IN THE NAME OF ALLAH
THE MOST BENEFICENT MOST MERCIFUL
SALT TOLERANCE AND LIFE HISTORY STRATEGIES OF *LIMONIUM STOCKSII*

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SALT TOLERANCE AND LIFE HISTORY STRATEGIES OF LIMONIUM STOCKSII

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Abstract

The purpose of the research presented in this dissertation was to determine the degree of salt tolerance and the life history characters of a perennial halophyte *Limonium stocksii* (Boiss.) Kuntz. during germination and growth. Seeds of *L. stocksii* were collected from a population located near Hawks Bay at the Arabian sea, Karachi.

Seed germination of *L. stocksii* was studied under controlled conditions in the laboratory at two light levels (12 h light/12 h dark and 24 h dark), two salinity sources (NaCl and seawater) with six concentrations (0, 10, 20, 30, 40, 50 and 60 dS m⁻¹), four temperature regimes (fluctuating night/day temperature regimes of 10/20, 15/25, 20/30, 25/35°C) and eight germination regulating chemicals (kinetin, GA, proline, betain, ethephon, nitrate, thiourea and Na-hypochlorite) using a completely randomized block design. Maximum seed germination of *L. stocksii* was obtained in non-saline control at all temperature regimes, however, 20/30°C appeared to be the optimal temperature where maximum germination was recorded up to 20 dS m⁻¹ NaCl. Seawater inhibited seed germination more than NaCl at low and moderate temperatures but at the highest temperature regime (25/35°C) seed germination in seawater was more than NaCl. Absence of light had little effect under non-saline condition, whereas, addition of salinity highly inhibited seed germination in dark and this inhibition was higher in seawater. Rate of germination was higher in all non-saline solutions and increase in NaCl and seawater salinity decreased the rate of germination. Rate of germination was low in the cooler temperature regime and an increase in temperature gradually increased rate of germination. Higher germination rates in NaCl solution occurred at 20/30°C while in seawater at 25/35°C. Seeds when transferred to distilled water after 20 d of either NaCl or seawater treatments recovered completely. Na-hypochlorite was identified as a successful germination
alleviating compound for salinity and temperature induced dormancy. Kinetin and ethephon partially alleviated salinity enforced seed dormancy in both light and dark conditions while other chemicals had no significant effect.

Salinity tolerance experiments of Limonium stocksii were conducted under ambient conditions. Plants were grown from seeds in plastic pots containing sandy soil and sub-irrigated with 0, 10, 20, 30, 40, 50 and 60 dS m$^{-1}$ solutions of both NaCl and seawater. Plants grown in non-saline control and in 10 dS m$^{-1}$ of both NaCl and seawater showed maximum growth (plant height, fresh and dry weight). Increasing salinity from 20 to 60 dS m$^{-1}$ led to growth inhibition. Tissue water content remained unaffected with increase in both NaCl and seawater salinity. Limonium stocksii accumulated considerable amounts of Na$^+$ and Cl$^-$ in comparison to K$^+$, Ca$^{2+}$ and Mg$^{2+}$ with increasing salinity. Na$^+$ accumulated more in stem than leaf and root. The selective ion transport capacity of stem was higher and was maximum for Na$^+$. 

Seed bank and demography of L. stocksii population were studied for one year. Three transects (300 ft. each) were laid across the population from landward to seaward zones. Soil pH of the study site ranged from 8.1 to 8.5 while soil conductivity varied from 55 dS m$^{-1}$ to 191 dS m$^{-1}$. The over all seed density was highest (5,887 seeds m$^{-2}$) in May and lowest in June. Seed density was highest in the landward zone and declined towards the seaward zone. Little variation was observed in different growth parameters throughout the year. Plant tissue water was lower in zones with high soil salinity. Leaves showed comparatively higher tissue water content from January to March. All growth parameters such as number of branches, root length, stem length and biomass were highest in the intermediate zone. Na$^+$ and Cl$^-$ accumulated more in leaves and stem than in roots while Mg$^{2+}$ and Ca$^{2+}$ were high only in leaves. K$^+$ levels were similar in all parts of L. stocksii. Ion accumulation
(except $\text{Ca}^{2+}$) was more in intermediate zone in comparison to landward and seaward zones.
مختصر

روحیات کا کالعدم و مرمت اور احیاء ضرورت اہم ہے۔ لکھو روپ میں اسلامی اخلاق کا منجیش ہے خاتمہ کا مفتی۔ روپ میں اسلامی اخلاق کے نجیاں خالیہ عرب کا سابل خالیہ کے اجرام بن کر اسٹا رہتا ہے۔

زمین میں نقصان کی کلیت کو پوچھنے کی ادائیگی اور افراد نے ایک مقدمہ پر پیش کیا ہے۔ لکھو روپ

اسلامی اخلاق کی روپ کے بہترہ محسوس کے لیے 1973ء میں 30لہ میں اسی محض اور افراد کے

کمک اور خوبصورتی کی کہانی اور مہم ہے کہ اسے کوئی کوارنیا اور سردار نے فیصلہ کی مہم کی کمیت

کے لیے انٹرکال (0.00244583، 0.0255 اور 0.0253 فیسر) اور انٹوکال (0.002586، 0.03 اور 0.035 فیسر) اور ہمکال (0.002586، 0.03 اور 0.035 فیسر) استعمال ہے گا۔ اس میں ہرار در پہچانے

زیرت پر انٹوکال اسلامی کے اضطرابات کی فضائی محدودیت میں بہترین بہترین دو کسی اسٹریٹ مزدور کے درجے

یہہ میں دوبارہ گارڈی میں 30لہ میں ہزار نواں ہے۔ اسے کارز کے سسٹک کو پیس کی

نہیں بیرون کے کم اور دور میں در جہاں چاہے تر ایک مسکن کا نہیں اسے کوئی کوارنیا اور

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لا ہو نے اسکاکی ای کے پوہل سے افزائش کے دوران تھا کہ برداشت کرنے کی صلاحیت قباحہ ہے۔

کبھی قبائل نے خود اپنی کھبرات کے ساتھ پوہل کر دی کہ ان کی انگریزی اور ان کے گملون سے خبرہ کیا جاتا تھا۔ ان کے زراعت گمیں زیر اپنے بہت سے مزاحمت کے لیے ان کے گملون کو کھلا کر ہوئے (0.00001 ملیمیٹر)۔

اور اور دیکھ کر حقیر (لگائے) نے لیا کہ ان کے گملون کو کھلا کر ہوئے (0.00001 ملیمیٹر)۔

بعض کو افزائش کا ذکر ہے کہ ان کا مقدار (20 دسمبر کے کننی میں) ہے۔ اور اور دیکھ کر حقیر مصیبتی ہے۔

مغرب کے علاقوں کے ذکر ہے۔ سالم کے میٹريک یا ضرورت کے ذکر ہے۔

کیا یہ مطالعہ کے ذکر ہے؟
CHAPTER 1

General Introduction
General Introduction

Soil salinization is one of major problems to achieve the sustainable development of agriculture in the world. Salinity adversely affects soil properties and the availability of essential nutrients to plants with detrimental impact on growth and yield of most crops. This problem exists in more than 100 countries of the world, particularly in arid and semi-arid regions. Salt-affected soils need immediate attention for efficient, low-cost and environmentally acceptable management to improve productivity of a range of crop species that can be grown on such soils. In recent years, the focus has been to a plant-based approach – phytoremediation. It involves the use of certain grasses, shrubs and trees with the potential to establish on salt-affected soils and to improve such degraded environments. Halophytes could play important roles in supplying high-quality fodder, food and fiber during seasonal feed shortages. They can produce fine turf to stabilize saline coastal dunes and ornamental plants to improve the aesthetics of saline ecosystems.

The oceans covering two third of the world’s surface are alternate sources of water. This water contains about 3-4.5 % of salts, mostly sodium chloride and can not be used directly for drinking, however, a large number of plants could grow when irrigated with this water. Lieth (1999) listed 2,750 species of halophytes, many of which could be used as cash-crops but only a few of these could tolerate seawater salinity. Traditional uses of halophytic plants are known from all continents. Since the past few decades, attempts have been initiated for developing systems to utilize treated wastewater and brackish water to irrigate crops and has been convincingly demonstrated to be a distinct possibility (Gul and Khan 2003). Such a system need work on the following aspects with high priority: 1) A quick test for screening of halophytes with economic and / or ecological value tolerating seawater irrigation 2)
Appropriate irrigation systems developed to suit the specific needs of plant species selected and 3) A model system to predict economic feasibility.

Halophytes grow vegetatively when rooted in salty soils and may also produce flowers and seeds (Baskin and Baskin 1998). Coping with salinity needs adaptations at several levels such as whole plant, tissue, cellular, sub-cellular and biochemical. Halophytes as a group have one or more of several physiological adaptations that allow survival in saline environment (Ungar 1991; Khan 2002). These include 1. Salt exclusion (from leaf, root), 2. Elimination (salt glands, bladder hairs and re-translocation), 3. Succulence, 4. Transport and compartmentalization and 5. Compatible solutes (Popp 1995). Of these, most common among halophytes is the ability of osmotic adjustment, allowing for the uptake of water despite high external salt concentration (Hasegawa et al. 2000; Bohnert et al. 2001). This is achieved not only by absorbing and sequestering salts but also by synthesizing organic osmotica (sugar, sugar alcohols, quaternary ammonium compounds etc.) (Popp 1995). It is estimated that over 450 halophytes occur throughout Pakistan in habitats ranging from coastal areas to mountain valleys (Khan 2002). About 108 species from 36 families occupy the coastal regions (Khan and Gul 2002). In general, halophyte diversity is attributed to 1) Habitat variation with respect to kind and concentration of salts, degree and duration of flooding and /or amount of annual precipitation and geographical location (Waisel 1972) and 2) Species variation in life forms and mechanisms of adapting to saline conditions (Zahran 1982).

The success of halophyte populations is greatly dependent on the germination responses of their seeds (Ungar 1995). 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). Germination
typically occurs between July and August, but individual species establish seedlings either early, late or throughout this germination window (Khan and Gul 2002). Maximum salt tolerance of desert species from Karachi coast at germination has been reported for dicotyledonous halophytes such as *Arthrocnemum macrostachyum* (10% germination at 1000 mM NaCl, Khan and Gul 1998), *Cressa cretica* (3% germination at 1000 mM NaCl, Khan 1999) and *Salsola imbricata* (6% germination at 800 mM, Mehrun-Nisa 2002). However a number of species including grasses could germinate between 300 to 500 mM NaCl (Khan 2002; Khan and Gulzar 2003).

Halophytes are exposed to a range of abiotic factors in the field during germination. Species differ in their sensitivity to salinity, light, temperature and moisture in nature (Ungar 1995; Noe and Zedler 2000). Interactions between variables determine optimal conditions for seed germination in halophytes. Halophytic species from Karachi coast show variable responses to temperature during germination under saline conditions. Halophytes such as *Arthrocnemum macrostachyum, Atriplex stocksii, Cressa cretica, Haloxylon stocksii* and *Suaeda fruticosa* showed better germination when exposed to high salinity at lower thermoperiod (15/25°C, Khan 1999). On the other hand, 20/30°C is reported to be the optimal temperature for seed germination of *Halopyrum mucronatum, Urochondra setulosa, Aeluropus lagopoides* and *Salsola imbricata* (Khan and Ungar 2001c; Gulzar et al. 2001; Gulzar and Khan 2001; Mehrun-Nisa 2002).

Light is another factor having profound effect on the seed germination of most species along with salinity and temperature (Agami 1986). Germination of many halophytes occurs at times with an optimal combination of day length, thermoperiod and salinity (Young et al. 1980; Khan and Weber 1986; Naidoo and Naicker 1992; Gutterman et al. 1995; Khan and Ungar 1997a).
Seeds of halophytes usually remain dormant during hypersaline conditions and germinate only after removal of these conditions. Korneef et al. (2002) suggested that seed dormancy is a complex adaptive trait of higher plants influenced by a large number of genes and environmental factors, especially those that affect the growth potential of a seed. Dormancy alleviating compounds such as proline, betaine, fusicoecin, GA₃, kinetin, nitrate, thiourea and ethephon can reduce the inhibitory effects of salinity on germination of halophytes (Bewely and Black 1994; Plyler and Proseus 1996; Khan and Ungar 1997b, 2001). Plant growth regulators are thought to exert their regulatory role by affecting gene expression and/or membrane function (Ho and Hagen 1993). However, the ability of these chemicals to relieve seed dormancy and stimulate germination varies with environmental factors such as temperature, light and from one species to another (Ungar 1991).

Survival of halophytes is largely dependent on the ability of seedlings to establish at their natural habitat and eventually grow to mature plants. High salinity causes hyper-ionic and hyper-osmotic stress effects and the consequence of these can be plant demise (Yeo 1998; Glenn and Brown 1999; Hasegawa et al. 2000). Most commonly stress is caused by high Na⁺ and Cl⁻ concentrations in the soil solution. Altered water status most likely brings about initial growth reduction however; the precise contribution of subsequent processes such as inhibition of cell division, cell expansion and acceleration of cell death has not been well understood (Ungar 1991; Munns 1993; Yeo 1998). Growth of most halophytic plants is inhibited by increase in salinity however; it is stimulated in some halophytes by different levels of salinity (Ungar 1991; Khan and Aziz 1998; Khan et al. 1999, 2000abc). Maximum growth of halophytes from Karachi coast is reported for Cressa cretica at 425 mM NaCl (Khan and Aziz 1998), Arthrocnemum macrostachyum at 400 mM NaCl (Khan and Gul
2002), *Suaeda fruticosa* at 200 mM NaCl (Khan et al. 2000b), *Haloxylon recurvum* at 180 mM NaCl (Khan et al. 2000c) and *Halopyrum mucronatum* at 90 mM NaCl (Khan et al. 1999). Growth was sub-optimal in control and at higher salinities used in the above mentioned experiments.

*Limonium stocksii* (Boiss.) Kuntze is a low branched, salt secreting, perennial, woody shrub from the family Plumbaginaceae. An almost pure population of *L. stocksii* is present at the farthest end of Manora creek near Hawks Bay, Karachi. It is usually found in association with few individuals of *Arthrocnemum macrostachyum, Aeluropus lagopoides, Urochondra setulosa, Suaeda fruticosa, Tamarix* spp. and *Atriplex stocksii*, however, relatively pure populations can also be seen on the drier and rocky areas of Karachi coast. Populations of *L. stocksii* at Hawks Bay face high salinity, water and temperature stresses but still produce a large number of seeds twice a year even in the absence of monsoon rains. These seeds become part of the seed bank where they are exposed to high temperature and salinity stress. Successful germination and establishment of seeds depends on monsoon rains during July and August (Khan and Gul 1998) and on the environmental variables including sand drift (dark environment), hot temperatures, high salinity and scarcity of water (Khan and Ungar 1997c).

The present study was conducted to better understand the eco-physiology of *L. stocksii*. I investigated salt tolerance of its seeds in dark, light and various temperature regimes during germination, the role of different chemicals on seed germination and the degree of salt tolerance and selective ion transport capacity during growth. Seed bank and demography were also studied in the coastal population over a one-year period to assess the temporal plant responses to abiotic stress factors.
Literature Cited


CHAPTER 2

Effect of light, salinity and temperature on the seed germination of *Limonium stocksii*
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 mM NaCl) and temperature regimes (10/20, 15/25, 20/30 & 25/35°C) in both a 12 h dark /12 h light photoperiod and in complete darkness. Highest germination (about 100%) was obtained at 0, 100 and 200 mM NaCl at 20/30°C and further increases in salinity decreased germination. Less than 5% seeds germinated at 500 mM NaCl at the optimal temperature. Germination was substantially inhibited by the lowest (10/20°C) and highest (25/35°C) temperature regime at all salinity levels except control and few seeds germinated above 200mM NaCl. Germination rate was highest at 20/30°C and lowest 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.
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 fresh water similar to glycophytes, but differ in their ability to germinate at higher salinities (Ungar 1995).

Perennial halophytes have variable salinity tolerance (Khan 2002) possibly due to factors such as light, temperature and moisture stress (Mahmoud et al. 1983; Baskin and Baskin 1998; Noe and Zedler 2000). Maximum salt tolerance at germination of sub-tropical species from the Karachi coast in Pakistan has been reported for *Arthrocnemum macrostachyum* (10% germination at 1000 mM NaCl, Khan and Gul 1998), *Cressa cretica* (3% germination at 1000 mM NaCl, Khan 1999) and *Salsola imbricata* (6% germination at 800 mM, Mehrun-Nisa 2002). However, a number of species could not germinate at NaCl concentrations higher than 400 mM (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 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 inhibited seed germination of *Sporobolus indicus* (L.) R. Br. (Andrews 1997) and *Triglochin maritima* L. (Khan and Ungar 1999) and 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), while seeds of *Atriplex stocksii* Boiss (Khan and Rizvi 1994) and *Suaeda fruticosa* (Khan and Ungar 1998) were not inhibited by the absence of light.

Most seeds are located near the soil surface, where salt concentration increases due to 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 during exposure to high salinity and they initiate germination when salinity is reduced (Keiffler 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 from the family Plumbaginaceae. It is distributed in high coastal marshes and rocky grounds near sea shore 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 few individuals of *Arthrocnemum macrostachyum, Aeluropus lagopoides, Atriplex stocksii, Suaeda fruticosa, Tamarix spp.* and *Urochondra setulosa*. Possible sources of moisture are the monsoon rains and oceanic seepage. Monsoon period lasts from June 15 to September 15. During this time, high tides increase oceanic seepage while rainfall (< 220 mm yr⁻¹) usually occurs during July and August. Storms are rarely reported from the Karachi coast.
Limonium stocksii flowers twice a year in June and November and a large number of seeds are produced in August and January. After dispersal seeds become a part of the seed bank and germinate after monsoon rains. The average ambient temperatures during monsoon are about 20°C at night and 30°C during the day. Limonium stocksii is a highly salt tolerant plant, which grows in the coastal areas under high salinity and is foraged by camels and goats. The economic potential of this species as a fodder crop for coastal saline areas and ornamental plant for beautification of beaches is great. Growing this species in the native habitat would preserve its population, would substantially reduce the grazing pressure and beautify coastal areas with plants irrigated with seawater.

The aim of the present study was to determine the seed germination, rate of germination and recovery responses of Limonium stocksii under various salinity, temperature and light conditions.
Materials and Methods

Seeds of *Limonium stocksii* were collected in February 2000, from a salt flat at the upper end of Manora Creek near Hawks Bay, Karachi, Pakistan (24°52'-647'N and 66°53'-321'E). Seeds were separated from the inflorescence, surface sterilized using sodium hypochlorite (0.52%) for one minute, thoroughly rinsed with distilled water and air-dried. Germination was carried out in 5 cm diameter tight fitting plastic Petri plates with 5 mL of test solution prepared in distilled and deionized water. Each dish was placed in a 10 cm-diameter plastic Petri plates as an added precaution against the loss of water by evaporation. Four replicates of 25 seeds each were used for each treatment. Seeds with radicles were considered to be germinated.

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 day temperatures (20°, 25°, 30°, 35°C) coincided with 12 h light period (Sylvania cool white fluorescent lamps, 25μmol m⁻² s⁻¹, 400-750 nm) and night temperatures (10°, 15°, 20°, 25°C) coincided with 12 h dark period. Seeds were germinated at six salinities (0, 100, 200, 300, 400 and 500 mM NaCl) in incubators after preliminary tests for the range of salinity tolerance. Percentage germination was recorded on every alternate days 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 at the same corresponding temperatures.

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

Rate of germination was estimated by using a modified Timson’s index of germination velocity, \( \Sigma G/t \), where \( G \) is the percentage of seed germination at 2 d
intervals, and \( t \) is the total germination period (Timson 1965; Khan and 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. The percentage recovery was determined by the formula \( \frac{(a-b)}{(c-b)} \times 100 \), 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 arcsine transformed before statistical analysis. These data were analyzed using SPSS for windows release 11 (SPSS 2002). A two way ANOVA was also used to demonstrate the interaction between various factors in affecting the rate, recovery and percentage germination. A Bonferroni test was used \( (P< 0.05) \) to determine significant differences between means of germination percentages among salinity treatments under various light and temperature regimes (Norusis 1994).
Results

Significant ($P < 0.0001$) two-way interactions were found for salinity, and temperature in affecting germination percentage, rate of germination and percent recovery of *Limonium stocksitii* seeds (Table 2.1).

Maximum seed germination in light was obtained in non-saline control at all temperature regimes (Fig. 2.1). Exposure to different levels of salinity resulted in a gradual decrease in germination percentage and this reduction varied with change in temperature regimes (Fig. 2.1). Best germination under saline condition were observed at $20/30^\circ$C where germination at 100 and 200 mM NaCl was not significantly different from distilled water control and a further increase in salinity decreased germination with only 5% seed germinating at 500 mM NaCl. Seed germination at 15/25°C was comparatively less than the germination at optimal temperature regime. Exposure to 10/20°C and 25/35°C temperature regimes substantially inhibited germination in all salinity treatments (Fig. 2.1). Only 25% seeds could germinate at 100 mM NaCl in the lowest temperature regime (Fig. 2.1).

Temperature also affected the velocity of germination under both saline and non-saline conditions (Fig. 2.2). Maximum germination in distilled water control was obtained after 2 d at all temperature regimes except for 10/20°C, where it took 14 d (Fig. 2.2). In saline solutions, maximum germination occurred in 6 to 18 d. At the optimal temperature regime, germination at lower salinity (100 & 200 mM NaCl) peaked in 6 d and in about 10 d at higher salinity treatments. However, seed germination peaked at 10 days for all salt concentrations at 15/25°C (Fig. 2.2).

Seed germination in distilled water control was not affected by darkness at all temperature regimes with the exception of 10/20°C, where germination percentage
Table 2.1. Two-way ANOVA of characteristics of *Limonium stocksii* due to salinity (S), temperature (T) and their interactions. Numbers indicate F values (***) = \( P < 0.001 \).

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>S</th>
<th>T</th>
<th>S x T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Germination</td>
<td>426.7***</td>
<td>127.7***</td>
<td>29.2***</td>
</tr>
<tr>
<td>Rate of germination</td>
<td>668.6***</td>
<td>229.5***</td>
<td>42.6***</td>
</tr>
<tr>
<td>Percent Recovery</td>
<td>359.6***</td>
<td>80.8***</td>
<td>23.7***</td>
</tr>
</tbody>
</table>
Fig. 2.1. Mean final germination percentage of *Limonium stocksii* in various salinity, temperature, light and dark conditions. Bars represent means (±S.E.). Values at each thermoperiod and light levels having same letters are not significantly different (*P* < 0.05) from the control (Bonferroni test).
Fig. 2.2. Cumulative mean germination percentage of *Limonium stocksii* seeds over time in 0, 100, 200, 300, 400 and 500 mM NaCl and in light/dark (12h/12h) photoperiod. Different symbols represent means (+S.E.).
was reduced to 50% in control (Fig. 2.1). Application of salinity under complete darkness greatly reduced germination percentages and the level of inhibition varied with temperature. About 2% seeds could germinate in 100 mM NaCl at the lowest (10/20°C) and highest (25/35°C) temperature regimes. Maximum germination (74%) was obtained at 20/30°C in 100 mM NaCl and it decreased to just 8% in 200 mM NaCl. At 15/25°C, 62% seeds germinated at 100 mM NaCl and 16% at 200 mM NaCl (Fig. 2.1).

Rate of germination was highest in non-saline controls except at 10/20°C and addition of NaCl slowed the rate of germination (Fig. 2.3). Temperature also influenced the rate of germination. Lower and higher temperatures showed a slower rate of germination from 100 to 300 mM NaCl than 20/30°C and 15/25°C (Fig. 2.3).

When light treated un-germinated seeds from salt treatments were transferred to distilled water, they recovered completely at all salinity and temperature regimes (Fig. 2.4).
Fig. 2.3. Rate of germination of *Limonium stocksii* seeds under various salinities and thermoperiods. Lines represent means (±S.E.).
Fig. 2.4. Mean recovery percent germination for *L. stocksii* in distilled water at different temperature regimes: 10/20°C (black bar); 15/25°C (horizontal bar); 20/30°C (vertical bar) and 25/35°C (diagonal bar) in various salinity treatments. Bars represent means (±S.E.).
Discussion

Plants native to subtropical maritime desert of Karachi are exposed to various levels of moisture and salinity stress due to an unpredictable monsoon period (Khan 2002). These conditions lead to differential life history strategies in desert 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 such as *Arthrocnemum macrostachyum* (1000 mM NaCl, Khan and Gul 1998), *Cressa cretica* (1000 mM NaCl, Khan 1999), *Salsola imbricata* (800 mM NaCl, Khan unpublished data), *Suaeda fruticosa* (500 mM NaCl, Khan and Ungar 1998), *Atriplex stocksii* (300 mM NaCl, Khan and Rizvi 1994) and some grasses like *Aeluropus lagopoides* (500 mM NaCl, Gulzar and Khan 2001), *Sporobolus iselados* (500 mM NaCl, Khan and Gulzar 2003), *Urochondra setulosa* (500 mM NaCl, Gulzar et al. 2001) and *Halopyrum mucronatum* (300 mM NaCl, Khan and Ungar 2001). *Limonium stocksii* is a moderately salt tolerant halophyte at germination when compared to other local halophytic species but it has the ability to germinate at salinity levels of up to 500 mM NaCl approaching seawater salinity (600 mM 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*) (Sheikh and Mahmood 1986; Khan and Gul 1998; Khan 1999). Seed germination of *L. stocksii* was also influenced by temperature. The optimal temperature for germination was 20/30°C and any increase or decrease in temperature...
regime inhibited germination. This inhibition progressively increased with salinity. Recruitment of *L. stocksii* in natural conditions through germination appears to take place only after monsoon rains. Germination of halophyte seeds in sub-tropical, coastal and inland salt marshes usually occurs after monsoon rains, which causes 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 germination percentage (West and Taylor 1981; Dudeck and Peacock 1985; Marcar 1987). Very rapid germination was reported for *Haloxylon recurvum* and *H. salicornicum* (Sharma and Sen 1989) and for *Limonium axillare* (Mahmoud et al. 1983) and it was considered 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 mM NaCl at 20/30 °C, a temperature regime similar to the average early summer period in Karachi.

*Limonium stocksii* seeds appear to have considerable tolerance to high salinity and temperature stress before germination. Seeds germinated within two days when transferred to non-saline medium from various salinity and temperature treatments. 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 of *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 moist 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 for about seven. 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 (Zia and Khan unpublished data). Although seeds could germinate only up to 500 mM NaCl, much lower salinity 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 like Arthrocnemum macrosiachyum and Sporobolus icladas lose viability when exposed to high temperature and salinity stress (Zia and Khan unpublished data). However, Suaeda fruticosa seeds maintain seeds viability similar to that of L. stocksii. Survival of seeds under extreme conditions provide this species a strategy for a successful recruitment in such a harsh environment. Limonium stocksii is a potential candidate to be used as an ornamental and fodder crop in coastal areas where only brackish or seawater is available.
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CHAPTER 3

Seed germination of *Limonium stocksii* under saline conditions
Abstract

*Limonium stocksii* (Boiss.) O. Kuntze (Plumbaginaceae), a salt secreting, perennial, shrub, is widely distributed in the intertidal zones of Karachi, Pakistan. Seeds were germinated both under a 12 h light/dark photoperiod and complete darkness in 0, 10, 20, 30, 40 and 50 dS m\(^{-1}\) seawater and sodium chloride at different temperature regimes (10/20, 15/25, 20/30 and 25/35°C). Seed germination decreased with an increase in salinity and few seeds germinated above 30 dS m\(^{-1}\) in seawater (25/35°C). Seawater appeared to inhibit seed germination more than NaCl at low and moderate temperatures, but at the highest temperature regime (25/35°C) seed germination in seawater was greater than NaCl. Absence of light had little effect under non-saline conditions, however, addition of salinity inhibited seed germination and this inhibition was higher in seawater. Seeds, when transferred to distilled water after 20 d of exposure to NaCl or seawater treatments recovered completely.
Introduction

*Limonium stocksii* (Boiss.) *Kuntze* is distributed throughout coastal Sindh and Balochistan in Pakistan (Khan and Gul 2002). Its habitat remains more or less wet all year long due to seepage of seawater. A relatively pure population 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 in soil. *Limonium stocksii* grows in association with *Arthrocnemum macrostachyum*, *Aeluropus lagopoides*, *Tamarix* spp. and *Suaeda fruticosa*. Unlike other co-occurring halophytes, recruitment of *L. stocksii* occurs primarily by seed germination after monsoon rains (Khan 2002). High temperature and salinity stress under natural conditions cause the death of other halophytic seeds except *Suaeda fruticosa* (Khan 2002). Wide spread recruitment of *L. stocksii* seeds after monsoon rains indicates the little effect of seawater on their viability.

Halophytes vary considerably in their level of salt tolerance (Khan 2002) which varies with stages of life cycle. Salt tolerance could be expressed as: 1) The ability of seeds to tolerate high salinity with out loosing viability while stored in the soil (seed bank), 2) The ability of seeds to germinate at high salinities and 3) The ability of a plant to complete its life cycle at high salinities (Khan and Gul 2002). Salt secreting halophytes germinating above seawater salinity include *Atriplex lacinata*, *A. rosea*, *A. tornabeni*, *Cressa cretica*, *Limonium vulgare* and *Tamarix pentandra*, (Binet 1965; Ungar 1967; Ignaciuk and Lee 1980; Woodell 1985; Khan 1991). Most salt secreting halophytes such as *Atriplex canescens*, *A. lentiformis*, *A. nummularia*, *A. polycarpa*, *A. prostrata* (triangularis) and *A. stocksii*, germinate at NaCl concentrations ranging from 0.34 to 0.52 M NaCl (Ignaciuk and Lee 1980; Khan and Ungar 1984; Uchiyama 1987; Mikheil et al. 1992; Katembe et al. 1998). While others
(Atriplex glabrifolia, 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).

Different abiotic factors such as temperature, photoperiod, soil salinity and moisture affect germination of halophytes (Noe and Zedler 2000; Khan 2002). However, the effect of soil salinity seems to dominate all other factors (Keiffer and Ungar 1997; Baskin and Baskin 1998). Experiments to test the effects of salinity on germination of halophytes have typically employed NaCl. Such tests may not be relevant to the field conditions because the source of moisture for the coastal plants is usually seawater, which is composed of different cations (Na⁺, Mg²⁺, Ca²⁺, K⁺ and Sr²⁺) and anions (Cl⁻, SO₄²⁻, Br⁻, F⁻, HCO₃⁻, H₂BO₃⁻), however, the concentrations of Na⁺ and Cl⁻ ions are higher (86 %). Weber and Antonio (1999) investigated the germination responses of three taxa of Carpobrotus in seawater and concluded that the rate and percent 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 and Iyengar 1985) and for Crithmum maritimum (Okusanya 1977). Germination of Salicornia bigelovii was inhibited at full strength seawater (4.02 %) (Rivers and Weber 1971) and was attributed to an interaction of seawater and temperature. Seed germination of Salvadora persica (Joshi et al. 1995) and Salicornia brachiata (Joshi and Iyengar 1982) was inhibited more by seawater in comparison to chlorides of Na⁺, K⁺ and Mg²⁺. They attributed this effect to other salts besides 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 and Gul 2001). Sub-tropical halophytes predominantly showed optimal seed germination at 20/30°C and any further increase or decrease in temperature affected the seed germination (Khan and Rizvi 1994; Khan and Ungar 1996; 1997; 1998; 1999; 2000; 2001; Gulzar and Khan 2001; Gulzar et al. 2001). Differential effects of temperature and seawater on seed germination are not widely reported. Rivers and Weber (1971) showed a slow but high germination at low temperature regimes in salinities tested and a faster rate and relatively low germination at high temperature regimes in seeds of temperate halophytes.

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

The present study investigates the effects of seawater and on the germination and recovery of *L. stocksii* seeds under various temperature and light regimes.
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, Pakistan (24°45'-25°N and 66°45'-67°E). Seeds were separated from inflorescence, surface sterilized using clorox (0.52%) for one minute, thoroughly rinsed with distilled water and allowed to air dry. Seeds were stored at the room temperature and germination experiments were initiated in April 2000. Germination was carried out in 5 cm diameter tight fitting plastic Petri dishes with 5 mL of test solution to investigate the effect of salinity, light and temperature on seeds of *L. stocksii* immersed NaCl and seawater. Germination was carried out in different dilutions (0, 10, 20, 30, 40 and 50 dS m⁻¹) of NaCl and seawater separately. The electrical conductivity for both salt solutions was maintained at the desired level with the help of a conductivity meter (CDM83, Radiometer). Four replicates of 25 seeds each were used for all treatments. Seeds with radicles were considered to have germinated.

To determine the effect of temperature, seeds were germinated in four alternating temperature regimes of 10/20, 15/25, 20/30 and 25/35°C in incubators (Percival Scientific). A 24 h cycle was used where day 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 night 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 incubators 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. Ungerminated 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 at the same corresponding temperatures. 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 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 the percentage of seed germination at 2 d intervals and \(t\) is the total germination period (Timson 1965; Khan and 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 were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analyzed using SPSS for windows release 11 (SPSS 2002). A multiple linear regression analysis was performed to compare seed germination and recovery response in NaCl and seawater in different light conditions and alternating temperature regimes. A Bonferroni post hoc test was used to determine significant differences \((P < 0.05)\) between means. Three-way and two-way ANOVA test were also performed to determine significance of various factors in affecting germination percentage and rate.
Results

A significant three-way interaction was found for salinity, light and temperature on seed germination of *Linonimum stocksii* (*P* < 0.0001) (Table 3.1). Highest germination was obtained in distilled water at all temperature regimes and at 10 dS m⁻¹ seawater except for 10/20°C thermoperiod where no seeds germinated in any salinity treatment except for the control (Fig. 3.1). Seed germination decreased with an increase in salinity and few seeds germinated at 50 dS m⁻¹ seawater in all temperature regimes (Fig. 3.1). The germination of seeds under saline conditions increased with an increase in temperature and maximum germination percentages were found for all salinity treatments at 25/35°C. Linear regression showed a promotion of germination with increasing temperature with $R^2$ values from 0.47 to 0.90 in seawater (Fig. 3.2). Complete darkness inhibited seed germination more than the light (Fig. 3.3).

Seed germination was inhibited more by seawater than by NaCl at cooler temperatures both in light and dark (Figs. 3.2 & 3.3). However, at warmer thermoperiod (25/35°C) seed germination in seawater was higher in comparison to NaCl solution (Fig. 3.2). A linear regression showed a promotion of germination in NaCl with $R^2$ values from 0.61 to 0.87 at different temperatures (Fig. 3.2). Seed germination under dark treatment was inhibited more by seawater than NaCl in all temperature regimes except for (25/35°C) (Fig. 3.3). However, light and dark treated seeds showed similar trends in affecting germination at different temperatures (Fig. 3.3). Germination showed a strong negative correlation with both seawater and NaCl salinities in both light and dark conditions (Figs. 3.2 & 3.3).
Table 3.1. Three-way ANOVA of germination percentage for salinity (S), temperature (T), light (L) and their interactions. Numbers are F-values significant at $P < 0.0001$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>NaCl</th>
<th>Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (T)</td>
<td>145.80***</td>
<td>61.40***</td>
</tr>
<tr>
<td>Light (L)</td>
<td>279.10***</td>
<td>365.40***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>661.20***</td>
<td>1070.50***</td>
</tr>
<tr>
<td>T X L</td>
<td>12.30***</td>
<td>0.3964***</td>
</tr>
<tr>
<td>L X S</td>
<td>40.90***</td>
<td>105.93***</td>
</tr>
<tr>
<td>T X S</td>
<td>26.81***</td>
<td>21.32***</td>
</tr>
<tr>
<td>T X L X S</td>
<td>18.40***</td>
<td>17.30***</td>
</tr>
</tbody>
</table>
Fig. 3.1. Seed germination mean (±S.E.) of *Limonium stocksii* in

- 0 d H₂O (control),
- 10,
- 20,
- 30,
- 40 and
- 50 dS m⁻¹ of seawater at 10/20, 15/25, 20/30 and 25/35°C light/dark (12h/12h) thermoperiod.
Fig. 3.2. Regression plot for mean final germination percentage of *Limonium stocksii* seeds germinated in non-saline control, 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. 3.3. Regression plot for mean final germination percentage of *Limonium stocksii* seeds germinated in d H$_2$O control 10, 20, 30, 40 and 50 dSm$^{-1}$ of NaCl (●) and seawater (○) at 10/20, 15/25, 20/30 and 25/35°C temperature regimes under complete darkness.
A two-way ANOVA for rate of germination for seawater showed significant differences with seawater salinity ($F = 376, P < 0.0001$), change in temperature ($F = 126, P < 0.0001$) and their interaction ($F = 20, P < 0.0001$). Rate of germination was higher in all non-saline solutions and decreased with an increase in NaCl and seawater salinity (Table 3.2). Rate of germination was low in 10/20°C and increased with increase in temperature. Optimal rate of germination in NaCl 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 3.2).

Un-germinated seeds from both NaCl and seawater solutions when transferred to distilled water recovered completely. A linear regression for recovery of germination at various temperature and salinity concentrations gave $R^2$ values from 0.62 to 0.84 for NaCl and from 0.49 to 0.84 for seawater (Fig. 3.4).
Table 3.2. Rate of germination (mean ± S.E.) at different temperature regimes in both seawater and NaCl solutions using modified Timson’s index (Timson 1965; Khan and Ungar 1984).

<table>
<thead>
<tr>
<th>EC (dS m⁻¹)</th>
<th>NaCl</th>
<th>Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10/20°C</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>32.30 ± 01.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.35 ± 00.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>06.10 ± 01.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>02.15 ± 00.32&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>01.95 ± 00.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>00.00 ± 00.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>00.00 ± 00.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>00.45 ± 00.45&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
<td>00.60 ± 00.34&lt;sup&gt;e&lt;/sup&gt;</td>
<td>00.00 ± 00.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>00.00 ± 00.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>00.00 ± 00.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>15/25°C</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>49.10 ± 00.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.20 ± 00.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>41.75 ± 02.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.95 ± 01.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>30.65 ± 03.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>03.05 ± 00.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>06.85 ± 01.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>00.75 ± 00.43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>40</td>
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<td>00.00 ± 00.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>00.10 ± 00.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>00.20 ± 00.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>20/30°C</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>49.80 ± 00.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.50 ± 00.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>47.10 ± 00.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.60 ± 02.79&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>20</td>
<td>42.95 ± 00.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>07.00 ± 03.18&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<tr>
<td>40</td>
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<td>00.00 ± 00.00&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>50</td>
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</tr>
<tr>
<td></td>
<td>25/35°C</td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>49.80 ± 00.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>33.70 ± 02.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.45 ± 01.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>20</td>
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<td>35.05 ± 04.83&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
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<td>21.00 ± 01.63&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>40</td>
<td>00.40 ± 00.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>07.95 ± 00.95&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>50</td>
<td>00.00 ± 00.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>03.60 ± 00.29&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: Values in both columns for every temperature regime having same letters are not significantly different at $P > 0.05$, Bonferroni test.
Fig. 3.4. Regression plot for percentage recovery germination of *Limonium stocksii* seeds germinated in d H₂O (control), 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.
Discussion

Coastal vegetation of Pakistan is primarily composed of perennial grasses and shrubs with few annuals (Khan and Gul 2001). Seeds, after dispersal, are typically subjected to high salinity and temperature stress and successful species are able to tolerate warm hypersaline conditions and retain their viability. However, most species do not recruit through seeds, and seed banks for many of these species are transient (Khan 1993). *Limonium stocksii*, *Suaeda fruticosa* and *Salsola imbricata* have persistent seed banks and a large number of seedlings were recorded after monsoon rains (Khan 2002). In the laboratory, seeds of *L. stocksii* germinated in both NaCl and seawater up to 50 dS m\(^{-1}\) and seawater was more inhibitory to seed germination at low to moderate temperature regimes. However, seed germination was inhibited more by NaCl at the warmest temperature.

Little information is available on the effect of seawater on the germination of halophytes (Rivers and Weber 1971; Joshi and Iyengar 1985; Woodell 1985; Joshi et al. 1995; McMillan 1998; Houle et al. 2001) and on the relative tolerance to seawater and NaCl solutions during seed germination (Tirmizi et al. 1993; Joshi et al. 1995). However, seeds that were able to germinate in NaCl at concentrations above seawater include *Arthrocnemum macrosostachyum*, *Cressa cretica* and *Salsola imbricata* (Khan 1991; Khan and Gul 1998; Mehrun-Nisa and Khan unpublished data), while others like *Aeluropus lagopoides*, *Haloxylon stocksii* (*recurvum*), *Sporobolus ioclados*, *Suaeda fruticosa* and *Urochondra setulosa* could germinate in up to 500 mM NaCl (Khan and Ungar 1996, 1998; Gulzar and Khan 2001, 2002; Gulzar et al. 2001). *Atriplex stocksii* and *Zygophyllum simplex* could only germinate in up to about 250 mM NaCl (Khan and Rizvi 1994; Khan and Ungar 1996). Seed germination of *Salvadora persica* (Joshi et al. 1995) and *Salicornia brachiata* (Joshi and Iyengar 1995) was also found to be more tolerant to NaCl than other species.
1982) was inhibited more by seawater in comparison to different chlorides of \( \text{Na}^+, \text{K}^+ \) and \( \text{Mg}^{++} \). They attributed this effect to seawater composition, which includes a combination of different salts with high concentration of \( \text{NaCl} \) (Joshi et al. 1995). However, Tirmizi et al. (1993) found that \( \text{NaCl} \) inhibited the germination of \( \text{Hipophae rhamnoides} \) more than seawater. Khan (unpublished data) compared the germination of various halophyte seeds in both seawater and \( \text{NaCl} \) and found the effect to be species specific.

Temperature is another critical factor involved in modulating seed germination responses under saline conditions (Khan 2002). Optimal germination of \( \text{L. stocksii} \) seeds under both seawater and \( \text{NaCl} \) was obtained at higher temperatures. Seed germination of \( \text{L. stocksii} \) was inhibited more by seawater as compared to \( \text{NaCl} \) at all thermoperiods (except for 25/35°C) and no seeds germinated above 30 dS m\(^{-1}\) seawater in comparison to 50 dS m\(^{-1}\) \( \text{NaCl} \) solution. Subtropical halophytes showed optimal germination at 20/30°C and any further increase or decrease in temperature negatively affected the germination (Khan and Rizvi 1994; Khan and Ungar 1996, 1997, 1998, 1999, 2000, 2001; Gulzar and Khan 2001; Gulzar et al. 2001). Interactions of temperature and seawater on seed germination are not widely reported. \( \text{Salicornia bigellevii} \) germinated faster and had high germination percentages in seawater when exposed to warmer thermoperiods (Rivers and 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 can 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 subtropical 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*) showed that *Limonium bellidifolium*, *L. humile* and *L. vulgare* recovered substantially when transferred from seawater to distilled water treatments. Similar recovery response from seawater is reported for *Carpobrotus* spp. (*Weber and Antonio 1999*), *Aster lauritentianus* (*Houle et al. 2001*) and *Holcus lanatus* (*Watt 1983*). Sub-tropical coastal halophytes like *Arthrocnemum macrostachyum* showed substantial recovery at 1000 mM NaCl (*Khan and Gul 1998*) while all others recovered in up to 600 mM NaCl. *Aeluropus lagopoides* (*Gulzar and Khan 2001*), *Atriplex stocksii* (*Khan 1999*) and *Urochondra setulosa* (*Gulzar et al. 2001*) showed about 75% recovery at 600 mM NaCl. While *Cressa cretica* (*Khan 1999*), *Haloxylon stocksii* (*Khan and Ungar 1996*), *Suaeda fruticosa* (*Khan and Ungar 1998*) and *Sporobolus ioclados* (*Khan and Gulzar 2003*) showed poor recovery responses.

Light requirements for seed germination of halophytes are quite varied (*Baskin and Baskin 1998*) and range from no effect to an obligate requirement for germination (*Thanos et al. 1991*; *DeVilliers et al. 1994*; *Khan and Rizvi 1994*; *Garcia et al. 1995*; *Andrews 1997*; *Khan and 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 treatments. This increased germination inhibition by seawater and NaCl could be due to the inactivity of Pfr in darkness under saline condition, which regulates several genes coding both for enzymes and structural proteins (*Bewley and Black 1994*).
*Limonium stocksii* is moderately salt tolerant among the coastal halophytes of Pakistan. It produces large numbers of showy flowers and viable seeds. Under laboratory conditions, seawater clearly prevented more seed germination as compared to NaCl solutions, particularly in complete darkness. Highest inhibition of seed germination was observed at the lowest thermoperiod, which explains the absence of *L. stocksii* seedlings in the field during winter despite the production of viable seeds. Recruitment of *L. stocksii* does not take place before monsoon, perhaps due to high ambient temperatures (around 40°C) and salinity. Germination after monsoon may primarily be due to a reduction in soil salinity and ambient temperatures. The high recovery of germination indicates that the seeds could remain viable under the higher temperatures and at increasing seawater concentrations. This enforced dormancy was immediately released when soil salinity was reduced. *Limonium stocksii* could germinate in conditions where most halophytes propagate vegetatively through rhizomes and stolons.
Literature Cited


Khan, M.A. 1999. Comparative influence of salinity and temperature on the germination of subtropical halophytes. In *Halophyte Uses in Different*


SPSS. 2002. SPSS 11 for Windows Update. SPSS Inc., USA.


CHAPTER 4

Effect of germination regulating chemicals in alleviating salinity induced germination inhibition of *Limonium stocksii* seeds
Abstract

Effect of different chemicals: kinetin (0.05 mM), ethephon (5 μM), GA₃ (0.3 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (10 mM) and thiourea (5 mM) was investigated in alleviating salinity enforced dormancy in *Limonium stocksii* seeds. Six salinity regimes (0-500 mM NaCl) were used in 12 h photoperiod and in complete darkness to study the effect of different germination regulating chemicals in inducing germination under saline condition. Only kinetin and ethephon successfully alleviated salinity enforced seed dormancy of *L. stocksii*. Kinetin was more successful than ethephon. Seed germination was substantially inhibited by salinity when germinated under complete darkness. Kinetin and ethephon appeared to be the only chemicals improving seed germination in complete darkness. All other germination regulating chemicals had no effect at low salinity and inhibited germination at high salinity in both light and dark.
Introduction

Seed dormancy is an adaptive mechanism to promote plant survival by distributing germination in both time and space (Lorenzo et al. 1999). It is influenced by a large number of genes and environmental factors, especially those that affect the growth potential of a seed (Korneef et al. 2002). In case of halophytes, seed germination is usually prevented by physiological causes (Khan 1996) induced by availability of less than optimal environmental conditions like water, light, oxygen, temperature and soil salinity (Corbineau and Come 1995; Ungar 1995). Exposure to osmotic stress causes plants to exhibit different morphological and developmental changes at the molecular, cellular and organism levels (Yeo 1998; Bohnert et al. 1999; Hasegawa et al. 2000) during the life cycle. These changes are due to the imbalance in growth regulators causing an increased level of endogenous ABA and other germination inhibitors and a decrease in endogenous growth promoters (Bewely and Black 1994).

Kabar (1987) suggested that endogenous hormone level is affected by many environmental stresses, however, external application of appropriate growth regulator optimizes physical metabolic conditions for germination. The role of various germination regulating chemicals such as proline, betaine, gibberellin, kinetin, nitrate, thiourea and ethephon in reducing the inhibitory effects of salinity on germination is reported for several halophytes (Kabar 1987; Bewely and Black 1994; Plyler and Proseus 1996; Gul and Weber 1998; Khan and Ungar 1997, 2000, 2001abc, 2002). Different regulatory roles are suggested for these chemicals in breaking seed dormancy in halophytes. They are thought to alleviate salinity effects on the germination by: 1) affecting gene expression and/or membrane function (Ho and Hagen 1993), 2) substituting for light and temperature (Khan and Weber 1986;
Bewely and Black 1994; Corbineau and Come 1995; Sutcliffe and Whitehead 1995), 3) acting as an osmoregulator or osmoprotectants of proteins in the cytoplasm (Poljakoff-Mayber et al. 1994; Gorham 1995) and 4) counteracting the effect of reduced promoter (cytokinins and gibberellins) and increased inhibitor substances, such as abscisic acid in seeds under high salinity (Kabar and Baltepe 1990). However, the ability of these chemicals to relieve seed dormancy and to stimulate germination varies with environmental factors such as temperature and light and from one species to another (Khan 2002).

Seeds of Limonium stocksii (Plumbaginaceae), a perennial halophytic shrub distributed in coastal areas at Karachi, are subjected to high salinity and temperature stress after dispersal. However, seeds germinate readily after monsoon rains under natural conditions. Salinity tolerance of seeds under laboratory conditions decreased sharply above 300 mM NaCl and only few seeds germinated at 500 mM NaCl (Zia and Khan 2004). The inhibition of germination may be due to an imbalance of critical germination regulating chemicals. This study was conducted to determine the role of germination regulating chemicals in alleviating salinity induced dormancy from seeds of L. stocksii.
Materials and Methods

Seeds of Limonium stocksii were collected from a salt flat at the upper end of Manora creek near Hawks Bay, Karachi (24°52-647′N and 66°53-321′E). Seeds were separated from the inflorescence, surface sterilized using sodium hypochlorite (0.52%) for one minute, thorough rinsed with distilled water and air-dried. Germination was carried out in 5 cm diameter tight fitting plastic Petri dishes with 5 mL of test solution. Four replicates of 25 seeds each were used for each treatment. Seeds with radicles were considered to be germinated.

Seeds were germinated in an incubator (Percival Scientific, USA) at a day/night temperature of 30/20°C with a 12 h photoperiod (Sylvania cool white fluorescent lamps, irradiance of 25μmol m⁻² s⁻¹, 400-700 nm) and in complete darkness in six salinity concentrations (0, 100, 200, 300, 400 and 500 mM NaCl). Dormancy relieving compounds: kinetin (0.05 mM), ethephon (5 μM), GA₃ (0.3 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (10 mM) and thiourea (5 mM) were used. The concentrations used were based on optimal level reported for promoting seed germination in most other species (Ungar 1998). Percent germination was recorded on alternate days for 20 d. Similarly seeds were germinated in complete darkness by placing Petri dishes in black plastic bags for 20 d and the germination was recorded after 20 d.

Rate of germination was estimated by using modified Timson's index of germination velocity, Σ G/t, where G is percentage of seed germinated at 2 d intervals, and t is total germination period (Timson 1965; Khan and 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 arcsine transformed before statistical analysis using SPSS for Windows release 11 (SPSS, 2002). Both two-way and three-way ANOVA tests were performed to determine significant effects of various factors and their interactions on rate and percentage germination. A Bonferroni test was used to determine significant differences between means.
Results

A three-way ANOVA of percentage germination of *L. stocksii* indicated significant (*P < 0.0001*) effects of salinity, light, chemicals and their interactions (Table 4.1). Best seed germination was obtained in distilled water control and in 100 mM NaCl under 12 h photoperiod. Further increase in salinity gradually decreased seed germination and only a few seeds could germinate in up to 500 mM NaCl (Fig. 4.1).

Application of kinetin and ethephon successfully alleviated inhibitory effect of salinity from seeds of *L. stocksii* (Fig. 4.1). Seed germination improved from 42% to 92% at 300 mM, from 4% to 56% at 400 mM and from 3 to 11% at 500 mM NaCl with kinetin. Ethephon enhanced seed germination in 300 mM NaCl and germination improved two-folds from 43% to 83% (Fig. 4.1). Seed germination in complete darkness showed an interaction with salinity (Table 4.1). All seeds germinated in non-saline condition under complete darkness but germination was highly inhibited in saline solutions (Fig. 4.1). Kinetin partially alleviated salinity effects only in low salinities whereas ethephon could not affect germination in complete darkness (Fig. 4.1).

Rate of germination in kinetin and ethephon was higher than non-saline control (Table 4.2). Significant two-way interactions of salinity and chemicals were obtained for rate of germination (Table 4.3).

Addition of other plant growth regulators used in saline solution could not alleviate salinity induced dormancy from the seeds of *L. stocksii* in both light and dark (Figs. 4.2 & 4.3).
Table 4.1. Results of three-way analysis of variance of germination by salinity (SAL), chemicals (CHEM) and light/dark (LD) conditions. F-values in table are highly significant at $P < 0.0001$ level.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>F</th>
</tr>
</thead>
<tbody>
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<td>Salinity (SAL)</td>
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<td>573.87***</td>
</tr>
<tr>
<td>Chemical (CHEM)</td>
<td>54753.63</td>
<td>7</td>
<td>57.95***</td>
</tr>
<tr>
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<td>1</td>
<td>506.52***</td>
</tr>
<tr>
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<td>28835.12</td>
<td>35</td>
<td>6.10***</td>
</tr>
<tr>
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<td>33555.88</td>
<td>5</td>
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</tr>
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<td>7</td>
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</tr>
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<td>40723.79</td>
<td>35</td>
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Fig. 4.1. Effect of kinetin and ethephon on seed germination of *Limonium stocksii* under various light and dark conditions. Means within each salinity treatment having different letters are significantly different from one another (*P < 0.05*), Bonferroni test.
Table 4.2. Effect of different germination regulating chemicals (thiourea, nitrate, proline, betaine, gibberellic acid, kinetin, and ethephon) on rate of germination of *Limonium stocksii* seeds in different salinity (0, 100, 200, 400 and 500 mM NaCl) treatments. Different letters in superscript represent significant ($P < 0.05$) differences within each salinity treatment, Bonferroni test.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Control</th>
<th>Kinetin</th>
<th>Ethephon</th>
<th>GA$_3$</th>
<th>Proline</th>
<th>Betaine</th>
<th>Nitrate</th>
<th>Thiourea</th>
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<td>0</td>
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<td>49.70$^a$</td>
<td>49.45$^a$</td>
<td>50.00$^a$</td>
<td>49.45$^a$</td>
<td>49.50$^a$</td>
<td>46.70$^a$</td>
<td>49.45$^a$</td>
</tr>
<tr>
<td></td>
<td>±0.47</td>
<td>±0.19</td>
<td>±0.55</td>
<td>±0.00</td>
<td>±0.55</td>
<td>±0.50</td>
<td>±1.74</td>
<td>±0.55</td>
</tr>
<tr>
<td>100</td>
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<td>49.55$^a$</td>
<td>48.90$^a$</td>
<td>37.30$^a$</td>
<td>40.70$^a$</td>
<td>46.60$^a$</td>
<td>37.25$^a$</td>
<td>43.95$^a$</td>
</tr>
<tr>
<td></td>
<td>±0.52</td>
<td>±0.22</td>
<td>±0.58</td>
<td>±12.43</td>
<td>±6.71</td>
<td>±2.00</td>
<td>±5.27</td>
<td>±2.50</td>
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<tr>
<td>200</td>
<td>37.90$^a$</td>
<td>47.10$^{ac}$</td>
<td>41.70$^a$</td>
<td>0.75$^b$</td>
<td>15.15$^{ad}$</td>
<td>11.85$^{b}$</td>
<td>16.95$^{ab}$</td>
<td>29.90$^a$</td>
</tr>
<tr>
<td></td>
<td>±4.47</td>
<td>±1.29</td>
<td>±6.25</td>
<td>±0.43</td>
<td>±6.79</td>
<td>±4.99</td>
<td>±5.36</td>
<td>±6.23</td>
</tr>
<tr>
<td>300</td>
<td>15.65$^a$</td>
<td>38.45$^b$</td>
<td>30.00$^b$</td>
<td>0.30$^a$</td>
<td>2.45$^a$</td>
<td>8.55$^a$</td>
<td>1.85$^a$</td>
<td>6.75$^a$</td>
</tr>
<tr>
<td></td>
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<td>±6.08</td>
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<td>±2.07</td>
<td>±5.14</td>
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<tr>
<td>400</td>
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<td>3.05$^a$</td>
<td>0.35$^a$</td>
<td>2.05$^a$</td>
</tr>
<tr>
<td></td>
<td>±0.58</td>
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<td>±1.38</td>
<td>±0.00</td>
<td>±1.41</td>
<td>±1.25</td>
<td>±0.35</td>
<td>±0.87</td>
</tr>
<tr>
<td>500</td>
<td>0.60$^a$</td>
<td>3.10$^{ac}$</td>
<td>0.00$^{ab}$</td>
<td>0.00$^{ab}$</td>
<td>0.25$^a$</td>
<td>0.60$^a$</td>
<td>0.20$^a$</td>
<td>1.30$^a$</td>
</tr>
<tr>
<td></td>
<td>±0.36</td>
<td>±1.23</td>
<td>±0.00</td>
<td>±0.00</td>
<td>±0.25</td>
<td>±0.60</td>
<td>±0.20</td>
<td>±0.90</td>
</tr>
</tbody>
</table>
Table 4.3. Results of two-way analysis of variance of rate of germination by salinity (SAL) and chemicals (CHEM). F-values are significant at $P < 0.0001$.

<table>
<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>F</th>
</tr>
</thead>
<tbody>
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<td>266.98***</td>
</tr>
<tr>
<td>Chemical (CHEM)</td>
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<td>7</td>
<td>20.37***</td>
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<td>SAL * CHEM</td>
<td>7625.55</td>
<td>35</td>
<td>4.35***</td>
</tr>
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</table>
Fig. 4.2. Effect of different germination regulating chemicals on the seed germination of *L. stocksii* under 12 h photoperiod in different NaCl salinities. Means within each salinity treatment having different letters are significantly different from one another (*P* < 0.05), Bonferroni test.
Fig. 4.3. Effect of different chemicals on seed germination of *L. stocksii* under complete darkness in different NaCl salinities. Means within each salinity treatment having same letters are not different from one another (*P > 0.05*), Bonferroni test.
Discussion

Sub-tropical halophytes usually face limited availability of good quality water due to low rainfall and high temperature (above 40°C). The only available water is brackish or seawater. Abiotic stresses like temperature, light and salinity result in a less than optimal individual plant performance in terms of germination, growth and reproduction (Ungar 1995). Halophytes have evolved several mechanisms to survive these conditions. Recruitment in the field is usually through vegetative propagation by stolons and rhizomes. Recruitment through seeds is noticed only after monsoon rains when soil salinity is low. Seeds of some local halophytes have the ability to withstand high saline periods in the soil and remain viable (Khan 2002).

Application of kinetin in saline condition reduced the stress response of \textit{L. stocksii} to a considerable extent and caused the alleviation of salinity-enforced dormancy. Low concentration of kinetin (0.05 mM) appeared to be effective while high concentration (5 mM) either inhibited or had no effect on germination. Kinetin may be a limiting factor under salinity stress for seed germination of \textit{L. stocksii}. Khan and Ungar (2002) also reported 0.05 mM kinetin to be effective in alleviating innate as well as salinity induced dormancy in \textit{Zygophyllum simplex} seeds. Seed germination of other species under saline conditions has also been reported to improve significantly with the application of kinetin (Khan and Ungar 1985; Khan and Rizvi 1994; Khan et al. 1998).

Ethephon is known to affect seed germination by releasing ethylene, which reverts the inhibiting effects of PEG and ABA (Kepczynski 1986). It is also known to break embryo or seed coat imposed dormancy (Sutcliffe and Whitehead 1995; Kepczynski and Kepczynska 1997). There are a number of reports on dormancy breaking ability of ethylene (Kepczynski 1986; Kepczynski and Kepczynska 1997).
raise the possibility that the production of ethylene may contribute to the breaking of dormancy in imbibed seeds (Bewely and Black 1994; Khan and Ungar 2000). Application of ethephon partially alleviated salinity-induced dormancy from the light exposed seeds of *L. stocksii*. Schonbeck and Egley (1981) demonstrated that ethylene action is dependent on availability of light. Partial alleviation of salinity-induced dormancy was also obtained for *Atriplex stocksii* (griffithii), *Sporobolus arabicus* and *Zygophyllum simplex* (Khan and Ungar, 2000, 2001b, 2002). Ethylene is known to lower the water potential of the germinating seeds causing the removal of dormancy. However, partial alleviation of dormancy indicates that under saline condition water availability is not the only constrain on the seed but an interaction of various abiotic factors is responsible for this inhibition.

Baskin and Baskin (1998) reported that most salt desert and salt marsh halophytes have some form of physiological dormancy, which is usually alleviated by the application of GA₃. Gibberellic acid is reported to be one of the potent plant growth regulators to alleviate salinity effect on the germination of dicotyledonous halophytes (Khan and Ungar 2001abc; Khan et al. 1998; Gul et al. 2000). However, GA₃ failed to alleviate salinity-enforced dormancy in seeds of *L. stocksii*. Similar results were obtained for *Salsola imbricata* (Mehrun-Nisa 2003), *Sporobolus arabicus* (Khan and Ungar 2001b) and *Triglochin maritima* (Khan and Ungar 2001a). Compatible osmotica like proline and betaine have some effect in alleviating dormancy (Khan et al. 1998). In *L. stocksii*, both the chemicals failed to alleviate salinity-enforced dormancy. Similar results were obtained by Gulzar and Khan (2002) in seeds of *A. lagopoides*, *Urochondra setulosa* and *Sporobolus iociados*. Poljakoff-Mayber et al. (1994) found that there were low levels of proline and significant amounts of betaine in dry seeds of *Kosteletzkya virginica*, which may alleviate the
inhibitory effects by acting as an osmoregulator or osmoprotectant of proteins in the cytoplasm. However, external application of proline and betaine had no effect on seed germination. Proline and betaine failed to revert high salinity induced dormancy in the seeds of *Zygophyllum simplex* (Khan and Ungar 1997), *Arthrocnemum macrostachyum* and *Salicornia rubra* (Khan et al. 1998) while dormancy was alleviated in seeds of *Atriplex stocksii* (Khan and Ungar 2000).

The promotion of seed germination by nitrogenous compounds such as thiourea, nitrite and nitrate has been reported (Bewely and Black 1994). They are known to counteract the effect of reduced promoter (cytokinins and gibberellins) and increased inhibitor substances, such as abscisic acid, in seeds when they are exposed to high salt stress (Kabar and Baltepe 1990). Treatment with thiourea was highly effective in alleviating the inhibition of germination by salinity or high temperature (Gul and Weber 1998). Nitrogenous compounds had no effect on the germination of *L. stocksii*. Similar results were obtained for *S. imbricata* (Mehrun-Nisa 2002), *S. iodados* and *U. setulosa* (Gulzar and Khan 2002).

*Limonium stocksii* is a perennial woody shrub distributed in coastal areas of Karachi. It produces large number of seeds twice a year, which become a part of the seed bank where they face high temperature and salinity stress. Seeds maintain viability and germinate soon after monsoon rains. Laboratory experiments related to seed germination of *L. stocksii* indicated that seeds germinate as soon as salinity stress is relieved. A wide range of plant growth regulating substances was used to examine their effect on salinity induced dormant seeds. Seed germination of *L. stocksii* was partially alleviated by the application of kinetin and ethephon while other chemicals remained ineffective. This complete or partial failure of dormancy reversal is reported for many subtropical halophytes. Failure to respond to growth regulators in
subtropical halophytes could be due to the loss of viability or osmotic and ionic effects of salinity. However, in *L. stocksii* it appears that seeds are prevented from germination due to osmotic effects only without any change to its metabolism, therefore, germination promoting chemicals had little effect. This study indicates the need to examine the differential effects of various germination regulating chemicals to better understand the germination inhibition mechanisms under saline conditions.


SPSS. 2002. SPSS 11 for Windows update. SPSS Inc. Chicago, USA.


CHAPTER 5

Alleviation of salinity effects on the seed germination of
*Limonium stocksii* by Na-hypochlorite.
Abstract

This is a report on the role of sodium hypochlorite (NaOCl) in alleviation of salinity effects on the seed germination of Limonium stocksii. Seeds were either pre-treated with sodium hypochlorite or it was included in the medium with and without salinity (0-400 mM NaCl) at various temperature regimes (10/20, 15/25, 20/30 & 25/35°C). Application of sodium hypochlorite improved seed germination under saline conditions. Pretreatment of one minute with 10% sodium hypochlorite appeared most effective in alleviating salinity-induced dormancy. Sodium hypochlorite alleviated salinity effect on seed germination at cooler thermoperiod and an increase in temperature progressively reduced its salinity alleviating effects.
Introduction

A wide range of surface disinfectants, such as ethanol, hydrogen peroxide, bromine water, mercuric chloride, silver nitrate, sodium hypochlorite and antibiotics are used for surface sterilization (Bewley and Black 1994). Sodium hypochlorite has been used most commonly as a disinfectant, which releases oxygen gas as a by-product and is highly effective against all kinds of bacteria, fungi and viruses. It kills microbes by oxidizing biological molecules such as proteins and nucleic acids (Bloomfield and Arthur 1991). Higher concentrations of sodium hypochlorite have negative effects both on the germination and growth (Hsiao 1979; Hsiao and Hans 1981; Hsiao and Quick 1984; Ilahi and Hussain 1988). The oxygen gas releasing property of sodium hypochlorite enhances the oxidative respiration, which results in promoting seed germination (Ogawa and Iwabuchi 2001; Bewely and Black 1994; Vujanovic et al. 2000). Sodium hypochlorite is also known to break dormancy (Bewely and Black 1994; Igbinnosa and Okonkwo 1992; Galleta et al. 1989) by decomposing germination inhibitors (Mackinnon and Alderton 2000; Ogawa and Iwabuchi 2001), bleaching of the additional coat (Böhm 2003; Vujanovic et al. 2000), lowering pH (Böhm 2003), and enhancement of α-amylase activity (Kanecko and Morohashi 2003). Böhm (2003) found that sodium hypochlorite released coat-imposed dormancy in orchid seeds by chemical bleaching of the additional coat present around the embryo. It was suggested that weak acids such as hypochlorite, overcome dormancy by lowering the pH and as a result promoting oxygen uptake. Clevering (1995) reported that the germination of Scirpus lacustris and S. maritimus was significantly improved when seeds were presoaked in sodium hypochlorite.

Limonium stocksii (Boiss.) Kuntze in the family Plumbaginaceae is a salt secreting, perennial halophyte, prevalent in high coastal marshes as well as rocky
grounds near the sea shores of Pakistan. Relatively pure populations of *L. stocksii* are found at the farthest end of Manora creek near Hawks Bay, Karachi, Pakistan (24°52'-647"N and 66°53'-321"E). *Limonium stocksii* have the unique ability to recruit through seed germination in the field usually after monsoon rains, on the contrary, other species such as *Aeluropus lagopoides, Arthrocnemum macrostachyum, Atriplex stocksii, Suaeda fruticosa, Tamarix* spp. and *Urochondra setulosa* present in the vicinity are reported to propagate vegetatively. Laboratory experiments showed that seeds of *L. stocksii* are moderately salt tolerant and only few seeds could germinate above 400 mM NaCl (Zia and Khan 2004) at optimal thermoperiod (20/30°C), while seeds of other species from the area could germinate in or above 800 mM NaCl (Khan and Gul 2002). Exposure to high salinity and temperature do not have any effect on seed viability of *L. stocksii* (Zia and Khan 2004). Seeds of *L. stocksii* washed with sodium hypochlorite were seen to germinate better in high salinity treatments. This prompted detailed investigations on the alleviating effect of sodium hypochlorite on the *L. stocksii* seeds germinating under saline conditions. The aim of present study was to determine the alleviation of salinity effects of sodium hypochlorite on the seed germination of *L. stocksii*. 

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Materials and Methods

Seeds of *Limonium 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 manually separated from inflorescence and stored at room temperature. Germination was initiated in May 2000 using 5 cm diameter tight fitting plastic Petri plates with 5 mL of test solution prepared by using distilled water. Four replicates of 25 seeds each were used for each treatment. Seeds with radicles were considered to be germinated.

Seeds were pretreated with 0, 10, 20, 30 and 40% sodium hypochlorite for 1, 5, 10 and 20 minutes prior to germination and washed thoroughly with distilled water and air dried. Seeds were germinated in five concentrations (0, 100, 200, 300 and 400 mM) of NaCl at 20/30°C in 12 h light photoperiod. Seed germination was recorded on alternate days for 20 d.

Seeds were also germinated in mixtures of sodium hypochlorite (2, 4, 6 and 8%) and NaCl solutions (0, 100, 200, 300 & 400 mM) under four alternating temperature regimes of 10/20, 15/25, 20/30 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. Percent germination was recorded on alternate days for 20 d.

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 (Timson, 1965; Khan and 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 were arcsine transformed before statistical analysis. These data were analyzed using SPSS for Windows release 11 (SPSS 2002). A three-way ANOVA was also used to demonstrate the interaction between various factors in affecting germination. A Bonferroni test was used ($P < 0.05$) to determine significant differences between individual means.
Results

Three-way ANOVA of percentage germination indicated significant effects of salinity, sodium hypochlorite, pretreatment time, and their interaction (Table 5.1). There was little effect of sodium hypochlorite in distilled water control and at low salinity treatments (Fig. 5.1). However, alleviation of salinity effects on germination was recorded at salinity concentrations at or above 200 mM NaCl. Best results were obtained when seeds were pretreated with 10% sodium hypochlorite for one minute at 200 mM NaCl (Fig. 5.1). Rate of germination was also higher when seeds were pretreated for one minute using 10% sodium hypochlorite solution (Table 5.2).

Three-way ANOVA of percentage germination indicated a significant main effect of salinity, temperature, sodium hypochlorite and their interaction (Table 5.3). The alleviation effects of all sodium hypochlorite concentrations were obvious at all salinity treatments and low temperatures. Best results were obtained at 200 mM NaCl with 6 and 8% sodium hypochlorite at 10/20°C (Fig. 5.2). Increase in temperature gradually decreased the alleviation effect. Sodium hypochlorite substantially improved the rate of germination, which was noted even at its higher concentrations (Table 5.4). Rate of germination increased with the increase in temperature peaking at 20/30°C both in the absence and presence of sodium hypochlorite (Table 5.4).
Table 5.1. Results of three-way ANOVA of final germination of *Limonium stocksii* seeds due to salinity (SAL), sodium hypochlorite (NaOCl), pretreatment time (T) and their interactions. *** = $P < 0.001$, * = $P < 0.05$, $^{ns}$ = non-significant

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<td>17.41***</td>
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<tr>
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</tr>
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</table>

$^{ns}$ = non-significant
Fig. 5.1. Effect of various pretreatment times and different concentrations of Na-hypochlorite on percent germination of *L. stocksii* in saline solutions. Bars within each salinity treatment with same letters are not significantly different (*P* > 0.05) from one another (Bonferroni test).
Table 5.2. Rate of germination of *L. stocksii* using modified Timson’s index in different salinities with various pretreatment times of different hypochlorite concentrations.

<table>
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<th>NaOCl (%)</th>
<th>NaCl (mM)</th>
<th>Control</th>
<th>One</th>
<th>five</th>
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<th>Twenty</th>
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<td>0</td>
<td>49.45±0.19</td>
<td>49.80±0.20</td>
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<td>46.75±1.26</td>
<td>48.55±1.06</td>
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<td>100</td>
<td>45.35±2.35</td>
<td>47.10±0.33</td>
<td>44.25±1.04</td>
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<td>46.30±1.40</td>
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<tr>
<td></td>
<td>200</td>
<td>26.25±3.49</td>
<td>42.95±0.83</td>
<td>38.60±0.93</td>
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<td>33.00±2.98</td>
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<tr>
<td></td>
<td>300</td>
<td>08.35±2.35</td>
<td>21.80±1.33</td>
<td>10.15±1.97</td>
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<tr>
<td></td>
<td>400</td>
<td>01.80±0.98</td>
<td>01.50±0.58</td>
<td>05.25±0.96</td>
<td>03.95±1.1</td>
<td>01.85±0.87</td>
</tr>
</tbody>
</table>

|           | 0         | 49.45±0.19 | 47.20±1.84 | 45.95±1.81 | 47.15±1.49 | 47.00±1.29 |
|           | 100       | 45.35±2.35 | 43.60±2.27 | 46.80±1.24 | 41.85±1.65 | 45.80±2.21 |
|           | 200       | 26.25±3.49 | 30.45±2.06 | 28.20±3.48 | 26.75±3.2  | 33.60±3.87 |
|           | 300       | 08.35±2.35 | 08.20±0.84 | 10.05±1.89 | 11.15±0.73 | 13.65±1.37 |
|           | 400       | 01.80±0.98 | 02.85±1.28 | 03.50±0.91 | 03.05±1.37 | 03.65±1.93 |

|           | 0         | 49.45±0.19 | 45.35±1.90 | 43.30±0.86 | 48.40±1.01 | 45.80±0.14 |
|           | 100       | 45.35±2.35 | 45.45±2.18 | 41.40±2.84 | 44.20±1.54 | 43.85±2.66 |
|           | 200       | 26.25±3.49 | 38.05±1.84 | 36.60±3.14 | 38.10±1.39 | 29.65±2.31 |
|           | 300       | 08.35±2.35 | 18.90±2.34 | 15.70±1.85 | 22.65±1.06 | 20.05±5.28 |
|           | 400       | 01.80±0.98 | 04.85±0.73 | 05.65±1.46 | 10.65±1.00 | 05.65±1.58 |

|           | 0         | 49.45±0.19 | 43.30±2.40 | 45.30±3.19 | 45.50±2.63 | 47.15±0.93 |
|           | 100       | 45.35±2.35 | 42.30±1.09 | 45.65±2.11 | 44.65±1.22 | 41.90±1.99 |
|           | 200       | 26.25±3.49 | 26.85±3.82 | 35.00±2.80 | 32.25±4.24 | 35.40±4.00 |
|           | 300       | 08.35±2.35 | 07.25±2.82 | 12.05±2.48 | 16.70±0.79 | 11.75±4.03 |
|           | 400       | 01.80±0.98 | 00.30±0.30 | 07.30±1.50 | 06.80±3.04 | 04.35±3.42 |
Table 5.3. Results of three-way ANOVA of final germination percentage of *Limonium stocksii* due to salinity (SAL), temperature (TEM), sodium hypochlorite (NaOCl), and their interactions. *** = \( P < 0.001 \), * = \( P < 0.05 \)

<table>
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<td>460.5***</td>
</tr>
<tr>
<td>NaOCl</td>
<td>6563.27</td>
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</tr>
<tr>
<td>TEM x SAL</td>
<td>14628.47</td>
<td>12</td>
<td>6.8***</td>
</tr>
<tr>
<td>TEM x NaOCl</td>
<td>34162.69</td>
<td>12</td>
<td>15.9***</td>
</tr>
<tr>
<td>SAL x NaOCl</td>
<td>20753.12</td>
<td>16</td>
<td>7.3***</td>
</tr>
<tr>
<td>TEM x SAL x NaOCl</td>
<td>13640.85</td>
<td>48</td>
<td>1.6*</td>
</tr>
</tbody>
</table>
Fig. 5.2. Germination percentage of *Limonium stocksii* seeds immersed in different salinity concentrations with various percent of Na-hypochlorite. Bars within each salinity treatment having same letters are not significantly different ($P > 0.05$) from one another (Bonferroni test).
Table 5.4. Rate of germination (Mean ± Standard Error) of *L. stocksii* in different salinities with various concentrations of Na-hypochlorite (NaOCl) at different temperature regimes.

<table>
<thead>
<tr>
<th>NaOCl (%)</th>
<th>Salinity (mM)</th>
<th>Thermoperiod (night-day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10/20°C</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>44.55±1.13</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>29.25±9.29</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>02.65±0.98</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>00.35±0.35</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>00.00±0.00</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>37.85±3.90</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>21.75±6.55</td>
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<tr>
<td></td>
<td>200</td>
<td>05.10±1.11</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>02.30±0.47</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>00.15±0.15</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>39.70±4.70</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>25.15±4.26</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>06.55±2.74</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>00.25±0.25</td>
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<td></td>
<td>400</td>
<td>00.55±0.55</td>
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<td>6</td>
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<td>100</td>
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<td></td>
<td>300</td>
<td>8.15±2.05</td>
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<tr>
<td></td>
<td>400</td>
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<td>8</td>
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<td>32.30±4.69</td>
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<td></td>
<td>100</td>
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<td>300</td>
<td>10.65±2.74</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>03.55±1.26</td>
</tr>
</tbody>
</table>
Discussion

Sodium hypochlorite is a potent disinfecting agent, widely used for surface sterilization of seeds (Bewley and Black 1994; Kaneko and Morohashi 2003). It is also known to improve seed germination or to break dormancy in different kinds of seeds (Frank and Larson 1970; Abdul-Baki 1974; Major and Wright 1974; Fieldhouse and Sasser 1975; Hsiao 1979; Hsiao et al. 1981; Drew and Brocklehurst 1984; Galletta et al. 1989; Igbinnosa and Okonkwo 1992; Clevering 1995; Fushing et al. 1998; Miyoshi and Mii 1998; Vujanovic et al. 2000). Yildiz and Celal (2002) reported that pretreating *Linum usitatissimum* with sodium hypochlorite for short period stimulated germination. They attributed this stimulation to the scarification of the seed coat resulting in improved water and oxygen penetration or to enhanced oxidative respiration by increased supply of oxygen and decomposition of sodium hypochlorite.

Pretreatment of *L. stocksii* seeds with sodium hypochlorite caused a substantial leaching of colored material but showed little effect on seed germination of *L. stocksii* at non-saline control and low saline conditions. However, pretreatment of seeds for one and five minutes with 10-30% sodium hypochlorite substantially alleviated salinity enforced seed dormancy. Inclusion of sodium hypochlorite (2%) to the medium also significantly improved *L. stocksii* seed germination at 200 and 300 mM NaCl and this effect did not change with any further increase in sodium hypochlorite concentration. Leaching of the chemicals noticed during pretreatment could be instrumental in increased imbibition of water from saline solution and resulted in improved germination under saline conditions. Germination promoting chemicals like GA3, kinetin, fusicoxcin, ethephon, thiourea, proline, betaine and potassium nitrate failed to improve seed germination of *L. stocksii* under saline
conditions, except for kinetin which had some alleviating effect (Zia and Khan 2003). Improvement of seed germination by sodium hypochlorite pretreatment could be due to increased availability of oxygen (Ogawa and Iwabuchi 2001), increase in amylase activity (Kaneko and Morohashi 2003), destruction of inhibitors (Makinnon and Alderton 2000; Ogawa and Iwabuchi 2001) and facilitation in leaching inhibitors (Zia and Khan unpublished data).

Effect of sodium hypochlorite in alleviating salinity effects on germination was optimal at cooler thermoperiod (10/20°C). Promotion of seed germination at cooler temperatures could be due to decreased penetration of sodium hypochlorite into seeds and decomposition to provide oxygen (Drew and Brockelhurst 1984; Yildiz and Celal 2002) while decrease in seed germination at higher temperatures could be caused through facilitation in penetration of sodium hypochlorite through seed coat and increased accumulation in the seeds. High concentrations of sodium hypochlorite may cause the oxidation of biological molecules such as proteins and nucleic acids, which could result in germination inhibition (Bloomfield and Arthur 1991).

Washing seeds with sodium hypochlorite appeared to be quite useful in getting successful recruitment of species. This compound not only protects germinating seeds from all kinds of pathogens but also facilitates the leaching of toxic compounds and scarification of seed coat to improve the permeability for water and oxygen. These properties could contribute in increased germination which otherwise would be difficult. This study reports that inhibiting effect of high salinity could also be alleviated both by pretreatment or inclusion of sodium hypochlorite in the medium. The exact mechanism of this alleviation is not known, however, it could be mediated through leaching and destruction of inhibitors produced by high salt stress and increased uptake of water from saline solution due to scarification of seeds. The
exact mechanism by which sodium hypochlorite alleviates salinity effects on germination need to be explored.
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CHAPTER 6

Growth and selective ion transport of *Limonium stocksii* under saline conditions
Abstract

*Limonium stocksii* is a perennial forb found at the Arabian sea coast near Karachi, Pakistan. Plants were grown in plastic pots using sub-irrigation under natural conditions. Growth parameters and plant succulence were highest at 0 and 10 dS m\(^{-1}\) salinity (both NaCl and seawater). Increase in salinity in the medium progressively decreased the growth and plants survived in up to 60 dS m\(^{-1}\). No significant difference was observed between NaCl and seawater treatments. Succulence did not change at low salinity (10 dS m\(^{-1}\)), however, a further increase in salinity substantially decreased the tissue water. Plant accumulates high quantities of Na\(^+\) and Cl\(^-\) with the induction of salinity in the medium. The selective transport capacity of *L. stocksii* for Na\(^+\) over other elements increased with an increase in salinity in both root and stem, however, roots displayed lower selective ion transport capacity compared to stem.
Introduction

There is a wide spectrum of salinity tolerance among higher plant species, and although many halophytes have been studied in great detail, the basic organismal and cellular mechanisms which clearly distinguish them are still obscure (Robinson et al. 1997). Growth of some halophytes is generally stimulated by various level of salinity (Walker 1989; Drake and Ungar 1989; Pfister 1999; Gul and Khan 1998; Khan et al. 2000a, 2000b; Onkware 2000; Pujol et al. 2001). However, growth of some less tolerant halophytes decreased substantially with an increase in salinity (Jaenicke et al. 1996; Köhl 1997; Wang et al. 1997; Fung et al. 1998; Wu et al. 1998; Lillebø et al. 2003). Greenhouse experiments on subtropical halophytes from Karachi coast indicate that species like *Atriplex nummularia* f. *nummularia*, *Cressa cretica*, *Haloxylon stocksii* (recurvum) and *Suaeda fruticosa* could grow at high salinity and their growth was stimulated by the low salinity (Khan and Aziz 1998; Gul and Khan 1998; Khan et al. 2000a, 2000b). However, species like *Atriplex stocksii* (griffithii) could tolerate only in up to 400 to 500 mM NaCl (Khan et al. 2000c).

Optimal growth of dicotyledonous halophytes may be associated with succulence of leaves and stem (Pfister 1999), Na⁺ exclusion at the root and ion accumulation and secretion through salt glands in leaves (Munns et al. 1983; Flowers 1986; Miyamoto et al. 1996; Hester et al. 2001). At the tissue level, relative amounts of Na⁺ and K⁺ appear to be important factors regulating growth of plants under saline conditions (Wang et al. 1997; Pujol et al. 2001). K⁺ plays a key role in several physiological processes such as osmotic regulation, protein synthesis and enzyme activation (Wang et al. 2002, 2004). The substitution of K⁺ by Na⁺ may lead to nutritional imbalance (Peng et al. 2004). Many halophytes however, maintain a high ratio of Na: K in plant tissues (at least 5 to 10) and still exhibit growth promotion
under saline conditions (Gorham et al. 1980; Rozema 1991). This is probably due to their ability to use Na\(^+\) instead of K\(^+\) in different metabolic processes. Flowers (2004) reported that Na\(^+\) replaced K\(^+\) in regulating stomatal mechanism in the halophyte Suaeda maritima. Halophytes also differ in their ability to absorb nutrients selectively from soil solutions dominated by Na\(^+\) and Cl\(^-\) (Wang et al. 2002) and in their capacity to transport and accumulate Na\(^+\) and K\(^+\) under saline condition (Wang et al. 2002, 2004).

Intensive research programs to identify ornamental halophyte species suited to saline environment and/or irrigation with saline water are being developed in different parts of the world (Lieth 1999) and some of these halophytic species, including Limonium spp. are already grown commercially. Better utilization of such halophytic species depends on knowledge of their degree of salt tolerance (Alarcon et al. 1999; Hester et al. 2001). Limonium stocksii (Boiss.) Kuntze is a good candidate for a coastal area ornamental plant. This perennial shrub is distributed in high coastal marshes as well as rocky grounds near the sea shores of Pakistan and India. This evergreen halophyte produces beautiful flowers twice a year (June and November) while facing high salinity, drought and temperature stress. After monsoon rains, L. stocksii recruit from seeds whereas, most other co-occurring species such as Aeluropus lagopoides, Arthrocnemum macrostachyum, Atriplex stocksii, Suaeda fruticosa, Tamarix spp. and Urochondra setulosa employ vegetative propagation (Khan 2002). This study reports growth and selective transport of ions of L. stocksii under increasing seawater and NaCl at the mature vegetative phase of life cycle.
Materials and Methods

Seeds of *L. stocksii* were collected in February 2000, from the study site, which is a flat area in between Manora Creek and Hawks bay, Karachi (24°52-647’N and 66°53-321’E). Seeds were separated from the inflorescence, surface sterilized using clorox (0.85 % sodium hypochlorite) and stored at 4°C. Growth experiments were initiated in August 2000 in a green house at University of Karachi under ambient atmospheric conditions. Plants were grown from seeds, in 10 cm x 8 cm plastic pots filled three fourth with sandy soil, for two months until they attained a height of about 2 cm. Similar sized plants were thinned to 10 in each pot. Half strength Hoagland and Amon solution No. 2 (Moore 1960) was provided to the plants treated with NaCl salinity while seawater treated plants were nourished by a nitrogen nutrition supplement (Popp and Polania 1989). Pots were sub-irrigated, and the water level was adjusted daily to correct for evaporation. Six pots each were grown in sand culture having NaCl and seawater solutions of 0, 10, 20, 30, 40, 50 and 60 dS m⁻¹. Salinities of the growing medium were selected after a preliminary test of salinity tolerance. Salt solutions were replaced once a week to avoid buildup of salinity in pots. At the initiation of the experiment, salinity concentrations were gradually increased by 10 dS m⁻¹ at 2 d intervals to reach the maximum salinity levels of 60 dS m⁻¹ after 12 d. Fresh and dry weight of root, stem and leaves and length of root and shoot were measured by harvesting plants 60 d after the highest salt concentration was reached. Plants were oven dried at 80°C for 48 h before dry weight was determined.

Chloride ion was measured with a Beckman specific ion electrode. Cation content of root shoot and leaves was analyzed using a Perkin Elmer model 360 atomic absorption spectrophotometer. The Na⁺ and K⁺ levels of plants were examined by
flame emission spectrometry and Ca\textsuperscript{2+} and Mg\textsuperscript{2+} levels by atomic absorption spectrometry.

Values for selective transport capacity (ST\textit{n}) by different parts of plant (root/stem = ST\textit{1} and stem/leaf = ST\textit{2}) for Na\textsuperscript{+} over K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+} and Cl\textsuperscript{-} at different salinity levels were estimated by the formula \( \text{STn} = \frac{A}{B} \), where \( A \) stands for ionic ratio in part A and \( B \) stands for ionic ratio in part B while \( n \) is the number of ST in various parts, \( n = 1, 2, 3, \ldots \). The bigger the ST value, the stronger the part A controls Na\textsuperscript{+} and promotes transport of other ion to part B, indicating a stronger selective transport capacity of part A (Wang et al. 2002).

A completely randomized ANOVA analysis was used to test for significant differences within mean values for growth and ion relations. A Bonferroni test was carried out to check for differences with in individual treatment means (SPSS, 2002).
Results

No significant differences were observed between NaCl and seawater treatments on morphological attributes of *L. stocksii*. Growth changed little by salinity increments up to 20 dS m\(^{-1}\) in both seawater and NaCl solutions. Further increases in salinity resulted in a decrease of stem length, fresh and dry weight of root, stem and leaf (Figs. 6.1, 6.2 & 6.3). Root length of *L. stocksii* was remained unaffected by type and level of salinity treatment (Fig. 6.1). A two-way ANOVA indicated significant \((P<0.0001)\) effects of salinity, plant part and their interaction on fresh weight of *L. stocksii*, however, non significant interaction was observed for dry weight (Table 6.1). Fresh and dry weight of leaf was substantially higher than either stem or root and varied little in up to 20 dS m\(^{-1}\). Further increase in salinity caused significant inhibition with little difference between types of salinity treatments (Figs. 6.2 & 6.3). Both root and stem exhibited no significant change in fresh and dry weight with increasing salinity of either NaCl or seawater (Figs. 6.2 & 6.3).

A two-way ANOVA indicated no significant difference in tissue water of any plant part with the type of salinity used in the experiment. Significant \((P<0.0001)\) interactions between salinity and plant parts were observed in affecting tissue water of *L. stocksii* (Table 6.1). Leaf tissue water was considerably higher than stem and root. Tissue water content of both leaf and stem remained unaffected with increasing salinity. Root tissue water declined sharply above 10 dS m\(^{-1}\) (Fig. 6.4).
Fig. 6.1. Comparative effect of NaCl (-----) and seawater (- - ) dilutions (0, 10, 20, 30, 40, 50 and 60 dS m⁻¹) on stem and root length of L. stocksii. Bars (means ±S.E.) having same letters along increasing salinity are not significantly different (P >0.05) from each other.
Fig. 6.2. Comparative effect of NaCl (clasps) and seawater (shaded) dilutions (0, 10, 20, 30, 40, 50 and 60 dS m$^{-1}$) on fresh weight of leaf, stem and root of L. stocksii. Bars represent means (±S.E.). Bars with same letters along salinity are similar ($P > 0.05$) from each other.
Fig. 6.3. Comparative effect of NaCl (■■■) and seawater (□□□) dilutions (0, 10, 20, 30, 40, 50 and 60 dS m⁻¹) on dry weight of leaf, stem and root of L. stocksii. Bars (means ±S.E.) having same letters between different salinity treatments are not significantly different (P >0.05) from each other.
Table 6.1. Results of two-way analysis of variance of characteristics by salinity (S) and plant part (P). (Numbers are F values at *P<0.01, ***P<0.0001, ns non-significant).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Salinity (S)</th>
<th>Plant part (P)</th>
<th>P x S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh weight</td>
<td>19.425***</td>
<td>96.864***</td>
<td>8.034***</td>
</tr>
<tr>
<td>Dry weight</td>
<td>7.625ns</td>
<td>34.105ns</td>
<td>1.825*</td>
</tr>
<tr>
<td>Tissue water</td>
<td>34.91***</td>
<td>289.10***</td>
<td>17.79***</td>
</tr>
</tbody>
</table>
Fig. 6.4. Comparative effect of different NaCl (‒‒‒) and seawater (■) dilutions (0, 10, 20, 30, 40, 50 and 60 dS m⁻¹) on tissue water content of leaf, stem and root of L. stocksii. Bars (means ±S.E.) having same letters along increasing salinity are not significantly (P > 0.05) different from one another. Bonferroni test.
A two-way ANOVA indicated significant ($P<0.05$) effects of salinity (except for $K^+$ and $Cl^-$) and plant parts (only for $Na^+$) and their interaction (except for $K^+$ and $Cl^-$) on ion content of *L. stocksii* (Table 6.2). High ion accumulation was recorded at highest salinity for all ions except $Na^+$, which accumulated more in 40 dS m$^{-1}$ than 50 and 60 dS m$^{-1}$ (Fig. 6.5). Accumulation of $Na^+$ appeared highest in stem than roots and leaves (Fig. 6.5). The selective transport capacity of *L. stocksii* for $Na^+$ over other elements increased with an increase in salinity in both root and stem (Table 6.3). Selective transport capacity (ST$_2$, stem/leaf) values of all the ionic ratios were greater than the ST$_1$ (root/stem) values at all salinity levels (Except for 30 dS m$^{-1}$) indicating that the selective transport capacity of stem is higher than roots and leaves (Table 6.3).
Table 6.2. Results of two-way analysis of variance of different ions by salinity (S) and plant part (P). (Numbers are F-values at *P<0.01, **P<0.001, ***P<0.0001, ns non-significant).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Salinity (S)</th>
<th>Plant part (P)</th>
<th>P x S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>164.4***</td>
<td>80.7***</td>
<td>27.8***</td>
</tr>
<tr>
<td>Potassium</td>
<td>10.4ns</td>
<td>56.8ns</td>
<td>7.2ns</td>
</tr>
<tr>
<td>Calcium</td>
<td>6.2***</td>
<td>24.3ns</td>
<td>3.2**</td>
</tr>
<tr>
<td>Magnesium</td>
<td>4.8***</td>
<td>39.9ns</td>
<td>4.3***</td>
</tr>
<tr>
<td>Chloride</td>
<td>7.937ns</td>
<td>55.513ns</td>
<td>5.788ns</td>
</tr>
</tbody>
</table>
Fig. 6.5. Ion content of root ( ), stem ( ) and leaf ( ) of Limonium stocksii grown in various levels of saline medium. Bars represent means (± Standard Error).
Table 6.3. The ST1 (Root: Stem) and ST2 (Stem: Leaf) values of different ions in *Limonium stocksii* at various salinity levels.

<table>
<thead>
<tr>
<th>Salinity (dS m⁻¹)</th>
<th>Na /K</th>
<th>Na /Ca</th>
<th>Na /Mg</th>
<th>Na /Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ST1</td>
<td>ST2</td>
<td>ST1</td>
<td>ST2</td>
</tr>
<tr>
<td>0</td>
<td>1.5</td>
<td>1.7</td>
<td>0.9</td>
<td>1.7</td>
</tr>
<tr>
<td>10</td>
<td>1.1</td>
<td>2.5</td>
<td>0.3</td>
<td>2.7</td>
</tr>
<tr>
<td>20</td>
<td>0.7</td>
<td>1.6</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>30</td>
<td>8.4</td>
<td>0.9</td>
<td>3.1</td>
<td>0.5</td>
</tr>
<tr>
<td>40</td>
<td>0.8</td>
<td>2.9</td>
<td>0.5</td>
<td>1.9</td>
</tr>
<tr>
<td>50</td>
<td>0.7</td>
<td>1.4</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>60</td>
<td>1.7</td>
<td>2.6</td>
<td>0.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>
Discussion

Seawater and NaCl both equally affected different growth parameters of L. stocksii. Plants growing in 10 dS m\(^{-1}\) salinity showed better growth in comparison to non saline control, however, this difference was not statistically significant. Species with high salinity tolerance show less morphological changes in the presence of low amount of salt (Sánchez-Blanco et al. 1991). Different Limonium species are reported to excrete salts directly via salt glands (Batanouny et al. 1992; Alarcon et al. 1999). Ungar (1991) suggested that the salt stimulated dry mass production in some halophytes acts as a dilution factor mechanism even though the level of succulence decreases. Little variation in dry weight of stem and root of L. stocksii with increasing salinity and low water content in both parts supports this view. Similar results were obtained for L. latifolium cv. avignon and a hybrid L. belaard and it was suggested that low dry weight reduction with regard to control involves a higher tolerance to saline stress (Alarcon et al. 1999).

*Limonium stocksii* could survive at 60 dS m\(^{-1}\), a concentration equal to full strength seawater. When compared to the salt tolerance of few co-occurring species such as Arthrocnemum macrostachyum (1000 dS m\(^{-1}\), Khan and Ungar unpublished data), Suaeda fruticosa (1000 dS m\(^{-1}\), Khan et al. 2000b), Aeluropus lagopoides (50 dS m\(^{-1}\), Gulzar et al. 2003a) and Urochondra setulosa (50 dS m\(^{-1}\), Gulzar et al. 2003b), *L. stocksii* appears to be a moderately salt tolerant and could be grouped with miohalophyte (Greenway and Munns 1980).

There are two main negative effects of high salt concentrations that influence plant growth and development: water deficit (Munns and Termaat 1986) and ion toxicity associated with excessive Cl\(^-\) and Na\(^+\) (Niu et al. 1995) leading to Ca\(^{2+}\) and K\(^+\) deficiency (Cramer et al. 1987) and to the nutrient imbalance (Marshner 1995; El-
Hamdaoui et al. 2003). However plants differ greatly in their responses to salinity (Hasegawa et al. 2000). Many halophytes accumulate and sequester Na\(^+\) and K\(^+\) balanced by Cl\(^-\) as the basic mechanism to adjust the osmotic potential of their internal tissue to the external salinity (Flowers and Yeo 1986; Cheeseman 1988) and therefore, a high Na\(^+\): K\(^+\) ratio in plant tissue may favor growth. Limonium stocksii accumulated a large amount of ion and their concentration increased with increase in salinity in different parts of plant. Increase in Na\(^+\) and Cl\(^-\) caused a decrease in their antagonistic solute i.e. K\(^+\). Dicotyledonous halophytes have the ability to maintain a high Na\(^+\): K\(^+\) ratio by storing most of the Na\(^+\) in their vacuole and thus require little K\(^+\) for cytosolic metabolism (Flowers and Yeo 1988; Glenn et al. 1999). Ion accumulation was highest in stem and lowest in roots. Low ion content of roots in comparison to leaves and stem were also obtained for Agriophyllum squarrosum, Artemisia sphaerocephala, Caragana korshinskii, Corispermum mongolicum and Zygophyllum xanthoxylum (Wang et al. 2004). Wang et al. (2002) suggested that the plants having salt glands in their aerial parts exhibit weak selective transport capacity in their roots with majority of Na\(^+\) transporting to stem and then leaves and finally secreted by salt gland as NaCl. While Tester and Davenport (2003) reported that roots tend to maintain fairly constant levels of NaCl over time and can regulate NaCl levels by export to the shoot. Generally, ion accumulation (Except for Mg\(^{2+}\)) and their selective transport to the upper parts increased with an increase in salinity. Highest concentration of all the ions was observed in stem at seawater level indicating its osmotic adjustment through ion accumulation. This response has also been considered as a mechanism of salt tolerance where the roots avoid the toxic effects of ions by transporting them toward upper parts and thus maintain their growth (Misra et al. 1996; Alarcon et al. 1999), while shoots may accumulate high concentrations of NaCl.
as an osmoticum (Flowers and Yeo 1986; Glenn et al. 1999). Munns (2002) suggested that salt tolerant plants may have a low rate of Na\(^+\) and Cl\(^-\) transport to leaves, and have the ability to compartmentalize these ions in vacuoles to prevent their buildup in cytoplasm or cell walls and thus avoid salt toxicity.

My results indicate that *L. stocksii* is a moderately salt tolerant halophyte that could grow in salt concentration above seawater. Growth of *L. stocksii* was equally affected both by NaCl and seawater. Roots of *L. stocksii* showed no effect of increasing salinity due to their low selective transport values. Plant accumulated a greater amount of K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and Cl\(^-\) than Na\(^+\) at lower salinities while a high concentration of Na\(^+\) and Cl\(^-\) was observed with increase in salinity. The high selective transport value of stem indicates that *L. stocksii* preferentially protects its photosynthetic and reproductive organs so as to survive and reproduce in saline environment. *Limonium stocksii* has good economic potential as an ornamental plant which could be cultivated using either brackish water or seawater along coast.
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CHAPTER 7

Population biology of *Limonium stocksii* from a salt flat near Arabian sea coast
Abstract

A population of *Limonium stocksii* was studied over a one-year period at the upper end of Manora Creek along the Arabian Sea coast, near Karachi, Pakistan. The community was divided into three zones. Soil pH of the study area varied from 8.1 to 8.5. Soil conductivity was maximum (191 dS m\(^{-1}\)) in September and minimum (55 dS m\(^{-1}\)) in June. Soil water content was relatively low and varied from 1.6% in February to 9.5% in June. *Limonium stocksii* maintained a persistent seed bank, which peaked at 5887 seeds m\(^{-2}\) in May. Very slow growth was observed during the study, however, plants in the intermediate zone showed better growth than the other two zones. Stem and leaf tissue water content were affected by salinity fluctuations but root tissue water remained unaffected. Na\(^+\) and Cl\(^-\) accumulated in stem and leaves more than other ions. Leaves also accumulated a very high amount of Mg\(^{++}\) than root and stem.
Introduction

The Arabian Sea coast near Karachi, Pakistan is extremely arid due to low rainfall and high temperature. The region is represented by about 108 halophytic species, mostly herbaceous perennials from 36 families (Khan and Gul 2002). Plumbaginaceae is represented by *Limonium stocksii* (Boiss) O. Kuntze, a salt secreting, low branched perennial. Populations of *L. stocksii* occupy salt flats and rocky grounds near the seashore at Karachi coast. Laboratory experiments on seed germination (Zia and Khan 2004) and growth of *L. stocksii* showed it to be a moderately salt tolerant halophyte which could be classified as miohalophyte (Zia and Khan unpublished data).

Coastal vegetation is broadly classified as salt marsh, coastal dunes, brackish and rocky salt planes (Day et al. 1989; Mitch and Gosselink 1993; Migahid and Elhaak 2001). Demographic studies of many halophytic populations show that halophytes exhibit both interspecific and intraspecific variation in salinity stress resistance depending on their location (Hester et al. 2001). Generally, salinity, water and temperature are critical environmental factors that determine the vegetation pattern in a region (Sen et al. 1982; Böer 1996) however, populations are predominantly affected by specific overriding physical factors such as sand burial (Middleton et al. 1991), inundation frequency (Hutchinson 1982; Leuschner et al. 1998; Gul and Khan 1999; Bockelmann et al. 2002) and pulsed changes in substrate salinity (Whigham et al. 1989; La Peyre and Rowe 2003; Lillevang et al. 2003).

Halophytes utilize a number of different mechanisms at the cellular level to achieve osmotic adjustment, including 1). Inorganic ion accumulation, 2). Synthesis or accumulation of organic compounds and 3). Water loss (Ungar 1991). Leaves of many halophytes play important role in combating saline stress by developing
succulence, reducing surface area, depositing thick cuticle or a cover of waxy layer on epidermis, developing hairs, sunken stomata, salt glands or a combination of these (Khan and Gul 2002).

Soil seed banks are important components of vegetation dynamics affecting both ecosystem resistance and resilience (Pugnaire and Lazaro 2000) and their relationship to plant population is crucial to understanding the development of plant desert communities (Kemp 1989). On the contrary, Gul and Weber (2001) questioned the role of soil seed banks in determining population dynamics of saline deserts, because recruitment from the seed bank is a rare phenomenon. In arid environments, both perennial and annual species accumulate seeds in the soil but distinction between persistent and transient seed banks is weak, as the extent of germination depends on rainfall (Baskin and Baskin 1998) and on the soil moisture threshold for germination, which vary among species (Parke and Venables 1996). Pugnaire and Lazaro (2000) suggested that rainfall variability strongly affects species composition. For halophytes, the ability to form seed banks has survival value under extreme conditions which mature plants would not be able to endure (Gul 1998). Seed bank studies from Karachi, Pakistan, show that 1. perennial shrubs and grasses dominating the vegetation maintain a persistent seed bank (Gulzar and Khan 1994; Aziz and Khan 1996) and 2. seed banks exhibit considerable spatial and temporal variation in size (Khan and Gul 1999). The significance of seed reserves in the soil in determining the establishment of plant population in hypersaline environment is not clearly understood.

The population is extended from the edge of Manora estuary to the farthest end of the creek. The area near the edge received regular inundation from seawater while inundation progressively decreased towards landward side. It may get inundated
sometimes during the year with high monsoons. Present study was designed to monitor the variation in demography and growth parameters along the inundation gradient.
Materials and Methods

The study was carried out from July 2000 to June 2001 in a salt flat near Hawks Bay, Karachi, Pakistan (24°52-647’N and 66°53-321’E). The main source of moisture for the population is seawater seepage from the backwaters of Manora Creek. The mean ambient summer and winter temperatures are around 36°C and 25°C, respectively (Gul and Khan 1999). Rains are received during monsoon season from June to September, however no rainfall was observed during the study year. The study site was dominated by Limonium stocksii along with few individuals of Aeluropus lagopoides, Arthrocnemum macrostachyum, Suaeda fruticosa, Tamarix spp. and Urochondra setulosa.

Three transects (300 ft) were laid across the population from landward to seaward sides. These transects were further divided into three zones perpendicular to the transects. Nine plants from each zone were randomly harvested each month to measure the morphological growth parameters (root and stem length, number of branches, fresh and dry weight).

For ion analyses 0.5 g of ground dry plant material was boiled in 25 ml of deionized distilled water for 2 h at 100°C using a dry heat bath. This hot water extract was cooled and filtered using Whatman no. 2 filter paper. One mL of this hot water extract was diluted with distilled water for ion analysis. Inorganic cations K⁺, Na⁺, Ca²⁺ and Mg²⁺ of the plant organs were analyzed by atomic absorption spectrometry and Cl⁻ by chloride meter.

Nine soil cores were randomly collected monthly from each zone using a soil corer (1.5 cm X 6 cm) for seed bank analysis. Seeds from these samples were manually extracted with the help of a binocular microscope and identified with the help of pre-collected seed samples. Nine surface soil samples were also collected
monthly for analysis of conductivity and moisture content. These results were analyzed using two-way and three-way ANOVA (SPSS 2002).
Results

Soil pH varied from 8.1 to 8.5 along transects, with lower pH in the intermediate zone in comparison to the other zones. Soil conductivity varied from 55 dS m\(^{-1}\) to 191 dS m\(^{-1}\) in different zones throughout the year (Fig. 7.1). Soil conductivity was highest in the intermediate zone and lowest in the landward zone (Fig. 7.1). Low soil moisture content was recorded as there was no rainfall during the study. Generally, soil moisture increased from landward to seaward zone (Table 7.1). Minimum soil moisture (1.6%) was recorded in February in the landward zone and maximum (9.4%) was in seaward zone during June (Table 7.1). Soil moisture was maintained at higher levels in the intermediate zone from July to November and in the seaward zone from December to June (Table 7.1).

*Seed* bank size of *L. stocksii* was generally small. Seed density decreased from the landward to seaward zone and was maximum in May and minimum in June (Fig. 7.2). Maximum seed density reached up to 5,887 seeds m\(^{-2}\) (Fig. 7.2). Mean annual average value for seed density was highest in the landward zone while it was almost equal in the other two zones. A few seeds of *Aeluropus lagopoides*, *Cyperus* spp. and *Suaeda fruticosa* were also encountered from the landward zone.

Growth parameters such as root and stem length (Fig. 7.3), number of branches (Fig. 7.4), fresh and dry biomass (Figs. 7.5 & 7.6) varied little in different zones. A two-way ANOVA indicated significant main effects of month (\(F = 34.152, P<0.0001\)), zone (\(F = 3.798, P<0.05\)) and their interaction (\(F = 1.88, P<0.05\)) in affecting the root length. Root length was highest in September in intermediate zone while stem length showed no variation throughout the year (Fig. 7.3). Two-way ANOVA of stem length indicated significant individual effect of month (\(F = 49.778, P<0.0001\)) while zone (\(F = 2.279^{*}\)) and its interaction with month (\(F = 1.109^{*}\)) was
Fig. 7.1. Soil conductivity of different zones in L. stocksii community along transect, from July 2000 to June 2001. Bars represent means (± Standard Error).
Table 7.1. Monthly variation in moisture content (%) of the soil in different zones of *Limonium stocksii* community during one-year study from July 2000 to June 2001.

<table>
<thead>
<tr>
<th>Months</th>
<th>Landward</th>
<th>Seaward</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>July</td>
<td>1.72 ± 0.26</td>
<td>3.37 ± 0.30</td>
<td>3.33 ± 0.53</td>
</tr>
<tr>
<td>August</td>
<td>5.44 ± 0.53</td>
<td>6.12 ± 0.46</td>
<td>5.56 ± 0.51</td>
</tr>
<tr>
<td>September</td>
<td>2.63 ± 0.46</td>
<td>4.03 ± 0.46</td>
<td>2.81 ± 0.24</td>
</tr>
<tr>
<td>October</td>
<td>3.98 ± 0.35</td>
<td>3.51 ± 0.54</td>
<td>6.56 ± 0.78</td>
</tr>
<tr>
<td>November</td>
<td>2.70 ± 0.15</td>
<td>2.93 ± 0.45</td>
<td>2.06 ± 0.25</td>
</tr>
<tr>
<td>December</td>
<td>7.76 ± 0.64</td>
<td>4.24 ± 0.65</td>
<td>4.67 ± 0.53</td>
</tr>
<tr>
<td>January</td>
<td>1.80 ± 0.25</td>
<td>3.79 ± 0.39</td>
<td>4.44 ± 0.39</td>
</tr>
<tr>
<td>February</td>
<td>1.67 ± 0.20</td>
<td>2.10 ± 0.26</td>
<td>2.00 ± 0.43</td>
</tr>
<tr>
<td>March</td>
<td>2.28 ± 0.28</td>
<td>3.13 ± 0.36</td>
<td>3.50 ± 0.41</td>
</tr>
<tr>
<td>April</td>
<td>2.69 ± 0.24</td>
<td>2.72 ± 0.31</td>
<td>3.69 ± 0.35</td>
</tr>
<tr>
<td>May</td>
<td>3.00 ± 0.35</td>
<td>2.91 ± 0.25</td>
<td>4.88 ± 0.51</td>
</tr>
<tr>
<td>June</td>
<td>6.78 ± 0.42</td>
<td>8.30 ± 0.99</td>
<td>9.49 ± 0.86</td>
</tr>
</tbody>
</table>
Fig. 7.2. Distribution and number of seeds of *Limonium stocksii* in the seed bank sampled during one year study from July 2000 to June 2001. Bars represent means (+ Standard Error).
non significant. Number of branches was highest in November and June (Fig. 7.4). A two-way ANOVA indicated a significant main effect of month ($F = 7.026, P<0.0001$), zone ($F = 2.55, P<0.05$) and their interaction ($F = 1.652, P<0.05$) in affecting the number of branches.

A three-way ANOVA indicated significant main effects of month, zone, plant part and their interactions in affecting fresh weight, dry weight and tissue water content of *L. stocksii* (Table 7.2). In general, leaf and stem biomass was much higher than roots. Biomass values varied in the order intermediate>landward>seaward zones. *Limonium stocksii* plants collected in June had the highest fresh and dry weight while lowest in December (Figs. 7.5 & 7.6). Fresh and dry weights were highest for leaf in the landward zone and for stem in the intermediate zone (Fig. 7.5 & 7.6). Tissue water content was not very high (Fig. 7.7). Leaf and stem accumulated more tissue water than root. Leaves tissue water was higher from January-March (Fig. 7.7). Stem exhibited highest tissue water content in July and decreased in August. Root tissue did not vary much throughout the year (Fig. 7.7).

A two-way ANOVA indicated significant individual effects of zone, plant part and their interactions in affecting the ion content of *L. stocksii* (Table 7.3). In general all ions accumulated more in leaf except for $K^+$, which had similar values between plant parts. This was followed by stem with slightly lower values except for $Mg^{++}$, which was substantially lower in comparison to leaf. Roots had comparatively much lower ion values than other plant parts except for $Cl^-$ and $K^+$ (Fig. 7.8). $K^+$ varied little in different plant parts and had low values in comparison to Na and $Cl^-$ (Fig. 7.8).
Fig. 7.3. Variation in root and stem length of *Limonium stocksii* in different zones along a transect. Bars represent means (± Standard Error).
Fig. 7.4. Number of branches of *Limonium stocksii* in different zones of *Limonium stocksii* community from July 2000 to June 2001. Bars represent means (± Standard Error).
Table 7.2. Results of three-way analysis of variance of characteristics by months (M), zones (Z) and plant part (P) in affecting the fresh and dry weight and tissue water content in *L. stocksii*. (Numbers are F-values at *P*<0.01, **P*<0.001, ***P*<0.0001, ns non-significant).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Tissue water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month (M)</td>
<td>11.8***</td>
<td>10.5***</td>
<td>6.3***</td>
</tr>
<tr>
<td>Zone (Z)</td>
<td>14.6***</td>
<td>10.6***</td>
<td>0.7ns</td>
</tr>
<tr>
<td>Plant part (P)</td>
<td>107.1***</td>
<td>62.1***</td>
<td>261.1***</td>
</tr>
<tr>
<td>M x Z</td>
<td>3.4***</td>
<td>2.8***</td>
<td>2.7***</td>
</tr>
<tr>
<td>M x P</td>
<td>2.0**</td>
<td>1.7*</td>
<td>3.9***</td>
</tr>
<tr>
<td>Z x P</td>
<td>5.6***</td>
<td>3.8**</td>
<td>1.6ns</td>
</tr>
<tr>
<td>M x Z x P</td>
<td>0.5ns</td>
<td>0.3ns</td>
<td>1.1ns</td>
</tr>
</tbody>
</table>
Fig. 7.5. Fresh weight of root, stem and leaves of *Limonium stocksii* in different zones of community from July 2000 to June 2001. Bars represent means (+ Standard Error).
Fig. 7.6. Variation in dry weight of different parts of *Limonium stocksii* from different zones along transect from July 2000 to June 2001. Bars represent means (± Standard Error).
Fig. 7.7. Tissue water (g g\(^{-1}\) dry wt) of root, stem and leaves of *Limonium stocksii* from different zones of community during July 2000 to June 2001. Bars represent means (± Standard Error).
Table 7.3. Two-way ANOVA of ions in *Limonium stocksii* due to Zone (Z), Plant Part (P) and their interactions. Numbers indicate F-values at \( ** = P < 0.0001 \), \( * = P < 0.001 \), \( * = P < 0.05 \), ns = non-significant

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Na(^+)</th>
<th>K(^+)</th>
<th>Ca(^{++})</th>
<th>Mg(^{++})</th>
<th>Cl(^-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone (Z)</td>
<td>3.1*</td>
<td>49.5 ns</td>
<td>14.0***</td>
<td>9.6***</td>
<td>11.3***</td>
</tr>
<tr>
<td>Plant part (P)</td>
<td>2.9*</td>
<td>65.9 ns</td>
<td>191.5***</td>
<td>65.5***</td>
<td>5.5**</td>
</tr>
<tr>
<td>Z x P</td>
<td>6.5***</td>
<td>70.8 ns</td>
<td>6.3***</td>
<td>6.9***</td>
<td>3.5**</td>
</tr>
</tbody>
</table>
Fig. 7.8. Values for various ions in different parts of *Limonium stocksii* collected from roadward (A), intermediate (B) and waterward (C) zones. Bars represent means (± Standard Error).
Discussion

The main source of water for *L. stocksii* population near Hawks Bay, Karachi was seepage from the sea as very little rain fall was recorded for the couple of years. Soil pH was basic in nature varying from 8.1 to 8.5. Soil conductivity varied from 55 dS m\(^{-1}\) to 191 dS m\(^{-1}\) throughout the year, many folds higher than the optimal salinity level for growth under greenhouse conditions (Zia and Khan unpublished data). Differences in salinity between laboratory conditions are seldom comparable to those encountered in the field due to the variation in salt concentration at and around root surface and the differences in soil salinity with changes in soil moisture (Barrilleaux and Grace 2000). In addition, salinity measurements are limited only to the salt concentrations and do not indicate variations either in the composition or combinations of salts. Variation in soil salinity increased from landward to seaward zone throughout the year. Coastal substrate salinity varies considerably, from 0.1 to 3% (Barbour et al. 1985) and depends on the time of year and proximity to the sea (Weber and D’Antonio 1999). The fluctuating substrate salinity is reported to affect growth of many halophytes (Whigham et al. 1989; La Peyre and Rowe 2003; Lillebø et al. 2003).

Seed bank studies from Karachi, Pakistan, demonstrated that dominant perennial shrubs and grasses maintain a persistent seed bank (Gulzar and Khan 1994; Aziz and Khan 1996). Most salt desert and salt marsh halophytes maintain a persistent seed bank which serves as a long term seed storage mechanism during unpredictable cold, dry or hypersaline periods (Ungar 1995). *Limonium stocksii* appears to have a persistent seed bank, however, seed bank size was very small with a maximum of 5,887 seeds m\(^{-2}\). Seeds when tested for viability germinated showing no sign of dormany. Seeds of *L. stocksii* are ready to germinate (Zia and Khan 2004) and remain
non-dormant even after long periods of storage at ambient temperature (Zia and Khan unpublished data). Lack of rainfall during the study period was the reason for absence of any seedling data, however, a large number of seedlings were observed in the same community after monsoon rains the following year (Zia personal observation). Seeds remained attached on the mother plant for 2-3 months before dispersal, where after, while still enclosed in bracts were seen to be collected by ants in large numbers. This predator activity may well be the reason for the patchy seed bank data and the pattern of seedlings emergence after rainfall.

Resources are often limited in saline environments. Plants allocate the available resources to various plant structures or functions according to several possible compromises maximizing their fitness (Harper 1977). Life history strategies partly depend on selection for optimal resource allocation to growth, reproduction or maintenance of vegetative structures (Abrahamson 1979). *Limonium stocksii* allocated most of its biomass to aboveground parts. Similar results were found in laboratory experiment on the growth of *L. stocksii* (Zia and Khan unpublished data). Clarke and Jacoby (1994) studied the biomass allocation in above ground parts of *Juncus kraussii, Sarcocornia quinqueflora and Sporobolus virginicus* and concluded that soil moisture and soil salinity were the major controlling factors. Growth parameters of *L. stocksii* varied little throughout the year probably due to both water deficit and a subsequent rise in soil salinity. Slow growth rate in saline habitats has survival value as there is little inter-specific competition (Adu et al. 1994). Stem length and fresh weight data correlate with soil moisture content. Tissue water content of *L. stocksii* was also not high in any plant part and remained unchanged throughout the year. Similar results for tissue water were also observed in greenhouse study (Zia and Khan unpublished data).
Plants growing in saline environment encounter salt toxicity, water deficit and nutrient deficiency or imbalance (Munns and Termaat 1986; Munns, 1993; Cramer et al. 1987; Adu et al. 1994; Niu et al. 1995). Many halophytes control high Na\(^{+}\) and Cl\(^{-}\) concentrations either through ion exclusion at the root or secretion of ions from the leaves through salt glands (Flowers 1985). Other halophytes accumulate salts in their tissues for osmotic adjustment and compartmentalize ions in the vacuole (Gorham et al. 1985). Ion accumulation data showed that *L. stocksii*, like most halophytes (Luttge and Smith 1984) accumulated high concentrations of Na\(^{+}\) in leaves and stem as an osmoticum. Accumulation of Na\(^{+}\) was two folds greater than the Cl\(^{-}\). The electrical and chemical gradient analysis across salt glands in *Limonium* spp. showed that it is an electrogenic Cl\(^{-}\) pump with affinity for K\(^{+}\) and Na\(^{+}\) (Long and Mason 1983). This could be the reason for low Cl\(^{-}\) content in *L. stocksii* or may be other anions like sulphates and organic acids are doing the balancing act. Donovan et al. (1997) correlated higher leaf Na\(^{+}\) with the dominance of Na\(^{+}\) nutrition in halophytes. Relationship of ion accumulation and soil moisture content showed an increase in soil moisture content and a decrease in Na\(^{+}\) accumulation in leaves and stem.

In conclusion, the *Limonium stocksii* population studied was found to be highly salt tolerant. It maintained a viable and persistent seed bank and after monsoon rains recruitment mainly takes place by seed germination. Growth of plants continued at a slow rate and most of the biomass accumulated in upper plant parts. *Limonium stocksii* accumulated large amounts of Na\(^{+}\) followed by Cl\(^{-}\) and K\(^{+}\). Na\(^{+}\) appears to have a role as an osmoticum.
Literature Cited


CHAPTER 8

General conclusions
Limonium stocksii is one of the less common halophytes found on the fringes of coastal salt marshes at the Arabian sea coast near Karachi, Pakistan. The plant completes its life cycle by producing flowers, fruits and seeds twice a year despite very high soil salinity, little precipitation and high rate of evapotranspiration. Growth of the plant is usually very slow due to the water, salinity and temperature stresses. Seeds start to disperse soon after maturity and form a persistent seed bank although myrmerochory was found in the population. Limonium stocksii produces beautiful pink flowers and could be used as an ornamental plant in areas where good quality water is scarce.

The research presented in this dissertation examined the effect of environmental factors (light, temperature, salinity) on the germination of a perennial halophyte Limonium stocksii, the only member from family Plumbaginaceae found on the coast of Pakistan. Various germination regulating chemicals were used to determine their role in alleviating salinity stress on seed germination. The effect of NaCl, seawater and density on the growth and ionic relations under controlled conditions was also studied. Life history strategies of L. stocksii population was followed for twelve months.

Limonium stocksii seeds germinated in distilled water and under low salinity, however, germination was inhibited with a further increase in salinity and few seeds germinated at 50 dS m⁻¹. Seawater generally inhibited seed germination more than isotonic solutions of NaCl. Optimal temperature regime for germination was 20/30°C for NaCl and 25/35°C for seawater treated seeds under both light and dark environments. Seeds germinated well under control conditions in dark, however, induction of salinity synergistically inhibited seed germination. Sodium hypochlorite, kinetin and ethephon partially alleviated salinity and temperature induced dormancy.
in *L. stocksii* seeds while proline, betain, gibberellic acid, fusicoccin, thiourea and nitrate had no significant effect.

Isotonic solutions of NaCl and seawater equally inhibited the growth of *L. stocksii* and plants survived concentrations in up to full strength seawater (60 dS m$^{-1}$). Tissue succulence did not change significantly due to increase in salinity but cation and anion concentrations increased in the tissue with the increase in salinity to attain osmotic equilibrium under highly saline conditions. The selective transport capacity of stem for Na$^+$ over other ions was higher than root.

Seeds maintained a small but persistent seed bank and recruitment took place after considerable rainfall during the monsoon season. Growth of the plant in natural conditions was relatively slow in comparison to green house which could be attributed to a combination of high water, salinity and temperature stresses.

My data clearly indicates that *Limonium stocksii* is salt tolerant both at germination and growth stages and germination could be promoted with the application of sodium hypochlorite, kinetin and ethephon to seeds. The population has the ability to survive extreme drought, salinity and temperature stress and produce beautiful flowers twice a year. *Limonium stocksii* could be used as an ornamental plant in areas where good quality water is not available for irrigation.
Contribution to Knowledge

The following are considered to be key contributions to original knowledge from the research described in this dissertation:

1. *Limonium stocksii* has no innate dormancy and seed germinate well under control and low salinity and could germinate in up to 50 dS m\(^{-1}\) salinity. It could germinate better at moderate temperatures and seeds would not be able to germinate under saline conditions in dark.

2. Sodium hypochlorite, kinetin and ethephon could alleviate salinity induced germination inhibition in *L. stocksii*.

3. Isotonic solutions of NaCl and seawater had similar effect on the growth of *L. stocksii* plants. Plant could survive full strength seawater irrigation.

4. Population maintained a persistent seed bank and despite slow growth produced flower and seed twice a year under high water, temperature and salinity stresses.

5. *Limonium stocksii* appears to be a highly salt, drought and temperature stress tolerant plant which could be utilized as an ornamental plant to develop the coastal landscape.
COMPARATIVE EFFECT OF NaCl AND SEAWATER ON SEED GERMINATION OF LIMONIUM STOCKSIII

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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.
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 (arc sine) 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 germination 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>
<thead>
<tr>
<th>Conductivity (dS cm⁻¹)</th>
<th>Index of germination velocity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl</td>
<td>Seawater</td>
</tr>
<tr>
<td>0</td>
<td>49.80 ± 0.2²</td>
<td>49.50 ± 0.2³</td>
</tr>
<tr>
<td>10</td>
<td>47.10 ± 0.3³</td>
<td>40.60 ± 2.8³</td>
</tr>
<tr>
<td>20</td>
<td>42.95 ± 0.8³</td>
<td>07.00 ± 3.2³</td>
</tr>
<tr>
<td>30</td>
<td>21.80 ± 1.3³</td>
<td>10.75 ± 5.7³</td>
</tr>
<tr>
<td>40</td>
<td>01.54 ± 0.6³</td>
<td>00.00 ± 0.0³</td>
</tr>
<tr>
<td>50</td>
<td>00.60 ± 0.4³</td>
<td>02.05 ± 0.9³</td>
</tr>
</tbody>
</table>

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. vulgaris* 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.

References


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EFFECT OF GERMINATION REGULATING CHEMICALS IN ALLEVIATING SALINITY INDUCED GERMINATION INHIBITION OF LIMONIUM STOCKSII SEEDS

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Abstract

Effect of different chemicals: kinetin (0.05 mM), ethephon (5 \textmu M), GA_3 (0.3 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (10 mM) and thiourea (5 mM) was investigated in alleviating salinity enforced seed dormancy from Limonium stocksii. Six salinity regimes (0-500 mM NaCl) were used in 12 h photoperiod and in complete darkness to study the effect of different germination regulating chemicals in inducing germination under saline condition. Only kinetin and ethephon successfully alleviated salinity enforced seed dormancy of L. stocksii. Kinetin was more successful than ethephon. Seed germination was substantially inhibited by salinity when germinated under complete darkness. Kinetin and ethephon appeared to be the only chemicals which improved seed germination in complete darkness. All other germination regulating chemicals had no effect at low salinity but inhibited germination at high salinity in both light and dark.

Introduction

Seed dormancy is an adaptive mechanism to promote plant survival by distributing germination in both time and space (Lorenzo \textit{et al.}, 1999). It is influenced by a large number of genes and environmental factors, especially by those which affect the growth potential of a seed (Korneef \textit{et al.}, 2002). In case of halophytes, seed germination is usually prevented by physiological causes (Khan, 1996) induced by availability of less than optimal environmental conditions like water, light, oxygen, temperature and soil salinity (Corbineau & Come, 1995; Ungar, 1995). Exposure to osmotic stress causes plants to exhibit different morphological and developmental changes at the molecular, cellular and organismal levels (Yeo, 1998; Bohnert \textit{et al.}, 1999; Hasegawa \textit{et al.}, 2000) during the life cycle. These changes are due to the imbalance in growth regulators causing an increased level of endogenous ABA and other germination inhibitors and a decrease in endogenous growth promoters (Bewely & Black, 1994).

Kudret (1987) suggested that endogenous hormone level is affected by many environmental stresses however, external application of appropriate growth regulators optimize physical metabolic conditions for germination. The role of various germination regulating chemicals such as proline, betaine, gibberellin, kinetin, nitrate, thiourea and ethephon in reducing the inhibitory effects of salinity on germination is reported for several halophytes (Kudret, 1987; Bewely & Black, 1994; Plyer & Proseus, 1996; Gul & Weber, 1998; Khan & Ungar, 1997, 2000, 2001a,b,c, 2002). Different regulatory roles are suggested for these chemicals in breaking seed dormancy in halophytes. They are thought to alleviate salinity effects on the germination by 1) affecting gene expression and/or membrane function (Ho & Hagen, 1993); 2) substituting for light and temperature (Khan & Weber, 1986; Bewely & Black, 1994; Corbineau & Come, 1995; Sutcliffe & Whitehead, 1995); 3) acting as an osmoregulator or osmoprotectant of proteins in the cytoplasm (Poljakoff-Mayber \textit{et al.}, 1994; Gorham, 1995); and 4) counteracting the effect
of reduced promoter (cytokinins and gibberellins) and increased inhibitor substances, such as abscisic acid in seeds under high salinity (Kabar & Baltepe, 1990). However, the ability of these chemicals to relieve seed dormancy and to stimulate germination varies with environmental factors such as temperature and light and from one species to another (Khan, 2003).

Seeds of *L. stocksii* are subjected to high salinity and temperature stress after dispersal. However, seeds germinate readily after monsoon rains under natural conditions. Salinity tolerance of seeds under laboratory conditions decreased sharply above 300 mM NaCl and only few seeds germinated at 500 mM (Zia & Khan, 2004). The inhibition of germination may be due to an imbalance of critical germination regulating chemicals. The present study was conducted to determine the role of germination regulating chemicals in alleviating salinity induced dormancy from seeds of *L. stocksii*.

**Materials and Methods**

Seeds of *Limonium 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, surface sterilized using sodium hypochlorite (0.52 %) 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. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated with the emergence of radicle.

Seeds were germinated in an incubator at a day/night temperature of 30/20 °C in incubators (Percival Scientific, USA) with a 12-h photoperiod (Sylvania cool white fluorescent lamps, irradiance of 25 μmol. m⁻²·s⁻¹, 400-700 nm) and in complete darkness in six salinity concentrations (0, 100, 200, 300, 400 and 500 mM NaCl). Dormancy relieving compounds: kinetin (0.05 mM), ethephon (5 μM), GA₃ (0.3 mM), proline (0.1 mM), betaine (0.1 mM), nitrate (10 mM) and thiourea (5 mM) were used. The concentrations used were based on optimal level promoting seed germination in most other species. Percent germination was recorded alternately for 20 days. Similarly seeds were germinated in complete darkness by placing petri-plates in black plastic bags for 20 days and the germination was recorded after 20 days.

Rate of germination was estimated by using modified Timson's index of germination velocity, ΣG/t, where G is percentage of seed germinated at 2-d intervals, and t is total germination period (Khan & 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. Germination data was arcsine transformed before statistical analysis using SPSS for Windows release 11 (SPSS, 2002). The 2-way and 3-way ANOVA were used to determine significant effects of various factors and their interaction on rate and percentage germination. A Bonferroni test was used to determine significant differences between means.

**Results**

Three way ANOVA of percentage germination of *L. stocksii* indicated significant (P < 0.0001) effect of salinity, light, chemicals and their interactions (Table 1). Best seed germination was obtained in distilled water control and in 100 mM NaCl under 12-h photoperiod. Further increase in salinity gradually decreased seed germination and only a few seeds could germinate in 500 mM NaCl (Fig. 1).
Table 1. Results of three-way analysis of variance of final germination by salinity (SAL), chemicals (CHEM) and light/dark (LD) conditions. (The F-values are highly significant at P < 0.0001 level).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (SAL)</td>
<td>387319.21</td>
<td>5</td>
<td>573.87***</td>
</tr>
<tr>
<td>Chemical (CHEM)</td>
<td>54753.63</td>
<td>7</td>
<td>57.95***</td>
</tr>
<tr>
<td>Light/Dark (LD)</td>
<td>68373.38</td>
<td>1</td>
<td>506.52***</td>
</tr>
<tr>
<td>SAL *CHEM</td>
<td>28835.12</td>
<td>35</td>
<td>6.10***</td>
</tr>
<tr>
<td>SAL *LD</td>
<td>33555.88</td>
<td>5</td>
<td>49.72***</td>
</tr>
<tr>
<td>CHEM *LD</td>
<td>41009.96</td>
<td>7</td>
<td>4.74***</td>
</tr>
<tr>
<td>SAL *CHEM *LD</td>
<td>40723.79</td>
<td>35</td>
<td>8.62***</td>
</tr>
</tbody>
</table>

Table 2. Effect of different germination regulating chemicals (thiourea, nitrate, proline, betaine, gibberellic acid, kinetin, and ethephon) on rate of germination of Limonium stocksii seeds in different salinity (0, 100, 200, 400 and 500 mM NaCl) treatments. (Different letters in superscript represent significant (P < 0.05) differences within each salinity treatment, Bonferroni test).

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Control</th>
<th>Kinetin</th>
<th>Ethephon</th>
<th>GA3</th>
<th>Proline</th>
<th>Betaine</th>
<th>Nitrate</th>
<th>Thiourea</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49.30 a</td>
<td>49.70 a</td>
<td>49.45 a</td>
<td>50.00 a</td>
<td>49.45 a</td>
<td>49.50 a</td>
<td>46.70 a</td>
<td>49.45 a</td>
</tr>
<tr>
<td>±0.47</td>
<td>±0.19</td>
<td>±0.55</td>
<td>±0.00</td>
<td>±0.55</td>
<td>±0.50</td>
<td>±1.74</td>
<td>±0.55</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>46.30 a</td>
<td>49.55 a</td>
<td>48.90 a</td>
<td>37.30 a</td>
<td>40.70 a</td>
<td>46.60 a</td>
<td>37.25 a</td>
<td>43.95 a</td>
</tr>
<tr>
<td>±0.52</td>
<td>±0.22</td>
<td>±0.58</td>
<td>±12.43</td>
<td>±6.71</td>
<td>±2.00</td>
<td>±5.27</td>
<td>±2.50</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>37.90 a</td>
<td>47.10 ac</td>
<td>41.70 a</td>
<td>0.75 b</td>
<td>15.15 ad</td>
<td>11.85 b</td>
<td>16.95 ab</td>
<td>29.90 a</td>
</tr>
<tr>
<td>±4.47</td>
<td>±1.29</td>
<td>±6.25</td>
<td>±0.43</td>
<td>±6.79</td>
<td>±4.99</td>
<td>±5.36</td>
<td>±6.23</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>15.65 a</td>
<td>38.45 b</td>
<td>30.00 b</td>
<td>0.30 a</td>
<td>2.45 a</td>
<td>8.55 a</td>
<td>1.85 a</td>
<td>6.75 a</td>
</tr>
<tr>
<td>±5.30</td>
<td>±4.97</td>
<td>±6.08</td>
<td>±0.30</td>
<td>±2.07</td>
<td>±5.14</td>
<td>±0.82</td>
<td>±2.72</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>1.50 a</td>
<td>17.60 b</td>
<td>3.20 a</td>
<td>0.00 a</td>
<td>2.30 a</td>
<td>3.05 a</td>
<td>0.35 a</td>
<td>2.05 a</td>
</tr>
<tr>
<td>±0.58</td>
<td>±4.50</td>
<td>±1.38</td>
<td>±0.00</td>
<td>±1.41</td>
<td>±1.25</td>
<td>±0.35</td>
<td>±0.87</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.60 a</td>
<td>3.10 ac</td>
<td>0.00 ab</td>
<td>0.00 ab</td>
<td>0.25 a</td>
<td>0.60 a</td>
<td>0.20 a</td>
<td>1.30 a</td>
</tr>
<tr>
<td>±0.36</td>
<td>±1.23</td>
<td>±0.00</td>
<td>±0.25</td>
<td>±0.60</td>
<td>±0.20</td>
<td>±0.90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Results of two-way analysis of variance of rate of germination by salinity (SAL) and chemicals (CHEM). (The F values are significant at P < 0.0001).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (SAL)</td>
<td>66829.57</td>
<td>5</td>
<td>266.98***</td>
</tr>
<tr>
<td>Chemical (CHEM)</td>
<td>7138.56</td>
<td>7</td>
<td>20.37***</td>
</tr>
<tr>
<td>SAL * CHEM</td>
<td>7625.55</td>
<td>35</td>
<td>4.35***</td>
</tr>
</tbody>
</table>

Application of kinetin and ethephon successfully alleviated inhibitory effects of salinity from seeds of L. stocksii (Fig. 1). Seed germination improved from 42 to 92% at 300 mM, from 4 to 56% at 400 mM and from 3 to 11% at 500 mM NaCl with kinetin. Ethephon enhanced seed germination in 300 mM NaCl and germination improved two-folds from 43 to 83% (Fig. 1). Seed germination in complete darkness showed an interaction with salinity (Table 1). All seeds germinated in non-saline condition under complete darkness but germination was highly inhibited in saline solutions (Fig. 1). Kinetin partially alleviated salinity effects only at low levels, whereas ethephon could not affect germination in complete darkness (Fig. 1).
Fig. 1. Effect of kinetin and ethephon in different light and dark conditions on seed germination of *Limonium stocksii*. (Mean within each salinity treatment having different letters are significantly different from one another (P<0.05), Bonferroni test).
Rate of germination in kinetin and ethephon was higher than non-saline control (Table 2). The 2-way ANOVA of rate of germination indicated significant effects of salinity, chemicals and their interactions (Table 3). Addition of other plant growth regulators used in saline solution could not alleviate salinity induced dormancy from the seeds of *L. stocksii* in both light and dark (Fig. 2 & 3).

**Discussion**

Sub-tropical halophytes usually face limitations in the availability of good quality water due to low rainfall and high temperature (above 40 °C). The only available water is brackish or seawater. These abiotic stresses (temperature, light and salinity) result in a less than optimal individual plant performance in terms of germination, growth and reproduction (Ungar, 1995). Halophytes have evolved several mechanisms to survive these conditions. Recruitment in the field is usually through vegetative propagation by stolons and rhizomes. Recruitment through seeds is noticed only after monsoon rains when soil salinity is low. Seeds of some local halophytes have the ability to withstand high saline periods in the soil and remain viable (Khan, 2003).

Application of kinetin in saline condition reduced the stress response of *L. stocksii* to a considerable extent and caused the alleviation of salinity enforced dormancy. Kinetin may be a limiting factor under salinity stress for seed germination of *L. stocksii*. Khan & Ungar (2002) also reported 0.05 mM kinetin to be effective in alleviating innate as well as salinity induced dormancy in *Zygophyllum simplex* seeds. Seed germination of other species under saline conditions has also been reported to improve significantly with the application of kinetin (Khan & Ungar, 1985; Khan & Rizvi, 1994; Khan et al., 1998).

Ethephon is known to affect seed germination by releasing ethylene, which reverts the inhibiting effects of PEG and ABA (Kepczynski, 1986). It is also known to break embryo or seed coat imposed dormancy (Sutcliffe & Whitehead, 1995; Kepczynski & Kepczynska, 1997). There are a number of reports on dormancy breaking ability of ethylene (Kepczynski, 1986; Kepczynski & Kepczynska, 1997). These reports raise the possibility that the production of ethylene may contribute to the breaking of dormancy in imbibed seeds (Bewely & Black, 1994; Khan & Ungar, 2000). Application of ethephon partially alleviated salinity induced dormancy form the light exposed seeds of *L. stocksii*. Schonbeck & Egley (1981) demonstrated that ethylene action is dependent on availability of light. Partial alleviation of salinity induced dormancy was also obtained for *Zygophyllum simplex*, *Sporobolus arabis* and *Atriplex giffithii* (Khan & Ungar, 1998 2000, 2001b, 2002). Ethylene is known to lower the water potential of the germinating seeds causing the removal of dormancy. However, partial alleviation of dormancy indicates that under saline condition water availability is not the only constrain on the seed but an interaction of various abiotic factors is responsible for this inhibition.

Baskin & Baskin (1998) suggested that most salt desert and salt marsh halophytes have some form of physiological dormancy, which is usually alleviated by the application of GA₃. Gibberellic acid is reported to be one of the potent plant growth regulators to alleviate salinity effect on the germination of dicotyledonous halophytes (Khan et al., 1998; Gul et al., 2000; Khan & Ungar, 2001a,b,c). However, GA₃ failed to alleviate salinity enforced dormancy in seeds of *L. stocksii*. Similar results were obtained for *Salsola imbricata* (Mehrun-Nisa, 2003), *Sporobolus arabicus* (Khan & Ungar, 2001b) and *Triglochin maritima* (Khan & Ungar, 2001a).
Fig. 2. Effect of different germination regulating chemicals on the seed germination of *L. stocksii* under 12-h photoperiod in different NaCl salinities. (Means within each salinity treatment having different letters are significantly different from one another (P<0.05), Bonferroni test).
Fig. 3. Effect of different germination regulating chemicals on seed germination of *L. stocksii* under complete darkness in different NaCl salinities. (Mean with in each salinity treatment having different letters are significantly different from one another (P<0.05), Bonferroni test).
Compatible osmotica like proline and betaine have some effects in alleviating dormancy (Khan et al., 1998). In *L. stocksii* both the chemicals failed to alleviate salinity-enforced dormancy. Similar results were obtained by Gulzar & Khan (2002) in seeds of *A. lagopoides, Urochondra setulosa* and *Sporobolus ioclados*. Poljakoff-Mayber et al. (1994) found that there were low levels of proline and significant amounts of betaine in dry seeds of *Kosteletzkya virginica*, which may alleviate the inhibitory effects by acting as an osmoregulator or osmoprotectant of proteins in the cytoplasm. However, external application of proline and betaine had no effect on seed germination. Proline and betaine failed to revert high salinity induced dormancy in the seeds of *Zygophyllum simplex* (Khan & Ungar, 1997), *Arthrocnemum macrostachyum* and *Salicornia rubra* (Khan et al., 1998) while dormancy was alleviated in seeds of *Atriplex stocksii* (Khan & Ungar, 2000).

The promotion of seed germination by Nitrogenous compounds such as thiourea, nitrite and nitrate has been reported (Bewely & Black, 1994). They are known to counteract the effect of reduced promoter (cytokinins and gibberellins) and increased inhibitor substances, such as abscisic acid, in seeds when they are exposed to high salt stress (Kabar & Baltepe, 1990). Treatment with thiourea was highly effective in alleviating the inhibition of germination by salinity or high temperature (Gul & Weber, 1998). Nitrogenous compounds had no effect on the germination of *L. stocksii*. Similar results were obtained for *S. imbricata* (Mehrun-Nisa, 2004), *S. ioclados* and *U. setulosa* (Gulzar & Khan, 2002).

*Limonium stocksii* is a perennial woody shrub distributed in coastal areas of Karachi. It produces large number of seeds twice a year, which become a part of the seed bank when they face high temperature and salinity stress. Seeds maintain viability and germinate soon after monsoon rains. Laboratory experiments related to seed germination of *L. stocksii* indicated that seeds germinate as soon as salinity stress is relieved. A wide range of plant growth regulating substances was used to examine their effect on salinity induced dormant seeds. Seed germination of *L. stocksii* was partially alleviated by the application of kinetin and ethephon while other chemicals remained ineffective. This complete or partial failure of dormancy reversal is reported for many subtropical halophytes. Failure to respond to growth regulators in subtropical halophytes could be due to the loss of viability or osmotic and ionic effects of salinity. However, in *L. stocksii* it appears that seeds are prevented from germination due to osmotic effects only without any change to its metabolism, therefore germination promoting chemicals had little effect. This study indicates the need to examine the differential effects of various germination regulating chemicals to understand better the germination inhibition mechanisms under saline conditions.

**Acknowledgement**

We would like to thank, **University of Karachi**, and **Higher Education Commission** for providing the research grant to **Ms. Sabahat Zia**.

**References**


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

Sabahat Zia and M. Ajmal Khan

Abstract: *Limonium stocksii* (Boiss.) Kunze (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 12:12 photoperiod under saline conditions. Recovery experiments showed that exposure of seeds to various salinity and temperature regimes had little effect on viability of seeds.

Keywords: germination, light, *Limonium stocksii*, NaCl, recovery, temperature.

Résumé: Le *Limonium stocksii* (Boiss.) Kunze (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 germent en présence de 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 germent aux concentrations de 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 observe avec une photopériode de 12 h, sous des conditions saines. 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.


[Traduit par la Rédaction]

Introduction

Halophytes are distributed in coastal and inland saline habitats throughout the world (Ahnin 1990, Ungar 1991). 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).

Percenual 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; Noc and Zeller 2000). Maximum salt tolerance for germination of subtropical species from the Karachi coast in Pakistan has been reported for *Arthrospermum novum* (Moric.) C. Koch (10% germination at 1000 mmol/L NaCl; Khan and Gil 1998), *Cresta cretica* L. (3% germination at 1000 mmol/L NaCl; Khan 1999), and *Salsola irinthera* Forsk. (5% 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...
when there is an optimal combination of day length, temperature regime, and salinity (Naidoo and Naicker 1992; Zitterman et al. 1995; Khan 2002). Absence of light almost completely inhibits seed germination of Triglochin marina L. (Khan and Ungar 1999) and Sporobolus indicus (L.) Br. (Andrews 1997) and partially inhibits germination in Sium gramineum L. (Garcia et al. 1995), Allium sativum L. & Sin. Brassica tournefortii Gouan, Cakile ritchii Scop., and Quononas nitida (L.) Hoffmanns & Link (Thomaz et al. 1991), while seeds of Atriplex stockii (Khan and Risvi 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 brackish 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 need to remain viable in high salinity and germinate when salinity decreases (Khan and Ungar 1997). Halophytes known to maintain viability for extended periods of time, such as Suaeda fruticosa and Triglochin marina, have a partial germination recovery when salinity stress is alleviated (Khan 2002).

Atriplex stockii (Beiss.) Kuntze is a low-branching, salt-loving, woody shrub in the family Plumbaginaceae. It is distributed in high coastal marshes as well as rocky grounds across the coast of Pakistan and India (Gujarat). In Karachi, populations of L. stockii are found at the farthest end of Manora Creek near Hawks Bay in association with a few individuals of Arthrocnemum macrostachyum, Aeluropus littoralis (L.) Trin. ex. Trin., Urochondra setulosa (Trin.) L. Hubbard, Suaeda fruticosa, Tamarix spp., and Atriplex stockii. Possible sources of moisture are the monsoon rains and oceanic advection. The monsoon period starts 15 June and ends on 15 September. Owing to high tides during this period, oceanic advection increases, while rainfall (220 mm/year) usually occurs during July and August. Storms are rarely recorded from the Karachi coast. Limonium stockii flowers generally begin in June and November, and a large number of seeds are produced in August and January. After rinsing seeds become part of the seed bank and germinate 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 stockii 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 still unknown. It is also an important component of littoral ecosystems in 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. stockii under various salinity, temperature, and light conditions.

Materials and methods

Seeds of L. stockii were collected in February 2006 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 florescence, 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 diameters, tightly 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 μmol·m⁻²·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 = 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—a, 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. stockii seeds (Table 1).
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 ± SE.

![Graph showing germination percentages at different conditions.](image)

<table>
<thead>
<tr>
<th>NaCl (mmol/L)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>10:20 °C Light</td>
</tr>
<tr>
<td>10:20 °C Dark</td>
</tr>
<tr>
<td>15:25 °C Light</td>
</tr>
<tr>
<td>15:25 °C Dark</td>
</tr>
<tr>
<td>20:30 °C Light</td>
</tr>
<tr>
<td>20:30 °C Dark</td>
</tr>
<tr>
<td>25:35 °C Light</td>
</tr>
<tr>
<td>25:35 °C Dark</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>S</th>
<th>T</th>
<th>S x T</th>
</tr>
</thead>
<tbody>
<tr>
<td>germination</td>
<td>426.7***</td>
<td>127.7***</td>
<td>29.2***</td>
</tr>
<tr>
<td>net of germination</td>
<td>668.6***</td>
<td>229.5***</td>
<td>42.6***</td>
</tr>
<tr>
<td>recovery</td>
<td>359.6***</td>
<td>80.8***</td>
<td>23.7***</td>
</tr>
</tbody>
</table>

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

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

Discussion

Limonium stocksii native to the subtropical maritime desert of Karachi is exposed to various levels of moisture and salinity stress due to an unpredictable monsoon period (Khan 2002). These conditions lead to different life history strategies in plants that allow the plants to maximize their fitness (Al-1995). Coastal areas of Pakistan are reported to have at least 100 species of halophytes (Khan and Gul 2002), and their tolerance of only a small number of these species is known. The available reports indicate that dicotyledonous species are more tolerant during germination, including species such as Arthrocnemum macrostachyum (1000 mmol/L NaCl; Khan and Gul 1998), Cressa cretica (1000 mmol/L NaCl; Khan 1999), Salicornia irminicata (800 mmol/L NaCl; Khan, unpublished data), Suaeda fruticosa (500 mmol/L NaCl; Khan and Unger 1998), Atriplex stocksii (500 mmol/L NaCl; Khan and Rizvi 1994), and some grasses as Aeluropus lagopoides (500 mmol/L NaCl; Gulzar and Khan 2001), Urochloa setacea (500 mmol/L NaCl; Gulzar and Khan 2001), Halopyrum mucronatum (300 mmol/L NaCl; Unger 2001), and Triglochin visianii (300 mmol/L NaCl; Khan and Gul 2003). Limonium stocksii is a moderately salt-tolerant halophyte at germination compared with other local halophytic species, but its ability to germinate at salinity levels of up to 500 mmol/L NaCl, which approaches seawater salinity (500 mmol/L NaCl), needs to be considered.

Temperature and salinity interact to affect the germination of halophytes (Khan and Rizvi 1994; Khan and Unger 1997, 1998; Khan and Gul 1998). Some species are more sensitive to changes in temperature (Cressa cretica and Zygophyllum plect) than others (Arthrocnemum macrostachyum and Suaeda fruticosa) (Khan 1999; Khan and Unger 1998; Sheikh Mahmood 1986). Seed germination of L. stocksii is 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 be high after monsoon rains. Germination of halophytes 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 Unger 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; Markat 1987). Very rapid germination was reported for Haloxylon recurvum and Haloxylon salicornicum (Sharma and Sen 1989) and Limonium axillare (Mahmood 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 germinate 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.
Limonium stackeii seeds displayed a greater tolerance to salt-salininity and temperature stress before germination, than from various salinity treatments and temperature recovery for L. aestivum, which showed 95% recovery for 0% seawater treatments. Khan and Ungar (1998) observed a quick recovery in Suaeda fruticosa seeds at temperature regimes. The ability of halophyte seeds to survive hypersaline conditions and germinate when salinity reduced provides them with multiple opportunities for newly establishment in unpredictable saline environments than and Ungar 1997).

Limonium stackeii usually grows in coastal salt marshes that remain more or less wet during all seasons due to deep-rooted seawater. Seed reserves in the soil are exposed to high-temperature stress (around 40 °C or higher) while immersed in seawater during seven months of the year. Seeds L. stackeii remain viable under natural conditions, after exposure to salinity and temperature stress and germinate readily when salinity and temperature stress are reduced under monsoon rains (S. Zia and M.A. Khan, unpublished data). Although seeds could only germinate in up to 1 mol/l NaCl, which is much lower in comparison to other associated halophytic species, their salinity tolerance may be stored in the seed bank, and thus a new reproductive strategy in these unpredictable conditions. Seeds of species such as Arthrocnemum macrostachyum and Spermenites indica 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. stackeii. Survival of seeds under extreme conditions provides this species with a strategy for successful recruitment in a harsh environment. Limonium stackeii is a potential candidate as an ornamental and forage crop in coastal areas, where only brackish water or water is available.

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**ferences**


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