

Seed Germination Strategies of *Desmostachya bipinnata*: A Fodder Crop for Saline Soils

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Abstract

Desmostachya bipinnata (L.) Stapf. (Poaceae), or drub, a perennial grass of near-coastal and inland deserts, is a potential fodder crop for either saline soils or where only brackish water is available for irrigation. The responses of *D. bipinnata* seeds under various salinity (0, 100, 200, 300, 400, and 500 mM NaCl), temperature (10°–20°, 15°–25°, 20°–30°, and 25°–35°C) and light (12:12-h dark:light and 24-h dark) regimes were investigated. All seeds germinated under nonsaline conditions, however, increase in salinity resulted in a progressive decrease in germination, and few seeds germinated at 500 mM NaCl. Change in temperature had little effect on seed germination under nonsaline conditions, however, seed germination was inhibited under cooler saline conditions. The germination under saline conditions improved at warmer temperature regimes. Seed germination under nonsaline control in dark was similar to those of seeds germinated in light. However, at high salinities, seed germination was substantially inhibited in dark in comparison to those germinated in light. When ungerminated seeds were transferred to distilled water they germinated immediately, and those from higher salinity and temperature regimes had higher recovery. The ability of seeds to germinate over a range of salinity and temperature regimes suggests possibilities for sustainable use of this species as a cash crop in saline soils.

Resumen

El *Desmostachya bipinnata* (L.) Stapf. (Poaceae), un zacate perenne que crece cerca de las costas y en el interior de los desiertos, es una especie con potencial forrajero tanto para suelos salinos como para áreas en las que solo se dispone de aguas residuales para irrigación. Se investigó la respuesta de semillas de *D. bipinnata* a varios regimenes de salinidad (0, 100, 200, 300, 400 y 500 mM NaCl), temperatura (10°–20°, 15°–25°, 20°–30° y 25°–35°C) y luz (12:12 h oscuridad: luz y 24 h de oscuridad). Todas las semillas germinaron bajo condiciones de no salinidad, pero, al incrementar la salinidad se redujo progresivamente la germinación y solo pocas semillas germinaron a 500 mM NaCl. Bajo condiciones de no salinidad, los cambios de temperatura tuvieron un efecto mínimo en la germinación de las semillas, sin embargo, la germinación se inhibió bajo condiciones de salinidad y bajas temperaturas. La germinación bajo condiciones salinas mejoró en los regimenes de temperaturas altas. La tasa de germinación bajo condiciones de no salinidad (control) fue igual en la oscuridad que en presencia de luz. Sin embargo, en altos niveles de salinidad, la germinación fue substancialmente inhibida en la oscuridad. Cuando las semillas sin germinar se transfirieron a agua destilada germinaron inmediatamente y las provenientes de altos niveles de salinidad y temperatura tuvieron una mayor recuperación. La capacidad de las semillas para germinar en un amplio rango de salinidad y temperatura sugiere la posibilidad de utilizar de esta especie como un cultivo generador de ingresos en suelos salinos.

Key Words: grass, halophyte, Karachi, light, recovery, temperature

INTRODUCTION

Perennial halophytic grasses constitute a valuable fodder and cash crop resource on saline and alkaline wastelands (Lieth et al. 1999; Dagar et al. 2004) besides ecological applications, such as landscaping or rehabilitation, of damaged ecosystems (Debez et al. 2004). Several salt-tolerant grasses are an easily available feed supplement, such as *Aeluropus lagopoides* (Linn.) Trin. Ex Thw. and *Sporobolus madraspatanus* Bor. in India and Pakistan (Joshi and Bhoite 1988; Gulzar 2002). These grasses could be used as fodder crops for saline areas or

where irrigation water is of poor quality. Direct-seeding propagation method is efficient and quick; therefore, better understanding of germination responses under saline conditions is essential for sustainable use of salt-tolerant grasses (Noe and Zedler 2000).

Halophytic grasses vary significantly in their response to salinity, which appears to influence their distribution along steep salinity gradients (Dodd and Donovan 1999; Khan and Gulzar 2003; Redondo et al. 2004). Generally, seed germination declines progressively with rising salinity levels (Mahmood and Malik 1996; Al-Khateeb 2006); seeds of some species show no effect at 100 mM NaCl (Gulzar 2002; Al-Khateeb 2006), whereas others showed no effect at 200 mM NaCl (Hester et al. 1998; Perez et al. 1998; Shen et al. 2003). Some grass species show salt stimulation at low salt concentrations e.g., 5 dS · m⁻¹ or about 50 mM NaCl (Mahmood and Malik 1996). Joshi et al. (2005) reported salt tolerance of up to 0.5% (107 mM NaCl) for *A. lagopoides* and *S. madraspatanus*, whereas none

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of their seeds germinated above that level. *Halopyrum mucronatum* (L.) Stapf. (Khan and Ungar 2001) and *Sporobolus virginicus* L. Kunth (Breen et al. 1997) could germinate in up to 350 mM NaCl, whereas some seeds of *Urochondra setulosa* (Trin.) C. E. Hubb., *A. lagopoides*, and *Sporobolus ioclados* Nees ex Trin. Nees, germinated at 500 mM NaCl (Gulzar and Khan 2001; Gulzar et al. 2001; Khan and Gulzar 2003).

Temperature is also an important factor affecting seed germination under saline and nonsaline conditions (Khan and Ungar 1997a) probably because of its effect on the structural integrity and activity of enzymes (Bewley and Black 1994). Seeds completely failed to germinate in the presence of moisture at low temperatures, whereas higher temperatures could also suppress seed germination (Gulzar and Khan 2001). The optimal temperature required for seed germination of most grasses as well as dicots from coastal and inland areas of Pakistan is 20°–30°C (Gulzar 2002; Khan and Gul 2006), which is similar to the prevailing temperature regime after the monsoon rains.

Halophytes show remarkable recovery of germination after exposure to hypersaline conditions (Keiffer and Ungar 1997; Shen et al. 2003) with values equal to those of nonsaline controls (Zia and Khan 2004) at optimal temperatures. Prolonged exposure to salinity could promote or inhibit seed germination and the recovery response may vary with the duration and intensity of exposure to salt stress in their natural habitat (Keiffer and Ungar 1997). Although in other cases, <10% of ungerminated seeds died due to salt toxicity (Khan and Gulzar 2003). Ability of seeds to recover from salt stress could be crucial for the successful establishment under optimal environmental conditions following reduction in salt levels by rainfall or flooding by irrigation water in agricultural systems (Shen et al. 2003). It could also provide multiple opportunities for cohort establishment in unpredictable saline environments (Khan and Ungar 1997a).

Light is known to regulate seed germination, and most local halophytic grasses showed lower germination in the absence of light, especially at higher salinities (Khan and Gulzar 2003). Seed burial in sand by wind in natural habitat or by tillage in cultivated fields would reduce light intensity, and measurable quantities of light do not penetrate depths greater than a few millimeters (Tester and Morris 1987). Light and salinity interact to reduce germination in a number of halophytes, such as *Allenrolfea occidentalis* (S. Wats.) Kuntze (Gul and Weber 1999), *Limonium stocksii* (Boiss.) O. Kuntze (Zia and Khan 2002), and in 4 desert shrubs and a forb (Khan and Ungar 1997b). Interaction of light with temperature is more inhibitory at constant temperature and in dark than diurnally fluctuating light and temperature conditions (Godoi and Takaki 2004).

D. bipinnata is a C₄ perennial grass, native to the Sudanian region, from Northern Africa (Morocco to Somalia), Sicily, and Cyprus through the Middle East to Central Asia, Pakistan, and India (Cope 1982), and has been introduced into Southeast Asia (Aronson 1989). The stem and root are used for Ayurvedic medicine in India. The plant occurs as a weed in agricultural areas and is considered to have salt tolerance of <5.6 dS · m⁻¹ (60 mM NaCl) on dilutions of Mediterranean Sea water (Aronson 1989). The plant propagates through its rhizome after monsoon rains and also produces numerous flowers and

seeds twice a year during June and October. Young leaves are browsed by cattle (Joshi and Bhoite 1988).

The present study tested the following hypotheses: 1) seeds of *D. bipinnata* germinate better under freshwater conditions, 2) seed germination occurs at NaCl concentrations higher than 400 mM, 3) changes in thermoperiod affect seed germination, 4) seeds germinate more (to a greater percentage) during photoperiods than during the dark, 5) seeds germinate rapidly under nonsaline conditions, 6) seeds exposed to high salinity germinate when the salinity stress is removed, and 7) changes in temperature regime affect seed viability under saline conditions.

MATERIALS AND METHODS

Spikelets of *D. bipinnata* were harvested from 3 different populations on the Karachi University campus during 2004 and 2006. Seeds were separated from the inflorescence, cleaned, and dry-stored at room temperature. Six salinity concentrations (0, 100, 200, 300, 400, and 500 mM NaCl) were used, based on preliminary test for salt tolerance of the species. Values for water potential (ψ_w) of corresponding NaCl solutions were measured with the help of the dew-point microvolt meter model HR-33T (Logan, Utah) on Whatman No. 2 filter paper disks dipped with the corresponding salt solution placed in a C-52 sample chamber at 35°C. Lang (1967) reported that ψ_w values may, however, vary a little with change in temperature. Germination was tested on freshly collected seeds in programmed incubators (Percival) at 10°–20°, 15°–25°, 20°–30°, and 25°–35°C temperatures at 2 photoperiod regimes: a 12:12 hour dark:light (25 $\mu\text{M} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 400–700 nm Sylvania cool-white fluorescent lamps) and a 24-hour dark photoperiod. The temperatures used were based on mean values from meteorological data recorded over the past 100 years. Twenty-five seeds each were placed in 4 airtight, sterilized, plastic petri plates with 5 mL of test solution. The resulting factorial experiment was 6 × 4 × 2 (salinity × thermoperiod × photoperiod) arranged in randomized-block design with 3 blocks (seed lots). Seeds were considered to have germinated at radicle emergence (Bewley and Black 1994), and germination was noted on alternate days. To evaluate rate of germination, mean germination time was calculated with the help of the Edmond and Drapala (1958) method denoted as mean days for emergence and expressed by

$$M_{\text{days}} = \frac{\sum N_i G_i}{\sum G_i} \quad [1]$$

where G_i is the number of seedlings emerged on the day of observation, and N_i is number of days between the day of sowing and the day of observation. After 20 days, all ungerminated seeds were placed in distilled water under the initial temperature regimes for another 20 days. The percentage recovery of ungerminated seeds (RU) was determined using the relation

$$RU = \frac{a-b}{c-b} \times 100 \quad [2]$$

Table 1. ANOVA of seed germination in 3 seed lots (Lot) of *Desmostachya bipinnata* by salinity (S), temperature (T), photoperiod (P), and their interactions.

Source	Sum of squares	Mean squares	df	F
Lot	356	178	2	2 ¹
S	112 204	22 441	5	229 ²
T	48 640	16 213	3	166 ²
P	20 176	20 176	1	206 ²
S × T	11 285	752	15	8 ²
S × P	5 087	1 017	5	10 ²
T × P	248	82	3	1 ¹
S × T × P	17 531	1 169	15	12 ³
Error	9 202	98	94	

¹Nonsignificant.

² $P < 0.0001$.

³ $P < 0.01$.

where *a* is total number of seeds germinated after being transferred to distilled water, *b* is the total number of seeds germinated in saline solution, and *c* is the total number of seeds. Rate of recovery was also calculated by the method of Edmond and Drapala (1958) using the percentage of recovery data. Germination and recovery germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance. Analysis of variance (ANOVA) was carried out to determine whether significant differences were present among means because of the individual and interactive effects of seed lot, salinity, temperature, and photoperiod (SPSS 2002). A post hoc Bonferroni test was used to determine whether significant differences were present among individual salinity treatments. Linear regression was plotted to compare the differences in germination (light and dark) and recovery responses in different temperature regimes (SPSS 2002).

RESULTS

The 3-way ANOVA indicated significant effects of salinity ($F_{5,94} = 229$, $P < 0.0001$), temperature ($F_{3,94} = 166$, $P < 0.0001$), photoperiod ($F_{1,94} = 206$, $P < 0.0001$), and their interactions on seed germination of *D. bipinnata* (Table 1). Seed germination was highest (96% at 25°–35°C) in the nonsaline control, and increases in salinity resulted in concomitant decreases in seed germination, with only 30% seeds germinating in 500 mM NaCl at the optimal temperature regime of 25°–35°C (Fig. 1). No seed germinated above 200 mM NaCl at 10°–20°C (Fig. 1). Seed germination was higher at all salinity concentrations at temperature regimes above 15°–25°C. The higher temperatures showed similar final germination percentages at most salinities except for 400 and 500 mM NaCl, where germination was about twice as much at 25°–35°C in comparison to 20°–30°C (Fig. 1).

Seed germination decreased substantially in both saline and nonsaline treatments in dark compared with germination in photoperiod (Table 1). Higher temperature regimes at higher salinity concentrations inhibited seed germination (Fig. 2).

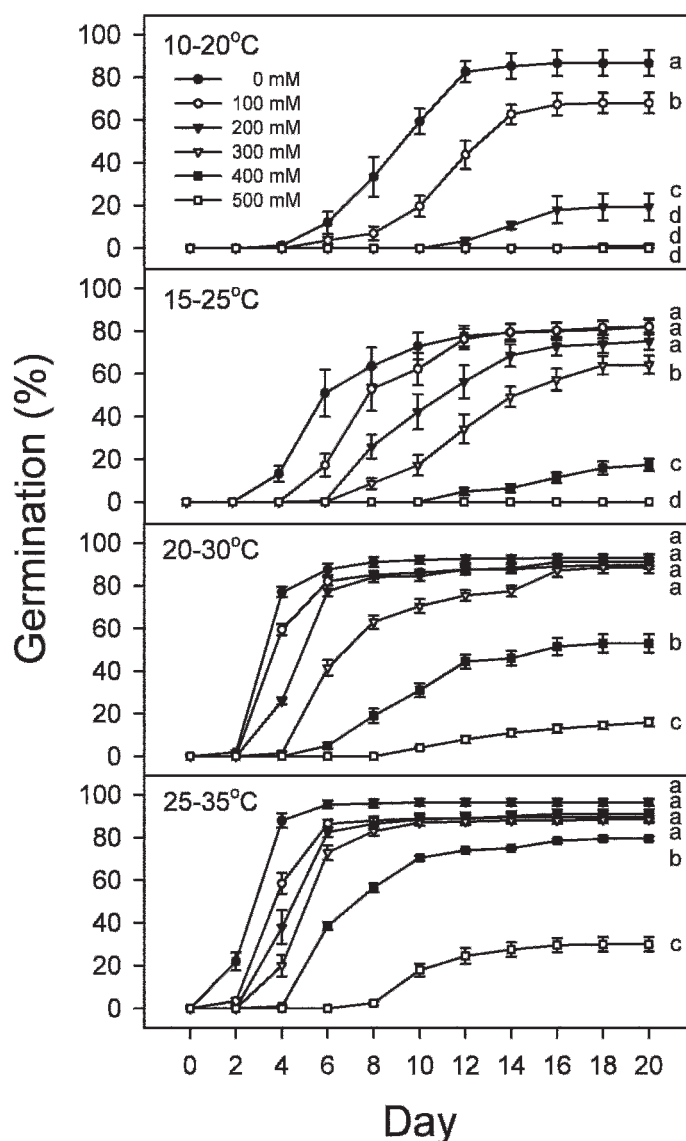


Figure 1. Percentage of seed germination of *Desmostachya bipinnata* in various salinity (0, 100, 200, 300, 400, and 500 mM NaCl) and temperature (10°–20°, 15°–25°, 20°–30°, and 25°–35°C) regimes. Data points represent mean (\pm SE; $n = 3$) of 3 replications for each temperature treatment with 4 subsamples of each salinity treatment.

When seeds were transferred to distilled water after 20 days of salinity treatment, the recovery germination percentages increased with increase in pretransfer salinity at all temperatures except for the lowest temperature regime of 10°–20°C (Fig. 3). Seeds subjected to higher temperatures had higher recovery percentages (Fig. 3), but at the lowest temperature (10°–20°C), recovery was lower (8% from 500 mM NaCl). Rate of germination (R_{Ger}) increased progressively with increasing salinity at all temperatures. Most seed germinated within 6 days of salinity treatment in up to 200 mM NaCl, whereas at higher salinities, seeds required about 14 days to achieve maximum seed germination (Fig. 1; Table 2). Rate of recovery germination (R_{Rec}) showed relatively higher values in seeds from the higher salt pretreatment concentrations and at lower temperatures (Table 2). Maximum recovery of about 80% was observed within 4 days of transfer from saline conditions.

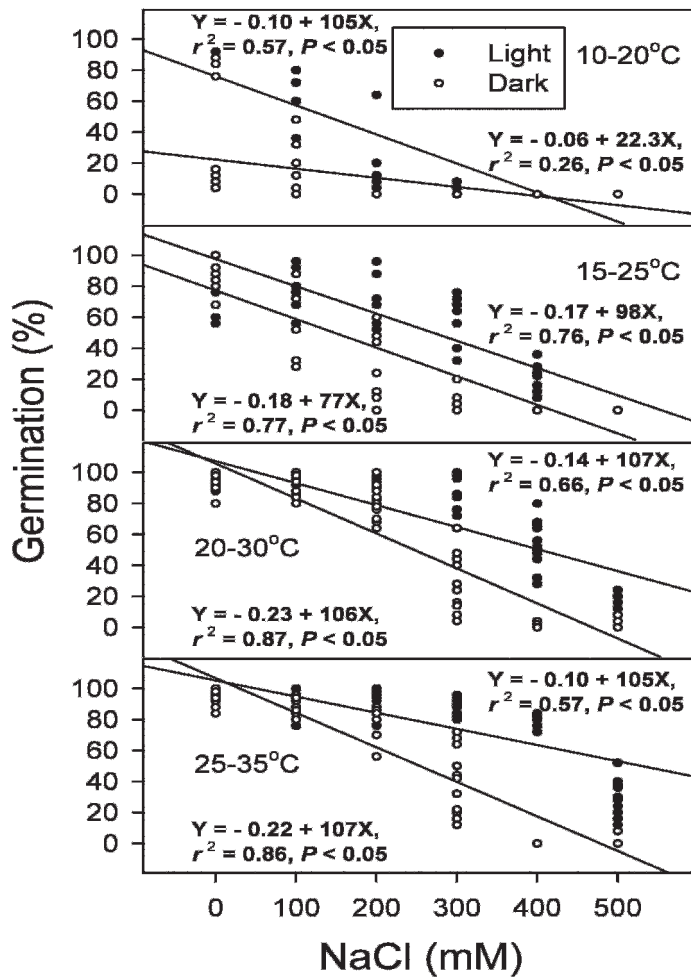


Figure 2. Linear regression for final germination percentages of *Desmostachya bipinnata* seeds after 20 days at various salinity (0, 100, 200, 300, 400, and 500 mM NaCl), temperature (10°–20°, 15°–25°, 20°–30°, and 25°–35°C), and photoperiod (12-h light: 12-h dark, and 24-h dark) regimes.

DISCUSSION

Seed germination is affected by many factors, such as temperature, light, and soil moisture (Zheng et al. 2004); however, the dominant abiotic factor affecting seed germination varies among species and partially accounts for the differences in timing of germination in the field (Noe and Zedler 2000). This study was designed to test 7 hypotheses, and we learned by testing those hypotheses that seeds of *D. bipinnata* germinate better under nonsaline conditions; however, some seeds could germinate at >400 mM NaCl. Temperature and light played a significant role in seed germination. Seeds maintained viability when exposed to high salinity and recovery was higher at higher-temperature regimes. Salinity, temperature, photoperiod, and their interactions significantly affected seed germination of *D. bipinnata*. Seeds appeared to have high salinity tolerance at higher temperatures in the presence of light.

Seeds of subtropical grasses are usually nondormant at maturity (Khan and Ungar 1999; Gulzar et al. 2001; Gulzar and Khan 2001) except for *S. ioclados* (Khan and Gulzar 2003), which shows <40% germination under nonsaline

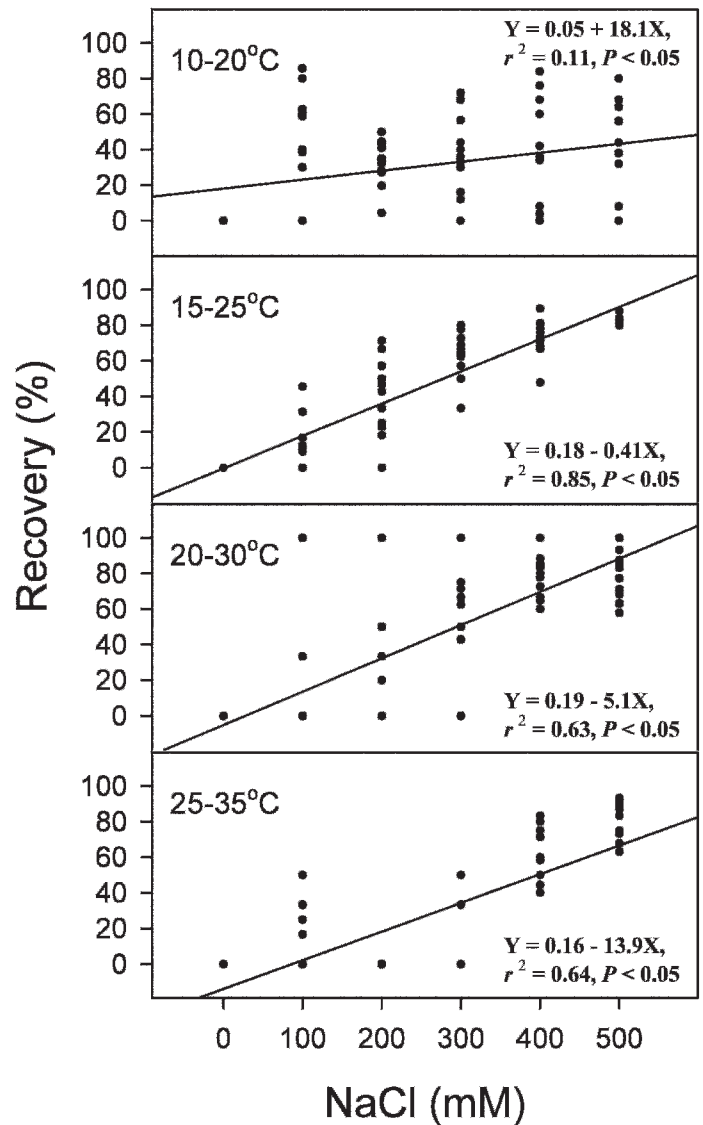


Figure 3. Linear regression for percentage of recovery germination for *Desmostachya bipinnata* seeds transferred to distilled water from various salinity (0, 100, 200, 300, 400, and 500 mM NaCl) and at the same temperature (10°–20°, 15°–25°, 20°–30°, and 25°–35°C) regimes.

conditions. Seeds of *D. bipinnata* were also nondormant, and almost all seeds germinated under optimal environmental conditions. Seed germination of halophytes tends to decrease with increase in salinity (Ungar 1995; Khan and Gul 2006) except for more salt-tolerant grasses, such as *Elytrigia elongata* (Host) Nevski., which had 60% germination at 445 mM NaCl and *Spartina alterniflora* Loisel., the most salt-tolerant grass reported, with salt tolerance of up to 6% (about 1027 mM) NaCl (Mooring et al. 1971). The threshold salinity for significant reduction in germination varies with grass species and mostly ranges between 100–500 mM NaCl and subtropical grasses appear to be quite salt-tolerant approaching seawater salinity (about 500 mM NaCl; Khan and Gulzar 2003). Inhibition or delay in germination under saline conditions is due to an osmotic effect (Khan and Ungar 1997a; Khan and Gulzar 2003), which limits the uptake of water during seed germination (Murillo-Amador et al. 2002) rather than ion toxicity (Hyder and Yasmin 1972) by hindering

Table 2. Rates of germination (R_{Ger}) and recovery (R_{Rec}) of *Desmostachya bipinnata* seeds in response to variable salinity (0, 100, 200, 300, 400, and 500 mM NaCl) and temperature (10°–20°, 15°–25°, 20°–30°, and 25°–35°C) regimes. Numbers in parentheses show water potential of each NaCl solution at 35°C. Letters for rates (mean \pm SE; $n = 3$) in columns are not significantly ($P < 0.05$) different from each other between different salinities, Bonferroni test.

NaCl (ψ_w) mM (–MPa)	10°–20°C		15°–25°C		20°–30°C		25°–35°C	
	R_{Ger}	R_{Rec}	R_{Ger}	R_{Rec}	R_{Ger}	R_{Rec}	R_{Ger}	R_{Rec}
0	9 a ± 0	0 a	8 a ± 1	0 a	5 a ± 2	0 a	4 a ± 0	0 a
100 (0.45)	12 b ± 0	6 b ± 1	10 b ± 1	2 b ± 1	5 a ± 0	1 b ± 1	5 b ± 1	1 b ± 1
200 (0.97)	14 c ± 0	5 b ± 0	12 b ± 2	4 c ± 0	6 a ± 1	1 b ± 1	6 b ± 0	0 a
300 (1.43)	0 d ± 1	3 c ± 1	13 b ± 1	4 c ± 0	9 b ± 0	4 c ± 1	6 b ± 0	1 b ± 0
400 (1.93)	0 d ± 1	9 d ± 1	16 c ± 1	4 c ± 0	11 c ± 0	4 c ± 0	8 c ± 0	4 c ± 0
500 (2.40)	0 d ± 1	9 d ± 1	0 d	4 c ± 0	14 d ± 1	4 c ± 0	11 d ± 1	4 c ± 0

activity of membrane or cytosolic enzymes and hormones (Bewley and Black 1994). However, both ionic and toxic effects may be involved to varying degrees in different taxa (Allen et al. 1986), and death is the cause of low seed germination in some species (Khan and Gulzar 2003). Mahmood and Malik (1996) found that the grasses *Cynodon dactylon* (L.) Pers., *D. bipinnata*, *Polypogon monspeliensis* (L.) Desf., and *Sporobolus arabicus* Boiss. showed a decrease in germination with increase in salinity from 3 to 20 dS \cdot m^{–1}. Only *D. bipinnata* exhibited salt stimulation at low salinity (5 dS \cdot m^{–1} or 52 mM NaCl) and had 40 % germination at 20 dS \cdot m^{–1} (206 mM NaCl) with no seed germinating beyond this level. Grasses like *P. coloratum* L. (Perez et al. 1998), *P. hemitimon* Schult. (Hester et al. 1998), *U. setulosa* (Gulzar et al. 2001), *A. lagopoides* (Gulzar and Khan 2001), and *S. ioclados* (Khan and Gulzar 2003) germinate well in up to 200 mM NaCl concentrations, but at higher salt concentrations, germination was substantially reduced. *H. mucronatum* also showed reduced germination above 200 mM NaCl, and seeds failed to germinate beyond 350 mM NaCl (Khan and Ungar 2001). In this study, *D. bipinnata* seeds had >80% germination at 200 mM NaCl.

Temperature has a major impact in regulating phenology and distribution of halophytes in arid and semiarid environments (Khan and Ungar 1997b; Khan and Gulzar 2003). Decreased germination at suboptimal temperatures may accelerate degradative metabolic processes (Bewley and Black 1994). Most subtropical grasses germinate best at 20°–30°C, and extreme thermoperiods inhibit seed germination and rate of germination of these species (Khan and Gulzar 2003). Seeds of *Panicum turgidum* Forssk. appeared to germinate better in the intermediate incubation temperatures of 15°–25° and 20°–30°C (Al-Khateeb 2006). Better germination as well as recovery was observed in the case of *D. bipinnata* at higher temperatures 25°–35°C would confer a competitive advantage over other co-occurring species either at the onset of, or soon after, monsoon

rains. Similar results were obtained for *Leptochloa chinensis* L. Nees., a weed of Italian rice fields (Benevenuti et al. 2004).

Seed germination response of halophytes to light is highly variable and may range from an obligate requirement to a slight stimulation or a slight inhibition, from no effect to complete germination inhibition (Baskin and Baskin 1998). This may be a mechanism of preventing germination when seeds are buried in the soil, covered by leaf litter or by a muddy layer, or flooded by a high water level (Schütz and Rave 1999; Hroudová and Zakravski 2003). Light is perceived by phytochrome (Borthwick et al. 1954); thus, germination onset or inhibition is a function of incident radiation capable of activating phytochrome that absorbs far red light (Pfr) or inactivating phytochrome that absorbs red light (Pr) (Benvenuti et al. 2004). Phytochrome-dependent light response is a frequent natural process within species that colonize open areas (Ballaré 1994). Lack of or lower germination during burial would allow seeds to escape fatal germination (Mapes et al. 1989) as in *L. chinensis* (Benvenuti et al. 2004). Absence of light did not affect germination of *U. setulosa* and *H. mucronatum*, slightly stimulated *A. lagopoides*, and greatly inhibited seed germination in *S. ioclados* and *S. arabicus* (Khan and Ungar 2001; Khan and Gulzar 2003). Light promoted seed germination of *D. bipinnata* at salinity treatments greater than 200 mM NaCl in the higher thermoperiod regimes, whereas at lower thermoperiod and salinity regimes, fewer differences were observed between the photoperiod and dark germinated seeds.

Halophytes show variable recovery of germination responses after exposure to high salinity and temperature stress (Shen et al. 2003). This variation in recovery responses could be helpful for survival of plants following salt stress. *A. lagopoides* (Gulzar and Khan 2001) and *U. setulosa* (Gulzar et al. 2001) showed a high (85%) recovery at 600 mM NaCl at 20°–30°C, whereas *S. ioclados* (Khan and Gulzar 2003) showed overall poor recovery response. Macke and Ungar (1971) found 87% recovery in seeds of *Puccinellia nuttalliana* (Schult.) Hitchc. in distilled water in comparison to 5.2% after 45 days of exposure to 2% NaCl. Percentage and rate of germination of *Hordeum jubatum* L. decreased after extended exposure to hypersaline conditions, and no recovery was obtained after 1 year in 10% NaCl (Keiffer and Ungar 1997). Most seeds of *D. bipinnata* recovered after 20 days exposure to 500 mM NaCl at 25°–35°C, which indicated an osmotic, rather than a toxic, effect of salinity. Recovery was also high at the higher thermoperiod as opposed to better recovery at 20°–30°C, the moderate thermoperiod regime for other co-occurring grasses.

MANAGEMENT IMPLICATIONS

D. bipinnata occurs in habitats ranging from riverbeds to moist salt flats under warm temperatures. This grass has relatively high protein content and could be a good fodder. Young plants are grazed; however, older plants with hard fibers injure the mouth of the animals. Our field trials indicate that this species could be used as a dry fodder and grown by using brackish water (up to 10 dS \cdot m^{–2} or about 100 mM NaCl) irrigation. Dry fodder, which is about 85% of the total food consumed by livestock, contributes significantly in developing livestock in areas where freshwater supply is limited. Production, nutrition

levels or grazing value, productivity, quality, and palatability of *D. bipinnata* are being investigated.

The high temperature and salinity tolerance of our test species would support late sowing in the field; however, because germination is only the first phase in establishment and growth, it is difficult to make inferences about irrigation with saline water and subsequent growth and productivity. Further research on *D. bipinnata* could confirm these traits and provide useful information in better using this grass as a fodder plant.

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