Our result clearly showed that selected species from the playa habitat display higher salinity tolerance during storage in the soil and are however ready to germinate and are prevented from germination due to high salinity and temperature stress. Temperate halophytes like *Kalidium foliatum*, *Halocnemum strobilaceum* (Song et al., 2006) and *Salsola affinis* (Wei et al., 2008) failed to germinate under high salinity stress and readily germinated when stress was removed.

In this study we tested how stresses like light, temperature and salinity affect individually or collectively the vitality of the investigated species. Our elaborate and complex experimental protocol clearly established that all of the above factors are playing a role in controlling seed germination by allowing seeds to germinate when conditions are conducive. None of these environmental factor causes seed mortality even at higher salt concentrations, or induces any secondary dormancy. Seeds were prevented from germination due to enforced dormancy caused by sub-optimal levels or combinations of these factors. Seed germination in the playa habitat during short and specific periods has an important role in successful seedling survival and population establishment (Bu et al., 2008). The seed germination of all test species required lower soil salinity, moderate temperatures and presence of light. Such characteristics may be similar to local environmental conditions during spring (March, April and May), when the snow starts to melt due to rising temperature associated with higher rain fall. In addition, salt is rinsed from the top layers of the soil due to excessive flooding. Consequently, seeds of the three species may germinate in late spring to mid-summer, when the temperature window is also suitable for it. Species of the present study maintain a seed bank throughout the autumn (September to November) and winter (December to February) seasons by enforced dormancy. Dormancy provides help reducing the risk of seedling mortality during unfavorable situations.

Our study validates the hypothesis that the final germination and germination velocity of all test species was optimum under non-saline condition in 12 h-photoperiod and at 20/30 °C temperature regime. The inhibitory effect of combined stresses on seed germination was more severe than an individual one; however, this effect varied with species. Selected species showed enforced dormancy and maintained viability during complete darkness and in the presence of extreme temperatures and salinity.

It can be concluded that these species may not be tolerant to extreme temperature and soil salinity during germination but are highly tolerant during storage in the soil. During this period they do not go into secondary or induced dormancy but were always ready to germinate pending the availability of favorable environmental conditions. Recruitment from seeds would be facilitated whenever the window of opportunity is available. This study provides some basic information related to suitability of environmental condition for better germination of *Halogeton glomeratus*, *Lepidium latifolium* and *Peganum harmala*, which may be helpful if these species are cultivated as cash-crops.

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