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SEED GERMINATION OF *LIMONIUM STOCKSII* UNDER SALINE CONDITIONS

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Abstract

Limonium stocksii (Boiss.) O. Kuntze, (Plumbaginaceae) a secreting, perennial shrub is widely distributed in the inter-tidal zones of Karachi, Pakistan. The effect of seawater and Sodium chloride showed that the seed germinated both under a 12-h photoperiod and complete darkness in 0, 10, 20, 30, 40 and 50 dS m^{-1} seawater and Sodium chloride at different temperature regimes (10^o:20^o, 15^o:25^o, 20^o:30^o and 25^o:35^oC). Seed germination decreased with an increase in salinity and few seeds germinated above 30 dS m^{-1} in seawater (25^o:35^oC). When seawater effect on germination was compared with that of NaCl, seawater appeared to inhibit seed germination more than NaCl at low and moderate temperatures but at the highest temperature regime (25^o:35^oC) seed germination in seawater was more than NaCl. Absence of light had little effect under non-saline condition, however, addition of salinity inhibited seed germination in dark and this inhibition was higher in seawater. Seeds when transferred to distilled water after 20-d of either NaCl or seawater treatments recovered completely.

Introduction

Limonium stocksii (Boiss.) Kuntze, is distributed in the coastal Sindh and Balochistan, Pakistan (Gul & Khan, 2002) which remain more or less wet during all seasons due to seepage of seawater. It is found at the farthest end of Manora Creek near Hawks bay, Karachi. *Limonium stocksii* produces a large number of seeds twice a year and the seed reserves face high salinity and temperature stresses during their storage in soil. Recruitment of *L. stocksii,* unlike other co-occurring halophytes takes place primarily by seed germination usually after monsoon rains (Khan, 2003). High temperature and salinity stress cause the death of other halophytic seeds except for S*uaeda fruticosa* under natural conditions (Khan, 2003) but the wide spread recruitment of *L. stocksii* seeds after monsoon rains indicate the little effect of seawater on their viability. *Limonium stocksii* grows in association with *Arthrocnemum macrostachyum*, *Aeluropus lagopoides*, *Tamarix spp*. and *Suaeda fruticosa. Limonium stocksii* has a tremendous economic value as ornamental plants particularly in the coastal areas with little fresh water availability.

Halophytes vary a great deal in their ability to tolerate salt (Khan, 2003). Tolerance also varies with stages of their life cycle, which could be expressed as 1). The ability to tolerate high salinity without loosing viability while stored in the soil (seed bank), 2). The ability to germinate at high salinities and 3). The ability to complete its life cycle at high salinities (Khan & Gul, 2002). Secreting halophytes which could germinate above seawater salinity are *Limonium vulgare, Cressa cretica, Tamarix pentandra, Atriplex rosea, A. tornabeni*, and *A. laciniata* (Binet, 1965; Ungar, 1967; Ignaciuk & Lee, 1980; Woodell, 1985; Khan, 1991). Most secreting halophytes viz., *Atriplex nummularia, A. triangularis, A. prostrata, A. canescense, A. lentiformis, A. polycarpa,* and *A. stocksii* show germination at NaCl concentrations ranging from 0.34 to 0.52 M NaCl (Ignaciuk & Lee, 1980; Khan & Ungar, 1984; Uchiyama, 1987; Mikheil *et al*., 1992; Katembe *et al*., 1998). While few of them viz., *Atriplex glabriuscula, A. rependa, Limonium axillare* and *Melulaca ericifolia* have low salt tolerance (< 0.2 M NaCl) during germination (Ladiges *et al*., 1981; Mahmoud *et al*., 1983; Fernandes *et al*., 1985).

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Different abiotic factors such as temperature, soil salinity, photoperiod and soil moisture affect germination of halophytes (Noe & Zedler, 2000; Khan, 2003). However the effect of soil salinity seems to dominate over all other factors (Keiffer & Ungar, 1997; Baskin & Baskin, 1998). Experiments determining the effect of salinity on the germination of halophytes are typically conducted with NaCl as a source of salinity. Such tests may not be relevant to the field conditions because the source of moisture for the coastal plants is usually seawater, which is combination of different cations (Na^{+}, Mg^{++}, Ca^{++}, K^{+} and Sr^{++}) and anions (Cl^{-}, SO_4^{-}, Br^{-}, F^{-}, HCO_3^{-}, H_3BO_3), however, the concentrations of Na and Cl ions are higher (86 %). Weber & Antonio (1999) investigated the germination responses of three taxa of *Carpobrotus* in seawater and concluded that the rate and percentage germination were reduced with seawater in comparison to non-saline control. They also observed that lower concentrations of seawater did not affect seed germination and that the inhibitory effect of seawater was only osmotic. Similar results were obtained for *Suaeda nudiflora* (Joshi & Iyengar, 1985) and for *Crithmum maritimum* (Okusanya, 1977). Germination of *Salicornia bigelovii* was inhibited at full strength seawater (4.02 %) (Rivers & Weber, 1971) and was attributed to a combined effect of seawater and temperature. Seed germination of *Salvadora persica* (Joshi *et al.*, 1995) and *Salicornia brachiata* (Joshi & Iyengar, 1980) was inhibited more by seawater in comparison to different chlorides of Na, K and Mg. They attributed this effect to seawater composition, which includes a combination of different salts with 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.

Temperature regimes have been shown to affect the germination of halophyte seeds at various salinities (Khan & Gul, 2001). Sub-tropical halophytes predominantly show optimal germination at 20:30°C and any further increase and decrease in temperature affected the germination (Khan & Rizvi, 1994; Khan & Ungar, 1996; 1997; 1998; 1999; 2000; 2001; Gulzar & Khan, 2001; Gulzar *et al*., 2001). Temperature interaction with seawater in affecting seed germination is not widely reported. Rivers and Weber (1971) showed a slow but high germination at low temperature regimes in salinities tested and faster rate and relatively low germination at high temperature regimes.

Recovery of germination of sub-tropical halophytes showed some variability in their salt tolerance when exposed to high salinity and temperature stress in the soil (Gul & Khan, 2002). *Arthrocnemum macrostachyum* showed a substantial recovery at 1000 mmol/L NaCl (Khan & Gul, 1998) and others like *Aeluropus lagopoides* (Gulzar & Khan, 2001), *Atriplex stocksii* (Khan, 1999), *Limonium stocksii* (Zia & Khan, unpublished data) and *Urochondra setulosa* (Gulzar *et al*., 2001) showed a high recovery at 600 mmol/L NaCl. While *Cressa cretica* (Khan, 1999), *Haloxylon stocksii* (Khan & Ungar, 1996), *Salsola imbricata* (Khan, unpublished data), *Suaeda fruticosa* (Khan & Ungar, 1998) and *Sporobolus ioclados* (Khan & Gulzar, 2003) showed poor recovery responses.

The present study gives an account of the effect of seawater on the germination of *L. stocksii* seeds under various temperature and light regimes and compares the differences in germination responses to NaCl and seawater.

Materials and Methods

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 were surface sterilized using clorox (0.52%) for one minute followed by thorough rinsing with distilled water and air-drying. Germination was carried out in 50 x 9-mm 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 replicate of 25 seeds each were used for all treatments. Seeds with the emerging radicals were considered to be germinated.

To determine the effect of temperature, seeds were germinated in four alternating temperature regimes of 10°:20°C, 15°:25°C, 20°:30°C and 25°:35°C in incubators (Percival Scientific). A 24 h cycle was used where higher temperatures (20°, 25°, 30°, 35°C) coincided with the 12-h light period (Sylvania cool white fluorescent lamps, 25µM m^{-2} s^{-1}, 400-750 nM) and lower temperatures (10°, 15°, 20°, 25°C) coincided with the 12-h dark period. Seeds were also germinated in complete darkness by placing Petri-plates in black plastic bags and then in germinators at the above-mentioned temperature regimes for 20 d. Percent germination was recorded at alternate days for 20 d for light germinated seeds and for dark germinated seeds it was only after 20 d. Un-germinated seeds from the salinity treatments 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. The recovery percentage was determined by the following formula: (a–b)/(c–b)*100, where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seed 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, $\Sigma G / t$, where G is percentage of seed germination at 2-d intervals, and t is total germination period (Khan & Ungar, 1999). 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 Bonferroni post hoc test was used to determine significant differences ($p<0.05$) between means. A three way and two way ANOVA test was also performed.

Result

A three way ANOVA indicated significant ($p<0.0001$) individual effects of salinity, light, temperature and their interactions on the seed germination of *Limonium stocksii* (Table 1). Highest germination was obtained in distilled water and at 10 dS m^{-1} seawater except for the cooler (10°:20°C) thermoperiod where no seed germinated in any salinity treatment (Fig. 1). Seed germination decreased with an increase in salinity and few seeds germinated at 50 dS m^{-1} seawater (Fig. 1). The germination of seeds under saline conditions improves with the increase in temperature and maximum germination percentages are found at 25°:35°C. A linear regression explains a high promotion of germination by increasing temperature with R^2 values ranging from 0.47 to 0.90 in seawater (Fig. 2). Dark germinated seeds show the similar germination in comparison to light germinated seeds.

Seed germination was inhibited more by seawater in comparison to NaCl at cooler temperatures both in light and dark (Figs. 2 & 3). However at warmer thermoperiod (25°:35°C) seed germination in seawater was higher in comparison to NaCl (Fig. 2) A linear regression explains a high promotion of germination while in NaCl solution R^2 Values ranged between 0.61 to 0.87 at different temperatures (Fig. 2). Similar effect of seawater and NaCl was reported in dark germinated seeds (Fig. 3). However, germination under dark condition was inhibited more by seawater than NaCl in all temperature regimes except for (25°:35°C). Germination showed a strong negative correlation with both seawater and NaCl salinities in both light and dark conditions (Figs. 2 & 3).

Table 1. A three-way ANOVA of germination percentage due to salinity (S), temperature (T), Light (L) and their interactions. Numbers indicate F-values significant at $p<0.05$.

Factor	NaCl	Seawater
Temperature (T)	145.80***	061.40***
Light (L)	279.10***	365.40***
Salinity (S)	661.20***	1070.50***
T X L	012.30***	039.64***
L X S	040.90***	105.93***
T X S	026.81***	021.32***
T X L X S	018.40***	017.30***

Table 2. Rate of germination (Mean ± S.E.) at different temperature regimes in both seawater and NaCl solutions using modified Timson's index (Khan and Ungar, 1984).

Conductivity (dS cm^{-1})	Test solutions	
	NaCl	Seawater
10 °:20°C		
0	32.30 ± 01.62 a	41.35 ± 00.67 b
10	06.10 ± 01.66c	02.15 ± 00.32 c
20	01.95 ± 00.32d	00.00 ± 00.00 e
30	00.00 ± 00.00e	00.45 ± 00.45 e
40	00.60 ± 00.34e	00.00 ± 00.00 e
50	00.00 ± 00.00e	00.00 ± 00.00 e
15 °:25°C		
0	49.10 ± 00.58a	46.20 ± 00.26 a
10	41.75 ± 02.44a	30.95 ± 01.91 b
20	30.65 ± 03.73b	03.05 ± 00.26 c
30	06.85 ± 01.28c	00.75 ± 00.43 c
40	00.60 ± 00.36c	00.00 ± 00.00 c
50	00.10 ± 00.10c	00.20 ± 00.20 c
20 °:30°C		
0	49.80 ± 00.20a	49.50 ± 00.21 a
10	47.10 ± 00.33a	40.60 ± 02.79 a
20	42.95 ± 00.83a	07.00 ± 03.18 b
30	21.80 ± 01.33c	10.75 ± 05.68 b
40	01.54 ± 00.58b	00.00 ± 00.00 b
50	00.60 ± 00.36b	02.05 ± 00.87 b
25 °:35°C		
0	50.00 ± 00.00a	49.80 ± 00.08 a
10	33.70 ± 02.45b	48.45 ± 01.02 a
20	06.70 ± 03.37c	35.05 ± 04.83 b
30	01.05 ± 00.38c	21.00 ± 01.63 d
40	00.40 ± 00.23c	07.95 ± 00.95 c
50	00.00 ± 00.00c	03.60 ± 00.29 c

Note: Values in both columns for every temperature regime having same letters are not significantly different at $p > 0.05$, Bonferroni test.

A two way ANOVA of rate of germination (Timson's index) with seawater showed significant ($p<0.0001$) differences with seawater salinity ($F = 376$), change in temperature ($F = 126$) and their interaction ($F = 20$, Table 1). Rate of germination, was higher in all non-saline solutions and increase in NaCl and seawater salinity decreased the rate of germination (Table 2). Rate of germination was low in the cooler temperature regime and increase in temperature gradually increased rate of germination. In NaCl peak rate under saline conditions was achieved at (20°:30°C) while in seawater treatment it was obtained at (25°:35°C). Rate of germination in NaCl was higher at all temperatures except for (25°:35°C) in comparison to seawater (Table 2).

Un-germinated seeds from both NaCl and seawater solutions when transferred to distilled water recovered completely. A Linear regression plot for recovery of germination at various temperature and salinity concentrations with R^2 values ranging between 0.62 to 0.84 for NaCl and from 0.49 to 0.84 for seawater (Fig. 4).

Discussion

Coastal vegetation of Pakistan is primarily perennial grasses and shrubs with few annuals (Khan & Gul, 2001) and their seeds after the dispersal are subjected to high salinity and temperature stress. Seeds of successful species should be able to tolerate warm hyper-saline conditions and retain their viability. The data available indicate that most of them do not recruit themselves through seeds and seed bank are of transient nature (Khan, 1993). *Suaeda fruticosa, Limonium stocksii* and *Salsola imbricata* have persistent seed bank and large number of seedlings are recorded after the monsoon rains (Khan, 2003). Seeds of *L. stocksii* germinated both in NaCl and seawater up to 50 dS m^{-1} and the seawater were inhibitorier to seed germination at low to moderate temperature regimes; however seed germination was inhibited more by NaCl at the warmest temperature.

Little data is available on the effect of seawater on the germination of subtropical halophytes (Joshi & Iyengar, 1985). However, seeds were able to germinate in NaCl at concentrations above seawater that includes *Cressa cretica, Arthrocnemum macrsostachyum, Salsola imbricata* (Khan, 1991; Khan & Gul, 1998; Mehrunnusa *et al*., 2007), while others like *Suaeda fruticosa, Haloxylon recurvum, Aleuropus lagopoides, Urochondra setulosa,* and *Sporobolus ioclados* (Khan & Ungar, 1996, 1998; Gulzar & Khan, 2001, 2002; Gulzar *et al*., 2001) could germinate in up to 500 mmol/L NaCl. *Atriplex stocksii* and *Zygophyllum simplex* could only germinate in upto about 250 mmol/L NaCl (Khan & Rizvi, 1994; Khan & Ungar, 1996). There is little information available on the effect of seawater on the germination of halophytes (Rivers & Weber, 1971; Joshi & Iyengar, 1980, 1985; Woodell, 1985; McMillan, 1988; Joshi *et al*., 1995; Houle *et al*., 2001) and on the relative tolerance of seawater and NaCl solutions during the seed germination (Tirmizi *et al*., 1993; Joshi *et al*., 1995). Seed germination of *Salvadora persica* (Joshi *et al.*, 1995) and *Salicornia brachiata* (Joshi & Iyengar, 1980) was inhibited more by seawater in comparison to different chlorides of Na, K and Mg. They attributed this effect to seawater composition, which includes a combination of different salts with 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. Khan (unpublished data) compared the germination of various halophyte seeds in both seawater and NaCl and found the effect to be species specific.

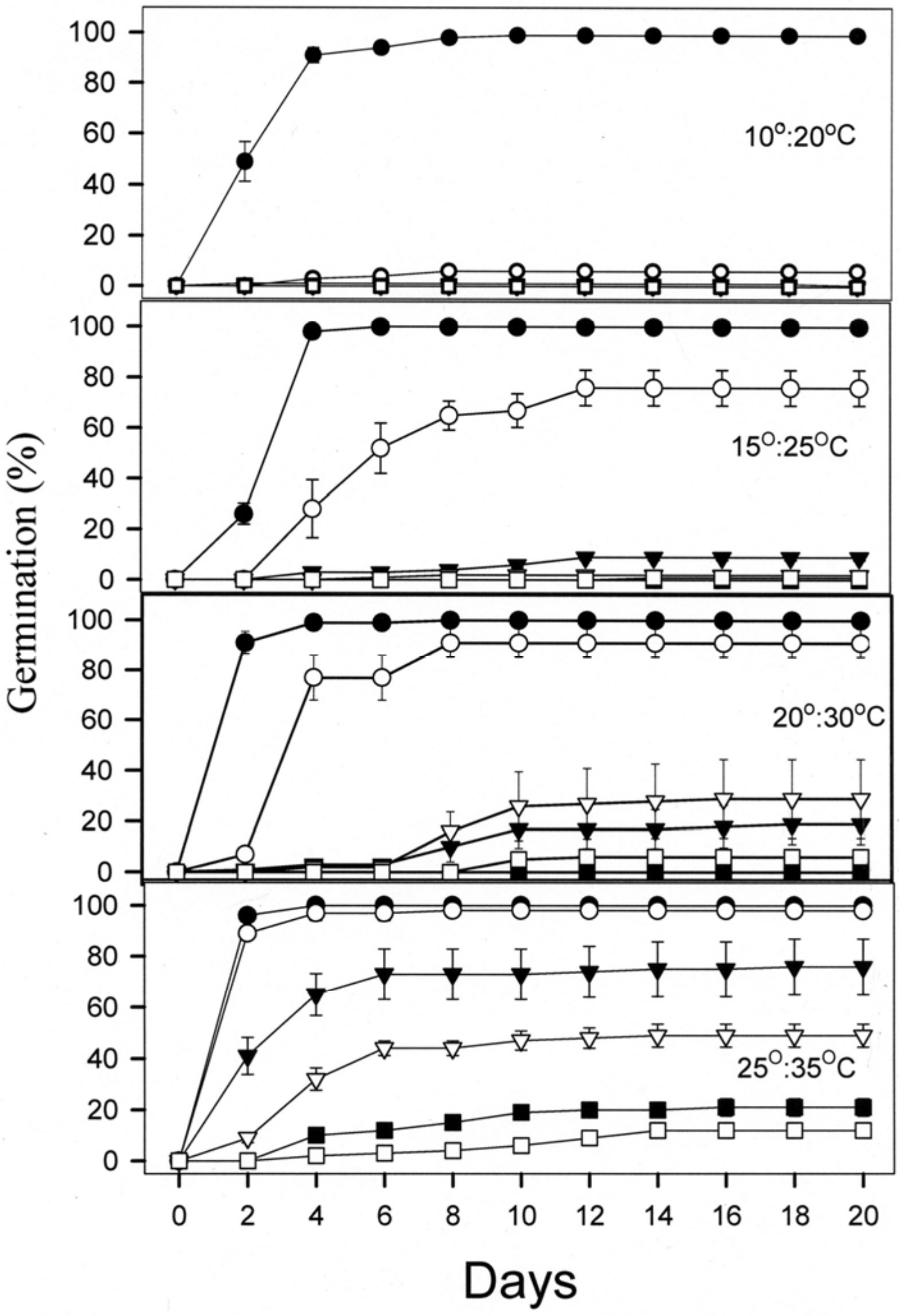

Fig. 1. Seed germination of *Limonium stocksii* in ● 0, ○ 10, ▼ 20, ▽ 30, ■ 40 and □ 50, dS m^{-1} of seawater at 10:20, 15:25, 20:30 and 25:35°C temperature regimes.

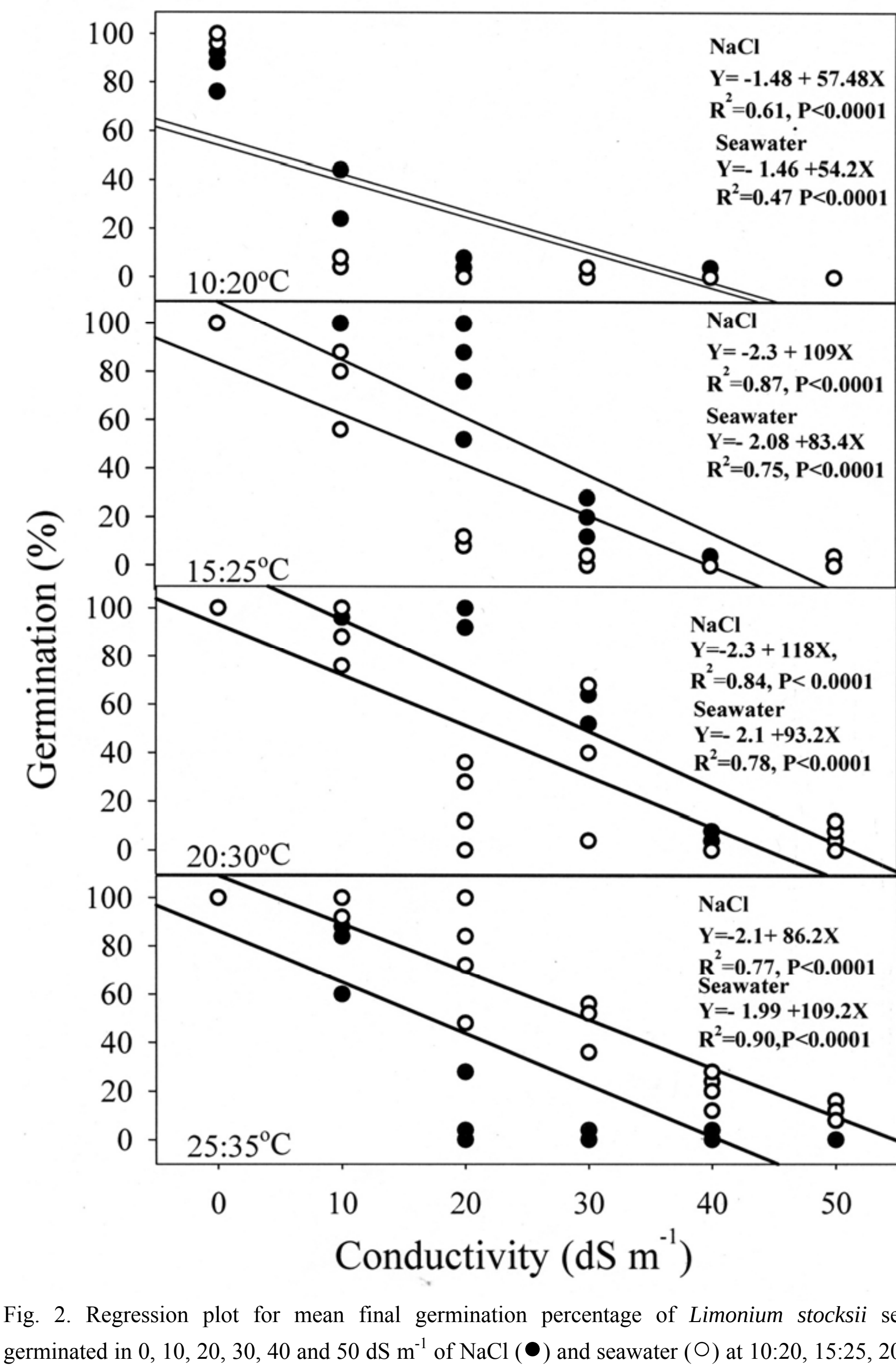

Fig. 2. Regression plot for mean final germination percentage of *Limonium stocksii* seeds germinated in 0, 10, 20, 30, 40 and 50 dS m^{-1} of NaCl (●) and seawater (○) at 10:20, 15:25, 20:30 and 25:35°C temperature regimes under 12-h photoperiod.

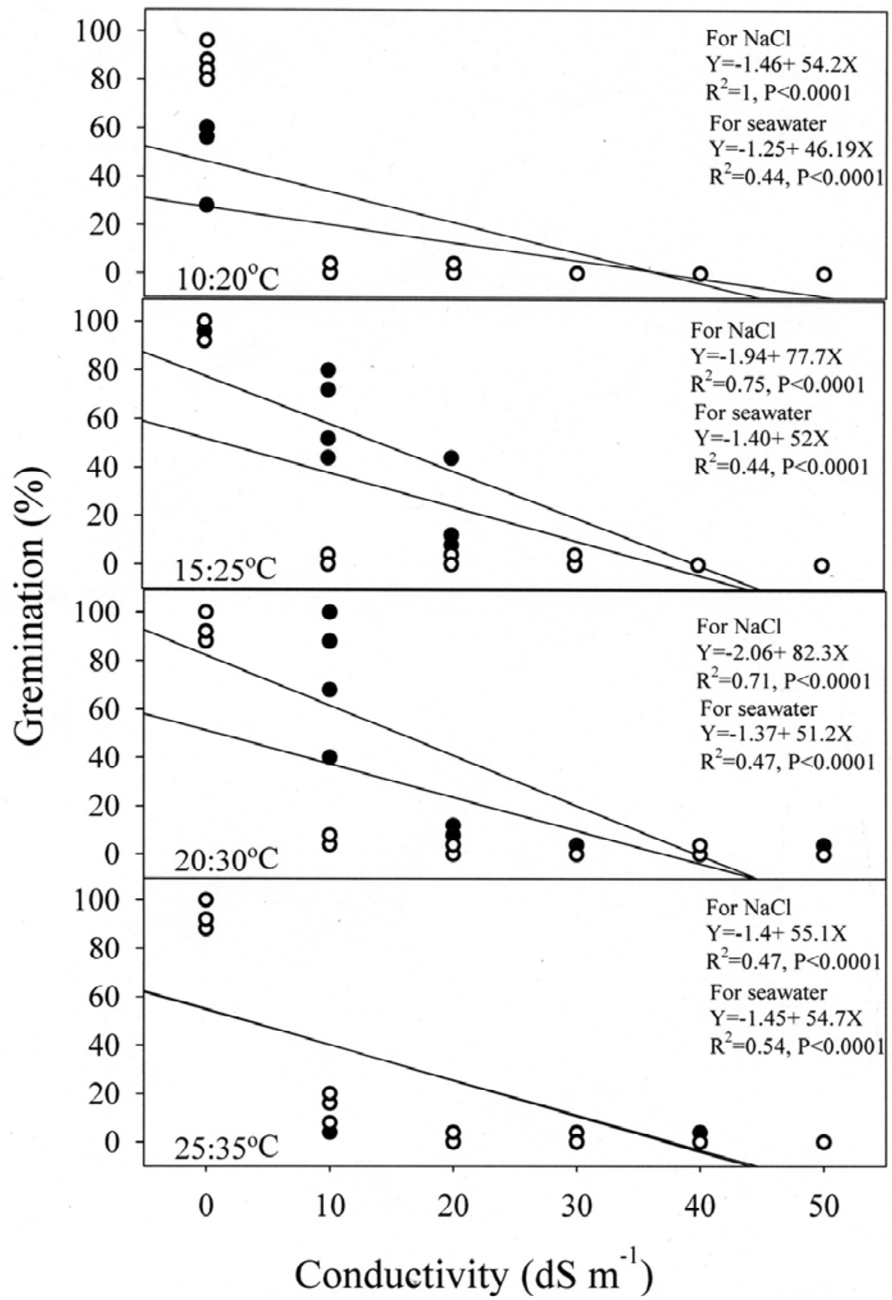

Fig. 3. Regression plot for mean final germination percentage of *Limonium stocksii* seeds germinated in 0, 10, 20, 30, 40 and 50 dS m^{-1} of NaCl (●) and seawater (○) at 10:20, 15:25, 20:30 and 25:35°C temperature regimes under complete darkness.

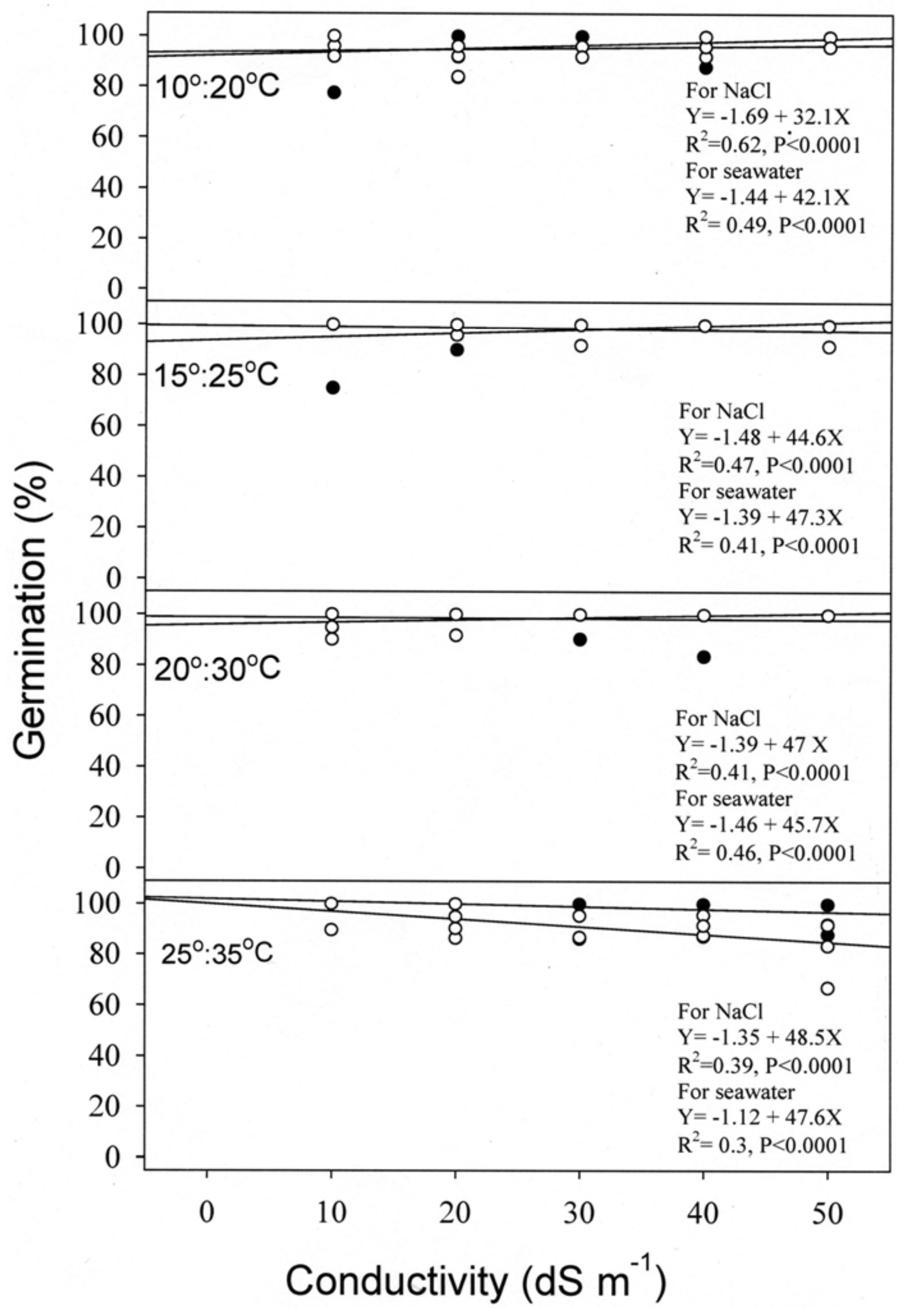

Fig. 4. Regression plot for percentage recovery germination of *Limonium stocksii* seeds germinated in 0, 10, 20, 30, 40 and 50 dS m^{-1} of NaCl (●) and seawater (○) at 10:20, 15:25, 20:30 and 25:35°C temperature regimes under 12-h photoperiod.

Temperature is another of critical factors involved in modulating seed germination responses under saline conditions (Khan, 2003). Optimal germination of *L. stocksii* seeds under both seawater and NaCl was obtained at higher temperatures. Seed germination of *L. stocksii* was inhibited more by seawater as compared to NaCl at all thermoperiods (with the exception of 25°:35°C) and no seed germinated above 30 dS m^{-1} seawater in comparison to 50 dS m^{-1} NaCl solution. Sub-tropical halophytes studied predominantly show optimal germination at 20°:30°C and any further increase and decrease in temperature affected the germination (Khan & Rizvi, 1994; Khan & Ungar, 1996, 1997, 1998, 1999, 2000, 2001; Gulzar & Khan, 2001; Gulzar *et al*., 2001). Temperature interaction with seawater in affecting seed germination is not widely reported. *Salicornia bigellovi* germinated faster and had high germination percentages in seawater when germinated at warmer thermoperiods (River & Weber, 1971).

The enforced dormancy response for halophyte seeds to saline conditions is of selective advantage to plants growing in highly saline habitats because seeds could withstand high salinity stress and provide a viable seed bank for recruitment of new individuals, but seed germination would be limited to periods when soil salinity levels were within the species tolerance limits (Ungar, 1982). However, halophyte seeds differ in their ability to recover from salinity stress and germinate after being exposed to hyper-saline conditions. Recovery of germination of sub-tropical halophytes also showed some variability and they appeared to be less salt tolerant while in the seed bank when compared with temperate desert species. Seeds of *L. stocksii*, recovered completely when transferred to distilled water after a 20-d treatment of seawater. Woodell (1985) has shown that various *Limonium* species viz. *L. bellidifolium, L. humile*, and *L. vulgare* recovered substantially when transferred to distilled water from seawater. Similar recovery response from seawater is reported for *Carpobrotus* spp (Weber & D'Antonio, 1999), *Aster laurentianus* (Houle *et al*., 2001) and *Holcus lanatus* (Watt, 1983). Other sub-tropical coastal halophytes like *Arthrocnemum macrostachyum* showed a substantial recovery at 1000 mmol/L NaCl (Khan & Gul, 1998) while all others recovered in up to 600 mmol/L NaCl. Some halophyte show about 75% recovery (Gulzar & Khan, 2001; Khan, 1999; Gulzar *et al*., 2001) however a number of them showed little or no recovery from high salinity stress (Khan, 1999; Khan & Ungar, 1996; Khan & Ungar, 1998; Khan & Gulzar, 2003).

Light requirements for seed germination of halophytes are quite varied (Baskin & Baskin, 1998) and it ranges from no effect to obligate requirement for germination (Thanos *et al*., 1991; DeVilliers *et al*., 1994; Khan & Rizvi, 1994; Garcia *et al*., 1995; Andrew, 1997; Khan & Ungar, 1997, 1998, 1999). Seeds of *L. stocksii* do not require light under non-saline conditions, however, absence of light caused substantial inhibition of seed germination both in NaCl and seawater. This 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).

Limonium stocksii is moderately salt tolerant among the coastal halophytes found around coastal areas of Pakistan. It produces large number of flowers and seeds which are ready to germinate round the year. Seawater under laboratory conditions clearly prevented more seeds from germination in comparison to NaCl solutions and this inhibition of germination markedly increased in the darkness. Highest inhibition of germination was observed at the lowest thermoperiod, which explains the failure of *L. stocksii* during winter despite of its readiness to germinate in laboratory conditions. Recruitment of *L.*

stocksii does not take place during early summer before monsoon perhaps due to high ambient temperature (around 35^{o}C). Germination after monsoon is primarily due to reduction in soil salinity and ambient temperature. Seeds germinated better at higher temperature, however, increase in seawater concentration simply prevented seeds from germination and they showed higher germination when salinity stress is released. This help seeds of *L. stocksii* to germinate under conditions where most halophytes like to propagate through rhizomes and stolons.

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