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COMPARATIVE EFFECT OF NaCl AND SEAWATER ON SEED GERMINATION OF *LIMONIUM STOCKSII*

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Abstract

Limonium stocksii (Boiss.) O. Kuntze, a secreting perennial in the family Plumbaginaceae, is widely distributed in the coastal marshes of Karachi, Pakistan. We are reporting here the effect of seawater and NaCl on the seed germination of *L. stocksii* both under a 12 h photoperiod and in complete darkness. Seed germination decreased with increase in salinity and few seeds germinated above 30 dS m^{-1} in both NaCl solution and seawater. Absence of light had little effect under non-saline condition however, addition of salinity synergistically inhibited seed germination. Seawater inhibited seed germination of *L. stocksii* more than NaCl solutions under both light and dark conditions. All un-germinated seeds when transferred to distilled water after 20 days of salinity treatments readily germinated.

Introduction

The coastal plants of Pakistan is represented by 108 species from 36 families (Khan & Gul, 2002). The vegetation is dominated by perennial grasses (20), sedges (14), chenopods (13), with only 13 annuals (Khan & Gul, 2002). Germination is highly punctuated due to the region's sub-tropical type of seasonality. Annual and some perennial plants typically germinate during the wet, cooler monsoon season. Germination typically occurs between July and August, but individual species establish seedlings either early or late in, or throughout this germination window. We have shown that temporal variance in soil salinity, temperature regimes and soil moisture explains a significant portion of the timing of germination of Karachi coastal wetlands (Gul & Khan, 2002). A large number of perennial halophytes dominating the coastal marshes and sand dunes do not usually germinate even in the monsoon periods when soil salinity is low (Khan & Gul, 1998; Khan & Ungar, 1997a, 1998; Gulzar & Khan, 2001). Laboratory studies showed that they were very highly tolerant to salinity and could germinate in 1000 mM NaCl (Khan & Gul 1998; Khan 1999). This failure of germination might be related to various combinations of salts present in seawater and perhaps have different effect on germination in comparison to NaCl (Ungar, 1978). Some studies have been made on the effect of seawater on the seed germination of halophytes (Rivers & Weber 1971; Joshi & Iyengar 1982, 1985; Woodell 1985; McMillan 1988; Joshi *et al.*, 1995; Baskin & Baskin 1998; Houle *et al.,* 2001) but little information is available on the relative tolerance of seawater and NaCl solutions on the seed germination of halophytes. The objective of the present investigation was to determine and compare the effects of NaCl solutions and seawater on seed germination of *L. stocksii*.

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Materials and Method

Limonium stocksii, a perennial woody shrub of the family Plumbaginaceae (Bokhari, 1972) is found near the seashore particularly in rocky and saline areas in India and Pakistan. Seeds of *L. stocksii* were collected 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 and stored at 4°C. Before storage they were surface sterilized using clorox (52%) for one minute followed by thorough rinsing with distilled water and air-drying. Germination was carried out in 50 mm diam., tight-fitting plastic Petri dishes with 5 ml of test solution. Germination was carried out in NaCl and seawater (0, 10, 20, 30, 40 and 50 dS m^{-1}) separately. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated with radicle emergence. Seeds were germinated in an incubator at a day/night temperature of 20/30 °C with 12-h photoperiod (*Sylvania* cool white fluorescent lamps, 25 μmol $m^{-2} s^{-1}$, 400-750 nm) while other set was placed in the same temperature but in complete darkness for 20 days. Percent germination was recorded on alternate days for 20 days for light germinated seeds. Dark germinated seeds were counted once after 20 days. Un-germinated seeds from the NaCl treatments were transferred to distilled water after 20 days to study the recovery of germination, which was also recorded at 2 day intervals for 20 days. The recovery percentage was determined by the following 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. The rate of germination was estimated by using modified Timson's index of germination velocity which is as follows:

$$\Sigma G/t$$

where G is percentage of seed germination at 2-d intervals, and t is total germination period (Khan & Ungar, 1984). 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. Germination data was transformed (arcsine) before statistical analysis. These data were analyzed using SPSS for Windows release 10 (SPSS, 2001). A Bonferonni post hoc test was used to determine significant differences ($p < 0.05$) between means.

Result

A one-way ANOVA indicated that germination of *L. stocksii* seed was significantly affected by light ($F=21.81$, $P<0.0001$), NaCl ($F=83.88$, $P<0.0001$) and seawater ($F=141.20$, $P<0.0001$).Sodium chloride solution had no effect on the seed germination in up to 20 dS m^{-1}, but a further increase in salinity inhibited germination. Few seeds germinated above 40 dS m^{-1} (Fig. 1). Seawater reduced germination from about 100% to 20% at 20 dS m^{-1}, and no seed germinated above 30 dS m^{-1}. Absence of light had no effect in non-saline control. However, germination inhibition caused by both NaCl and seawater increased considerably in the dark, and seawater inhibited seed germination more than NaCl solution.

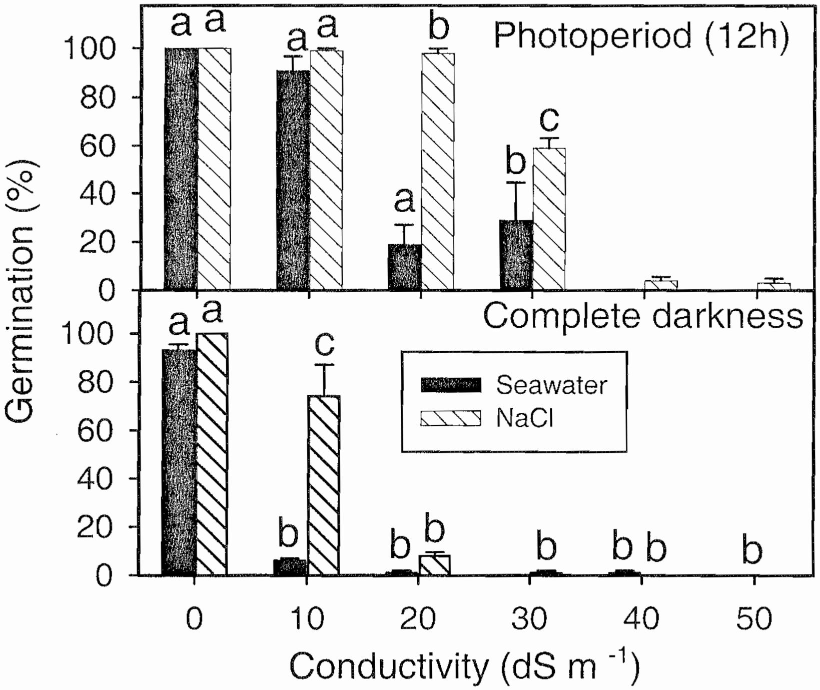

Fig. 1. Seed germination of *Limonium stocksii* in dS m^{-1} of NaCl and seawater. Values for each of the salinity treatment having the same letters are not significantly different ($p > 0.05$) from one another (Bonferroni test).

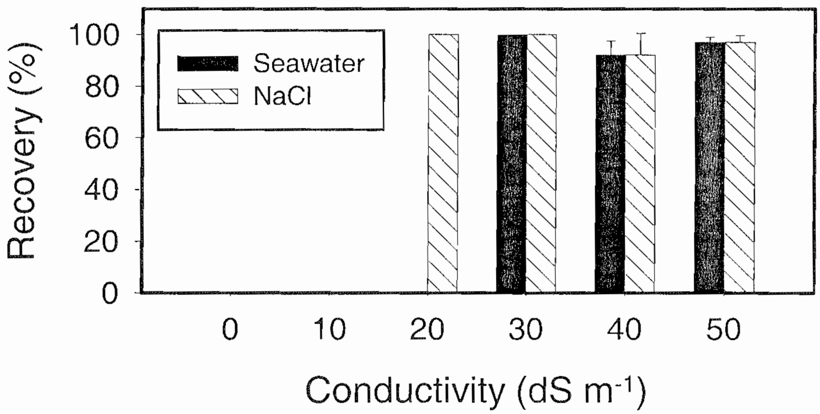

Fig. 2. Recovery of seed gremination of *Limonium stocksii* in NaCl and seawater under 12 h photoperiod.

A one-way ANOVA indicated that the rate of germination of *L. stocksii* seed was significantly affected by NaCl ($F= 174.48$, $P<0.0001$) and seawater ($F=130.21$, $P<0.0001$). The rate of germination showed the same pattern as that of percent germination (Table 1).

Table 1. Index of germination velocity of *Limonium stocksii* seeds (Mean ± S.E.) using a modified Timson's index.

Conductivity ($dS\ cm^{-1}$)	Index of germination velocity	
	NaCl	Seawater
0	49.80 ± 0.2^{a}	49.50 ± 0.2^{a}
10	47.10 ± 0.3^{a}	40.60 ± 2.8^{b}
20	42.95 ± 0.8^{a}	07.00 ± 3.2^{c}
30	21.80 ± 1.3^{b}	10.75 ± 5.7^{c}
40	01.54 ± 0.6^{c}	00.00 ± 0.0^{d}
50	00.60 ± 0.4^{d}	02.05 ± 0.9^{d}

Different letters in superscript represent significant ($P<0.05$) differences between treatments at each type of salinity. ANOVA, Bonferroni test.

A one-way ANOVA indicated that the recovery of germination of *L. stocksii* seed was significantly affected by NaCl ($F= 74.57$, $P<0.0001$) and seawater ($F=117.6$, $P<0.0001$). When un-germinated seeds from both NaCl and seawater solutions were transferred to distilled water, they recovered completely (Fig. 2).

Discussion

Seeds of *Limonium stocksii* demonstrated the absence of innate dormancy as all seeds germinated in non-saline control. Increase in NaCl concentration gradually decreased germination and few seeds germinated at 50 $dS\ m^{-1}$ NaCl. The halophytes found around the Karachi coast could be grouped into three categories based on their salt tolerance (Khan & Gul, 2002). The first category includes species like *Atriplex stocksii* (Khan & Rizvi, 1994) and *Zygophyllum simplex* (Khan & Ungar, 1997a) which could germinate at or below 300 mM NaCl. The second category include halophytes like *Aeluropus lagopoides* (Gulzar & Khan, 2001), *Haloxylon stocksii* (Khan & Ungar, 1996), *Suaeda fruticosa* (Khan & Ungar, 1998), *Sporobolus ioclados* (Gulzar & Khan, unpublished data), and *Urochondra setulosa* (Gulzar *et al.*, 2001) which could germinate in up to 500 mM NaCl, while the third category include species like *Arthrocnemum macrostachyum* (Khan & Gul, 1998), *Cressa cretica* (Khan, 1999) and *Salsola imbricata* (Khan, unpublished data) which have the ability to germinate in up to 100 $dS\ m^{-1}$ NaCl. *Limonium stocksii* appears to be a moderately salt tolerant species and belong to the second group.

Seed germination of *Limonium stocksii* was inhibited more by seawater and no seed germinated above 30 $dS\ m^{-1}$. There is little information available on the effect of seawater on the germination of halophytes (Rivers & Weber, 1971; Joshi & Iyengar, 1982, 1985; Woodell, 1985; McMillan, 1988; Joshi *et al.*, 1995; Houle *et al.*, 2001) and on the relative tolerance of seawater and NaCl solutions during seed germination (Joshi *et al.*, 1995; Tirmizi *et al.*, 1993). Seed germination of *Salvadora persica* (Joshi *et al.*, 1995) and *Salicornia brachiata* (Joshi & Iyengar, 1982) was inhibited more by seawater in comparison to different chlorides of Na, K, and Mg. They attributed this effect to seawater composition, which included a combination of different salts with a high concentration of NaCl (Joshi *et al.*, 1995). However, Tirmizi *et al.*, (1993) found that NaCl inhibited the germination of *Hipophae rhamnoides* more than seawater.

Light requirements for seed germination of halophytes are quite varied (Baskin & Baskin, 1998) and it ranges from no effect to obligate requirement for germination (Andrews, 1997; DeVilliers *et al.*, 1994; Garcia *et al.*, 1995; Khan & Rizvi, 1994; Khan & Ungar, 1998; Khan & Ungar, 1997b; Khan & Ungar, 1999; Thanos *et al.*, 1991). Seeds of *L. stocksii* do not require light under non-saline conditions, however, absence of light synergistically inhibited seed germination both under NaCl and seawater. Increased germination inhibition by seawater and NaCl could be due to the inactivity of Pfr in darkness which regulates several genes coding both for enzymes and structural proteins (Bewley & Black, 1994).

Seeds of *L. stocksii*, when transferred to distilled water after a 20-day treatment of both NaCl and seawater completely recovered. Woodell (1985) showed that various *Limonium* species viz., *L. bellidifolium, L. humile* and *L. vulgare* recovered substantially when transferred to distilled water. Similar recovery response from seawater is reported for *Carpobrotus* spp., (Weber & D'Antonio, 1999), *Aster laurentianus* (Houle *et al.*, 2001), *Holcus lanatus* (Watt, 1983). Similar recovery responses of seed germination were also reported from NaCl treatments (Khan & Ungar 1996, 1997a, 1998; Khan & Gul, 1998).

Limonium stocksii is a moderately salt tolerant plant among the coastal halophytes found around coastal areas of Pakistan. Seawater clearly prevented more seed from germination in comparison to NaCl solutions of the similar concentrations and this inhibition of germination markedly increased in the dark. Seeds appear to have enforced dormancy when present under saline conditions and germinated when salinity stress was removed.

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