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Germination and recovery responses of heteromorphic seeds of two co-occurring *Arthrocnemum* species to salinity, temperature and light

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ABSTRACT

Arthrocnemum macrostachyum and *A. indicum* are succulent halophytes found in the coastal marshes of southern Pakistan and many other countries, where they act as primary producers and also have many potential economic usages. They produce large quantities of heteromorphic seeds every year. Many aspects of the seed biology of these congeneric plants are not known. This comparative study was conducted to determine the germination and subsequent recovery of ungerminated seeds on distilled water of heteromorphic seeds of these halophytes to salinity (0, 200, 400, 600, 800 and 1000 mM NaCl), thermoperiod (15/25, 20/30 and 25/35 °C), photoperiod (12 h light/12 h dark and 24 h dark) and their interactions. Seeds of *A. macrostachyum* were of two colors (i.e. black and brown), while seeds of *A. indicum* differed in size (i.e. small and large). Seeds of the two species lacked primary/innate dormancy and germinated quickly in distilled water at low to moderate thermoperiods, while 25/35 °C was more inhibitory. Seeds were positively photoblastic and failed to germinate in dark. Seeds of *A. macrostachyum* showed higher tolerance to salinity (germinating at 600 mM NaCl, equivalent to sea water salinity) than those of *A. indicum* (not germinating above 400 mM NaCl). Germination of black but not brown seeds of *A. macrostachyum* was higher in 200 mM NaCl compared to control at 25/35 °C. Whereas in case of *A. indicum,* large seeds showed higher germination in distilled water than small seeds but were sensitive to salinity. Ungerminated seeds from high salinity and dark, when transferred to distilled water and 12 h photoperiod respectively, showed recovery of germination. Hence, our data indicate many similarities and differences in the germination responses of heteromorphic seeds of *A. macrostachyum* and *A. indicum.*

1. Introduction

Many plants of arid, semi-arid and saline habitats produce heteromorphic seeds, which differ in color, size and/or shape (Harper 1977; Imbert 2002; Matilla et al. 2005; Gul et al. 2013; Bhatt and Santo, 2016). These differences in seed morphology often accompany differences in dispersal, germination responses, dormancy status, longevity in seed bank and seedling growth responses (Khan and Ungar 1984; Imbert 2002; Brändel 2004; Yao et al. 2010; El-Keblawy et al., 2015). Seed heteromorphism is therefore considered an adaptation to maximize fitness of plants under harsh and unpredictable environments, whereby two/more seed morphs would germinate under different sets of environmental conditions (Ungar 1987; Song et al. 2008; Yao et al. 2010; El-Keblawy et al., 2015). About 200 species mostly from the Amaranthaceae, Brassicaceae, Caryophyllaceae and Poaceae are known to produce dimorphic seeds (Imbert 2002; Matilla et al. 2005). A number of halophytic genera including *Arthrocnemum*, *Atriplex*, *Chenopodium*, *Cakile*, *Salicornia*, *Salsola*, *Spergularia*, *Suaeda* and *Trianthema* also contain species with heteromorphic seeds (Ungar 1977, 1987; Morgan and Myers 1989; Matilla et al. 2005; Yao et al. 2010; Gul et al. 2013; Bhatt and Santo, 2016).

Responses of heteromorphic seeds to salinity may vary among species (Gul et al. 2013). In many cases heteromorphic seeds show similar responses to salinity such as in *Atriplex rosea* (Khan et al. 2004) and *Halopyrum mucronatum* (Khan and Ungar 2001). While, in others, heteromorphic seeds show differential salinity tolerance responses (Gul et al. 2013). For example, salinity tolerance limit of brown seeds of *Suaeda aralocaspica* was higher (1400 mM NaCl) compared to black (400 mM NaCl) seeds (Wang et al. 2008). Similarly, large seeds of *Salicornia europaea* germinated in as high as 856 mM NaCl whereas small seeds only in up to 514 mM NaCl (Ungar 1979). Hence, responses of heteromorphic seeds to salinity appear variable and inconclusive.

Heteromorphic seeds of halophytes may also differ in their requirements for thermoperiod and photoperiod for optimal germination. For example, large seeds of *Atriplex prostrata* germinated optimally at 20/35 °C, whereas optimal thermoperiod for small seeds was 5/25 °C (Carter et al. 2003). Likewise, optimal thermoperiod for the germination brown (20/30 °C) and black (25/35 °C) seeds of *Halopyrum mucronatum* also differed (Khan and Ungar 2001). In contrast, heteromorphic seeds

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of many halophytes like *Atriplex centralasiatica* (Li et al. 2008) and *Suaeda splendens* (Redondo-Gómez et al. 2008) showed similar thermoperiod requirements for optimal germination. Generally, a positive link exists between light requirement and small seed size probably to restrict germination only in the top soil layers, as small seeds possess less food reserves thereby cannot support seedling emergence from deeper soil layers (Grime et al., 1981; Gul et al. 2013). This kind of information about the light requirements of heteromorphic seeds is scanty. Wang et al. (2012) reported that heteromorphic seeds of *Suaeda acuminata* showed higher germination under light compared to dark. In contrast, black but not brown seeds of *S. corniculata* required light to germinate (Cao et al. 2012). These data thus show diverse responses of heteromorphic seeds to temperature and photoperiod.

Arthrocnemum is a small genus of succulent Salicornioideae halophytes (Kühn et al. 1993; Freitag et al. 2001), which are commonly found in the coastal marshes of South Africa, Asia, Mediterranean Europe and Northern America (Shepherd et al. 2005). Two species of *Arthrocnemum* namely *A. macrostachyum* and *A. indicum* are found in the salt marshes along coast of Pakistan (Freitag et al. 2001; Khan and Qaiser 2006). These succulent halophytes co-occur along salt marshes of Gadani area of the Balochistan province of Pakistan (Khan and Qaiser 2006). *Arthrocnemum macrostachyum* produces black and brown seeds (Khan and Gul 1998; Gul et al. 2013), whereas *A. indicum* produces large and small seeds (Personal observation; Fig. 1). Khan and Gul (1998) have reported differences in salinity tolerance of dimorphic seeds of *A. macrostachyum* under various temperature but not photoperiod regimes. While, Saeed et al. (2011) have compared the effects of NaCl and sea water salinities on seed germination of *A. indicum* under various thermoperiods and photoperiods; however, no discrimination was made between responses of dimorphic seeds. These halophytes not only act as important primary producers of the coastal marshes but also hold immense economic potentials particularly as biodiesel, camel fodder and medicinal crops (Khan and Qaiser 2006; Qasim et al., 2011; El-Wahab et al. 2008).

The aim of this study was to compare germination and recovery responses of heteromorphic seeds of these congeneric species to salinity, thermoperiod, photoperiod and their interactions. The following questions were addressed: (1) Is there any dormancy in the heteromorphic seeds of two species? (2) What are salinity tolerance limits of the heteromorphic seeds of two species? (3) Do heteromorphic seeds of two congeners differ in their thermoperiod and photoperiod requirements for optimal germination? (4) Can ungerminated seeds, if any, from high salinity recover their germination capacity when transferred to distilled water? and (5) What are the probable ecophysiological significances of the tested responses of heteromorphic seeds of the two species?

2. Materials and methods

2.1. Plant habitat and seed collection

Seed bearing inflorescence of *A. macrostachyum* and *A. indicum* were collected from a halophyte community growing along dry-marsh pan around Gadani ship-breaking yard (Latitude: 25° 4′36.62″N; Longitude: 66°42′35.91″E; Distance from seafront: ~300 m) of the Lasbela District, Balochistan, Pakistan during June 2015 and transported to the laboratory. The study area is part of Saharo-Sindian phytogeographic region where summer temperature may exceed 40 °C and annual precipitation is generally <350 mm (Ali and Qaiser 1986; Baloch et al. 2016). Seeds were collected randomly from large number of plants of each test species. Seeds were separated from the inflorescence husk manually, surface sterilized with 1% (*v*/*v*) sodium hypochlorite for 1 min, followed by thorough rinsing with distilled water and air-drying. Cleaned seeds were stored at room temperature (≈25 °C) in dry clear petri plates until used. Seeds were used in experiments within three months after collection (which resulted in no significant change in viability/germination of seeds during this period; data not given).

2.2. Seed morpho-physical characteristics

Size of the dimorphic seeds of two test species was estimated with the help of color photographs of randomly chosen 100 seeds by using

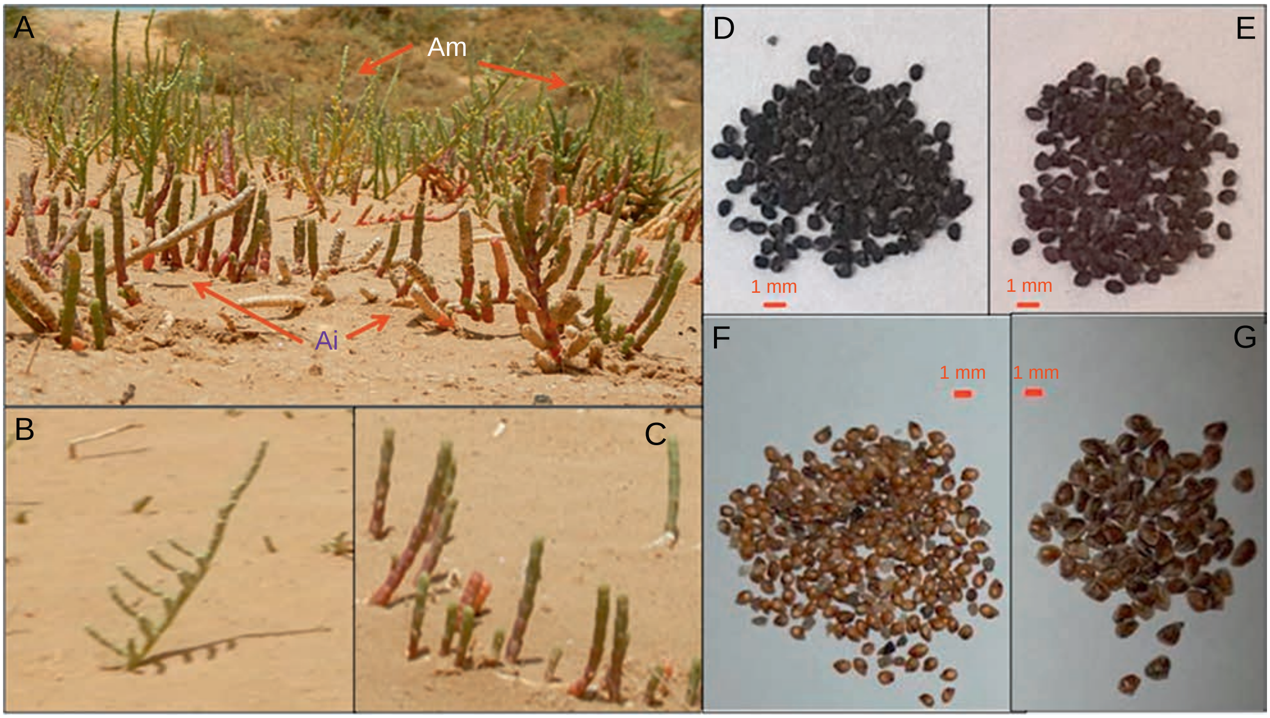

Fig. 1. Co-occurring populations (A) of *A. macrostachyum* (*Am*) and *A. indicum* (*Ai*) in natural habitat. Plant morphology of *A. macrostachyum* (B) and *A. indicum* (C). Heteromorphic seeds of *A. macrostachyum* (D-E) and *A. indicum* (F-G).

Image J software (http://imagej.nih.gov/ij/images/). Seed color was identified with the help of catalog of Ralcolors (www.ralcolors.com). Texture of the testa was determined by observing seeds under a hand-held magnifying lens. Fresh weight (FW) of 1000 freshly collected seeds of each species was noted with the help of an analytical balance (precision 0.0001 g). Dry weight (DW) was measured after drying the seeds in an oven at 105 °C for 48 h. Moisture content was calculated by using the formula: $\text{Moisture}\ (\%) = [(\text{FW} - \text{DW}) \times 100]/\text{FW}$. Initial viability of the collected seeds was assessed with the help of 2, 3, 5-triphenyl tetrazolium chloride (TTC) test (MacKay 1972; Bradbeer, 1998).

2.3. Seed germination experiments

Seed germination experiments were conducted in a programmed incubator with different thermoperiods (15/25, 20/30 and 25/35 °C; common thermoperiods reported for the region; Gul et al. 2013), where low temperatures coincided with 12 h dark and high temperatures with 12 h light ($\approx 25\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$; 400–700 nm, Philips cool-white fluorescent lamps). Germination was carried out in tight fitting clear plastic Petri dishes (5 cm Φ) with 5 ml of test solution. Seeds were exposed to different salinity levels (0, 200, 400, 600, 800 and 1000 mM NaCl) with 4 replicates of 25 seeds per treatment. Germination percentage (i.e. protrusion of embryonic axis; sensu Bewley and Black 1994) was recorded every alternate day for 20 days (i.e. time sufficient for maximum germination in highest salinity based on preliminary trials). Rate of germination was calculated by using a modified Timson index of germination velocity (i.e. $\Sigma G/t$; where G is percent germination at 2d interval and t is the total germination time) proposed by Khan and Ungar (1984). An additional set of experiment using aforementioned thermoperiods and salinity levels was conducted under complete (24 h) dark to study role of light in the germination of test species. Germination of this set was recorded once after 20 days.

2.4. Germination recovery experiments

After 20 days, all ungerminated seeds from different NaCl treatments under 12 h photoperiod were thoroughly rinsed with distilled water and transferred to distilled water for another 20 days to study recovery of germination from salinity stress. While, ungerminated seeds from dark experiment were transferred to 12 h photoperiod in same test solutions and thermoperiods for another 20 days to examine germination recovery from dark. Recovery of germination was noted on every alternate day for 20 days. Recovery of germination was calculated as percent of the ungerminated seeds from salinity or dark treatment germinated after transfer to distilled water or light respectively. Rate of germination recovery was estimated using the Timson Index mentioned above. At the end of the recovery experiments, all remaining ungerminated seeds were tested for their viability by using 1% (*w*/*v*) 2, 3, 5-triphenyl tetrazolium chloride (TTC) solution (MacKay 1972; Bradbeer, 1998).

2.5. Statistical analysis

All data were statistically analyzed with the help of SPSS version 16.0 (SPSS, 2007). Analysis of variance (ANOVA) was performed to find out whether different experimental factors (salts, thermoperiods and photoperiods) affected germination, recovery, viability and mortality significantly. Germination data were transformed (arcsine) to meet ANOVA assumptions. Post-hoc Bonferroni Test ($p < .05$) was conducted to indicate significant differences among mean values across salinity treatments. While, *t*-test was performed to compare two mean values within a salinity treatment.

3. Results

3.1. Morphology of the dimorphic seeds

Both *A. macrostachyum* and *A. indicum* produced dimorphic seeds (Fig. 1). Seeds of *A. macrostachyum* were of two colors (i.e. black and brown), while dimorphic seeds of *A. indicum* differed in size and mass (i.e. small and large). Generally, seeds of the two species were smooth textured and had low moisture content (<6%). Seeds of *A. macrostachyum* were oblong, while those of *A. indicum* were oval in shape (Table 1). Initial viability of the collected seeds was 70 and 80% for black and brown seeds of *A. macrostachyum*, while of large and small seeds of *A. indicum* was 79 and 53% respectively (Table 1).

3.2. Seed germination responses

Analysis of variance (ANOVA) indicated significant effects of seed morphology (M), thermoperiod (T), salinity (S) and interaction of T and S on seed germination of *A. macrostachyum* (Table 2). Generally, both black and brown seeds of *A. macrostachyum* germinated maximally in distilled water in thermoperiod-dependant manner. In distilled water, highest (Black = 70%, Brown = 80%) germination occurred at the low thermoperiod (15/25 °C) and lowest germination (Black = 50%, Brown = 60%) was observed at the high thermoperiod (25/35 °C) (Fig. 2). Rate of germination also decreased with increases in temperature (Fig. 3). Dimorphic seeds of *A. macrostachyum* were positively photoblastic and completely failed to germinate in the dark. Germination of black and brown seeds was comparable to non-saline control in up to 400 mM NaCl under low (15/25 °C) to moderate (20/30 °C) thermoperiods (Fig. 2). Interestingly, at 25/35 °C both final and rate of germination of black seeds were higher in 200 mM NaCl as compared to distilled water control (Figs. 2 and 3). Black seeds also had higher germination in 400 mM NaCl as compared to brown seeds at

Table 1
Morpho-physical characteristics of heteromorphic seeds of *A. macrostachyum* and *A.indicum*.

Characteristics	Details			
	A. macrostachyum		*A. indicum*	
	Morph 1	Morph 2	Morph 1	Morph 2
Color	⬛ Jet Black	🟫 Chocolate Brown	🟫 Olive Brown	🟫 Olive Brown
Perianth/Hairs	Absent	Absent	Absent	Absent
Shape	Oblong	Oblong	Oval	Ovate
Size (diameter, mm)	0.82	0.73	1.12	0.76
FW (mg 1000^{-1} seeds)	119.9	93.6	478.8	196.8
DW (mg 1000^{-1} seeds)	116.0	90.0	452.4	188.5
Moisture (% of FW)	3.3	3.8	5.5	4.2
Initial Viability (%)	70	80	79	53

Table 2
Analysis of variance (ANOVA) indicating effects of seed morphology, thermoperiods, salinity and their interactions on mean final germination (MFG), germination rate (GR), recovery from salinity (RS), rate of recovery from salinity (RRS), recovery from dark, rate of recovery from dark (RRD) of *A. macrostachyum* and *A. indicum* seeds. Numbers in the table are *F* and *P* values.

Species	Parameters	MFG		GR		RS		RRS		RD		RRD	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
A. macrostachyum	Morphology (M)	4.110	0.046	4.010	0.049	9.479	0.003	9.983	0.002	7.749	0.007	4.225	0.043
	Thermoperiod (T)	4.614	0.013	10.715	0.000	2.399	0.097	6.374	0.003	37.085	0.000	10.394	0.000
	Salinity (S)	78.763	0.000	114.852	0.000	83.445	0.000	88.788	0.000	31.818	0.000	71.748	0.000
	M × T	1.398	0.254	1.059	0.352	0.083	0.921	0.259	0.773	0.313	0.732	0.787	0.459
	M × S	2.724	0.050	2.856	0.043	2.479	0.050	2.564	0.044	0.733	0.536	1.348	0.266
	T × S	4.578	0.001	5.808	0.000	1.498	0.169	1.551	0.151	6.950	0.000	5.849	0.000
	M × T × S	0.907	0.495	0.647	0.692	0.394	0.921	0.270	0.974	0.471	0.828	0.376	0.892
A. indicum	Morphology (M)	9.521	0.003	2.788	0.101	433.804	0.000	401.626	0.000	148.770	0.000	100.117	0.000
	Thermoperiod (T)	6.663	0.003	8.313	0.001	6.754	0.002	8.690	0.000	0.655	0.523	3.539	0.036
	Salinity (S)	119.215	0.000	138.895	0.000	4.020	0.005	5.732	0.000	124.224	0.000	124.978	0.000
	M × T	2.124	0.129	0.650	0.526	2.293	0.107	3.259	0.043	5.550	0.006	11.450	0.000
	M × S	15.886	0.000	11.454	0.000	1.366	0.252	1.270	0.288	20.386	0.000	17.653	0.000
	T × S	4.622	0.003	8.552	0.000	2.258	0.030	3.664	0.001	5.362	0.001	7.061	0.000
	M × T × S	4.904	0.002	2.585	0.047	1.546	0.153	2.241	0.031	0.365	0.833	2.946	0.028

this thermoperiod. However, both black and brown seeds could germinate up to 600 mM NaCl.

Seed morphology (*M*), thermoperiod (*T*), salinity (*S*) and their interactions (except *M* x *T*,) had significant effects on germination percentage of *A. indicum* seeds (ANOVA; Table 2). Large seeds showed significantly ($p < .05$) higher rate and final germination as compared to small seeds in distilled water at all temperature regimes (Figs. 2 and 3). Generally, the high thermoperiod was more inhibitory than other thermoperiods for the germination of large seeds. While, small seeds germinated optimally at the low thermoperiod and an increase in thermoperiod was inhibitory (Fig. 2). However, at the moderate thermoperiod germination of small seeds remained comparable to non-saline control in up to 400 mM NaCl. Seeds of *A. indicum* could germinate only in up to 400 mM NaCl, hence had lower salt tolerance than the seeds of *A. macrostachyum*. However, similar to *A. macrostachyum* seeds, there was no germination of *A. indicum* seeds in the dark.

3.3. Recovery responses

Ungerminated seeds of *A. macrostachyum* from different NaCl treatments showed high recovery of germination when transferred to distilled water irrespective of seed morph and thermoperiod (Figs. 4 and 5). Although germination of seeds occurred in up to 600 mM NaCl, recovery occurred from as high as 1000 mM NaCl solution in either seed type. Likewise, seeds of *A. macrostachyum* also showed recovery of germination when transferred from dark to light (12 h-photoperiod) with higher and faster recovery values at the moderate thermoperiod than others (Figs. 6 and 7).

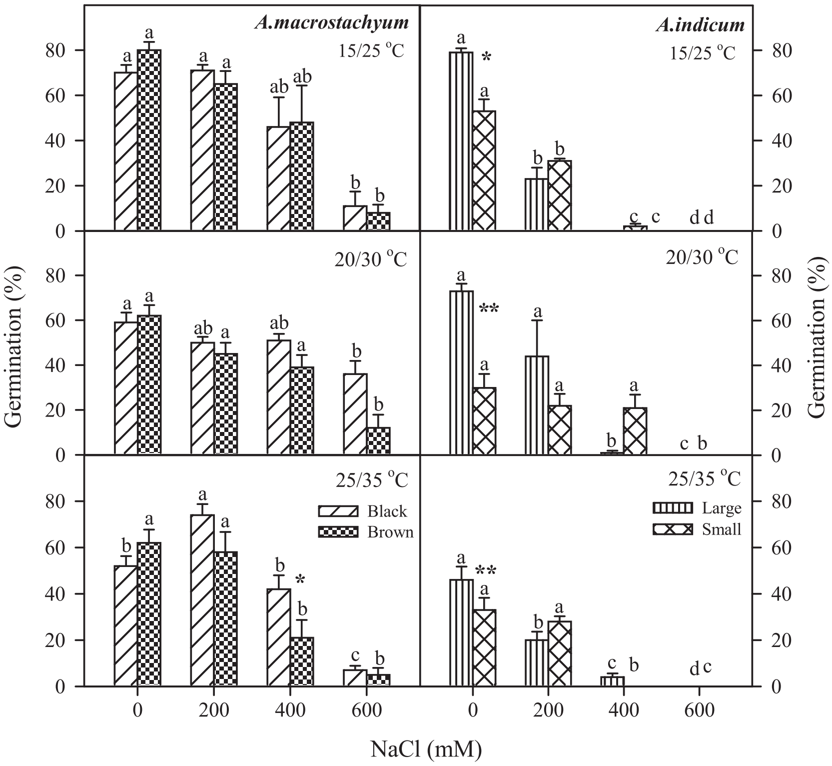

Fig. 2. Mean (±S.E.) final germination percentages of heteromorphic seeds of *A. macrostachyum* and *A. indicum*, in different salt concentrations under different temperature regimes. Similar bars across salinity concentrations with same letter are not significantly different ($p < .05$). Asterisks (*) indicates significant between two bars within a salinity concentration (*t*-test; where * = $p < .05$, ** = $p < .01$, *** = $p < .001$).

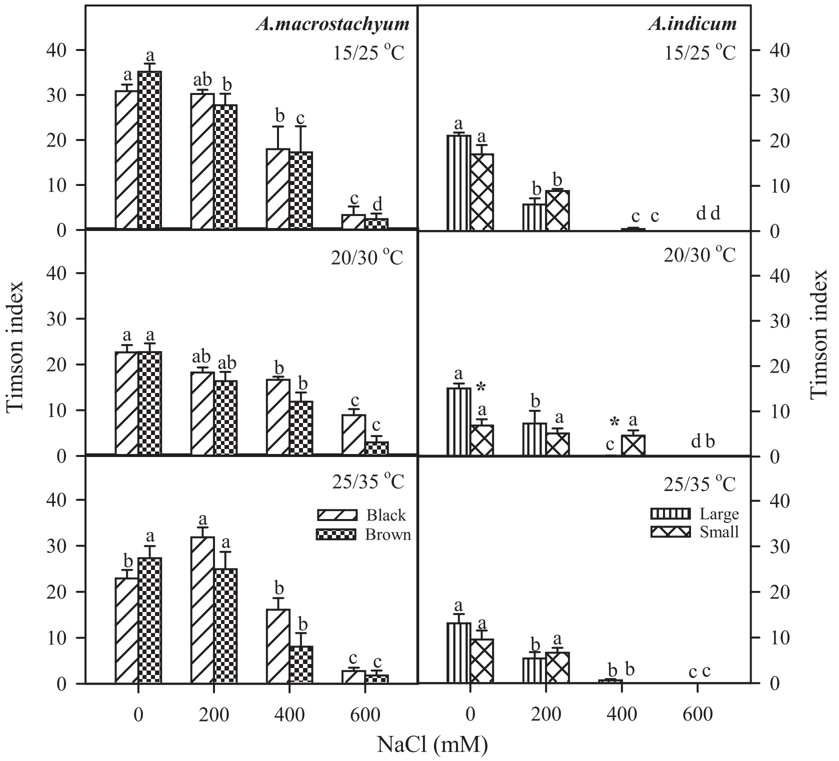

Fig. 3. Timson index of germination velocity for heteromorphic seeds of *A. macrostachyum* and *A. indicum*, in different salt concentrations under different temperature regimes. Similar bars across salinity concentrations with same letter are not significantly different (p < .05). Asterisks (*) indicates significant between two bars within a salinity concentration (*t*-test; where * = p < .05, ** = p < .01, *** = p < .001).

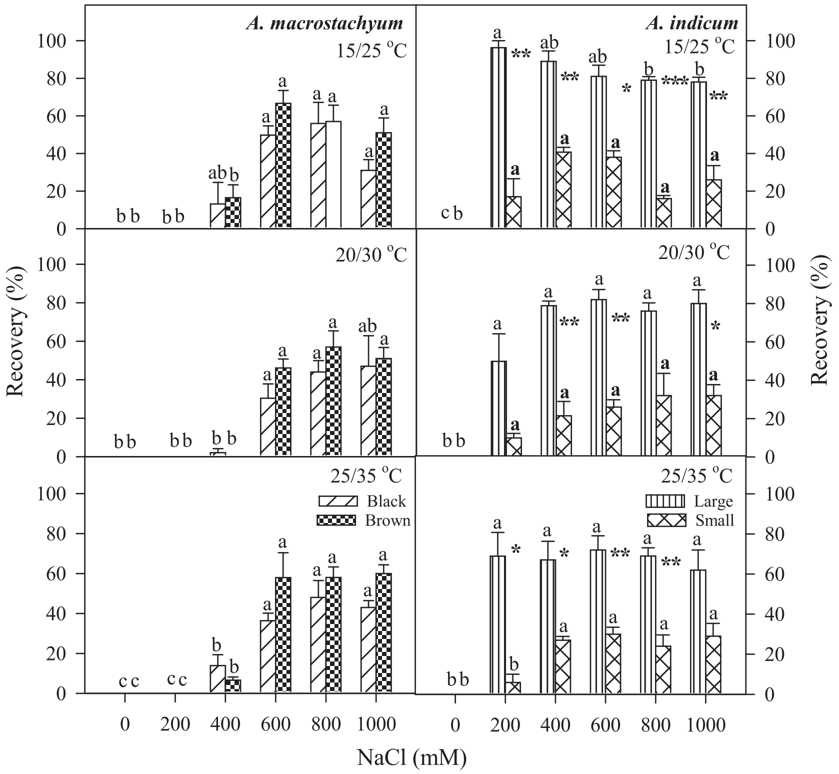

Fig. 4. Mean (±S.E.) recovery percentages of heteromorphic seeds of *A. macrostachyum* and *A. indicum* after transfer from different salt concentrations to distilled water under different temperature regimes. Similar bars across salinity concentrations with same letter are not significantly different (p < .05). Asterisks (*) indicates significant between two bars within a salinity concentration (*t*-test; where * = p < .05, ** = p < .01, *** = p < .001).

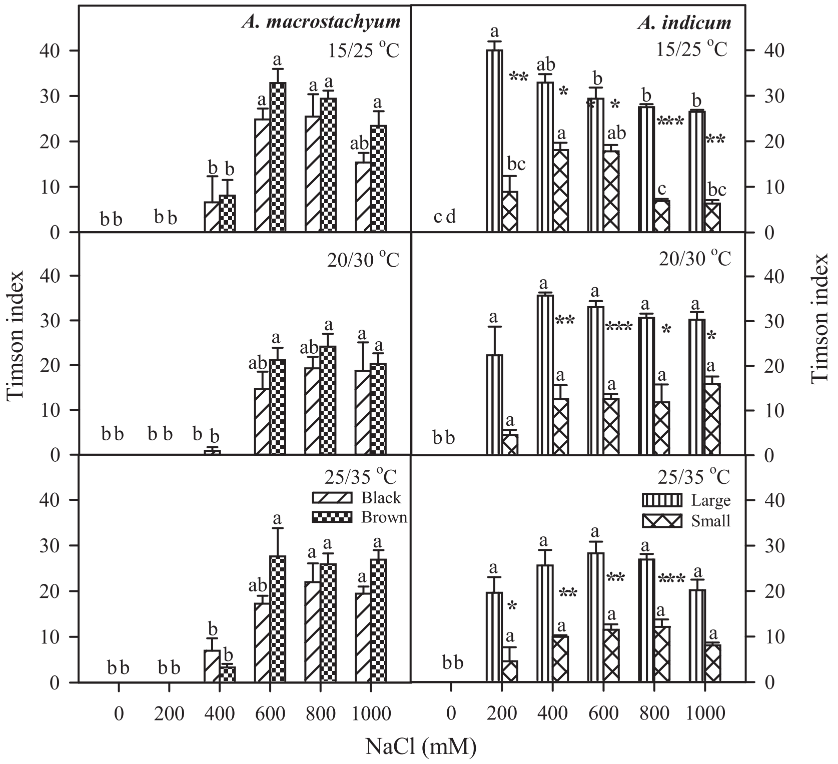

Fig. 5. Timson index for recovery of germination of *A. macrostachyum* and *A. indicum* seeds after transfer from different salt concentrations to distilled water under different temperature regimes. Similar bars across salinity concentrations with same letter are not significantly different ($p < .05$). Asterisks (*) indicates significant between two bars within a salinity concentration (*t*-test; where * = $p < .05$, ** = $p < .01$, *** = $p < .001$).

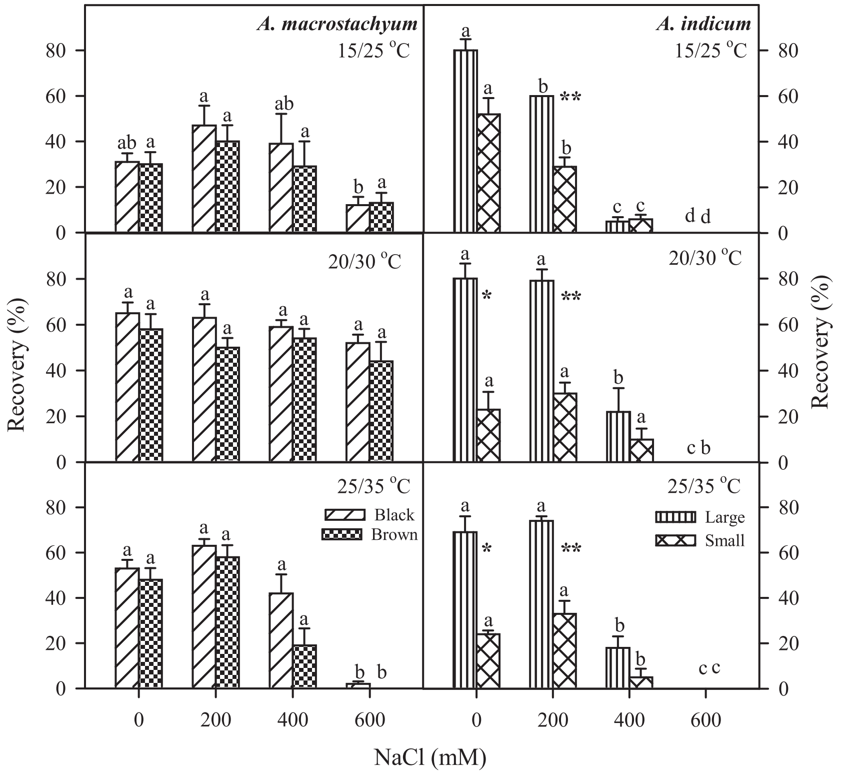

Fig. 6. Mean (±S.E.) recovery percentages of seeds of *A. macrostachyum* and *A. indicum* after transfer from dark to light under respective salt concentrations and temperature regimes. Similar bars across salinity concentrations with same letter are not significantly different ($p < .05$). Asterisks (*) indicates significant between two bars within a salinity concentration (*t*-test; where * = $p < .05$, ** = $p < .01$, *** = $p < .001$).

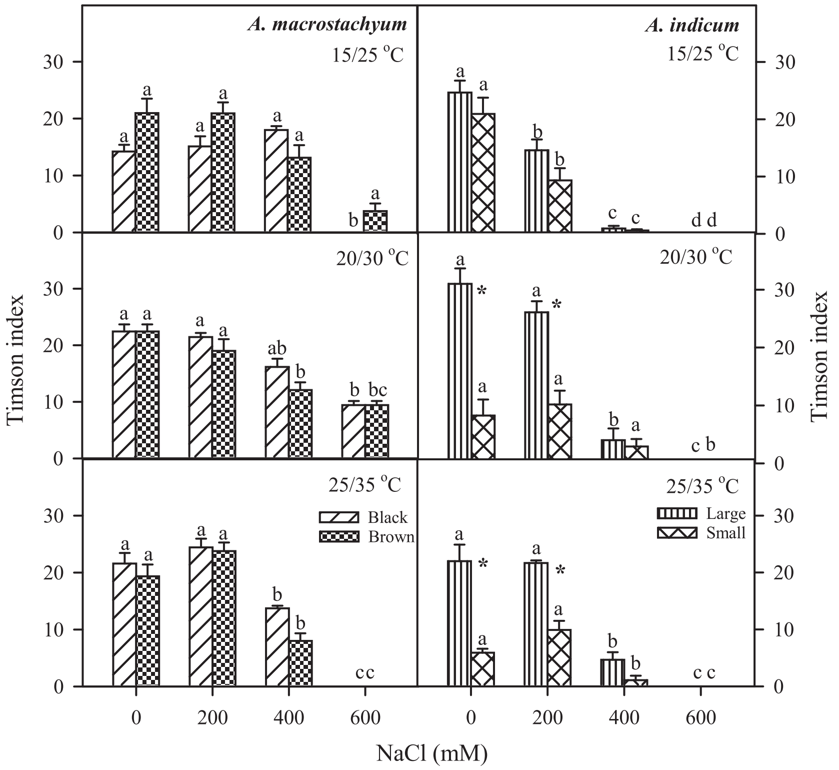

Fig. 7. Timson index for recovery of germination of *A. macrostachyum* and *A.indicum* seeds after transfer from dark to light under respective salt concentrations and temperature regimes. Similar bars across salinity concentrations with same letter are not significantly different ($p < .05$). Asterisks (*) indicates significant between two bars within a salinity concentration (*t*-test; where * = $p < .05$, ** = $p < .01$, *** = $p < .001$).

Ungerminated seeds of *A. indicum* also showed recovery of germination when transferred from salt solutions to distilled water (Fig. 4). There was substantially higher and faster recovery of large ungerminated seeds than the small seeds (Figs. 4 and 5). Seeds of *A. indicum* could also recover their germination from as high as 1000 mM NaCl. Seeds of *A. indicum* also showed recovery from dark, when transferred to light, with significantly ($p < .05$) higher and faster values in large as compared to small seeds (Figs. 6 and 7). All remaining ungerminated seeds of both *A. macrostachyum* and *A. indicum* after recovery experiments were stained with TTC and found dead (data not given).

4. Discussion

Seeds of *A. macrostachyum* and *A. indicum* displayed heteromorphism for color (black and brown) and size (large and small) respectively. About 200 plants including many halophytes are known to produce heteromorphic seeds, which may differ in color, size and/or shape (Imbert 2002; Matilla et al. 2005; Gul et al. 2013). For instance, many halophytes such as *Atriplex canescens* (Bhatt and Santo 2016), *Atriplex rosea* (Khan et al. 2004), *Chenopodium album* (Yao et al. 2010), *Suaeda acuminata* (Wang et al. 2012) and *Suaeda splendens* (Redondo-Gómez et al., 2008) produce heteromorphic seeds which differ in color. Similarly, several halophytes such as *Salicornia europea* (Philipupillai and Ungar 1984), *Salicornia ramosissima* (Ameixa et al., 2016) and *Atriplex tringularis* (Khan and Ungar 1984) produce heteromorphic seeds which differ in size. These differences in seed morphology often lead to differences in germination time and requirements and hence affect dormancy, longevity in seed bank and seedling growth (Khan and Ungar 1984; Imbert 2002; Brändel 2004; Yao et al. 2010; El-Keblawy et al. 2015; Nisar, unpublished data). Seed heteromorphism thus acts as an adaptation in habitats, where environmental variations are extreme and the intermediate phenotype has low fitness (Venable 1985; Imbert 2002; Gul et al. 2013). Seeds of the test species were generally small (here not indicating the small seed morph), had low moisture content (<6%) and lacked any perianth/hairs similar to the seeds of many other coastal halophytes such as *Halopeplis perfoliata* (Rasool et al. 2017), *Limonium stocksii*, and *Suaeda fruticosa* (Hameed et al. 2014). Small seed size is often described as a common adaptive feature of the plants inhabiting disturbed and wet environments (Stromberg and Boudell 2013), where it may facilitate dispersal and reduce chances of seed predation (Fenner 1985; Shepherd et al. 2005). While, low moisture content often accompanies high seed longevity (Pieta-Filho and Ellis 1991; Bewley and Black 1994). Coastal marshes of the subtropical region experience large spatiotemporal variations in sediment moisture and salinity levels (Böer 1996; Khan and Gul, 1998). High seed longevity appears essential for halophytes inhabiting such habitats, as their seeds have to remain in soil seed banks until sufficient rain that reduces high temperature, moisture and salinity stresses (Ungar, 2001; Khan and Gul 2006; Rasool et al. 2017). Hence production of small (here not indicating the small seed morph) heteromorphic seeds with low moisture in *A. macrostachyum* and *A. indicum* appear important adaptations for survival under fluctuating marsh environment.

Heteromorphic seeds of *A. macrostachyum* and *A. indicum* lacked primary/innate dormancy (Sensu Harper 1959, 1977; Baskin and Baskin 1998) and germinated maximally in distilled water, as those of *Atriplex triangular* (Khan and Ungar 1984), *Salicornia ramosissima* (Ameixa et al. 2016) and *Synedrella nodiflora* (Souza Filho, and Takaki 2011). Small seeds of *A. indicum* had low initial viability (53%) compared to the large seeds but this was not attributed to dormancy as all ungerminated seeds were non-viable. At least one seed morph of *Suaeda aralocaspica* (Wang et al. 2008) and *S. corniculata* (Cao et al. 2012) showed some type/degree of dormancy. Although under non-saline conditions and at low to moderate thermoperiods black and brown seeds of *A. macrostachyum* germinated to similar percentages, heteromorphic seeds of *A. indicum* differed in their germination responses with substantially higher germination for large as compared to small seeds. Likewise, large seeds of *Cakile edentula* (Zhang 1993), *Atriplex sagittata* (Mandák and Pyšek 2005) and *Salsola komarovii* (Takeno and Yamaguchi 1991) also showed higher or faster germination than the small seeds. This higher germination might be ascribed to higher food reserves in large compared to small seeds (Yao et al. 2010; Siddiqui and Khan 2011).

Heteromorphic seeds of *A. macrostachyum* and *A. indicum* were thermoperiod sensitive, similar to the seeds of *Halopyrum mucronatum* (Khan and Ungar 2001) and *Atriplex prostrata* (Carter et al. 2003). In contrast, heteromorphic seeds of *A. centralasiatica* (Li et al. 2008) and *Suaeda splendens* (Redondo-Gómez et al. 2008) were less sensitive to temperature during germination. Hence, responses of heteromorphic seeds to thermoperiod appear variable. Under non-saline conditions, germination of heteromorphic (black and brown) seeds of *Arthrocnemum macrostachyum* and small seeds of *A. indicum* decreased with increases in the thermoperiod, with highest germination value at low (15/25 °C) and least at high (25/35 °C) thermoperiod. While, large seeds of *A. indicum* germinated equally well at low to moderate (20/30 °C) thermoperiod and their germination inhibited only at high thermoperiod. Gul et al. (2013) reviewed that high (25/35 °C) thermoperiod is generally inhibitory for the germination of most subtropical halophytes. Hence, sensitivity of seeds to high thermoperiod appears a strategy to prevent germination of most seeds in case of pre-monsoon rain when temperature is still high and not conducive for seedling survival. However, under low salinity (200 mM NaCl) black seeds of *A. macrostachyum* showed enhanced germination in comparison to that in distilled water control at 25/35 °C. This improved germination might be related to the phenomenon of cross-tolerance to high temperature by low salinity. Cross-tolerance is defined as phenomenon of increased tolerance for a stress upon exposure to certain levels of another stress (Sabehat et al. 1998; Bowler and Fluhr 2000; Pastori and Foyer 2002). Evidences are gathering that exposure to certain levels of salinity often enhance tolerance of halophytes to other stresses. For instance, tolerance of *Sesuvium portulacastrum* to mannitol-induced water stress improved upon exposure to salinity (Slama et al. 2007).

Seeds of *A. macrostachyum* showed higher salinity tolerance limit (600 mM NaCl, equivalent to sea water salinity) than those of *A. indicum* (400 mM NaCl). This high salinity tolerance during germination might be related to high abundance and broad distribution of *A. macrostachyum* in the coastal areas of Pakistan and other countries as compared to *A. indicum*, which has a confined distribution only in a few areas. Germination of black and brown seeds of *A. macrostachyum* was comparable to non-saline control in up to 400 mM NaCl under low to moderate thermoperiods. Heteromorphic seeds of *Salicornia ramossissima* (Ameixa et al. 2016) also displayed comparable germination in up to ≈257 mM NaCl. However, germination of black seeds of *A. macrostachyum* was comparatively higher than that of brown seeds at 400 mM NaCl at the highest thermoperiod. Ameixa et al. (2016) also reported that the differences in the germination response of heteromorphic seeds of *S. ramossissima* were evident only at high (≈342 and 428 mM NaCl) salinity levels. In case of *A. indicum,* large seeds were sensitive to salinity, while germination of small seeds remained comparable to non-saline control in up to 400 mM NaCl at moderate thermoperiod. Hence, it appears that the large seeds could confer a competitive advantage by quickly producing lots of seedlings in community gaps with low/no salinity after sufficient rains, small seeds could contribute towards dispersal and also ensure continuity of lineage by producing some seedlings in community gaps with high salinity.

All ungerminated seeds of the test species from different NaCl treatments (up to 1000 mM NaCl; twice as high as sea water salinity) showed high recovery of germination when transferred to distilled water, with comparable recovery value in two seed morphs of *A. macrostachyum* and higher/faster recovery in large compared to small seeds of *A. indicum*. Dimorphic seeds of *Atriplex canescens* (Bhatt and Santo 2016) and *Suaeda aralocaspica* (Wang et al. 2008) also showed recovery of germination from various salinity treatments, with higher values for brown than the black seeds. Similarly, ungerminated seeds of another marsh halophyte *Halopeplis perfoliata* also displayed high recovery from as high as 2000 mM NaCl (Rasool et al. 2017). These data about high recovery hint that the germination inhibition under saline condition was an 'enforced dormancy' (Sensu Harper 1959; 1977) condition probably resulting from the osmotic constraint rather than ionic toxicity of salinity. Our results also indicate that seeds of both *A. macrostachyum* and *A. indicum* have the ability to remain viable under high salinity of the natural habitat and will germinate once the salinity decreases by rainfall. However, there is a need to investigate differences, if any, in the persistence of dimorphic seeds of test species under natural conditions.

Heteromorphic seeds of both *A. macrostachyum* and *A. indicum* showed an absolute light requirement (i.e. positive photoblastic response) for germination and completely failed to germinate under dark. Germination of *Murdannia nudiflora* seeds also seized completely under dark (Ahmed et al. 2015). Similarly, seeds of *Viscum album*, *V. cruciatum* (Kuijt and Hansen 2015) and *Eragrostis ciliaris* (Khan et al. 2017) also displayed an absolute light requirement for germinating. This information on heteromorphic seeds of halophytes is generally confined to few studies. For instance, black but not brown seeds of *Suaeda corniculata* required light for germination (Cao et al. 2012). Presence of light enhanced germination of black (both unstratified and stratified) but not of brown (stratified) seeds of *Atriplex patula* (Nurse et al. 2008). Similarly, germination of heteromorphic seeds of *Suaeda acuminata* was inhibited substantially by dark (Wang et al. 2012). Ungerminated seeds of both *A. macrostachyum* and *A. indicum* from dark treatment also showed high recovery of germination when transferred to 12 h photoperiod, which is also an indicative of their positive photoblastic nature, as discussed above. Information about recovery from dark is very scarce. Recently, Rasool et al. (2017) reported that ungerminated seeds of another salt marsh halophyte *H. perfoliata* from dark also show high recovery when exposed to 12 h photoperiod. Often such acute light requirement is linked to small seed size, which is probably needed to prevent germination of small seeds under deep burial in soil because small seeds possess less food reserves to support seedling emergence from deeper soil layers (Khan and Gul 2006; Flores et al. 2011; Xia et al. 2016). 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. Hence germination inhibition under dark might be an adaptation of the seeds of the test species to prevent germination when buried deep in the marsh sediments and recovery of germination upon exposure to light might allow seeds buried in marsh sediments to germinate whenever drawn to surface due to sediment movement or erosion.

5. Conclusions

Arthrocnemum macrostachyum and *A. indicum* produce heteromorphic seeds, which differ in color and size, respectively. Seeds of two species lacked primary/innate dormancy that may result in prompt germination to take advantage of brief monsoon rainfall. High temperature (25/35 °C) was most inhibitory for germination that would help to prevent germination in early dry summer. Dark (burial) resulted in "enforced dormancy" (Sensu Harper 1959; 1977) probably to restrict seed germination only near the soil surface. Exposure to salinity also caused germination inhibition by "enforced dormancy" in the seeds probably via osmotic constraint. However, seeds of *A. macrostachyum* showed higher salinity tolerance limit than those of *A. indicum* that coincides well with broader distribution of *A. macrostachyum* compared to *A. indicum*. Our data also hints at greater contribution of black than brown seeds of *A. macrostachyum* to seedling establishment after occasional pre-monsoon showers which dilute sediment salinity while temperatures are still high. Whereas in case of *A. indicum,* large seeds could help in occupying community gaps with low/no salinity, while small seeds could contribute towards dispersal and in occupying community gaps with high salinity.

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