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SEED GERMINATION ECOLOGY OF *CYPERUS ARENARIUS* – A SAND BINDER FROM KARACHI COAST

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Abstract

Cyperus arenarius Retz. (Cyperaceae) is a low stoloniferous perennial of coastal sand dunes. We examined the effects of salinity (0, 100, 200, 300, 400 and 600 mM NaCl), temperature (10/20, 15/25, 20/30 and 25/35 °C) and light (12/12 h dark/light and 24 h dark) on seed germination of *C. arenarius*. Freshly collected seeds showed highest germination in distilled water and 100 mM NaCl at 25/35 °C. However, germination in 100 mM NaCl solution was about 25% slower than in distilled water. About 50% germination inhibition was observed at 200 mM NaCl and no seed germinated above 300 mM NaCl. Under optimal conditions, germination in complete darkness was about half of the 12-h photoperiod. About 50% of the un-germinated seeds from 400 and 600 mM NaCl recovered when transferred to distilled water (enforced dormancy), 35% remained un-germinated but viable (induced dormancy), while 15% were dead. Seeds from non-saline and complete dark treatment showed dark-induced dormancy (about 40%) whereas, seeds from saline conditions had about 20-25% higher salt-enforced dormancy than the 12 h photoperiod. Ecological significance of the effects of salinity, temperature, light and their interactions on seed germination and recovery of *C. arenarius* is discussed.

Introduction

Coastal sand dunes are formed by the gradual accumulation of oceanic sediments from the erosion of cliffs and bluffs (Maun, 2009). Coastal dunes are fragile (Gallego-Fernández *et al.*, 2011), ecologically interesting transitional environments linking terrestrial and marine ecosystems (Carboni *et al.*, 2009). This is particularly true for coastal dunes of subtropics such as those in southern Pakistan where halophytic grasses and sedges are an effective natural means of coastal sand dune stabilization (Khan *et al.*, 1999; Aziz *et al.*, 2005; Gallego-Fernández *et al.*, 2011).

Cyperus arenarius Retz., a stoloniferous perennial in the family Cyperaceae is widely distributed along the coastal sand dunes of Pakistan and is a good candidate for dune stabilization. It propagates rapidly after light monsoon showers and produces seeds twice a year. However, recruitment from seeds is rare (personal observations). Freshly collected seeds of most dicotyledonous and monocotyledonous sub-tropical halophytes are non-dormant (Khan & Gul, 2006) but many of these tend to become dormant and remain viable for a few months to several years after they enter the seed bank. Seeds preserved in these heterogeneous sand dune environments may confer an evolutionary advantage as a source of genetic variability (Dong & Alaten, 1999; Khan & Gul, 2006). Leck & Schütz (2005) reported persistent seed banks in members of the family Cyperaceae. This study seeks to address the following questions: 1) Do *C. arenarius* seeds exhibit innate dormancy? 2) How salt tolerant are the seeds at germination stage? 3) Does high salinity induce seed dormancy? 4) Do interactions of temperature, salinity and light influence seed germination? and 5) Is dormancy enforced by salinity reversible?

Materials and methods

Seed collection: Mature inflorescence of *Cyperus arenarius* Retz., were collected from coastal sand dunes of Sandspit, Karachi, Pakistan. Seeds were separated from inflorescence, cleaned and surface sterilized by using 1% sodium hypochlorite for 1 minute followed by thorough rinsing with distilled water and air drying.

Germination experiments: Germination was carried out in tight fitting plastic Petri-plates (5 cm Φ) using 7 ml of test solution. Germination experiment was conducted in programmed incubators (Percival Scientific, Boone, Iowa, USA) with alternating temperature regimes of 10/20, 15/25, 20/30 and 25/35°C, where low temperatures coincided with 12- hours dark and higher temperatures with 12- hours light period (25 µmol m^{-2} s^{-1}; 400-700 nm, Philips cool-white fluorescent tubes). Four NaCl concentrations (0, 100, 200 and 300 mM) were used. There were four replicates of 25 seeds each for each treatment. Germination (protrusion of embryonic axis) was recorded every alternate day for 20 days. Rate of germination was calculated according to Khan & Ungar (1984). Seed germination was also carried out in Petri-plates which were kept in dark-plastic bags separately to study the effect of dark on germination and salt tolerance at different temperature regimes. Seed germination was noted once after 20 d.

Recovery experiments: Two experiments were carried out by using different NaCl concentrations (0, 200, 400 and 600 mM) at 25/35°C (Optimum) under 12-hours photoperiod and in 24-hours dark conditions separately. After 20 d un-germinated seeds in both experiments were transferred to distilled water in 12 h photoperiod to study recovery of germination from salt and dark stress for 10 d.

Statistical analyses: Three-way analysis of variance (ANOVA) was conducted to determine if the experimental factors affect seed germination significantly. Post-hoc testing of ANOVA was conducted by using Bonferroni test ($p<0.05$). Statistical analyses were carried out with the help of SPSS version 11 (SPSS Inc.).

Results

Seed germination: A three-way ANOVA indicated that all the environmental factors tested and their interactions had a significant effect on seed germination of *C. arenarius* (Table 1). Highest germination was observed in distilled water and increases in NaCl concentration decreased germination at all temperature regimes, except 100 mM at 25/35°C (Fig. 1). Warmer temperature regimes were better for the germination of test species with the highest germination percentages at 25/35°C while the lowest seed germination occurred at 10/20°C. At the optimal temperature regime (25/35°C), 100% seed germination occurred within 10 d in distilled water with about 5% germination at 300 mM NaCl. Rate of germination showed a trend similar to percent germination except for a transient decrease in 100 mM NaCl at 25/35 °C (Fig. 2). Germination was substantially higher under 12 h photoperiod at all temperature regimes and NaCl concentrations than under 24-hours darkness (Fig. 1).

Recovery of germination: About 50% of the ungerminated seeds from salt treatments (200, 400 and 600 mM NaCl) in light germinated indicating a salt-enforced dormancy (Fig. 3). At high salt concentrations (400 and 600 mM NaCl) about 35% of the seeds were viable but did not germinate in distilled water while about 15% seeds died. The un-germinated seeds from 24-hours dark and NaCl (Dark + Salt) treatments showed higher recovery when transferred to distilled water under 12 h photoperiod conditions (Fig. 4). Relatively lower induced dormancy was observed in un-germinated dark-treated seeds from high salt (400 and 600 mM NaCl) treatments. The dark-treated un-germinated seeds from non-saline conditions (distilled water) showed relatively higher (about40%) induced dormancy than those from saline conditions under 12-hours darkness.

Table 1. Three-way ANOVA indicating the effects of salinity (SAL), temperature (T) and photoperiod (P) on final germination of *Cyperus arenarius* seeds.

Factor	*F* – value	*P*
SAL	9.94	<0.001
T	85.26	<0.001
P	5.41	<0.050
SAL * T	29.87	<0.001
SAL * P	98.74	<0.001
T * L	260.80	<0.001
SAL * T * L	13.85	<0.001

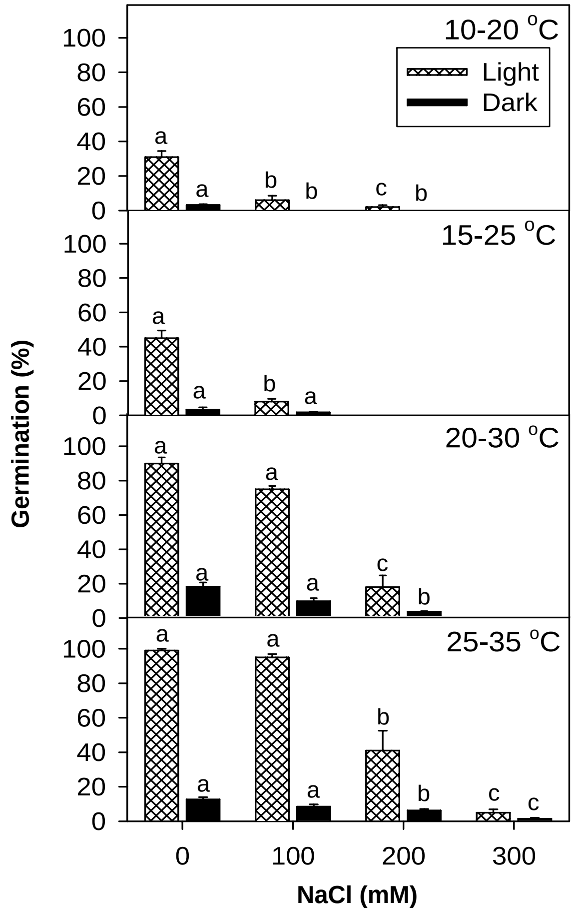

Fig. 1. Effect of light, salinity and temperature on the seed germination of *Cyperus arenarius*. Similar letters over bars represent mean germination percentages which are not significantly different ($p<0.05$) from each other at each NaCl concentration, (Bonferroni test.)

Discussion

Seeds of *Cyperus arenarius* readily germinated under optimal temperature, light and salinity regimes indicating the absence of innate dormancy. Our data is similar to a report in which about 80% seed germination was reported in *C. conglomeratus* under optimal conditions (El-Keblawy *et al.*, 2011). Salinity is reported to Inhibit seed germination of both crop plants (Anwar *et al.*, 2011; Arshad *et al*., 2012) as well as halophytes (Gul *et al.*, 2010; Saeed *et al.*, 2011). However, a number of sub-tropical halophytes appear to tolerate up to 500 mM NaCl and undergo salinity enforced seed dormancy possibly as a strategy to exploit the brief period of moisture available during monsoon showers (Khan & Gulzar, 2003; Khan & Gul, 2006). Only few seeds of the test species could germinate at 300 mM NaCl under optimum temperature and light conditions and it is comparatively more salt-sensitive than most of the other coastal halophytes of Pakistan (Khan & Gul, 2002). *Pancratium maritimum*, a monocot in the family Amaryllidaceae showed similar final germination percentage at 0 and 0.4% NaCl (Balestri & Cinelli, 2004). While only 10% seeds of *Cyperus conglmeratus* germinated at 100 mM NaCl (El-Keblawy *et al.*, 2011). Increases in salt concentration also inhibited germination in *Cyperus capitatus* seeds and no germination observed above 1% NaCl (Redondo-Gómez *et al.*, 2011). *Halopyrum mucronatum*, a coastal dune species in the family Poaceae is little affected by 100 mM NaCl at germination stage compared to non-saline control at 25/35°C. However, subsequent increases in salinity substantially inhibited its seed germination by 25% and 10% NaCl, at 200 mM and 300 mM NaCl, respectively.

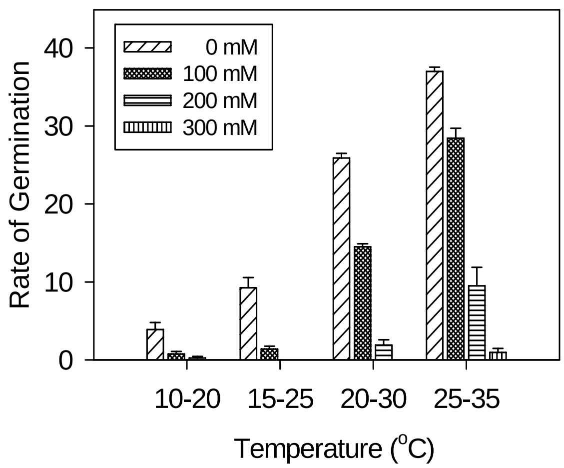

Fig. 2. Rate of germination of *Cyperus arenarius* in response to salinity (0, 100, 200 and 300 mM NaCl) and temperature (10-20, 15-25, 20-30 and 25-35°C) regimes.

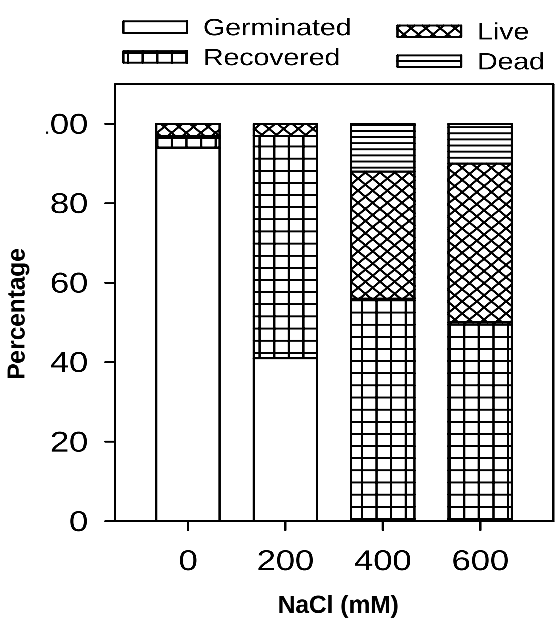

Fig. 3. Percentage of germinated, recovered, alive and dead seeds of *Cyperus arenarius* in response to salinity (0, 200 400 and 600 mM NaCl) at 25/35°C temperature regime in 12-h photoperiod.

Recovery of seed germination upon alleviation of salt stress is reported for a number of halophyte species (Shen *et al.,* 2003; Khan & Gul, 2006). The recovery responses of monocotyledonous species from saline conditions appear to be as variable as their germination responses.

Increases in salinity results in a progressive decrease in seed germination of most halophytes but the detrimental effects are less severe at optimum temperature (Khan *et al.*, 2000; Khan & Gulzar, 2003; Al-Khateeb, 2006; Khan & Gul, 2006). *Desmostachia bipinnata* seeds appeared to germinate better at 25/35°C than at lower temperature regimes (Gulzar *et al.*, 2007), that is similar to our test species, while most co-occurring species preferred moderate temperature regime of 20/30°C (Khan & Gulzar, 2003; Khan & Gul, 2006). Germination preference for higher temperatures in the dune environment could be due to niche separation to avoid competition with other species along the coast which occupied more stable soils with finer texture.

Light requirement of halophytes is quite variable among species (Baskin & Baskin 1998). It prevents germination when seeds are either buried in soil or shaded by dense vegetation or litter (Schütz & Rave, 1999; Hroudova´ & Zakravski, 2003). Baskin and Baskin (1998) reviewed that seed germination in members of Cyperaceae was higher in light than in dark, with 18 of 43 species germinating only in light. In this study, dark substantially inhibited the seed germination in comparison to 12 h photoperiod, irrespective of salinity and temperature treatments. This dark-induced germination inhibition appears a strategy to avoid germination in deep layers of the soil due to burial, which could be fatal for emerging seedlings.

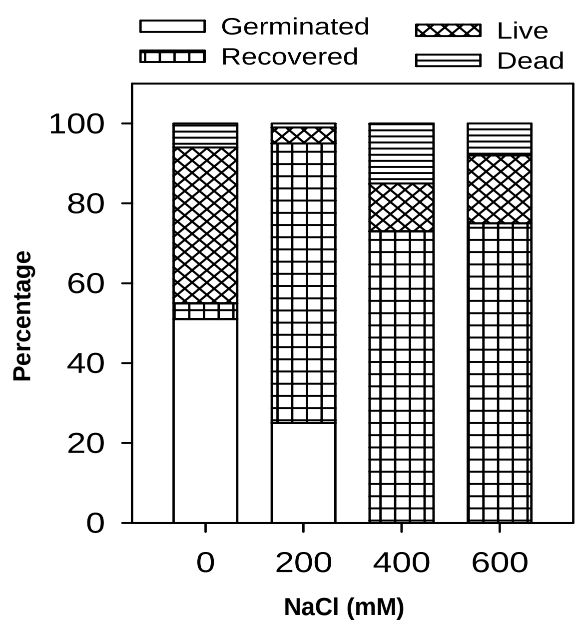

Fig. 4. Percentage of germinated, recovered, alive and dead seeds of *Cyperus arenarius*, in response to salinity (0, 200 400 and 600 mM NaCl) at 25/35°C temperature regime in complete dark.

For instance, *Aeluropus lagopoides* (Gulzar & Khan, 2001) and *Urochondra setulosa* (Gulzar *et al.,* 2001) showed a high (85%) recovery at 600 mM NaCl at their optimum temperature regime, whereas *Sporobolus ioclados* (Khan & Gulzar, 2003) recovered poorly due to innate dormancy. Germination responses of *C. arenarius* appear to fall between the above two categories. About 50% recovery after removal of salt stress under optimum temperature and photoperiod regimes indicates enforced seed dormancy in *C. arenarius*, due to osmotic stress. Whereas, about 35% of the un-germinated seeds from high salt treatments were viable (induced dormancy) and 15% were dead. Complete darkness and non-saline conditions caused induced dormancy in about 40% seeds which did not germinate after transfer to 12/12h photoperiod regime. Un-germinated seeds from highly saline conditions (400 and 600 mM NaCl) and complete darkness showed 20-25% more enforced dormancy and a similar reduction in induced dormancy in comparison with seeds from the 12 h photoperiod regime.

Conclusion

Cyperus arenarius is sensitive to saline conditions, particularly in the absence of light. However, it can tolerate high temperatures and can recover from up to seawater salinity under optimal temperature and light regimes. Seed germination appears to be tightly regulated by interactive effects of the major environmental factors. Similar responses of other sand dune species in the genus *Cyperus* (Thullen & Keeley, 1979; El Keblawy *et al.*, 2011) as well as other monocotyledonous halophytes of coastal sand dunes can be considered as indicators of a common adaptive response for long term survival in stressful conditions.

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