Effect of light, salinity, and temperature on seed germination of *Limonium stocksii*

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Abstract: *Limonium stocksii* (Boiss.) Kuntze (Plumbaginaceae) is a perennial, woody shrub distributed at Hawks Bay, Karachi, Pakistan. Experiments were carried out to investigate seed germination responses of *L. stocksii* at different salinities (0, 100, 200, 300, 400, and 500 mmol/L NaCl) and under different temperature regimes (10:20, 15:25, 20:30, and 25:35 °C), both in a 12 h dark : 12 h light photoperiod and in complete darkness. The highest percentage of germination (about 100%) was obtained at 0, 100, and 200 mmol/L NaCl at 20:30 °C, and a further increase in salinity resulted in a gradual decrease in germination. Less than 5% of seeds germinated at 500 mmol/L NaCl. Germination under salinity treatment at 15:25 °C was slightly more inhibitory than the optimal temperature regime, whereas under both 10:20 and 25:35 °C temperature regimes, seed germination was substantially reduced and few seeds germinated at concentrations higher than 200 mmol/L NaCl. Germination rate was fastest at 20:30 °C and slowest at 10:20 °C. Relatively low seed germination was obtained in the dark in comparison to seeds germinated in a 12-h photoperiod under saline conditions. Recovery experiments showed that exposure of seeds to various salinity and temperature regimes had little effect on viability of seeds.

Key words: germination, light, *Limonium stocksii*, NaCl, recovery, temperature.

Résumé : Le *Limonium stocksii* (Boiss.) Kuntze (Plumbaginaceae), est une plante pérenne ligneuse arbustive, qui se retrouve à Hawks Bay, près de Karachi, au Pakistan. Les auteurs ont conduit des essais pour étudier le comportement de la germination des graines du *L. stocksii* en présence de différentes concentrations de sel (NaCl 0, 100, 200, 300, 400 et 500 mmol/L), sous différents régimes de température (10:20, 15:25, 20:30 et 25:35 °C), et sous une photopériode de 12 h d'obscurité : 12 h de lumière, ainsi qu'en obscurité totale. On observe la plus forte germination (près de 100 %) en présence de NaCl 0, 100 et 200 mmol/L, et à 20:30 °C; une augmentation plus poussée de la salinité conduit à une diminution graduelle de la germination, seulement 5 % des graines germant en présence NaCl 500 mmol/L. La germination en présence de sel et à 15:25 °C est légèrement plus faible qu'au régime de températures optimum, alors qu'à 10:20 et 25:35 °C, la germination des graines est considérablement réduite, seulement quelques graines germant aux concentrations en NaCl supérieures à 200 mmol/L. Les taux de germination sont les plus rapides à 20:30 °C et les plus lents à 10:20 °C. Dans l'obscurité, la germination des graines est relativement plus faible comparativement à celle qu'on obtient avec une photopériode de 12 h, sous des conditions salines. Des essais de récupération montrent que l'exposition des graines à divers régimes de salinité et de températures a peu d'effet sur la viabilité des graines.

Mots clés : germination, lumière, *Limonium stocksii*, NaCl, récupération, température.

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Introduction

Halophytes are distributed in coastal and inland saline habitats throughout the world (Adam 1990; Ungar 1991), and their populations are subjected to high mortality risks because of the direct action of high-salinity stress or other associated abiotic factors (Ungar 1991). Seeds of halophytes usually show optimal germination in freshwater similar to glycophytes, but differ in their ability to germinate at higher salinities (Ungar 1995).

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Perennial halophytes vary in their ability to tolerate salinity (Khan 2002), and this variation could be due to a number of factors such as light, temperature, and moisture stress (Baskin and Baskin 1998; Mahmoud et al. 1983; Noe and Zedler 2000). Maximum salt tolerance for germination of subtropical species from the Karachi coast in Pakistan has been reported for *Arthrocnemum macrostachyum* (Moric.) C. Koch (10% germination at 1000 mmol/L NaCl; Khan and Gul 1998), *Cressa cretica* L. (3% germination at 1000 mmol/L NaCl; Khan 1999), and *Salsola imbricata* Forssk. (6% germination at 800 mmol/L; M.A. Khan, unpublished data). However, a number of species could not germinate at NaCl concentrations higher than 400 mmol/L (Khan 2002).

Temperature interacts with salinity to affect the germination of halophyte seeds (Khan et al. 2001). The adverse effect of high salinity is further aggravated by either an increase or decrease in temperature (Khan and Rizvi 1994; Khan 2002). Germination of many halophytes occurs at

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times when there is an optimal combination of day length, temperature regime, and salinity (Naidoo and Naicker 1992; Gutterman et al. 1995; Khan 2002). Absence of light almost completely inhibits seed germination of *Triglochin maritima* L. (Khan and Ungar 1999) and *Sporobolus indicus* (L.) R. Br. (Andrews 1997) and partially inhibits 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), while seeds of *Atriplex stocksii* Boiss. (Khan and Rizvi 1994) and *Suaeda fruticosa* Forssk. (Khan and Ungar 1998) are not inhibited by the absence of light.

Most seeds are located near the soil surface, where salt concentration changes because of continuous evaporation of ground water (Ungar 1991). Rainfall can quickly leach salt from the surface and supply water to the seed. Thus, for successful establishment of plants in saline environments, seeds must remain viable in high salinity and germinate when salinity decreases (Khan and Ungar 1997). Halophyte seeds are known to maintain viability for extended periods of time during exposure to high salinity, and they initiate germination when salinity is reduced (Keiffer and Ungar 1995; Khan and Ungar 1998, 1999; Khan 2002). Recovery germination of seeds from hypersaline conditions is affected by the temperature regime to which seeds are exposed (Khan and Ungar 1997). Halophytic species show a range of responses from partial to complete germination recovery when salinity stress is alleviated (Khan 2002).

Limonium stocksii (Boiss.) Kuntze is a low-branched, salt-secreting, woody shrub in the family Plumbaginaceae. It is distributed in high coastal marshes as well as rocky grounds near the coast of Pakistan and India (Gujarat). In Karachi, pure populations of *L. stocksii* are found at the farthest end of Manora Creek near Hawks Bay in association with a few individuals of *Arthrocnemum macrostachyum*, *Aeluropus lagopoides* (L.) Trin. ex. Thw., *Urochondra setulosa* (Trin.) C.E. Hubbard, *Suaeda fruticosa*, *Tamarix* spp., and *Atriplex stocksii*. Possible sources of moisture are the monsoon rains and oceanic seepage. The monsoon period starts 15 June and ends 15 September. Owing to high tides during this period, oceanic seepage increases, while rainfall (220 mm/year) usually occurs during July and August. Storms are rarely reported from the Karachi coast. *Limonium stocksii* flowers twice a year beginning in June and November, and a large number of seeds are produced in August and January. After dispersal seeds become part of the seed bank and germinate only after the monsoon rains. The average ambient temperature during the monsoon period ranges from 20 °C at night to 30 °C during the day. *Limonium stocksii* is a highly salt-tolerant plant that grows in coastal areas under high salinity, and it is occasionally foraged. The economic potential of this species as an ornamental plant for coastal saline areas is great. It is also an important component of littoral ecosystem of Karachi, Pakistan. Growing this species in its native habitat would preserve its population and reduce grazing pressure.

The aim of the present study was to determine percent germination, rate of germination, and recovery responses of *L. stocksii* under various salinity, temperature, and light conditions.

Materials and methods

Seeds of *L. stocksii* were collected in February 2000, from a salt flat at the upper end of Manora Creek near Hawks Bay, Karachi (24°45′–25°N and 66°45′–67°E). Seeds were separated from the inflorescence, surface sterilized using sodium hypochlorite (0.52%) for 1 min, followed by thorough rinsing with distilled water and air drying. Germination was carried out using 5 cm diameter, tight-fitting plastic Petri plates with 5 mL of test solution prepared by using distilled and deionized water. Each dish was placed in a 10 cm diameter plastic Petri plate as an added precaution against the loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated at the emergence of the radicle.

To determine the effect of temperature, seeds were germinated in incubators at four alternating temperature regimes of 10:20, 15:25, 20:30, and 25:35 °C. A 24-h cycle was used, where higher temperatures (20, 25, 30, and 35 °C) coincided with a 12-h light period (Sylvania cool white fluorescent lamps, 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 400–750 nm) and lower temperatures (10, 15, 20, and 25 °C) coincided with a 12-h dark period. Seeds were germinated at six salinities (0, 100, 200, 300, 400, and 500 mmol/L NaCl) as a result of preliminary tests, which determined the range of salinity tolerance. Percent germination was recorded on every alternate day for 20 d. Ungerminated seeds were transferred to distilled water after 20 d to study the recovery of germination, which was also recorded at 2-d intervals for 20 d.

Seeds were also germinated in complete darkness by placing Petri plates in black plastic bags and then in incubators at the above-mentioned temperature regimes for 20 d. Percent germination was recorded after 20 d.

Rate of germination was estimated by using a modified Timson's index of germination velocity, germination velocity = $\Sigma G/t$, where G is the percentage of seed germination at 2-d intervals and t is the total germination period (Khan and Ungar 1997). The maximum value possible for our data using this index was 50 (i.e., 1000/20). The higher the value, the more rapid the germination. The percent recovery was determined by the formula $(a-b)/(c-b) \times 100$, where a is the 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.

Germination data were transformed (arcsine) before a statistical analysis was performed. These data were analyzed using SPSS Version 6.1 for Windows (SPSS Inc. 1994). A two-way ANOVA was also used to demonstrate the interaction between various factors in affecting the rate, recovery, and percent germination. A Bonferroni test was used (P < 0.05) to determine significant differences between means of percent germination among salinity treatments under various light and temperature regimes (SPSS Inc. 1994).

Results

A two-way ANOVA indicated significant (P < 0.0001) individual effects of salinity, temperature, and their interaction on percent germination, rate of germination, and percent recovery of *L. stocksii* seeds (Table 1).

Fig. 1. Mean final percent germination of *Limonium stocksii* in various salinity, temperature, and light and dark conditions.Bars having the same letter within each light treatment are not significantly different ($P < 0.05$) from the control (Bonferroni test). Bars represent mean ± SE.

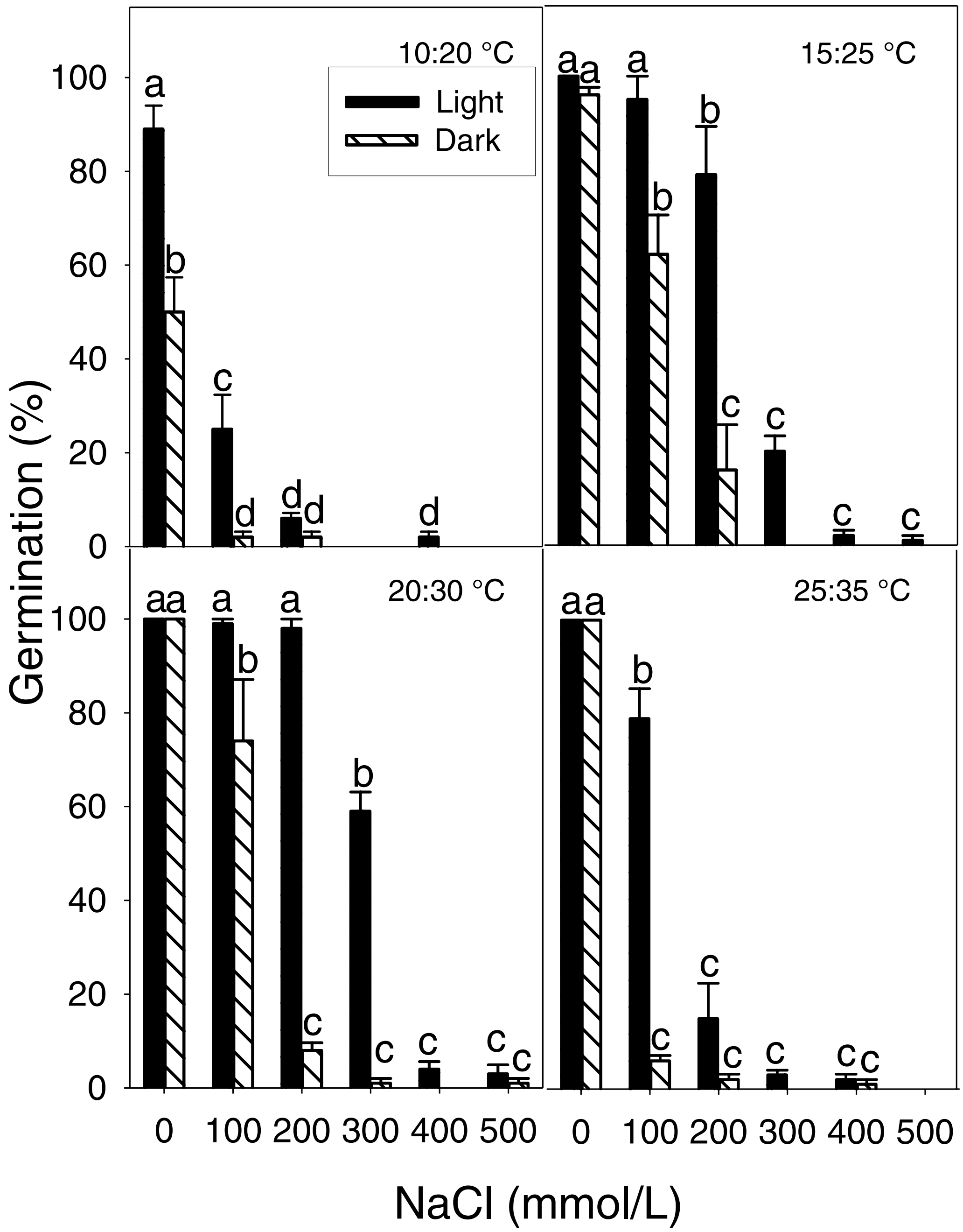


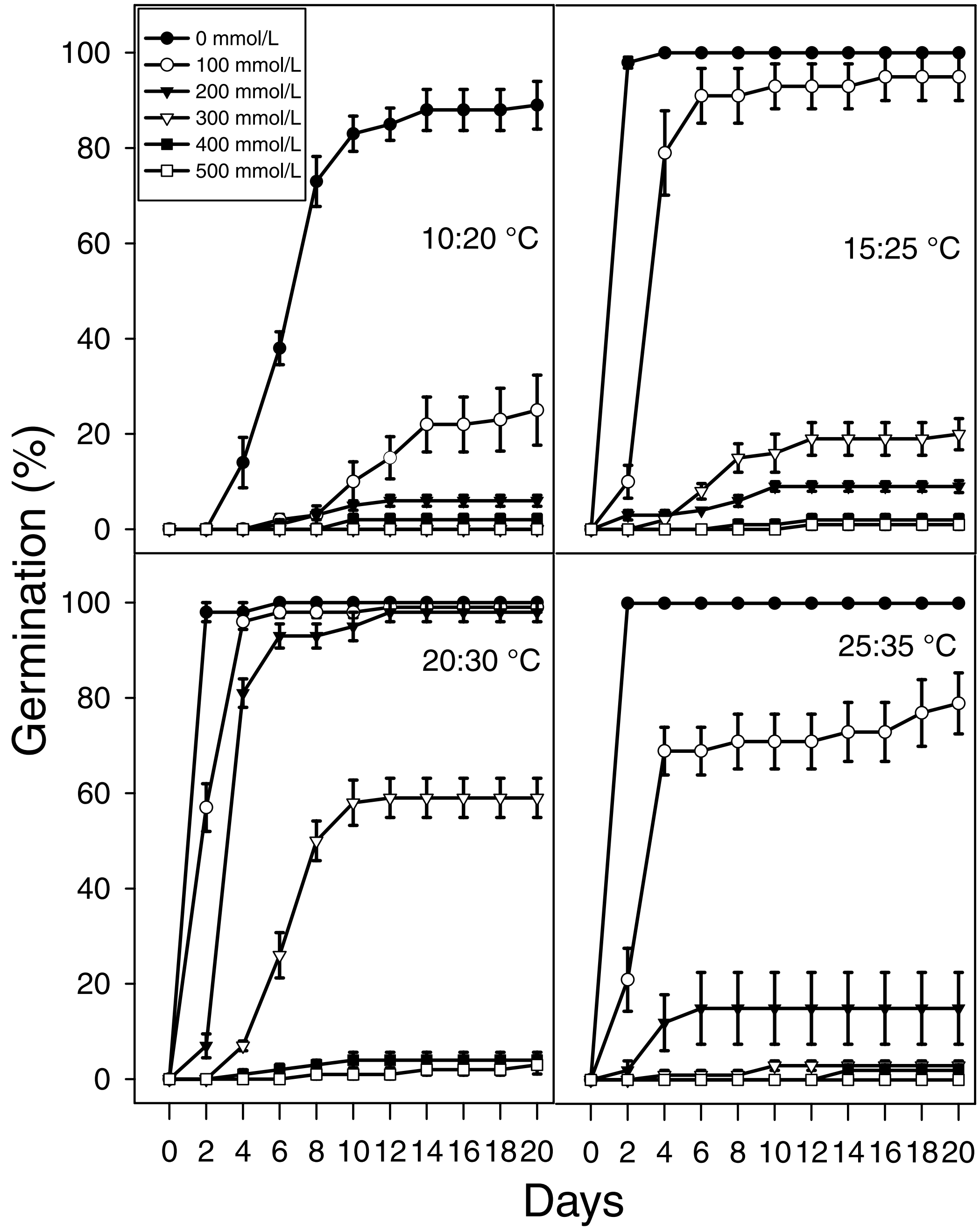
Table 1. A two-way ANOVA of the effects of salinity (S), temperature (T), and their interaction on germination of *Limonium stocksii*

Dependent variable	S	T	S × T
% germination	426.7***	127.7***	29.2***
Rate of germination	668.6***	229.5***	42.6***
% recovery	359.6***	80.8***	23.7***

Note: Numbers indicate F values (***, $P < 0.001$).

Maximum seed germination in light was obtained in non-saline control under all temperature regimes (Fig. 1). Exposure to different salinity levels resulted in a gradual decrease in percent germination, and this reduction varied with the change in temperature regime (Fig. 1). Best germination under saline conditions was observed at 20:30 °C treatment, where germination in 100 and 200 mmol/L NaCl was not significantly different from the control. A further increase in salinity decreased germination and only 5% of seeds germinated at 500 mmol/L NaCl. Seed germination at 15:25 °C was comparatively lower than germination under the optimal temperature regime. Exposure to lower (10:20 °C) and

Fig. 2. Cumulative mean percent germination of *Limonium stocksii* seeds over time in 0, 100, 200, 300, 400, and 500 mmol/L NaCl in 12 h light : 12h dark photoperiod. Bars represent mean ± SE.



higher (25:35 °C) temperature regimes substantially inhibited germination in all salinity treatments (Fig. 1), and the lowest germination was obtained at 10:20 °C, where 25% seed germination was obtained in 100 mmol/L NaCl.

Temperature also affected speed of germination under both saline and nonsaline conditions (Fig. 2). Maximum germination in the distilled water control was obtained after 2 d under all temperature regimes except for 10:20 °C, where it was attained in 14 d (Fig. 2). In saline solutions, maximum germination varied from 6 to 18 d. Under the optimal temperature regime, germination at lower salinity (100 and 200 mmol/L NaCl) peaked in 6 d and it was about 10 d in higher salinity treatments. However, seed germination peaked at 10 d for all salt concentrations at 15:25 °C (Fig. 2).

Seed germination in the distilled water control was not affected by darkness under any temperature regime with the exception of 10:20 °C, where percent germination was reduced to 50% (Fig. 1). Application of salt under complete darkness greatly reduced percent germination and the level of inhibition varied with temperature. About 2% of seeds could germinate in 100 mmol/L NaCl under lower (10:20 °C) and higher (25:35 °C) temperatures. Maximum germination (74%) was obtained at 20:30 °C in 100 mmol/L NaCl, and it decreased to just 8% in 200 mmol/L NaCl. At

15:25 °C, 62% of seeds germinated in 100 mmol/L NaCl, and 16% germination was obtained in 200 mmol/L NaCl (Fig. 1).

Rate of germination was highest in nonsaline controls except at 10:20 °C, and addition of NaCl slowed the rate of germination (Fig. 3). Temperature also influenced rate of germination. At lower and higher temperatures, seeds showed a slower rate of germination from 100 to 300 mmol/L NaCl than at 20:30 °C and 15:25 °C (Fig. 3).

When light-treated, ungerminated seeds from salt treatments were transferred to distilled water, they recovered completely under all salinity and temperature regimes (Fig. 4).

Discussion

Plants native to the subtropical maritime desert of Karachi are exposed to various levels of moisture and salinity stress because of an unpredictable monsoon period (Khan 2002). These conditions lead to different life history strategies in desert plants that allow the plants to maximize their fitness (Kigel 1995). Coastal areas of Pakistan are reported to have around 100 species of halophytes (Khan and Gul 2002), and salinity tolerance of only a small number of these species is known. The available reports indicate that dicotyledonous species vary in their tolerance during germination, including species such as *Arthrocnemum macrostachyum* (1000 mmol/L NaCl; Khan and Gul 1998), *Cressa cretica* (1000 mmol/L NaCl; Khan 1999), *Salsola imbricata* (800 mmol/L NaCl; M.A. Khan, unpublished data), *Suaeda fruticosa* (500 mmol/L NaCl; Khan and Ungar 1998), *Atriplex stocksii* (300 mmol/L NaCl; Khan and Rizvi 1994), and some grasses such as *Aeluropus lagopoides* (500 mmol/L NaCl; Gulzar and Khan 2001), *Urochondra setulosa* (500 mmol/L NaCl; Gulzar et al. 2001), *Halopyrum mucronatum* (300 mmol/L NaCl; Khan and Ungar 2001), and *Sporobolus ioclados* (500 mmol/L NaCl; Khan and Gulzar 2003). *Limonium stocksii* is a moderately salt-tolerant halophyte at germination when compared with other local halophytic species, but it has the ability to germinate at salinity levels of up to 500 mmol/L NaCl, which approach seawater salinity (600 mmol/L NaCl).

Temperature and salinity interact to affect the germination of halophytes (Khan and Rizvi 1994; Khan and Ungar 1997, 1998; Khan and Gul 1998). Some species are more sensitive to changes in temperature (*Cressa cretica* and *Zygophyllum simplex*) than others (*Arthrocnemum macrostachyum* and *Suaeda fruticosa*) (Khan 1999; Khan and Gul 1998; Sheikh and Mahmood 1986). Seed germination of *L. stocksii* was also influenced by temperature. We found 20:30 °C to be the optimal temperature for germination and any increase or decrease in temperature inhibited germination. This inhibition progressively increased with salinity. Recruitment of *L. stocksii* in natural conditions through germination appears to take place after monsoon rains. Germination of halophyte seeds in subtropical coastal and inland salt marshes usually occurs after monsoon rains, which causes a reduction in temperature and lowering of soil salinity (Khan and Gul 1998; Khan and Ungar 1998).

Several reports have indicated that the rate of germination is more sensitive to salinity than is overall percent germination (West and Taylor 1981; Dudeck and Peacock 1985; Marcar 1987). Very rapid germination was reported for *Haloxylon recurvum* and *Haloxylon salicornicum* (Sharma and Sen 1989) and *Limonium axillare* (Mahmoud et al. 1983), and they considered it to be a strategy to utilize the brief period of water availability after rainfall. Rogers et al. (1995) suggested that fast germination ensures rapid seedling establishment, which can minimize competition. Seeds of *L. stocksii* germinated rapidly in control and in up to 200 mmol/L NaCl at 20:30 °C, a temperature regime similar to the average early summer period in Karachi.

Fig. 3. Rate of germination of *Limonium stocksii* seeds under various salinity and thermoperiod treatments. Bars represent mean ± SE.

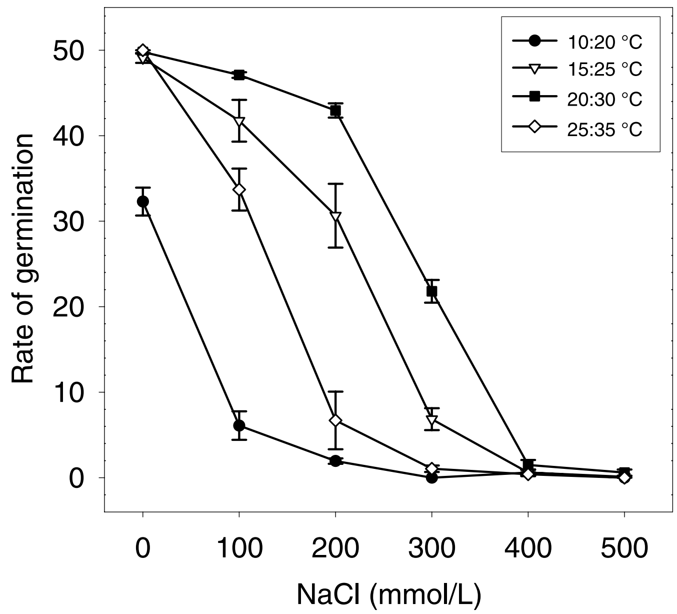
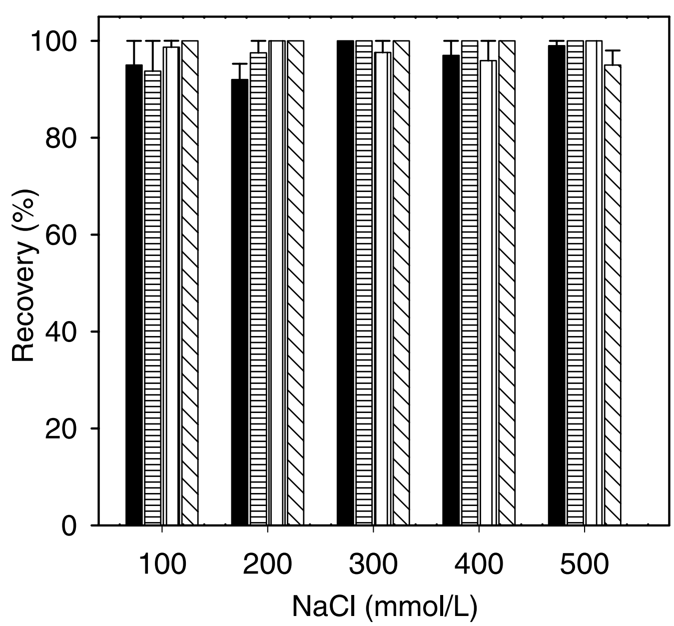


Fig. 4. Mean recovery percent germination for *Limonium stocksii* in distilled water under different temperature regimes in various salinity treatments. Bars represent mean ± SE.



Limonium stocksii seeds displayed a greater tolerance to high-salinity and temperature stress before germination. Seeds germinated within 2 d when transferred to nonsaline media from various salinity treatments and temperature regimes. Similar results were obtained by Mahmoud et al. (1983) for *L. axillare*, which showed 95% recovery for 60%–100% seawater treatments. Khan and Ungar (1998) also observed a quick recovery in *Suaeda fruticosa* seeds at all temperature regimes. The ability of halophyte seeds to survive hypersaline conditions and germinate when salinity is reduced provides them with multiple opportunities for cohort establishment in unpredictable saline environments (Khan and Ungar 1997).

Limonium stocksii usually grows in coastal salt marshes that remain more or less wet during all seasons due to seepage of seawater. Seed reserves in the soil are exposed to high-temperature stress (around 40 °C or higher) while imbibed in seawater during seven months of the year. Seeds of *L. stocksii* remain viable under natural conditions after extended exposure to salinity and temperature stress and germinate readily when salinity and temperature stress are reduced after monsoon rains (S. Zia and M.A. Khan, unpublished data). Although seeds could only germinate in up to 500 mmol/L NaCl, which is much lower in comparison to other associated halophytic species, their salinity tolerance during storage in the seed bank confers a successful reproductive strategy in these unpredictable conditions. Seeds of species such as *Arthrocnemum macrostachyum* and *Sporobolus ioclados* lose viability when exposed to high-temperature and salinity stress (S. Zia and M.A. Khan, unpublished data). However, *Suaeda fruticosa* seeds maintain a viability similar to that of *L. stocksii*. Survival of seeds under extreme conditions provides this species with a strategy for successful recruitment in a harsh environment. *Limonium stocksii* is a potential candidate as an ornamental and fodder crop in coastal areas where only brackish water or seawater is available.

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