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Light, salinity, and temperature effects on the seed germination of perennial grasses[1]

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The germination requirements of four perennial halophytic grasses, *Aeluropus lagopoides*, *Halopyrum mucronatum*, *Sporobolus ioclados*, and *Urochondra setulosa*, were studied under control conditions in the laboratory. Treatments included two light levels (12 : 12 h light : dark period and 24-h dark environment), six salinity concentrations (0, 100, 200, 300, 400, and 500 mmol/L NaCl), and four temperature regimes (fluctuating day : night temperature regimes of 10° : 20°, 15° : 25°, 20° : 30° and 25° : 35°C), using a completely randomized block design. Best seed germination of all grasses was obtained in a distilled water control. Increase in salinity progressively inhibited germination of all species. For example, few seeds of *H. mucronatum* germinated above 300 mmol/L NaCl, while seeds of the other grasses germinated in up to 500 mmol/L NaCl. Optimal temperature regime for germination for all species was 20° : 30°C both for light- and dark-germinated seeds. At higher temperatures differences between light and dark treatments were not significant. Absence of light had no effect on the seed germination of *U. setulosa* and *H. mucronatum*; however, germination was lower in all salinity treatments. In the case of *A. lagopoides*, absence of light substantially inhibited the germination both in control and saline conditions. The light effect was marked in the case of *S. ioclados*, which showed very low germination in the absence of light both under saline and nonsaline conditions.

Key words: *Aeluropus lagopoides*; germination of halophytes; *Halopyrum mucronatum*; Karachi, Pakistan; light, salinity, and temperature; *Sporobolus ioclados*; *Urochondra setulosa*.

Halophytic perennial grasses (18) and annual grasses (5) are reported from the Pakistani coast (Khan and Gul, 2002); however, the region is dominated by four perennial grasses i.e., *Aeluropus lagopoides*, *Halopyrum mucronatum*, *Sporobolus ioclados*, and *Urochondra setulosa*. The vegetation on the Karachi coast, the study area, is distributed along an inundation gradient in which *Avicennia marina* (Forssk.) Vierh dominates the seafront, followed by *Arthrocnemum macrostachyum* (Moric.) C. Koch, which receives daily inundation. The next zone with monthly inundation is occupied by monospecific stands of perennial grasses (Gul and Khan, 1998). All four species reproduce by vegetative propagation from rhizomes and through seedling establishment. While maintenance and growth of monospecific stands are mainly by vegetative propagation, the establishment of the species in new sites may depend on seed dispersal, germination, and establishment of seedlings (Gulzar, 2002). Successful germination and establishment of the species are dependent on annual monsoon rains during July and August (Gul and Khan, 1998). The environmental conditions experienced by seeds germinating on these surfaces include coverage by sand drift (dark environment), warm temperatures, and drought (Khan and Ungar, 1997).

Best germination of halophytic grasses is obtained under nonsaline conditions and their germination decreases with increases in salinity (Gulzar, 2002). Germination of grasses is usually inhibited at concentrations ranging from 250 to 350 mmol/L NaCl (Lombardi, Fochetti, and Onnis, 1998), while *Spartina alterniflora* Lois. had up to 8% germination at 1027 mmol/L NaCl (Mooring, Cooper, and Seneca, 1971). Variation in light and temperature under saline conditions also affected the germination of halophytic grasses (Myers and Morgan, 1989; Lorenzen et al., 2000; Khan and Ungar, 2001). Absence of light almost completely inhibited seed germination of *Triglochin maritima* L. (Khan and Ungar, 1999) and *Sporobolus indicus* (L.) R. Br. (Andrews, 1997), partially inhibited germination in *Apium graveolens* L. (Garcia et al., 1995), *Allium staticiforme* Sibth. & Sm., *Brassica tournefortii* Gouan, *Cakile maritima* Scop., and *Onanthus maritimus* (L.) Hoffmanns & Link (Thanos et al., 1991); however, *Atriplex stocksii* Boiss (Khan and Rizvi, 1994) was not affected by the absence of light.

The present study reports the effects of salinity and temperature both under light and dark conditions on the germination of perennial halophytic grasses.

MATERIALS AND METHODS

Inflorescences of *Aeluropus lagopoides* (Linn.) Trin. Ex Thw., *Halopyrum mucronatum* (Linn.) Stapf, *Sporobolus ioclados* (Nees ex Trin.) Nees, and *Urochondra setulosa* (Trin.) C.E. Hubbard were collected from their populations at Sands pit along the Arabian sea coast, Karachi. Seeds were separated from inflorescences, cleaned, and dry stored at room temperature after surface sterilization with 0.85% sodium hypochlorite for 1 min. Six salinity concentrations (0, 100, 200, 300, 400, and 500 mmol/L NaCl) were used based on a preliminary test for salt tolerance of the species. Seeds were germinated in two folds of Whatman number 1 filter paper placed in 2.5 × 18 cm glass test tubes with 5 mL of test solution to prevent evaporation. The tubes were sealed using parafilm. Four replicates of 25 seeds each were used for each treatment. A seed was considered to have germinated at the emergence of the radicle (Bewley and Black, 1994). Germination was tested in a programmed incubator (Percival Scientific, Boone, Iowa, USA) at (dark : light) 10° : 20°, 15° : 25°, 20° : 30°, and 25° : 35°C temperature regimes with a 12-h photoperiod (25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 400–700 nm Sylvania cool-white fluorescent lamps [Danvers, Massachusetts, USA]). Similarly, seeds were incubated in photographic envelopes for the dark treatment under the same conditions. Germination was noted after 20 d. Germination data (20 d) were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS, version 9.0 (SPSS, 1999). The effects of light, salinity, and temperature on the germination and rate of germination were examined using

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TABLE 1. Results of four-way analysis of variance of characteristics by light (L), salinity (SAL), species (SP), and temperature (TEM). All values are significant at the $P < 0.0001$ level.

Source	Sum of squares	df	*F*
L	26028.4	1	382.0
SAL	380428.6	5	1116.5
SP	63279.7	3	309.6
TEM	48027.3	3	235.0
L × SAL	10637.2	5	31.2
L × SP	4662.1	3	22.8
SAL × SP	80356.1	15	78.6
SAL × TEM	14967.5	15	14.6
SP × TEM	21601.7	9	35.2
L × TEM	23738.6	3	116.2
L × SAL × SP	7302.1	15	7.1
L × SAL × TEM	6290.7	15	6.2
L × SP × TEM	7797.9	9	12.7
SAL × SP × TEM	15808.0	45	5.2
L × SAL × SP × TEM	17002.8	45	5.6

analysis of variance (ANOVA). A Bonferroni test was carried out to determine whether significant ($P < 0.05$) differences occurred between individual treatments (SPSS, 1999).

RESULTS

A four-way ANOVA indicated significant ($P < 0.0001$) individual effects of salinity, light, temperature, species, and their interactions on seed germination (Table 1).

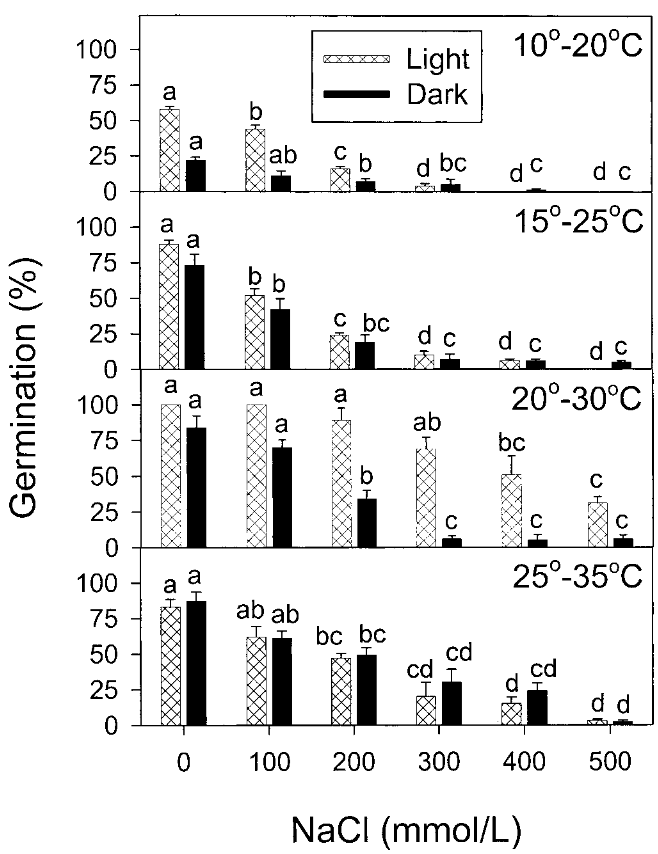

Fig. 1. Effect of light, salinity, and temperature on the germination of *Aeluropus lagopoides*. Bars represent means + 1 SE. Values at each NaCl concentration having the same letter are not significantly different ($P < 0.05$), Bonferroni test.

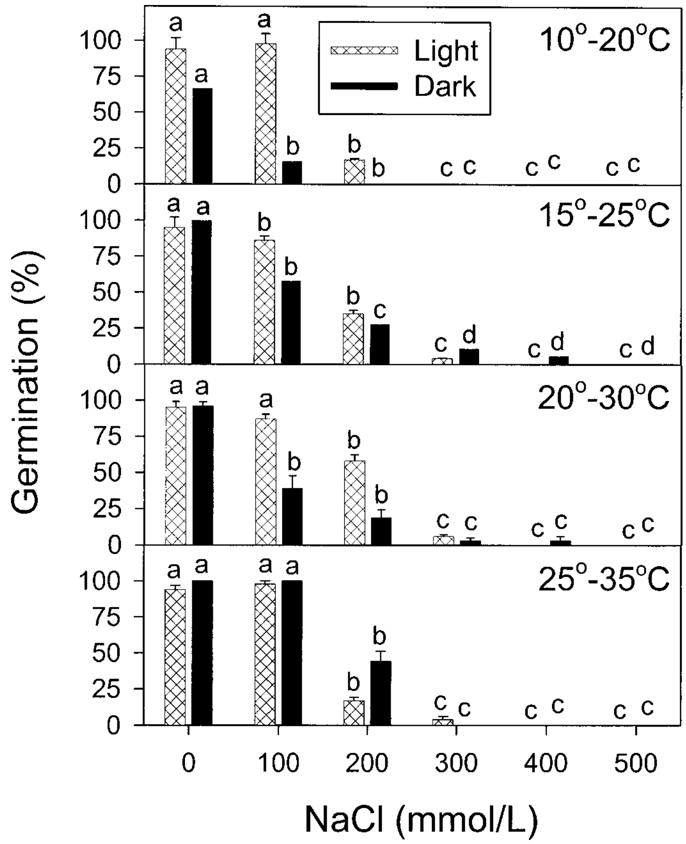

Fig. 2. Effect of light, salinity, and temperature on the germination of *Halopyrum mucronatum*. Bars represent means + 1 SE. Values at each NaCl concentration having the same letter are not significantly different ($P < 0.05$), Bonferroni test.

Seed germination in *Aeluropus lagopoides* was highest in distilled water in the 20° : 30°C temperature regime (Fig. 1). Thirty percent of the seeds germinated in 500 mmol/L NaCl at 20° : 30°C while no seed germinated at 10° : 20°C (Fig. 1). Germination was affected by temperature and was reduced from 100% at 20° : 30°C to only 60% at 10° : 20°C in the nonsaline control. Seeds showed higher germination in all treatments at 15° : 25°C and 20° : 30°C, with 100% germination in the nonsaline control; however, a further increase in temperature (25° : 35°C) inhibited germination. In darkness, germination was significantly inhibited at all salinity treatments at cooler thermoperiod (10° : 20°C) and was not affected by light regime at 15° : 25°C and 25° : 35°C; however, at optimal temperature regime (20° : 30°C), an increase in salinity progressively inhibited germination in dark (Fig. 1).

Germination of *Halopyrum mucronatum* seeds remained unaffected with change in thermoperiod under nonsaline conditions (Fig. 2). Germination was inhibited with increase in salinity, and few seeds germinated at concentrations higher than 300 mmol/L NaCl. This salinity-induced germination inhibition was greater at lower (10° : 20°C) and higher (25° : 35°C) temperature regimes. Absence of light had little effect in nonsaline controls except at 10° : 20°C, while under saline conditions it significantly inhibited germination under lower thermoperiod (10° : 20°C), and some inhibitory effects were noticed in yet other temperature regimes (Fig. 2).

Germination of *Sporobolus ioclados* seeds decreased with

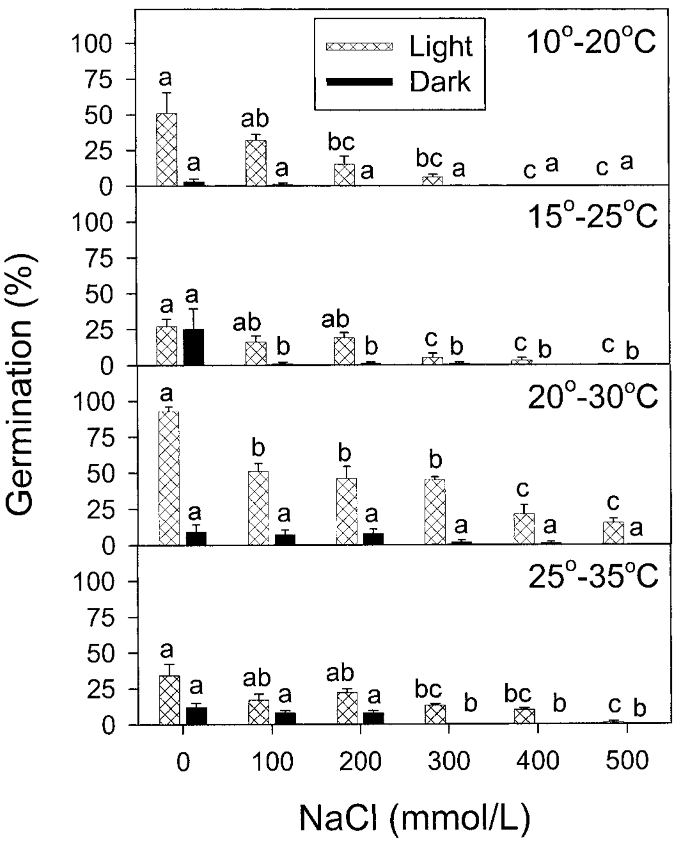

Fig. 3. Effect of light, salinity, and temperature on the germination of *Sporobolus ioclados*. Bars represent means + 1 SE. Values at each NaCl concentration having the same letter are not significantly different ($P < 0.05$), Bonferroni test.

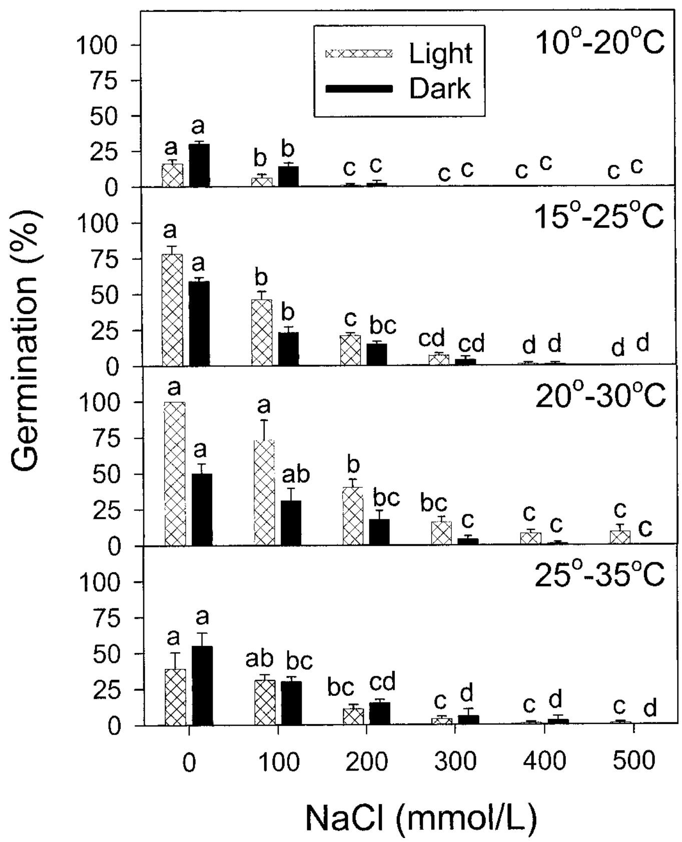

Fig. 4. Effect of light, salinity, and temperature on the germination of *Urochondra setulosa*. Bars represent means + 1 SE. Values at each NaCl concentration having the same letter are not significantly different ($P < 0.05$), Bonferroni test.

an increase in salinity (Fig. 3). Germination was greatly reduced in the 500 mmol/L NaCl treatment. Alternating temperature regimes of 20° : 30°C had maximum germination at all salinities (Figs. 1 and 2). Germination at other temperature regimes, i.e., 10° : 20°, 15° : 25°, and 25° : 35°C, had a similar pattern of germination at or above 300 mmol/L NaCl. Absence of light substantially inhibited germination under all thermoperiod and salinity regimes (Fig. 3).

Seed germination of *Urochondra setulosa* decreased with an increase in salinity and was maximal at 20° : 30°C temperature regime (Fig. 4), while both the highest and lowest temperatures inhibited germination. However, greater inhibition of germination occurred at the lowest temperature regime (10° : 20°C), where no seeds germinated at salinities above 200 mmol/L NaCl. Absence of light had no effect at higher and lower thermoperiods; however, at optimal temperature regimes dark germination was lower in all salinity treatments (Fig. 4).

DISCUSSION

The success of halophytic grasses under warm and dry ecosystems is primarily dependent on optimal conditions for germination and recruitment (Khan and Ungar, 2001). Seed germination of halophytic species is regulated by factors such as water, temperature, light, soil salinity, and their interactions (Noe and Zedler, 2000); however, each species responds to the abiotic environment in a unique manner. Halophytes vary greatly in their ability to tolerate salt, and this variation could be due to variation in the temperature regimes and availability of light (Khan and Ungar, 1997).

The test species differed greatly in their response to salinity, as quantified by the percentage germination. Best germination was obtained in distilled water controls in all grasses. Increase in salinity progressively inhibited germination, and few seeds of *H. mucronatum* germinated above 300 mmol/L NaCl. The seeds of other three grasses showed some germination even at 500 mmol/L NaCl. This high salt tolerance may be related to their zone of occurrence along the coast where they are regularly inundated by seawater or exposed to underground brackish water or seawater the year round. *Halopyrum mucronatum* on the other hand always occupies high ground on the sand dunes and is rarely exposed to high tides. Grasses usually are not very highly tolerant of salinity at germination (Khan and Ungar, 2001), and the maximum salt tolerance usually ranges between 250 and 350 mmol/L NaCl (Myers and Morgan, 1989; Lombardi, Fochetti and Onnis, 1998). *Spartina alterniflora* is an exception; it can germinate at concentrations higher than 400 mmol/L NaCl (Mooring, Cooper, and Seneca, 1971). It appears from our data that halophytic grasses from coastal areas of Karachi, Pakistan, are among the highly salt tolerant grasses at germination stage and have the ability to germinate in warm conditions. After the successful recruitment they establish themselves by utilizing the underground seawater or brackish water. *Sorobolus ioclados* and *U. setulosa*, however, require rainfall for the proper seed set. The distribution of *H. mucronatum* seeds on the sand dunes, where they are rarely inundated by seawater, can be attributed to the low salinity tolerance of the seeds during germination.

Halophytes vary in their response to light during germination. Some have an obligate requirement for germination, while in others presence of light enhances seed germination to varying degrees, and still others do not require light for germination (DeVilliers et al., 1994; Andrews, 1997; Khan and Ungar, 1997). Light requirements for germination were reported for 23 halophytic species by Baskin and Baskin (1998). Seeds of seven species required light for germination, five germinated to higher percentages in light than in darkness, two germinated equally well in light and darkness, eight germinated to higher percentages in darkness than in light, and one required darkness (Baskin and Baskin, 1998). All test species when germinated in the dark showed variable responses. Absence of light had some effect on the seed germination of *Urochondra setulosa* and *H. mucronatum*, but germination was inhibited in all salinity treatments. In the case *A. lagopoides*, absence of light substantially inhibited germination both in control and saline conditions. Light effect was marked in the case of *S. ioclados*, which showed little germination in the absence of light both under saline and nonsaline conditions. Light is an extremely important factor in releasing seed from dormancy (Bewley and Black, 1994).

It is expected that light-requiring seeds germinate at a time when habitat temperature stress and moisture stress are relatively low (Baskin and Baskin, 1998). Germination of coastal halophytic grasses from Pakistan is affected by variation in temperature both under saline and nonsaline conditions. The optimal temperature regime for the germination of grasses was 20° : 30°C studied both under light and dark environments. At higher temperatures differences between light and dark germinated seeds were not significant. The ability of seeds to germinate at increased levels of salinity was partly dependent on the test temperature (Khan and Ungar, 1999). In a number of halophytic grass species, including *Hordeum jubatum* (Badger and Ungar, 1989), *Iva annua* (Ungar and Hogan, 1970), *Puccinellia festucaeformis* (Onnis, 1981), *Diplachne fusca* (Myers and Morgan, 1989), and *Briza maxima* (Lombardi, Fochetti, and Onnis, 1998), germination percentages of the seeds incubated at high salinity levels increased at the optimal temperature and decreased when temperature was further increased or decreased. This optimal temperature for germination of halophytic grasses is similar to that of the ambient temperature after monsoon rains around Karachi, Pakistan. It seems that these grass species are well adapted to germinate under a seasonal temperature regime when availability of moisture and soil salinity levels are favorable for germination.

Burial in sand, temperature, and soil salinity play crucial roles during germination. It would be interesting to investigate the suitability of wave length and intensity of light during germination and whether they change with the change in temperature, and salinity or both. Present study points to some ecological strategies employed by seeds of sub-tropical coastal grasses to deal with high salinity and temperature stresses both under buried and exposed conditions.

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