Flora 236–237 (2017) 76–83

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

Original research

Seeds of *Halopeplis perfoliata* display plastic responses to various abiotic factors during germination

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ARTICLE INFO

Edited by Hermann Heilmeier

Keywords:
Dormancy regulating chemical
Photoperiod
Hypersalinity
Thermoperiod
Viability

ABSTRACT

Halopeplis perfoliata is a coastal marsh halophyte with several ecological and economic usages. Information about seed germination ecology of *Halopeplis* species is limited. Germination and recovery responses to NaCl (0, 0.1–0.6 mol L^{-1}), light (12 h light/12 h dark and 24 h dark), alternating temperature regimes (10/20, 15/25, 20/30 and 25/35 °C), NaCl exposure time (20, 40 and 60 days) and dormancy regulating chemicals (DRCs) were therefore studied. Fresh seeds were non-dormant and showed highest germination (≈90%) in distilled water, irrespective of incubation temperature. Seeds germinated better in 12 h photoperiod than 24 h dark. Salinity increment decreased germination; however some seeds could germinate in 0.6 mol L^{-1} NaCl under 12 h photoperiod. High salinity (≥0.3 mol L^{-1} NaCl) and 24 h dark enforced dormancy in seeds and they showed high recovery when transferred to distilled water and to light respectively. Seeds showed enforced dormancy under hyper-salinity (up to 2.0 mol L^{-1} NaCl) at all temperature regimes except at 25/35 °C where seed mortality was high. All DRCs (except proline) alleviated enforced dormancy due to dark but were generally ineffective in reversing inhibitory effects of salinity. Our data indicate that seeds of *H. perfoliata* are sensitive to variations in different abiotic factors, which may thus act as possible cue for germination in its habitats. Results of DRC application indicate that germination inhibition under high salinity and dark might be due to osmotic and biochemical reasons respectively.

1. Introduction

Salt marshes in sub-tropical regions such as those around Arabian Peninsula receive insufficient rain which leads to increased sediment salinity (Böer, 1996; Boorman, 2003). In some cases, salinity of the upper soil layer could be about twice as high as seawater salinity, which potentially influences survival and regeneration of native species (Böer, 1996; Ungar, 1999). Most seeds of coastal plants are deposited in top soil layers hence are under direct influence of salinity (Ungar, 1978; Gul and Khan, 1998). Gul et al. (2013) pointed out that salinity is one of the major factors influencing seed germination of halophytes such as those inhabiting coastal marshes. Generally, seeds of salt marsh halophytes are highly tolerant and can germinate in as high as seawater (≈0.6 mol L^{-1} NaCl) or even higher salinity (Gul et al., 2013). Seeds of coastal halophytes maintain viability under hyper-salinity by entering in a state of enforced dormancy, hence show high recovery of germination after sufficient rains (Gul et al., 2013; El-Keblawy et al., 2016).

Fluctuations in temperature and photoperiod also influence germination and dormancy status of halophyte seeds (Gul et al., 2013). Generally, sub/supra-optimal temperatures and dark (mainly due to burial) decrease both germination and salinity tolerance of halophyte seeds (Zia and Khan, 2004; El-Keblawy and Al-Rawai, 2006; Hameed et al., 2013). However, large variation exists in germination responses of the seeds of salt marsh halophytes to different temperature and salinity regimes. For example, seeds of only four out of eight salt marsh halophytes responded to changes in temperature and light during germination (Noe and Zedler, 2000), whereas, seeds of another salt marsh halophyte *Arthrocnemum indicum* responded to changes in temperature but not to light (Saeed et al., 2011). These reports indicate that the seed germination responses of coastal halophytes may vary among species.

Often the interactions of an environmental variable with others intensify its effects on germination (Gul et al., 2013). For example, adverse effects of high salinity were further aggravated at higher or lower than the optimal temperatures in many halophytes such as *Limonium stocksii* (Zia and Khan, 2004), *Atriplex cordobensis* (Aiazzi et al., 2002) and *Allenrolfea occidentalis* (Gul and Weber, 1999). Similarly, inhibitory effects of salinity on seed germination were aggravated more under dark as compared to light in several halophytes like *Suaeda heterophylla* (Hameed et al., 2013), *Halogeton glomeratus, Lepidium latifolium* and *Peganum harmala* (Ahmed and Khan, 2010). Similarly,

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http://dx.doi.org/10.1016/j.flora.2017.09.010
Received 4 May 2017; Received in revised form 7 September 2017; Accepted 27 September 2017
Available online 05 October 2017
0367-2530/

collective interaction of high salinity, high temperature and dark caused higher inhibition of seed germination in *Halogeton glomeratus* than predicted from a linear combination of the effects of these factors (Ahmed and Khan, 2010). Hence, interactive effects of different environmental factors seem to have great potential to influence germination of halophytes.

Germination inhibition under stress conditions is reportedly linked to changes in the endogenous chemical balance of the seeds (Bewley and Black, 1994; Li et al., 2016). For example, salinity caused inactivation/suppression of gibberellins (Hedden and Thomas, 2012) and enhanced biosynthesis of abscisic acid (Li et al., 2016), which coincided with germination inhibition. Hence, exogenous application of gibberellins (GAs) has been reported to improve seed germination of a wide variety of species including halophytes (Khan and Gul, 2006; Cao et al., 2014; Wu et al., 2015). Likewise, many other phyto-hormones and chemicals such as kinetin and thiourea have also been reported to mitigate salinity effects and improve seed germination of halophytes (Khan and Gul, 2006; Gul and Khan, 2008; El-Keblawy et al., 2010). However, efficacy of these exogenous treatments varies with the type of chemicals, nature of stress and/or plant species (Ahmed et al., 2014; Wu et al., 2015).

Halopeplis perfoliata (Forssk.) Bunge ex Asch. & Schweinf. is a succulent halophyte from the Amaranthaceae family that occupies coastal salt marshes of the Arabian Peninsula (Al-Oudat and Qadir, 2011). This species has many ecological and potential economic uses. For instance, it can be utilized for sand dune stabilization in coastal deserts (Turkelboom and Mehdi, 2009) and in soap and glass manufacturing industries (Al-Oudat and Qadir, 2011). This plant is an important intertidal/terrestrial producer and provides habitat structure for wild life (Pilcher et al., 2003). Seeds of this succulent halophyte are small ($\approx$0.09 mg per 100 seeds; El-Keblawy and Bhatt, 2015). El-Keblawy and Bhatt (2015) reported that the seeds of *H. perfoliata* from Sharjah, UAE can germinate in up to 0.4 mol L^{-1} NaCl. Mahmoud et al. (1983) reported that the seeds of *H. perfoliata* obtained from Red Sea coast of Saudi Arabia could germinate in up to 0.25 mol L^{-1} NaCl. El-Keblawy and Bhatt (2015) also reported that the short-term aerial seed banks can protect seeds of this species from effects of high soil salinity. However, information about effects of various temperatures, hyper-saline conditions and exogenous chemical treatments on seed germination of this halophyte is not known. We tested the following hypotheses on the seed germination of *Halopeplis perfoliata*: 1) Seeds of *H. perfoliata* will germinate in a wide range of salinity and temperatures, as indicated by the occurrence of this plant in highly saline marshes of the Arabian Peninsula. 2) Dark (indicating burial) will inhibit seed germination of the test species, as small seeds prefer to germinate at/near soil surface (i.e. presence of light) to ensure successful seedling establishment. 3) Due to interaction of environmental factors the effects of individual factors will be aggravated. 4) Exogenous application of dormancy regulating chemicals (DRCs) would improve germination under stress conditions.

2. Materials and methods

2.1. Study site and seed collection

Mature inflorescence of Halopeplis perfoliata were collected from a salt marsh (16° 44′ 23.05″ N, 42° 02′ 22.47″ E) near the coast of Jizan, Saudi Arabia in 2012. Seeds were collected from a large number of plants randomly to ensure adequate representation of the population's genetic diversity. Seeds were separated from their inflorescence husk manually and brought to laboratory in Pakistan. Seeds were surface sterilized using 1% bleach solution for 1 min, followed by thorough rinsing with distilled water and air-drying. Sterilized seeds were then used in experiments within four weeks after their collection.

2.2. Seed characteristics

Fresh weight (F_W) of 1000 seeds was measured using an analytical balance (precision 0.0001 g). Dry weight (D_W) of the seeds was determined by placing 1000 seeds in an oven at 105 °C for 48 h. Moisture content was then calculated as the difference between F_W and D_W. Ash or inorganic content of the seeds was determined by igniting oven dried seeds in a furnace at 550 °C for 3 h. Size of the seeds was measured using photographs of about 100 seeds randomly chosen with the help of Image J software (http://imagej.nih.gov/ij/images/). Seed texture was determined by observing seeds under a mini-microscope (Dini-Lite digital Microscope, ANMO Electronics Corporation). Seed color was matched against the catalog of Ralcolors (www.ralcolors.com).

2.3. Experiment 1: salinity tolerance limit and optimal germination conditions

Germination tests under a factorial combination of salinity, temperature and photoperiod were carried out in plastic petri dishes (50 mm diameter × 9 mm depth) with tight-fitting lids (to avoid evaporative loss of solution). Seven NaCl solutions (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mol L^{-1}, based on a preliminary trial) were used. There was only 5 mL of the solution in each petri plate to avoid oxygen stress. Petri plates were placed in germination chambers set at temperature regimes of 10/20, 15/25, 20/30 and 25/35 °C (common temperature regimes of the region; Gul et al., 2013), where low temperature coincided with 12 h dark and high temperature with 12 h light ($\approx$25 μmol m^{-2} s^{-1}, 400–750 nm; Philips cool-white fluorescent lamps). Under the same temperature and salinity treatments seeds were also incubated in complete darkness (24 h dark) by using dark photographic envelops. Four replicates with twenty-five seeds per petri plate were used for each treatment. Petri plates placed under 12 h photoperiod were monitored at 2 d intervals for 20 d and percent seed germination was noted. Emergence of the radical was the criterion for germination scoring (Bewley and Black, 1994). After 20 d of incubation rate of germination was calculated by using a modified Timson's index of germination velocity:

$$\text{Germination velocity} = \Sigma G/t$$

where, G is the percentage of seed germination at 2 d intervals and t is the total germination period (Khan and Ungar, 1985). The greater the value, the more rapid was germination. Germination of the seeds incubated in dark was noted once after 20 d. After 20 d un-germinated seeds from various salt treatments under 12 h light were transferred to distilled water, while un-germinated seeds from dark were exposed to 12 h light in order to study the recovery of germination. Seed germination during the recovery experiment was recorded at 2 d intervals for another 20 d period. The recovery percentage was calculated by using the following formula:

$$\text{Recovery (\%)} = (A - B/C) \times 100$$

where, A is total number of seeds germinated after being transferred to distilled water respectively light, B is the number of seeds germinated in saline solution respectively dark and C is total number of seeds. The seeds which did not germinate were further tested for their viability using 1% (w/v) 2,3,5-triphenyle-tetrazolium chloride solution (MacKay, 1972; Bradbeer, 1998).

2.4. Experiment 2: viability and germination responses of seeds to hyper-saline storage

To study tolerance of *H. perfoliata* seeds to hyper-saline conditions, seeds were exposed to four high salinity treatments (0.5, 1.0, 1.5 and

2.0 mol L^{-1} NaCl solutions; Keiffer and Ungar, 1997) at two temperature regimes (20/30 °C corresponding to optimal temperature for seed germination, and 25/35 °C, corresponding to summer temperature) under 12 h dark: 12 h light photoperiod for different time periods (20, 40 and 60 days). Absence of salinity (0 mM NaCl) served as control. There was only 5 mL of the test solution in each petri plate to avoid hypoxia stress. Germination, recovery, viability and mortality of the seeds were examined according to the methods described above for experiment 1.

2.5. Experiment 3: salinity tolerance and germination responses to dormancy regulating chemicals

To study if salinity tolerance of *H. perfoliata* seeds can be improved using different dormancy regulating chemicals (DRCs), seeds were germinated in different salinity treatments (0, 0.3 and 0.6 mol L^{-1} NaCl) in light (12 h photoperiod) as well as in complete darkness under optimal temperature (20/30 °C) in presence and absence of different DRCs. Betaine (1 mM), ethaphon (10 mM), fusicoccin (5 μM), kinetin (0.05 mM), proline (0.1 mM) and thiourea (10 mM) were used. Germination and recovery were noted as described above for experiment 1.

2.6. Statistical analyses

Germination data were arcsine transformed before statistical analysis. Analyses of Variance (ANOVAs) were used to determine if treatments (salinity, temperature, photoperiod, hyper-saline storage, and DRCs) had a significant effect on seed parameters. A Bonferroni test was carried out to compare mean values for significant ($P < 0.05$) differences. Software SPSS Version 11.0 (SPSS, 2011) was used for data analysis. Student *t*-test ($P < 0.05$) was performed to compare mean values for experiment 3 data.

3. Results

3.1. Seed characteristics

Seeds of *H. perfoliata* were ellipsoid, small (0.28 mm diameter) and rough textured (Fig. 1). Fresh weight of 1000 seeds was 92.9 mg and they had about 4.9% moisture. There were no perianth or hairs attached to the fully mature seeds (Table 1).

3.2. Salinity tolerance limit and optimal germination conditions

Three-way analysis of variance (ANOVAs) indicated significant ($P < 0.001$) effects of photoperiod, temperature, salinity and their interactions on mean final germination (MFG), rate of germination (G_{Rate}), recovery of germination from salinity (S_R) and recovery of germination from dark (D_R) of *H. perfoliata* seeds (Table 2).

Seeds were non-dormant and germinated maximally (≈90%) in distilled water irrespective of incubation temperature in the light (Fig. 2). However, increases in salinity (NaCl concentration) decreased MFG at all temperature regimes and there was < 10% MFG in the 0.6 mol L^{-1} NaCl treatment. Comparatively high MFG and G_{Rate} values were observed in ≥0.5 mol L^{-1} NaCl treatments at 20/30 °C than for other temperatures (Figs. 2 and 3). There was substantially low MFG in dark in comparison to 12 h photoperiod in all salinity and temperature treatments (Fig. 2). Interaction of photoperiod and salinity resulted in significantly ($P < 0.05$) lower germination at low salinity in dark compared to light. In addition, overall interaction of salinity, photoperiod and temperature (Table 2) caused substantial reduction in seed germination at low salinity and in dark especially at the highest temperature (Fig. 2). Salinity and temperature treatments had a similar effect on G_{Rate} as in case of MFG (Fig. 3). When un-germinated seeds from salinity and 12 h photoperiod were transferred to distilled water and incubated at the respective temperature for another 20 d, almost all seeds were germinated (S_R), hence total germination (MFG under saline condition + S_R) approached the level of MFG in distilled water. Likewise, when un-germinated dark incubated seeds were exposed to 12 h photoperiod for 20 d, they germinated (D_R) and total germination (MFG in dark + D_R) was comparable to MFG in respective salinity treatments under 12 h photoperiod (Fig. 2).

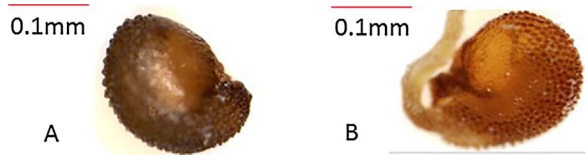

Fig. 1. Morphology of a) un-germinated and b) germinated seeds of *Halopeplis perfoliata* as seen under a light microscope.

Table 1
Characteristic features of *Halopeplis perfoliata* seeds collected from a coastal salt marsh in Jizan, Saudi Arabia.

Characteristic	Description
Color	Terra Brown[a]
Texture	Rough
Perianth/Hairs	Absent
Shape	Ellipsoid
Size (diameter, mm)	0.28
FW (mg 1000^{-1} seeds)	92.86
DW (mg 1000^{-1} seeds)	88.33
Moisture (% of FW)	4.88
Ash (% of FW)	3.87

[a] RAL-8028, www.ralcolors.com.

Table 2
Three-way ANOVA indicating effects of photoperiod (Phot), temperature (Temp), hyper-salinity (Salt) and their interactions on mean final germination (MFG), rate of germination (G_{Rate}), recovery from salinity (S_R) and recovery from dark (D_R) of *Halopeplis perfoliata* seeds.

Factor	d.f.	MFG	G_{Rate}	S_R	D_R
Phot	1	2270.3^{***}	–	–	–
Temp	3	106.866^{***}	118.662^{***}	21.863^{***}	17.982^{***}
Salt	6	298.232^{***}	504.267^{***}	15.218^{***}	229.734^{***}
Phot * Temp	3	56.192^{***}	–	–	–
Phot * Salt	6	138.699^{***}	–	–	–
Temp * Salt	18	11.492^{***}	17.157^{***}	2.166^{**}	11.947^{***}
Phot * Temp * Salt	18	11.585^{***}	–	–	–
Error	260	–	–	–	–

Numbers are *F*-values and asterisks in superscript are significance levels at $P < 0.05$ (***, $P < 0.001$).

3.3. Germination and viability responses of seeds to hyper-saline storage

A three-way ANOVA indicated significant individual effects of duration, temperature, hyper-salinity and their interaction on MFG, S_R, viability and mortality of the seeds of *H. perfoliata* (Table 3). Maximum (85%) germination occurred in distilled water within 20 days (Fig. 4). Some seeds (≈20%) germinated in 0.5 mol L^{-1} NaCl treatment and no seed could germinate in hyper-salinity treatments (1.0, 1.5 and 2.0 mol L^{-1} NaCl) even after 60 days (Fig. 4). Comparatively higher MFG was found in distilled water and 0.5 mol L^{-1} NaCl treatments at 20/30 °C than at 25/35 °C. About 70% of the un-germinated seeds from hyper-salinity germinated when transferred to distilled water (S_R) at 20/30 °C, even after 60 d of exposure. Exposure of seeds to hyper-salinity for 60 d caused significant ($P < 0.05$) reduction in S_R value at higher incubation temperature 25/35 °C (Fig. 4). About 10–15% seeds

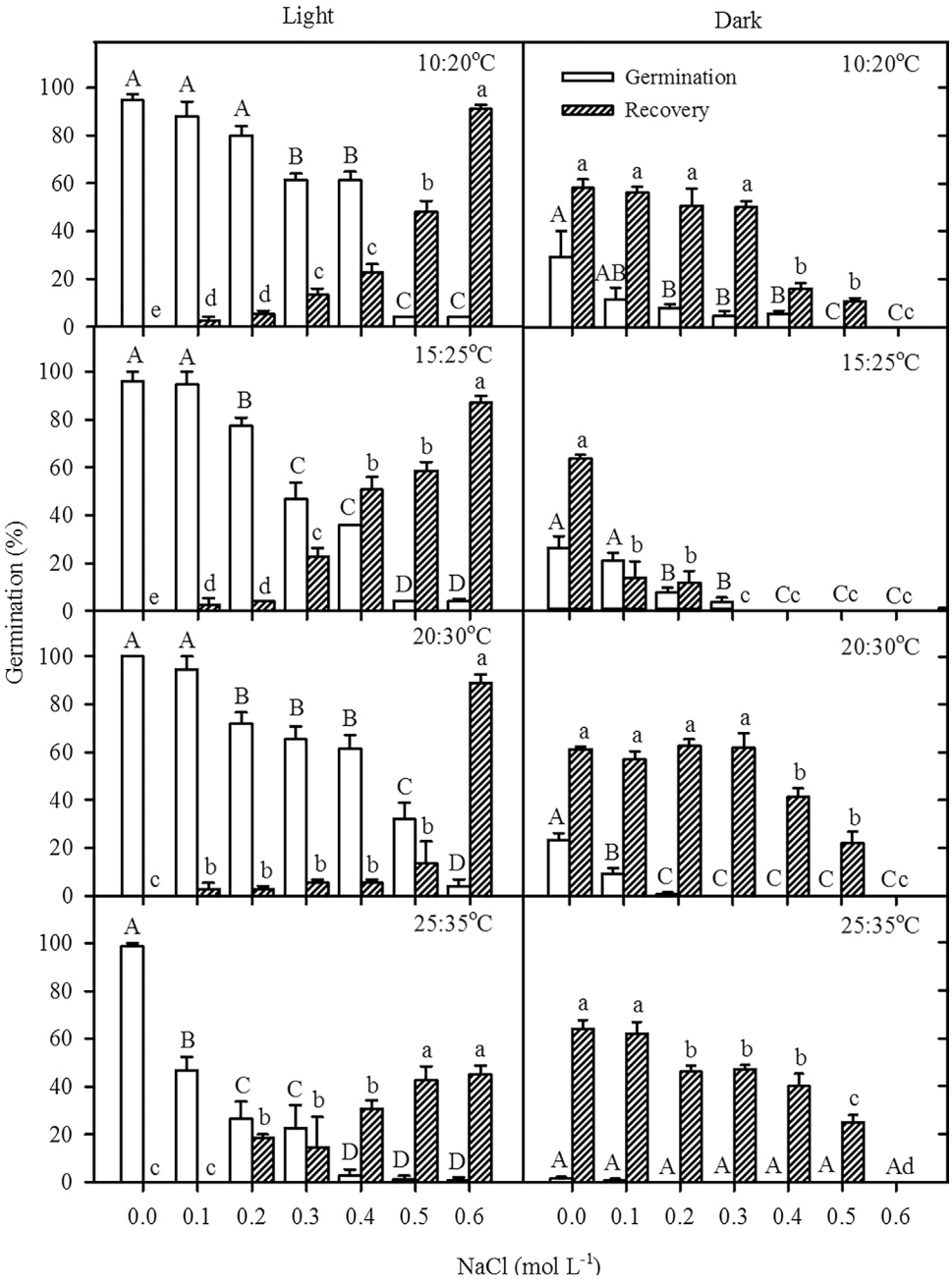

Fig. 2. Germination and recovery percentages of *Halopeplis perfoliata* seeds under different salinity treatments (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mol L^{-1} NaCl); temperatures (10/20, 15/25, 20/30 and 25/35 °C) and light (12 h photoperiod and 24 h darkness) regimes. Vertical bars are mean ± s.e, n = 4. Different letters (upper cases for germination, lower cases for recovery) on corresponding bars show significant differences among salinity treatments (Bonferroni test; $P < 0.05$).

were found dead in all salinity treatments at 20/30 °C after various exposure times. Interaction of hyper-salinity (1.0–2.0 mol L^{-1} NaCl), extended exposure (60 d) and high temperature (25/35 °C) caused highest seed mortality (up to 50%; Fig. 4).

3.4. Salinity tolerance and germination responses to dormancy regulating chemicals

There was a significant effect of dormancy regulating chemicals (DRCs; F = 4.07**; d.f. = 6), photoperiod (F = 119.73***; d.f. = 1), salinity (F = 470.31***; d.f. = 2) and their interaction (F = 3.80***; d.f. = 12) on MFG of *H. perfoliata* (error d.f.: 125). Ethephon improved MFG in high salinity (0.6 mol L^{-1} NaCl) only; kinetin was inhibitory, while betaine, fusicoccin, proline and thiourea had no effect on MFG of *H. perfoliata* in distilled water as well as in saline condition under 12 h photoperiod (Fig. 5a). However, all DRCs (except proline) improved MFG under complete darkness in distilled water as well as under saline condition (Fig. 5b). There was no effect of DRCs on recovery (both S_R and D_R) responses of seeds (data not given).

4. Discussion

4.1. Seed size and moisture content of Halopeplis perfoliata

Seeds of *H. perfoliata* were small (< 1 mm in diameter) like those of *H. amplexicaulis* and *H. pygmaea* (Shepherd et al., 2005; Anonymous, 2014) and had low (< 5%) moisture as reported for the seeds of many other halophytes such as *Limonium stocksii* and *Suaeda fruticosa* (Hameed et al., 2014). A number of reports suggest that in the absence of hairs or perianth, small size could be an important adaptation for dispersal and also reduces seed predation (Fenner, 1985; Shepherd et al., 2005). Stromberg and Boudell (2013) found that small seed size was associated with adaptation to disturbed and wet environments. Low moisture content on the other hand is reportedly essential for

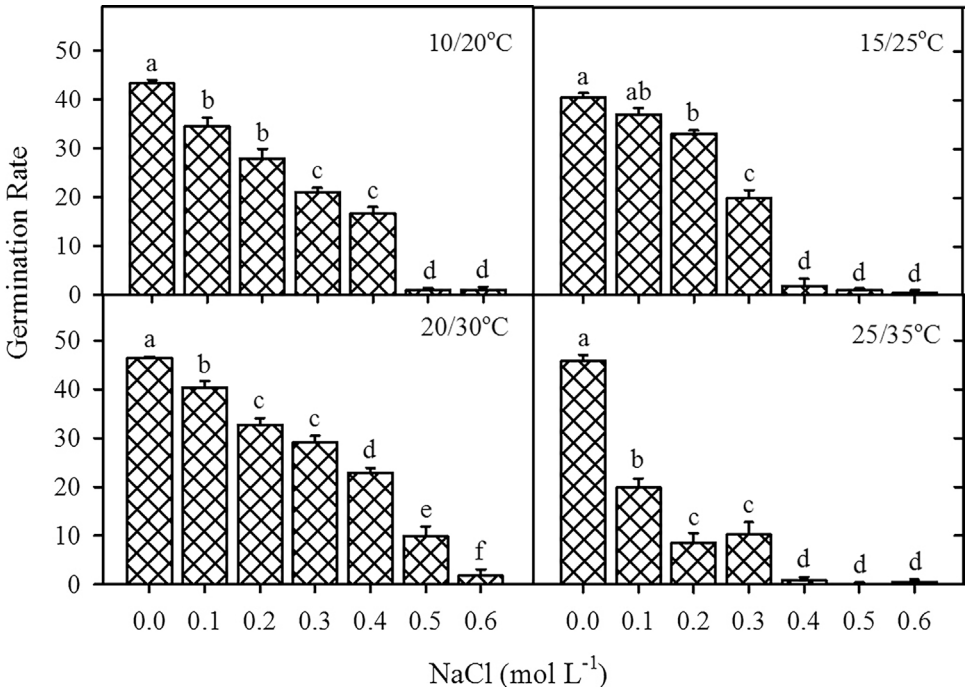

Fig. 3. Rate of germination of *Halopeplis perfoliata* seeds after different salinity treatments (0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mol L^{-1} NaCl); temperatures (10/20, 15/25, 20/30 and 25/35 °C) and light (12 h photoperiod) regimes. Vertical bars are means ± s.e (n = 4). Different letters show significant differences among salinity treatments (Bonferroni test; $P < 0.05$).

Table 3
Three-way ANOVA indicating effects of storage time (Days), temperature (Temp), hypersalinity (Salt), and their interactions on mean final germination (MFG), recovery from salinity (S_R), viability (Viab.) and mortality (Mort.) of *Halopeplis perfoliata* seeds.

Factor	d.f.	MFG	S_R	Viab.	Mort.
Days	2	4.98**	7.54***	58.64***	10.3***
Temp	1	49.36***	54.04***	44.85***	0.27^{ns}
Salt	4	1280.74***	6.31***	8.045***	6.84***
Days * Temp	2	5.46**	2.98^{ns}	17.79***	11.63***
Days * Salt	8	1.96^{ns}	1.92^{ns}	4.32***	1.05^{ns}
Temp * Salt	4	33.64***	6.45***	1.70^{ns}	1.26^{ns}
Days * Temp * Salt	8	2.10*	1.37^{ns}	1.70^{ns}	2.44*
Error	88	–	–	–	–

Numbers are *F*-values and asterisks in superscript are significance levels at $P < 0.05$ (ns, non-significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

prolonged longevity of the seeds (Ellis, 1991; Rajjou and Debeaujon, 2008). Hence, small size and low moisture content of the seeds appear important adaptations of *H. perfoliata* for survival in salt marshes, as reported for another halophyte *Arthrocnemum macrostachyum* (Gul and Khan, 1998).

4.2. Seed germination responses of Halopeplis perfoliata to variations in salinity, temperature and photoperiod

Seeds of *H. perfoliata* were non-dormant and germinated maximally (≈90%) in distilled water under 12 h photoperiod irrespective of incubation temperature. Gul et al. (2013) indicated that innate dormancy is generally absent in perennial halophytes, which may be an adaptive strategy to take advantage of water availability after rain (Song et al., 2005). Increases in salinity decreased both rate and final seed germination of *H. perfoliata;* however, a few seeds could also germinate in 0.6 mol L^{-1} NaCl (equivalent to seawater salinity) under 12 h photoperiod. Some other Salicornioideae species such as *Arthrocnemum macrostachyum* (1.0 mol L^{-1} NaCl; Khan and Gul, 1998) and *Sarcocornia ambigua* (0.77 mol L^{-1} NaCl; Freitas and Costa, 2014) also showed high salinity tolerance during seed germination. In contrast, ungerminated seeds of *H. perfoliata* in salinity showed a high recovery when transferred to distilled water, as reported for most other halophytes (Gul et al., 2013). This enforced dormancy (i.e. inability to germinate under stress condition while maintaining their viability) due to salinity is an important adaptive feature of halophyte seeds that distinguishes them from glycophytes (Khan and Gul, 2006). Enforced dormancy is also a key to constitute a persistent soil seed bank so that there is a chance for seedling recruitment after sufficient rain (Khan and Ungar, 1984; Cao et al., 2014).

Changes in temperature affect a number of processes related to seed germination such as membrane permeability, activity of membrane-bound proteins and cytosol enzymes (Gul and Weber, 1999; Bewley et al., 2013). Changes in temperature may also act as a germination timing sensor (Ekstam et al., 1999). In addition, interaction of temperature with salinity affects dormancy and tolerance of halophyte seeds (Gul et al., 2013; Hameed et al., 2013). In this study, although germination of the seeds of *H. perfoliata* was temperature insensitive under non-saline conditions, under saline conditions germination was clearly temperature dependent. Relatively high germination and salinity tolerance was observed at 20/30 °C. However, interaction of high temperature (25/35 °C) and salinity caused synergistic inhibition of germination compared to lower temperatures. Similarly, adverse effects of high salinity were further intensified at sub-/supra-optimal temperatures in several halophytes such as *Limonium tabernense* (Fernández et al., 2016), *L. stocksii* (Zia and Khan, 2004), *Atriplex cordobensis* (Aiazzi et al., 2002) and *Allenrolfea occidentalis* (Gul and Weber, 1999). Hence, our first hypothesis is partially true. Changes in temperature also influence recovery of germination of halophyte seeds (Khan and Ungar, 1997). Recently Gul et al. (2013) indicated that seeds of most subtropical halophytes germinate better at 20/30 °C, which resembles the post-rain ambient temperature in many subtropical areas. Recovery of germination in *H. perfoliata* in the light and under highest salinity was inhibited more at 25/35 °C than at the other temperatures, as in case of *A. indicum* (Saeed et al., 2011). Hence, higher temperature and salinity appear to act as co-regulatory factors by preventing germination of *H. perfoliata* in summer when there is high salinity in marsh sediments owing to no/low precipitation and high evapotranspiration.

Light has also been recognized as an important factor regulating seed germination of many plant taxa including most halophytes (Pons,

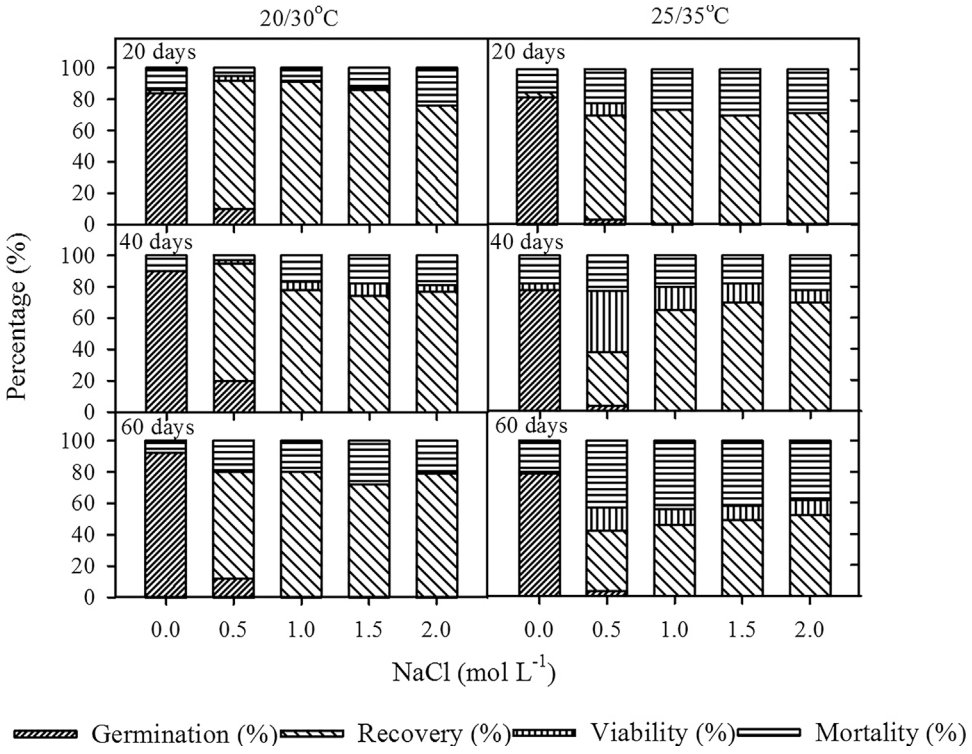

Fig. 4. Germination, recovery, viability and mortality percentages of *Halopeplis perfoliata* seeds after prolonged exposure (20, 40 and 60 days) in hypersaline (0.5, 1.0, 1.5 and 2.0 mol L^{-1} NaCl) treatment, temperatures (20/30 and 25/35 °C); and light (12 h photoperiod) regimes. Values are means of 4 replicates.

2000; Baskin and Baskin, 2001; Flores et al., 2006; Kettenring et al., 2006; Gul et al., 2013). However, halophytes vary in their light requirement for seed germination (Gul et al., 2013). Interestingly, most light requiring (positive photoblastic) seeds are small (Grime et al., 1981). Seeds of *H. perfoliata* are also small and germinated better in presence of light (12 h photoperiod) than in dark. In addition un-germinated seeds from dark treatment showed recovery of germination when transferred to 12 h photoperiod, thus indicating their positive photoblastic nature. Therefore our second hypothesis appears true. Milberg et al. (2000) suggested that light requirement for seed germination co-evolved with small seed size to ensure germination close to the soil surface. Light requirement is important for small seeds because they contain too little food reserves to support seedling emergence from deep burial in soil (Winn, 1985; Bonfil, 1998; Seiwa, 2000). Light also interacts with salinity to influence stress tolerance of halophyte seeds. For example, interactive effect of dark and high salinity caused more severe inhibition of germination of *H. perfoliata* seeds than salinity in the light. This finding is also in accordance with germination responses of *Suaeda heterophylla* (Hameed et al., 2013), *Halogeton glomeratus, Lepidium latifolium* and *Peganum harmala* (Ahmed and Khan, 2010). Hence it appears that light requirement for germination in our test species would favor germination of seeds at/near soil surface, to ensure light access to nascent seedlings, thereby maximizing their survival in marsh environments.

4.3. Seeds of Halopeplis perfoliata can endure hyper-salinity

Seeds of the salt marsh halophyte *H. perfoliata* showed high (80%) recovery when transferred from hyper-salinity (2.0 mol L^{-1} NaCl) to distilled water after 60 days of exposure at 20/30 °C. Similarly, seeds of another chenopod halophyte *Salsola affinis* also showed 37% recovery of germination, after 14 days of exposure to 2.0 mol L^{-1} NaCl (Wei et al., 2008). Seeds of some other halophytes *Suaeda depressa*, *S. linearis, Salicornia europaea*, and *Spergularia marina* could also withstand 0.85 mol L^{-1} NaCl treatment for 30 days in state of enforced dormancy and showed substantial recovery in distilled water (Ungar, 1995). Hence, germination inhibition under saline conditions and subsequent high recovery indicate osmotic effect rather than ionic toxicity of salinity on seeds of most halophytes including our test species. Interactive effect of long exposure time, high (25/35 °C) incubation temperature and salinity was highly detrimental to seed viability and about 50% seeds of *H. perfoliata* died after 60 d of exposure to NaCl at 25/35 °C as compared to 20/30 °C and distilled water (at which there was only ≤20% seed mortality each). This finding supports our third hypothesis. High storage temperatures reportedly reduce longevity of seeds, especially under moist conditions (Egley, 1990; Rajjou and Debeaujon, 2008).

4.4. Application of dormancy regulating chemicals could mitigate dark but not salinity effects

Various dormancy regulating chemicals (DRCs) such as betaine, ethephon, fusicoccin, kinetin, proline and thiourea are often reported to increase germination and stress tolerance of halophyte seeds (Khan et al., 1998; Gul et al., 2000; Khan et al., 2004; Mehrun-Nisa et al., 2007; Atia et al., 2009; El-Keblawy et al., 2010; Zehra et al., 2013; Ahmed et al., 2014). However, actions of these DRCs could be both species and stress specific (Gul et al., 2000; Ahmed et al., 2014). In this study, different DRCs generally had little/no effect on salinity tolerance of *H. perfoliata* but all (except proline) improved MFG under complete darkness as compared to un-treated seeds. These data thus partially support our fourth hypothesis. Ethephon and thiourea (Gul and Weber, 1998), betaine and kinetin (Gul et al., 2000) and fusicoccin (El-Keblawy et al., 2010; El-Keblawy, 2013; Zehra et al., 2013) could substitute light requirement for seed germination of some halophytes. As in this study, proline did not improve salinity as well as dark enforced dormancy in two other halophytes *Halogeton glomeratus* and *Peganum harmala* (Ahmed et al., 2014).

5. Conclusions

Our data indicate that sensitivity of *Halopeplis perfoliata* seeds to extremes of salinity, temperature and darkness could be an adaptation to confine germination only under optimum environmental conditions such as after sufficient rainfall, which would increase the chances of seedling survival. Seeds also have the potential to maintain viability

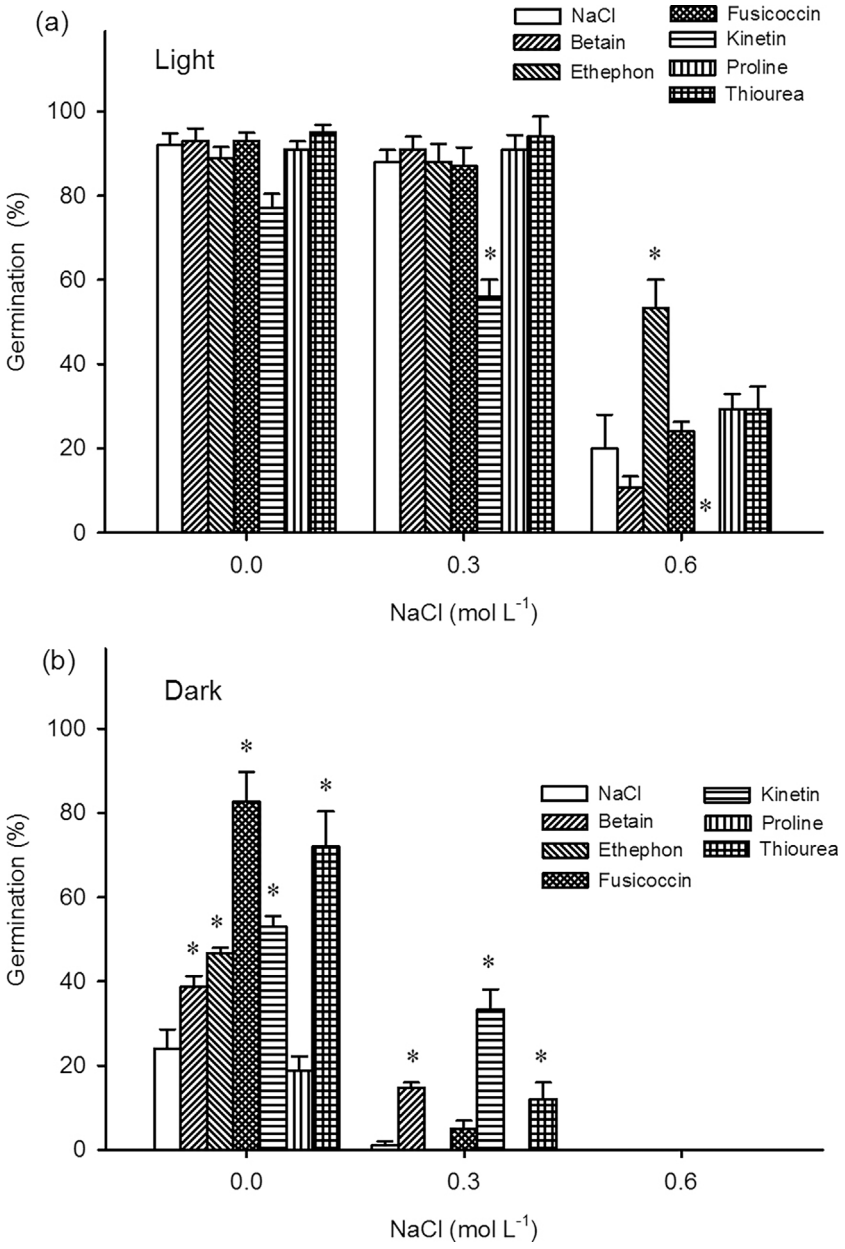

Fig. 5. Germination percentages of *Halopeplis perfoliata* treated with different dormancy regulating chemicals (DRCs) under different salinity treatments (0.3 and 0.6 mol L^{-1} NaCl) at 20/30 °C temperature and 12 h photoperiod (a) or 24 h darkness (b). Vertical bars are means ± s.e (n = 4). Asterisks (*) show significant differences in germination response with and without a DRC at a particular salinity level (Student's *t*-test; $P < 0.05$).

when buried in marsh sediments (hyper-saline conditions). Exogenous treatments indicated that salinity enforced dormancy has an osmotic, while dark enforced dormancy has a biochemical basis in this species.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

This research was partly supported by a grant from the Higher Education Commission of Pakistan under Pakistan-U.S. Science and Technology Cooperation Program.

References

Ahmed, M.Z., Khan, M.A., 2010. Tolerance and recovery responses of playa halophytes to light, salinity and temperature stresses during seed germination. Flora 205, 764–771.

Ahmed, M.Z., Gulzar, S., Khan, M.A., 2014. Role of dormancy regulating chemicals in alleviating the seed germination of three playa halophytes. Ekoloji 23, 1–7.

Aiazzi, M.T., Carpane, P.D., Di Rienzo, J.A., Argüello, J.A., 2002. Effects of salinity and temperature on the germination and early seedling growth of *Atriplex cordobensis* Gandoger et Stuckert (Chenopodiaceae). Seed Sci. Technol. 30, 329–338.

Al-Oudat, M., Qadir, M., 2011. The halophytic flora of Syria. international center for agricultural research in the dry areas. Syria 186.

Anonymous. 2014. http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=200006870.

Atia, A., Debez, A., Barhoumi, Z., Smaui, A., Abdelly, C., 2009. ABA, GA_3 and nitrate may control seed germination of *Crithmum maritimum* (Apiaceae) under saline conditions. C.R. Biol. 332, 704–710.

Böer, B., 1996. Plants as soil indicators along the Saudi coast of the Arabian Gulf. J. Arid Environ. 33, 417–423.

Baskin, C.C., Baskin, J.M., 2001. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. Academic Press San Diego.

Bewley, J.D., Black, M., 1994. Seeds: Physiology of Development and Germination, second ed. Plenum Press, New York and London.

Bewley, J.D., Bradford, K.J., Hilhorst, H.W., Nonogaki, H., 2013. Seeds: Physiology of Development, Germination and Dormancy, third ed. Springer, New York.

Bonfil, C., 1998. The effect of seed size cotyledon reserves, and herbivory on seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae). Am. J. Bot. 85, 79–87.

Boorman, L.A., 2003. Salt Marsh Review. An Overview of Coastal Saltmarshes, Their Dynamic and Sensitivity Characteristics for Conservation and Management. pp.

1–116 Joint Nature Conservation Committee Report, Peterborough 334.
Bradbeer, J.W., 1998. Seed Dormancy and Germination. Chapman and Hall, New York.
Cao, D.C., Baskin, C.C., Baskin, J.M., Yang, F., Huang, Z., 2014. Dormancy cycling and persistence of seeds in soil of a cold desert halophyte shrub. Ann. Bot. 113, 171–179.
Egley, G.H., 1990. High temperature effects on seed germination and survival of weed seeds in soil. Weed Sci. 3, 429–435.
Ekstam, B., Johannesson, R., Milberg, P., 1999. The effects of light and number of diurnal temperature fluctuations on germination of *Phragmites australis*. Seed Sci. Res. 9, 165–170.
El-Keblawy, A., Al-Rawai, A., 2006. Effects of seed maturation time and dry storage on light and temperature requirements during germination in invasive *Prosopis juliflora* (Sw.). Flora 201, 135–143.
El-Keblawy, A., Bhatt, A., 2015. Aerial seed bank affects germination in two small-seeded halophytes in Arab Gulf desert. J. Arid Environ. 117, 10–17.
El-Keblawy, A., Al-Ansari, F., Al-Shamsi, N., 2010. Impact of dormancy regulating chemicals on salinity induced dormancy in *Lasiurus scindicus* and *Panicum turgidum*: two desert glycophytic grasses. Plant Growth Regul. 62, 163–170.
El-Keblawy, A., Gairola, S., Bhatt, A., 2016. Maternal salinity environment affects salt tolerance during germination in *Anabasis setifera*: A facultative desert halophyte. J. Arid Land 8, 254–263.
El-Keblawy, A., 2013. Impacts of dormancy-regulating chemicals on innate and salinity-induced dormancy of four forage grasses native to Arabian deserts. Grass Forage Sci. 68, 288–296.
Ellis, R.H., 1991. The longevity of seeds. HortScience 26, 1119–1124.
Fenner, M., 1985. Seed Ecology, reprint ed. Chapman and Hall, University of Michigan.
Fernández, I.C.D., Luque, E.G., Mercado, F.G., Pedrosa, W., 2016. Influence of temperature and salinity on the germination of *Limonium tabernense* erben from tabernas desert (Almería SE Spain). Flora − Morphology, distribution. Funct. Ecol. Plants 218, 68–74.
Flores, J., Jurado, E., Arredondo, A., 2006. Effect of light on germination of seeds of Cactaceae from the Chihuahuan Desert, México. Seed Sci. Res. 16, 149–155.
Freitas, R.F., Costa, C.S., 2014. Germination responses to salt stress of two intertidal populations of the perennial glasswort *Sarcocornia ambigua*. Aquat. Bot. 117, 12–17.
Grime, J.P., Mason, G., Curtis, A.V., Rodman, J., Band, S.R., 1981. A comparative study of germination characteristics in a local flora. J. Ecol. 69, 1017–1059.
Gul, B., Khan, M.A., 1998. Population characteristics of a coastal halophyte *Arthrocnemum macrostachyum*. Pak. J. Bot. 30, 189–197.
Gul, B., Khan, M.A., 2008. Effect of compatible osmotica and plant growth regulators in alleviating salinity stress on the seed germination of *Allenrolfea occidentalis*. Pak. J. Bot. 40, 1957–1964.
Gul, B., Weber, D.J., 1998. Effect of dormancy relieving compounds on the seed germination of non-dormant *Allenrolfea occidentalis* under salinity stress. Ann. Bot. 82, 555–560.
Gul, B., Weber, D.J., 1999. Effect of salinity, light and thermoperiod on the seed germination of *Allenrolfea occidentalis*. Can. J. Bot. 77, 1–7.
Gul, B., Khan, M.A., Weber, D.J., 2000. Alleviation salinity and dark-enforced dormancy in *Allenrolfea occidentalis* seeds under various thermoperiods. Aust. J. Bot. 48, 745–752.
Gul, B., Ansari, R., Flowers, T.J., Khan, M.A., 2013. Germination strategies of halophyte seeds under salinity. Environ. Exp. Bot. 92, 4–18.
Hameed, A., Ahmed, M.Z., Gulzar, S., Gul, B., Alam, J., Hegazy, A.K., Alatar, A.R.A., Khan, M.A., 2013. Seed germination and recovery responses of *Suaeda heterophylla* to abiotic stresses. Pak. J. Bot. 45, 1649–1656.
Hameed, A., Rasheed, A., Gul, B., Khan, M.A., 2014. Salinity inhibits seed germination of perennial halophytes *Limonium stocksii* and *Suaeda fruticosa* by reducing water uptake and ascorbate dependent antioxidant system. Environ. Exp. Bot. 107, 32–38.
Hedden, P., Thomas, S.G., 2012. Gibberellin biosynthesis and its regulation. Biochem. J. 444, 11–25.
Keiffer, C.H., Ungar, I.A., 1997. The effect of extended exposure to hypersaline conditions on the germination of five inland halophyte species. Am. J. Bot. 84, 104–111.
Kettenring, K.M., Gardner, G., Galatowitsch, S.M., 2006. Effect of light on seed germination of eight wetland *Carex* species. Ann. Bot. 98, 869–874.
Khan, M.A., Gul, B., 1998. High salt tolerance in germinating dimorphic seeds of *Arthrocnemum indicum*. Int. J. Plant Sci. 159, 826–832.
Khan, M.A., Gul, B., 2006. Halophyte seed germination. In: Khan, M.A., Weber, D.J. (Eds.), Ecophysiology of High Salinity Tolerant Plants. Springer Press, pp. 11–30.
Khan, M.A., Ungar, I.A., 1984. Effects of salinity and temperature on the germination and growth of *Atriplex triangularis* Willd. Am. J. Bot. 71, 481–489.
Khan, M.A., Ungar, I.A., 1985. The role of hormones in regulating germination of polymorphic seeds and early seedling growth of *Atriplex triangularis* under saline conditions. Physiol. Plant. 63, 109–113.
Khan, M.A., Ungar, I.A., 1997. Effects of light, salinity, and thermoperiod on the seed germination of halophytes. Can. J. Bot. 75, 835–841.
Khan, M.A., Ungar, I.A., Gul, B., 1998. Action of compatible osmotica and growth regulators in alleviating the effect of salinity on the germination of dimorphic seeds of *Arthrocnemum indicum* L. Int. J. Plant Sci. 159, 313–317.
Khan, M.A., Gul, B., Weber, D.J., 2004. Action of plant growth regulators and salinity on the seed germination of *Ceratoides lanata*. Can. J. Bot. 82, 37–42.
Li, W., Yamaguchi, S., Khan, M.A., An, P., Liu, X., Tran, L.S.P., 2016. Roles of gibberellins and abscisic acid in regulating germination of *Suaeda salsa* dimorphic seeds under salt stress. Front. Plant Sci. 6, 1235. http://dx.doi.org/10.3389/fpls.2015.01235.
MacKay, D.B., 1972. The measurement of viability. In: Roberts, E.H. (Ed.), Viability of Seeds. Chapman and Hall, London, pp. 172–208.
Mahmoud, A., El-Sheikh, A.M., Abdul-Baset, S., 1983. Germination of two halophytes: *Halopeplis perfoliata* and *Limonium axilare* from Saudi Arabia. J. Arid Environ. 6, 87–98.
Mehrun-Nisa, Khan, M.A., Weber, D.J., 2007. Dormancy, germination and viability of *Salsola imbricate* seeds in relation to light, temperature and salinity. Seed Sci. Technol. 35, 595–606.
Milberg, P., Andersson, L., Thompson, K., 2000. Large seeded species are less dependent on light for germination than small seeded ones. Seed Sci. 10, 99–104.
Noe, G.B., Zedler, J.B., 2000. Differential effects of four a-biotic factors on the germination of salt marsh annuals. Am. J. Bot. 87, 1679–1692.
Pilcher, N.J., Phillips, R.C., Aspinall, S., Al-Madany, I., King, H., Hellyer, P., Beech, M., Gillespie, C., Wood, S., Schwarze, H., Al Dosary, M., Al Farraj, E., Khalifa, A., Böer, B., 2003. Hawar Islands Protected Area (Kingdom of Bahrain). (First draft. Management Plan).
Pons, T.L., 2000. Seed responses to light. In: Fenner, M. (Ed.), Seeds: the Ecology of Regeneration in Plant Communities, second ed. CABI Publishing, UK, pp. 237–260.
Rajjou, L., Debeaujon, I., 2008. Seed longevity: survival and maintenance of high germination ability of dry seeds. C.R. Biol. 331, 796–805.
Saeed, S., Gul, B., Khan, M.A., 2011. Comparative effects of NaCl and sea-salt on seed germination of *Arthrocnemum indicum*. Pak. J. Bot. 43, 1091–1103.
Seiwa, K., 2000. Effects of seed size and emergence time on tree seedling establishment: importance of developmental constraints. Oecologia 123, 208–215.
Shepherd, K.A., Macfarlane, T.D., Colmer, T.D., 2005. Morphology, anatomy and histochemistry of Salicornioideae (Chenopodiaceae) fruits and seeds. Ann. Bot. 95, 917–933.
Song, J., Fengi, G., Tian, C., Zhang, F., 2005. Strategies for adaptation of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* to a saline environment during seed-germination stage. Ann. Bot. 96, 399–405.
Stromberg, J.C., Boudell, J.A., 2013. Floods, drought, and seed mass of riparian plant species. J. Arid Environ. 97, 99–107.
Turkelboom, F., Mehdi, S., 2009. A Frame Work for Integrated Wetland Management of Jabbul Agro-Ecosystem. ICARDA, Syria p. 12.
Ungar, I.A., 1978. Halophyte seed germination. Bot. Rev. 44, 233–264.
Ungar, I.A., 1995. Seed germination and seed-bank ecology of halophytes. In: Kigel, J., Galili, G. (Eds.), Seed Development and Germination. Marcel and Dekker, New York, pp. 599–628.
Ungar, I.A., 1999. Ecophysiology of Vascular Halophytes. C.R.C. Press, Florida.
Wei, Y., Dong, M., Huang, Z.Y., Tan, D.Y., 2008. Factors influencing seed germination of *Salsola affinis* (Chenopodiaceae), a dominant annual halophyte inhabiting the deserts of Xingjiang, China. Flora 203, 134–140.
Winn, A.A., 1985. Effects of seed size and microsite on seedling emergence of Prunella vulgaris in four habitats. J. Ecol. 831–840.
Wu, Y.P., Chen, F., Hu, X.W., Baskin, C.C., Baskin, J.M., 2015. Alleviation of salinity stress on germination of *Leymus chinensis* seeds by plant growth regulators and nitrogenous compounds under contrasting light/dark conditions. Grass Forage Sci. 71, 497–506.
Zehra, A., Gul, B., Ansari, R., Alatar, A.R., Hegazy, A.K., Khan, M.A., 2013. Action of plant growth regulators in alleviating salinity and temperature effects on the germination of *Phragmites karka*. Pak. J. Bot. 45, 1919–1924.
Zia, S., Khan, M.A., 2004. Effect of light salinity, and temperature on seed germination of *Limonium stocksii*. Can. J. Bot. 82, 151–157.