EFFECTS OF SALINITY ON SEED GERMINATION, SEEDLING GROWTH AND SURVIVAL OF *SPARTINA CILIATA* BRONG.

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ABSTRACT – (Effects of salinity on seed germination, seedling growth and survival of *Spartina ciliata* Brong.) The effects of different salinities on the germination of *Spartina ciliata* seeds were tested during 40 days in the laboratory, using six concentrations of NaCl (0, 45, 80, 130, 170 and 215 mM) besides three concentrations of seawater (25, 30 and 35‰). In addition, the survival and growth of seedlings were tested in five constant salinity regimes (0, 45, 80 and 170 mM NaCl) as well as in five regimes of simulating seawater overwash. The results showed that seeds possed a high germination capacity over a wide range of salinities (0 to 215 mM NaCl). Although concentrations close to full-strength seawater completely inhibited seed germination, the transfer of the ungerminated seeds to freshwater immediately stimulated this. The effects of salinity on seedling growth showed that shoot height and root length were the variables most sensitive to salt stress. Frequent overwash with seawater significantly reduced the survival and growth of Spartina ciliata seedlings. The success in seed germination, survival, and growth of Spartina ciliata in salinities above those normally found in the natural environment could explain the species being distributed throughout salt-stressed foredune habitats in southern Brazil.

Key words – salinity stress, seed germination, seedling growth, coastal dune plant, *Spartina ciliata*

RESUMO – (Efeitos da salinidade sobre a germinação, crescimento e sobrevivência das plântulas de *Spartina ciliata* Brong.) O efeito de diferentes salinidades sobre a germinação das sementes de *Spartina ciliata* foi testado em laboratório ao longo de 40 dias, usando seis concentrações de NaCl (0, 45, 80, 130, 170 e 215 mM NaCl) e em três concentrações de água do mar (25, 30 e 35‰). Adicionalmente a sobrevivência e crescimento das plântulas foram testados em cinco regimes de salinidade (0, 45, 80, 130 e 170 mM NaCl) assim como, em cinco regimes de inundação com água do mar. Os resultados mostraram que as sementes apresentam alta capacidade germinativa numa grande amplitude de salinidades (0 a 215 mM NaCl). Apesar de concentrações próximas as da água do mar terem inibido completamente a germinação, a transferencia das sementes que não germinaram para água doce, estimulou, imediatamente, a germinação. O efeito da salinidade no crescimento das plântulas mostrou que a altura das hastes e comprimento das raízes foram as variáveis mais sensíveis ao estresse salino. Frequentes alagamentos com água do mar reduziram significativamente a sobrevivência e o crescimento das plântulas de Spartina ciliata. O sucesso na germinação, sobrevivência e crescimento de *Spartina ciliata* em salinidades acimas daquelas normalmente encontradas no ambiente natural poderiam explicar a distribuição da espécie ao longo dos hábitats estressados por sal nas dunas costeiras no extremo sul do Brasil.

Palavras-chave – estresse salino, germinação de sementes, crescimento das plântulas, plantas de dunas costeiras, *Spartina ciliata*.

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Introduction

Substrate salinity is one of the environmental factors that sand dune plants may encounter during their life cycles. Coastal sand dunes, particularly in hot and dry areas, are subjected to salinities ranging from its absence, after a heavy rainfall, up to 500 mM NaCl, after a sea overwash (Sykes & Wilson 1989). In southern Brazilian coastal dunes a maximum of 80mM NaCl was found in interstitial soil solution during the dry season (Cordazzo 1985). Substrate salinity can act as a major selective force, determining seed germination, seedling establishment and survival in coastal areas (Barbour 1970; Seneca 1972; Ungar 1978). The adverse effects of salinity on plant growth and development are complex, and may result from a combination of factors of nutritional, toxic and osmotic nature (Flowers *et al.* 1977; Greenway & Munns 1980; Grattan & Grieve 1993). The consequences of these complex effects are the reduction in leaf size, internode, and culm length, leading to a miniaturization of salt marsh and sand dune plants (Blits & Gallagher 1991; Cordazzo 1994). However, most halophytes have developed adaptations, i.e. succulence, osmotic adjustment, salt glands, ionic compartmentation, to dilute or counter-balance the effects of salinity. The effects of salinity on seed germination and plant growth have been studied for more than a century (Darwin 1857). Seed germination and seedling establishment under various salinities have been investigated in many halophytes (Ungar 1962, 1974; Bazzaz 1973; Woodell 1985), and particularly in some sand dune species (Seneca 1969; Sykes & Wilson 1989; Greipsson & Davy 1994,). However, *Spartina ciliata*, a perennial grass of southwestern Atlantic coastal sand dunes, has received no attention despite its wide-spread occurrence and importance. Therefore, the aim of this study was to examine the effects of salinity on seed germination, seedling growth and the survival of *S. ciliata*.

Material and methods

S. ciliata Brong. seeds were collected from a plant population of south Brazilian coastal dunes near Sarita Lighthouse (32°27'S; 52°21' W), in December/1992. All seeds were stored in a refrigerator and kept at 4°C until used in the experiments.

Seed germination - Seeds were surface sterilized in 1.25% sodium hypochlorite solutions for 15 min (Seneca 1969), rinsed with distilled water, and sown on two layers of filter paper (Whatman no. 1) in 9-cm Petri dishes wetted with 5ml of 0, 45, 80, 130, 170 and 215mM NaCl solutions. Each Petri dish was then sealed with Nescofilm to prevent evaporation, and randomly distributed in temperature-controlled incubators at 12h day (30°C) and 12h night (15°C) for 40 days. During this period the position of the Petri dishes in the incubators was changed daily, and the solutions and seeds were changed weekly. The germination of seeds was observed daily, the seed being considered as germinated when its radicle or plumule had emerged.

Seeds of coastal vegetation are often dispersed by seawater, and imbibition under these conditions (high salinity) may lead to seed germination. In order to test this, seeds of *S. ciliata* were soaked in seawater (25, 30 and 35‰, about 340, 430 and 600mM NaCl, respectively), corresponding to the ranges of salinities found in coastal waters of southern Brazil. Seed germination was evaluated after 30 days, and the ungerminated seeds were transferred to Petri dishes soaked with distilled water in order to observe the residual seed viability after 40 days from planting in the absence of salinity. All other seed germination conditions were the same as described above.

Seedling growth - To quantitatively evaluate the effect of increased salinity upon seedling growth, fifty seedlings of a similar size and age (7-days old), which had been germinated in distillated water, were transplanted to individual 9-cm diameter plastic pots filled with 4:1 sand and a potting compost mixture. Seedling growth (ten seeds for each salinity) was tested under five salinity treatments: 0, 45, 80, 130 and 170mM NaCl. Solutions were prepared by adding the appropriate amount of NaCl to Hoagland's nutrient solution. Application of 30ml of the solution was sufficient to wet the substrate completely. To prevent salt built up in the substrate, pots were flushed with an equal volume of deionized water prior to treatment. In addition, pots were flooded once a week with deionized water and no subsequent salt treatment was given. All plants were maintained in a glasshouse for 60 days. Afterwards, all plants were harvested and gently washed. The dry mass of leaves, roots and rhizomes was determined after 48 hours in an oven at 80°C. The height, leaf number and length of root were recorded.

Overwash with seawater - One hundred and twenty five seedlings (7-days old) were planted in individual plastic pots, as describe above, and cultivated in a glasshouse for 12 weeks. Plants were divided into five groups which were flooded with 50ml of seawater (salinity = 600mM NaCl) at intervals of 1, 2, 3, and 4 weeks, besides a control (without seawater). All pots were flushed with an equal volume of deionized water prior to treatment. Additionally, pots received 20ml of Hoagland's nutrient solution every 2 weeks. At the end of the experiment, the height, dry mass of leaves and survival were measured to determine seedling response to an overwash effect.

Statistical analysis - The seed germination data were transformed, using an arcsin square root transformation for normalization before statistical analysis. However, data shown in tables were not transformed. A one-way analysis of variance (ANOVA), using Tukey's multiple range test (Sokal & Rohlf 1981), was conducted to compare total seed germination and germination rate among treatments. Additionally, the lag time "L" (the time in days between the beginning of the experiment and the commencement of germination) and 't (50)" which indicates the time in which 50% germination was achieved. The use of the lag time, germination rate, and final germination proved to be a satisfatory definition for most species (Shipley & Parent 1991).

Results

Seeds of *S. ciliata* germinated in all NaCl concentrations (Tab. 1). A significant lower germination occurred only at a concentration of 215 mM NaCl (F=2.87; P<0.05). However, an increase in NaCl concentration delayed the germination rate t(50) and prolonged the lag time (L).

Table 1. Effect of salinity on Spartina ciliata seed germination.

Salinity (mM NaCl)	L[1]	%G[2]	t(50)[3]
0	3	96 (ab)	5.5
45	4	99 (a)	7.3
80	4	92 (ab)	7.7
130	4	90 (ab)	8.0
170	4	89 (ab)	9.2
215	5	88 (b)	11.0
		F = 2.87	

[1] L = time indays to the start of seed germination;
[2] %G = mean cumulative percentage of germination; values followed by the same letter in the column arenot statistically different according to Tukey's multiple range test, at 5% significance level;
[3] t(50) = mean time in days to reach 50% of the mean cumulative germination.

Seeds of *S. ciliata* did not germinate in all seawater concentrations within 30 days. However, when non-germinated seeds were transferred to distilled water they showed a percentage of germination of 93, 90 and 86% for salinities of 25, 30 and 35‰, respectively. The analysis of variance indicated no significant differences (F = 1.62) in seed germination between the three concentrations of seawater. However, the t(50) was reduced from 5.5 (seeds that germinated in salinity = 0) to 2 days (seeds non-germinated in seawater and transferred to distilled water).

Symptons of salinity stress were displayed by seedlings mainly in higher concentrations of NaCl. The leaves became twisted and tightly rolled and the tips of older leaves became yellowish.

At lower salinities, the length of root and height of seedlings of *S. ciliata* were a more sensitive indicator of salinity stress than were the number of leaves, or the leaf and root mass (Tab. 2). There was a highly significant relationship (F = 21.99; P < 0.001) between the height and salinity and between length of root (F=38.8; P<0.001) and salinity. The number of leaves was not significantly different with increasing salinity. Leaf dry mass was significantly lower above 130mM NaCl when compared to control seedlings which grew without salt. Root dry mass was significantly lower than that of the control

Table 2. Effect of salinity on *Spartina ciliata* seedling growth[1].

Variable	Sailinity (mM NaCl)[2]					
	0.0	45	80	130	170	F
Height (cm)	70.2(a)	58.7(b)	56.3 (b)	43.6(c)	37.8(c)	21.9
Length of root (cm)	33.8(a)	30.1(b)	22.8(c)	20.6(c)	15.1(d)	38.8
No. of leaves	5.6	5.2	5.5	4.9	5.0	2.3
Leaf Dry Mass (mg)	259.5(a)	204.7(ab)	201.1(ab)	141.1(bc)	119.4(c)	10.5
Root Dry Mass (mg)	125.0(a)	106.0(b)	81.7(bc)	67.1(cd)	46.4(d)	15.8

[1]. seven days old seedlings were transplanted into a greenhouse and grown for 60 days.
[2]. Mean values of 10 seedlings; values followed by the same letter in the row are not statiscitally different according to Tukey's

(0.0mM NaCl) only when NaCl concentration exceeded 45mM. At the end of the experimental period, leaves of seedlings grown in salinities above 85mM NaCl showed a fine layer of small white salt crystals on their surface.

Seedlings of *S. ciliata* showed a gradual decrease in survival and dry biomass in relation to the frequency of flooding with seawater containing 600mM NaCl (Tab. 3). Seedlings suffered high mortality (greater than 50%) when they were flooded with seawater once a week. There was a significant decrease in final height (F=129.2; P<0.001) and dry biomass (F=49.87; P<0.01) with an increasing frequency of flooding with seawater. Some seedlings showed typical symptoms of salinity stress (leaf rolling, chlorosis, and necrosis of the tips) at the end of the experimental period.

Discussion

Coastal dune plants are exposed to varying amounts of salt, from complete immersion in seawater during storm tides, to occasional salt spray (Cordazzo 1994). In general, the germination of halophytes and glycophytes responds in a similar way to increasing salinity with a reduction in both the final percentage and rate of germination, and an increasing delay in the initiation of the germination process. The precise salinity concentrations which cause a delay and reduction in the proportion of seeds germinating depends on the salt tolerance of each species (Ungar 1982; Martinez *et al.* 1992).

The results reported here demonstrate that *S. ciliata* exhibits a germination behaviour similar to halophytes (Ungar 1962, 1978; Rozema 1975) and glycophytes (Bazzaz 1973; Redmann 1974; Rozema 1975). Seed germination remained relatively unaffected by salinity up to 215mM NaCl; however it was completely inhibited at 340mM NaCl. Germination in distilled water of previously ungerminated salt-treated seeds indicated that the inhibitory effect of NaCl on seed germination is due mainly to osmotic effects. Thus, seed viability was not affected by a high NaCl concentration per se. The rapidity of germination

Table 3. Performance of *S. ciliata* seedlings[1]. when subjected to different conditions of overwash with seawater (600 mM NaCl).

Overwash	Initial Height(cm)	Final Height(cm)	Biomass(mg)	Survival(%)
Once per week	3.63	7.31 (a)	5.9 (a)	46
Once per 2 weeks	3.67	19.91 (b)	41.7 (b)	80
Once per 3 weeks	3.64	22.70 (bc)	41.2 (b)	86
Once per 4 weeks	3.59	25.00 (c)	59.0 (b)	94
Control 3.58 (no seawater)	41.81 (d)	112.2 (c)	100	
F=0.19(ns)	F=129.26	F=49.87		

[1]. 25 seedlings planted in individual pots for each treatment and cultivated for 12 weeks; means with the same letter in the column are not statistically different according to Tukey's multiple range test at 5% significance level. (ns = not significant).

in *S. ciliata* suggests some sort of stimulation effects of NaCl, which was also reported in some coastal plants (Woodell 1985). The rapid germination of seeds after salt pre-treatment has been attributed to weakening of the testa by solutions of low osmotic potential (Ungar 1978) and was also reported for *Triglochin bulbosa* and *T. striata* (Naidoo & Naicker 1992) as well as *Blutaparon portulacoides* (Cordazzo 1994).

The capacity of seeds of maritime species to remain dormant when subjected to high salinity is probably of adaptive value since seeds are often dispersed by the seawater and germination would be a disadvantage or lethal under such conditions (Lesko & Walker 1969; Ignaciuk & Lee 1980). An additional importance of this dormancy response to high salt stress in seeds of coastal species is that it permits seed survival during dry periods, when salinity hazards increases (Ungar 1978), and during periods of temporary flooding with highly saline waters (Ungar & Hogan 1970). However, after rainfall has leached or diluted the excess salts, seeds will still be capable of germinating, as was reported in *Cakile maritima* (Hocking 1982; Cordazzo 1994), *C. edentula* (Maun *et al.* 1990) and Blutaparon portulacoides (Cordazzo 1994). Another effect of the release of dormancy with the alleviation of salt stress is that it determines the salinity level at the period of seedling development, which is probably one of the most sensitive periods in the life of coastal plants (Ungar 1982).

S. ciliata seedling growth was more sensitive to salinity than seed germination, with significant reduction for salinities above 130mM NaCl. Although seedlings showed a significant reduction in growth at the highest salinity, the species tolerated salinities up to 130mM NaCl, which was higher than natural salt concentrations in the soil (Cordazzo 1985). Members of the subfamily Chloridoideae (*S. ciliata*) are generally salt resistant and withstand relatively high salinity in their growth medium (Liphschitz & Waisel 1982). Such tolerance is achieved either by salt excretion or by salt endurance. The first is usually found in plants like *Spartina* sp. with sunken salt glands, which are present mainly in the furrows of the adaxial surface (Bastos *et al.* 1993). In addition, some higher plants accumulate glycine betaine, besides other methylated quaternary ammonium compounds or proline particularly when growing in a saline media (Stewart *et al.* 1979). Thus, the accumulation of these compounds compensate for the osmotic disequilibrium between the cytoplasm and the vacuole (Flowers *et al.* 1977) and have either a neutral of beneficial influence on enzyme activity (Brandy *et al.* 1984). The presence of high levels of these organic compounds in coastal dune plants may be surprising since species such as *Ammophila arenaria*, and *Elymus arenaria*, are not characteristic of highly salinized soils (Stewart *et al.* 1979). Therefore, these compounds, apart from their osmoregulatory role, probably act as protectors of the macromolecular structure (Larher & Hamelin 1975).

Other characteristics, commom to most species of the Chloridoideae are the presence of a Kranz-type anatomy and the possession of the C4 syndrome (Smith & Brown 1973), which have been observed in *S. ciliata* (Cordazzo 1994). Many C4 plants were shown not only to tolerate sodium but also to require it as a micronutrient (Brownell & Crossland 1972), small quantities activating PEP carboxylase (Bidwell 1979). These adaptive halophytic characteristcs, common in the genus *Spartina*, could explain the relatively good performance of *S. ciliata* in saline soils. Concluding, the results obtained in the laboratory on seed germination and the growth of *S. ciliata* in salinities above those found in their natural environment, could explain why it is a dominant species in sand flats subject to an overwash by seawater, and in the most saline areas of southern Brazilian coastal dunes.

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