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COMPARATIVE EFFECTS OF NACL AND SEASALT ON SEED GERMINATION OF COASTAL HALOPHYTES

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Abstract

The coastal and near coastal habitats of Karachi, Pakistan are characterized by dominant stands of perennial halophytes like *Aeluropus lagopoides*, *Desmostachya bipinnata*, *Haloxylon stocksii* and *Suaeda fruticosa*. Experiments were carried out to investigate comparative effects of NaCl and seasalt salinity on both rate and final seed germination of these species. Salinity tolerance range of halophytes varied among species and also between salt types. Seed germination of all test species decreased with increase in salinity of both salt types. *Aeluropus lagopoides* was the most tolerant at 50 dS m^{-1} whereas, *S. fruticosa* the least salt tolerant at 30 dS m^{-1}, although the tolerance range was in following sequence: A. *lagopoides* > *D. bipinnata* > *H. stocksii* > *S. fruticosa*. While rate of germination showed a different pattern *D. bipinnata* > A. *lagopoides* = *H. stocksii* > *S. fruticosa.* Seasalt affected seed germination as well as rate of germination of *A. lagopoides, D. bipinnata* and *S. fruticosa* more adversely than NaCl especially at higher concentration. However, in *H. stocksii* seed germination was not significantly different in either salt type. Rate of germination also followed same pattern

Keywords: *Aeluropus lagopoides*, *Desmostachya bipinnata*, *Haloxylon stocksii*, Karachi, *Suaeda fruticosa*

Introduction

Halophytes are distributed in coastal and inland saline habitats and face direct and secondary effects of salinity stress at every stage of their life cycles (Adam, 1990; Khan & Gul, 2002*a*; Khan & Qaiser, 2006; Ungar, 1991). Germination is reported to be more vulnerable stage to salinity than others (Khan & Gul, 2002*a*, 2006). Halophytes show optimal seed germination under non-saline conditions like glycophytes but differ in their ability to germinate in presence of even higher levels of salinity (Ungar, 1995; Noe & Zedler, 2000; Khan & Gul, 2002a). Salinity tolerance of halophytes at germination varies among species (Yokoishi & Tanimoto, 1994; Ungar, 1995; Khan *et al*. 2002, 2006; Liu *et al*. 2006). Threshold tolerance level to salt stress at germination stage depends on the type of species, e.g., for *Salicornia herbacea* it is 1700 mM (Chapman, 1960) while *Spartina alterniflora* can tolerate 1030 mM NaCl stress (Mooring *et al*., 1971). Khan & Gul (2006) reviewed that approximately 83 % Dicotyledonous halophytes can germinate at or above sea water salinity (60 dS m^{-1}), whereas only 17 % of monocotyledonous species can tolerate such a high salinity at germination stage. However, information about seed germination of halophytes is far from complete, since actual halophytic diversity is unknown (Khan & Qaiser, 2006). Many of these can be potential candidates for producing food, fodder or forage using saline agriculture, which is likely to stay in future. However, success of saline agriculture greatly depends upon the knowledge of seed

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germination eco-physiology of halophytes, since most of halophytic species propagate through seeds.

Most of the studies on effect of salinity on seed germination of halophytes have been conducted using NaCl solutions. Such investigations may not infer germination responses of plants under field conditions, because in field soil contains different salts, which collectively influence seed germination in a different way from their individual effects (Ungar, 1978). Seasalt however mimic in composition to soil solution and can be used to analyze synergistic effect of different salts on seed germination (Liu *et al*. 2006). Unfortunately, little is known about how seawater affects on seed germination of halophytes (Baskin & Baskin, 1998; Houle *et al*. 2001; Khan *et al*. 2006). It appears that seawater inhibits germination more in comparison to NaCl (De Villiers *et al*. 1994; Houle *et al*. 2001; Zia & Khan, 2002). However, Tirmizi *et al*. (1993) found that NaCl inhibited the germination of *Hipophae rhamnoides* more than seawater. Similarly Atia *et al*. (2006) also reported that NaCl inhibits seed germination more in comparison to sea-salt.

Pakistani coastal areas are inhabited by 108 halophytic species belonging to 36 families. Most are perennial including grasses (20), sedges (14) and chenopods (13) (Khan & Gul, 2002*b*). *Aeluropus lagopoides, Desmostachya bipinnata*, *Haloxylon stocksii,* and *Suaeda fruticosa* are halophytes found in coastal habitats of Karachi, Pakistan. These halophytic species with wide ecological range are of medicinally important or used as forage or could also be used as a coastal dune stabilizer. Study under consideration was designed to investigate comparative effects of NaCl and seasalt salinity on seed germination of these potentially important halophytes.

Material and Methods

Seeds of test species were collected from a various saline habitats of Karachi Coast during March to April, 2005. Seeds were separated from the inflorescence and dry - stored at room temperature. Before storage they were surface sterilized using 0.85 % Clorox solution for one minute followed by thorough rinsing with autoclaved distilled water and air-drying. Germination was carried out in 5 cm diameter, 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 ^{o}C with 12-h photoperiod (Sylvania cool white fluorescent lamps, 25 µmol m^{-2} s^{-1}, 400-750 nm). Percent germination was recorded on alternate days for 20 days. 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 more rapid the germination. Germination data was acrsine transformed before statistical analysis to ensure homogeneity of variance. Entire experiment was repeated twice. These data were analyzed using SPSS for Windows release 11 (SPSS 2001). The effect of NaCl and seasalt on the germination and rate of germination of test species was examined using analysis of variance (ANOVA). A Bonferonni post hoc test was used to determine significant differences ($p < 0.05$) between means.

Results

Three-way analyses of variance show that the interaction of species (SP), salt type (ST) and concentration (CON) were significantly differed, although their individual effects except concentration were non-significant for both final ($F = 1.97$, $P < 0.01$) and rate ($F = 1.80$, $P < 0.01$) of seed germination. The interactive effect of SP and ST was also significant at both final ($F = 9.17$, $P < 0.001$) and rate ($F = 8.40$, $P < 0.001$) of seed germination level (Table 1).

In non-saline control *Aeluropus lagopoides* and *Desmostachya bipinnata* showed 100 % seed germination whereas only 80 % seeds germinated for *Haloxylon stocksii* and *Suaeda fruticosa* (Figure 1). Increase in salinity linearly decreased germination for *H. stocksii* and *S. fruticosa*, while in *A. lagopoides* and *D. bipinnata* threshold salinity for germination suppression was 20 dS m^{-1}. Salinity tolerance limit of test species decreased in following sequence: A. *lagopoides* > *D. bipinnata* > *H. stocksii* > *S. fruticosa*.

Table 1. Three-way ANOVA showing significance of the effect of various factors on final and rate of germination of halophytes.

	Source	Type III Sum of squares	df	F
Final Germination	SPECIES (SP)	25527	3	4.32 ns
	SALT TYPE (ST)	5188	1	1.81 ns
	CONCENTRATION (CON)	157678	5	22.23 **
	SP × ST	5424	3	9.17 **
	SP × CON	5361	15	1.81 ns
	ST × CON	6294	5	6.40 **
	SP × ST × CON	2956	15	1.97 *
Rate of Germination	SPECIES (SP)	2098	3	3.25 ns
	SALT TYPE (ST)	428	1	1.55 ns
	CONCENTRATION (CON)	31354	5	50.33 ***
	SP × ST	588	3	8.40 **
	SP × CON	644	15	1.83 ns
	ST × CON	525	5	4.50 *
	SP × ST × CON	351	15	1.80 *

Where, ns = non-significant, * = P< 0.01, ** = P< 0.001 and *** = P< 0.0001.

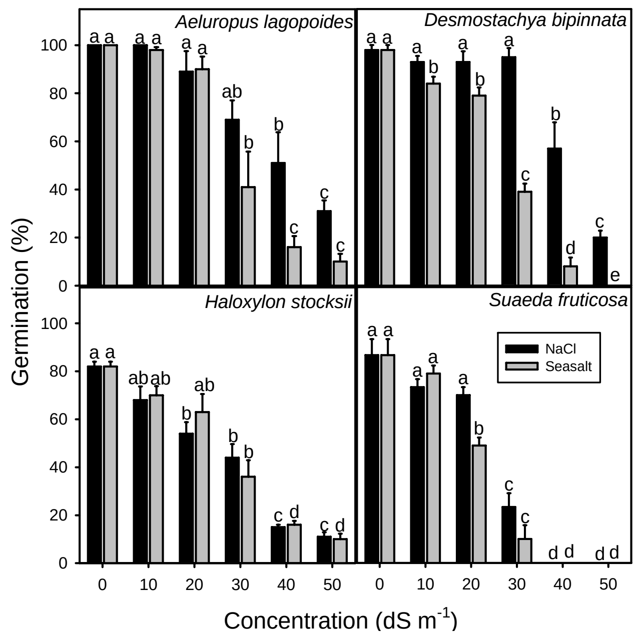

Fig. 1. Effect of NaCl and Seasalt on mean final seed germination of coastal halophytes.

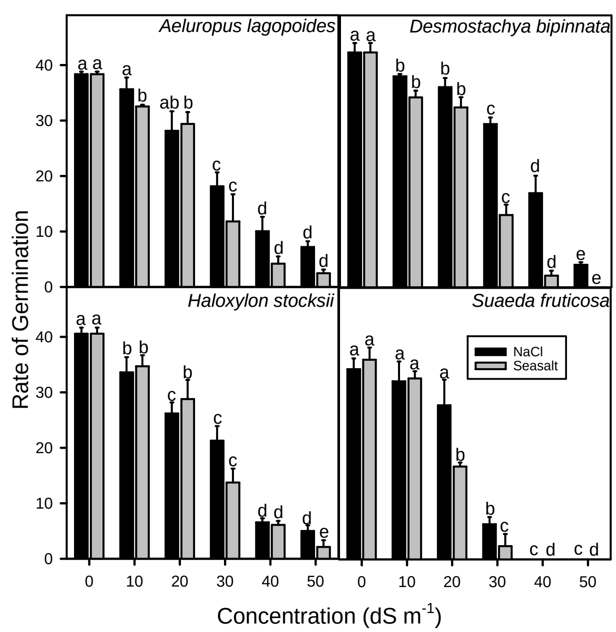

Fig. 2. Effect of NaCl and Seasalt on rate of seed germination of coastal halophytes.

For *A. lagopoides* mean final germination was not significantly different ($P < 0.05$) from non-saline control in either salt type up to 20 dS m^{-1}, but a further increase in salinity linearly decreased germination in both salts. However, seasalt decreased germination more in comparison to NaCl and 25% and 10 % seeds germinated at 50 dS m^{-1} of NaCl and seasalt respectively (Figure 1a). Seed germination of *D. bipinnata* linearly decreased with increasing saesalt salinity, but it was non-significantly ($P< 0.05$) different from control in NaCl up to 30 dS m^{-1} and at 50 dS m^{-1} of seasalt there was no seed germination, while 20 % seeds germinated at similar salinity level of NaCl (Figure 1b). In *H. stocksii* mean final germination progressively decreased with increase in salinity, with no germination above 50 dS m^{-1} and it was also not affected significantly ($P<0.05$) by salt type (Figure 1c). However, mean final germination in *S. fruticosa* significantly ($P<0.05$) differed between salt types, only above 10 dS m^{-1} with comparatively more adverse effects in seasalt than NaCl and no seed germination was found above 30 dS m^{-1} of both salts (Figure 1d).

Rate of seed germination for all test species was highest in distilled water controls. Increasing salinity linearly decreased rate of germination, however, seasalt delayed rate of germination more than NaCl at or above 30 dS m^{-1}in all except *H. stocksii*. Rate of germination of test species was in following oreder: *D. bipinnata* > A. *lagopoides* = *H. stocksii* > *S. fruticosa* (Figure 2).

Discussion

Salinity tolerance range of halophytes varied among species. Khan & Gul (2002) categorized the halophytes and found that halophytic species around the Karachi coast can be categorized into three groups on the basis of their salinity tolerance: a) Group-I that includes species like *Atriplex stocksii* (Khan & Rizvi, 1994), and *Zygophyllum simplex* (Khan & Ungar, 1997) which can germinate in up to 29.1 dS m^{-1} NaCl, b) Group-II that includes halophytes such as *Aeluropus lagopoides* (Gulzar & Khan, 2001), *Haloxylon stocksii* (Khan & Ungar, 1996), *Limonium stocksii* (Zia & Khan, 2002), and *Desmostachya bipinnata* (Gulzar *et al*. 2007) which have ability to tolerate up to 48.5 dS m^{-1} NaCl salinity at germination, and c) Group-III that includes species like *Arthrocnemum macrostachyum* (Khan & Gul, 1998), and *Cressa cretica* (Khan, 1999) which can germinate in up to 97 dS m^{-1} NaCl salinity. All test species appear to be moderately salt tolerant at germination and belong to Group-II, except *Suaeda fruticosa*, where no seed could germinate above 30 dS m^{-1}, hence can be included in Group-I.

Aeluropus lagopoides was the most tolerant, where comparatively more seeds germinated with higher seed germination rate at 50 dS m^{-1} in either salt type than other halophytic species. Gulzar & Khan (2001) reported identical results for *A. lagopoides*. Seeds of *Desmostachya bipinnata* and *Haloxylon stocksii* also germinated in up to 50 dS m^{-1} but rates as well as mean final germination in *D. bipinnata* were higher as compare to *H. stocksii*. *Suaeda fruticosa* was least salt tolerant among all species and showed no germination above 30 dS m^{-1} in both salts. Similar findings have been reported by Khan *et al*. (2006) for these species with seasalt salinity. However, Khan & Ungar (1998) in contrast reported that few seeds of *S. fruticosa* could germinate in up to 500 mM NaCl salinity. This difference might be attributed to genetic variability among populations. Therefore, a detailed study on intra-specific variation in seed germination responses among different populations of *S. fruticosa* is suggested.

There is little information is available on comparative influence of NaCl and seasalt on seed germination of halophytes (Joshi *et al*. 1995; Baskin & Baskin, 1998; Houle *et al*. 2001; Zia & Khan, 2002; Atia *et al*. 2006; Liu *et al*. 2006). Seed germination of *A. lagopoides* and *Desmostachya bipinnata* inhibited more in seasalt especially at higher concentration in comparison to *H. stocksii.* However, seed germination of *S. fruticosa* also reduced more in seasalt than NaCl especially above 10 dS m^{-1}. Zia & Khan (2002) also reported similar results for *Limonium stocksii*, where seasalt suppressed germination more as compared to NaCl. In contrast, Tirmizi *et al*. (1993) have reported that NaCl inhibits seed germination of *Hipophae rhamnoides* more in comparison to seasalt. Duan *et al*. (2003) also reported that NaCl inhibits germination of *Suaeda salsa* more than sea-salt. However, De Villiers *et al*. (1994) and Houle *et al*. (2001) reported inhibitorier effect of seasalt than NaCl on seed germination. This can be attributed to the fact that seasalt is a combination of several salts with leading concentration of NaCl, and seasalt induced germination suppression might be a consequence of synergistic lowering of osmotic potential of the medium than NaCl alone. However, a thorough physiological investigation is needed to understand why seasalt is more inhibitory in some species and not in others.

In summary, halophytic vegetation of Karachi coast varied in their salinity tolerance range and shows various effect in response to NaCl and seasalt at germination stage. *Aeluropus lagopoides*, *Desmostachya bipinnata* (Monocots) and *Haloxylon stocksii* (Dicots) were found to be moderately salinity tolerant (50 dS m^{-1}), while *Suaeda fruticosa* (Dicots) was low salt-tolerance (30 dS m^{-1}) under both salt condition. Salinity tolerance of test species was in following order: A. *lagopoides* > *D. bipinnata* > *H. stocksii* > *S. fruticosa*. The effect of both salts (NaCl and Seasalt) on germination was also varied among species. Seasalt affected seed germination of *A. lagopoides*, *D. bipinnata* (Monocots) and *S. fruticosa* (Dicots) more adversely than NaCl especially at higher concentration. However, seed germination of *H. stocksii* (Dicots) was not significantly different in either salt type. Variation in rate of seed germination of *Desmostachya bipinnata* and *Suaeda fruticosa* under NaCl and seasalt present only at high doses of salinity, whereas difference was absent for *Aeluropus lagopoides* and *Haloxylon stocksii*.

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