Running title: Responses to salt stress in *Juncus*

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Responses to salt stress in *Juncus acutus* and *J. maritimus* during seed germination and vegetative plant growth

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Abstract

Responses to increasing salinity, during seed germination and vegetative plant growth, were studied in two related species of *Juncus*, *J. maritimus* and *J. acutus*. In both species, germination was optimal in the absence of salt, reduced by about 50% in the presence of 200 mM NaCl, and completely inhibited by NaCl concentrations above 300 mM. Previous exposure of the seeds to salt, up to 500 mM NaCl, did not affect the germination capacity in *J. acutus*, and clearly enhanced it in *J. maritimus*. A concentration-dependent inhibition of plant growth was observed in the presence of NaCl for both species, together with the parallel accumulation of sodium ions in the leaves, as determined by cation exchange HPLC. Regarding the levels of divalent cations, in *J. acutus* Ca^{2+} and Mg^{2+} increased up to about two-fold in plants treated with 500 mM NaCl, as compared to control plants, whereas in *J. maritimus* they were three to four-fold higher than in *J. acutus* in the absence of salt, and did not change significantly with increasing NaCl concentrations. These results suggest that Ca^{2+} and Mg^{2+} participate in defence mechanisms against salt stress, which would be constitutive in *J. maritimus* and salt-inducible in *J. acutus*.

Keywords: abiotic stress, cation accumulation; ion exchange HPLC, *Juncus*, salt tolerance; seed germination.

The genus *Juncus*, distributed throughout the world in humid environments, includes more than 300 species (Mabberley, 1997), some of them halophytic. The two taxa included in the present study, *Juncus maritimus* Lam. (sea rush) and *J. acutus* L. (spiny rush), are taxonomically and ecologically related. Both are grass-like, perennial, rhizomatous wetland plants. *Juncus maritimus* is smaller, up to 1 m high, with a thick and long rhizome and a lax inflorescence, whereas *J. acutus* is more vigorous, reaching heights of 2 m, with a short rhizome, a compact inflorescence and a long pungent bract. Both species are classified within the subgenus *Juncus* and often grow in the same communities in salt marshes. *Juncus maritimus* has a Paleotemperate-Mediterranean distribution, whereas *J. acutus* is subcosmopolitan. Both species are common in Spanish coastal marsh communities developed on permanently humid soil, rich in chlorides, especially NaCl, and with alkaline carbonates (Fernández-Carvajal, 1982). The species *J. maritimus*, characteristic of the phytosociological class *Juncetea maritime* Br.-Bl. 1931, is frequent in coastal wetlands experiencing temporary flooding, mostly in spring in Central Europe (Woodell, 1985) and in autumn and spring in the Mediterranean region (Boira, 1988). *Juncus maritimus* is a salt-tolerant species (Boira, 1988) that also grows in conditions of higher salinity, such as in halophylous plant communities of the class *Arthrocnemetea* Br.-Bl. & R. Tx. 1943 (Costa & Boira, 1981; Costa et al., 1986; Llorens, 1986). *Juncus acutus* is a species with a wider ecological range, but less salt tolerant and less competitive than *J. maritimus* in conditions of flooding and edaphic humidity. It tolerates soils with high levels of sulphates and chlorides (Rivas Goday, 1945) and soils with sandy texture and hydric stress during the dry summer season in the Mediterranean areas. It also appears in gypsophile facies of some communities of the class *Juncetea maritime* (Bolós, 1967). *Juncus acutus* is also frequent on dunes in

zones of estuaries (Fernández-Carvajal, 1982). Both species have a fast germination rate, which, combined with vegetative propagation, allow them to spread rapidly.

Different authors have studied germination responses to salt stress in *Juncus acutus* (Greenwood & MacFarlane, 2006; Martínez-Sánchez et al., 2006; Vicente et al., 2007) and in *J. maritimus* (Clarke and Hannon, 1970; Woodell 1985; Partridge & Wilson 1987a). However, there are few reports on plant growth and cation accumulation upon salt treatments and, as far as we know, no comparative studies including both species from the same geographic area.

The purpose of this study was to compare the patterns of germination, vegetative plant growth and cation accumulation in leaves in *Juncus acutus* and *J. maritimus* treated with NaCl solutions of increasing concentration. We expected that the responses to salt stress under in vitro and greenhouse conditions would reflect the ecological differences which have been observed between these two closely related species, and therefore to provide new information on their mechanisms of salt tolerance, which appear to be more efficient in *J. maritimus* than in *J. acutus*.

Material and Methods

Plant material

Seeds of *Juncus acutus* and *J. maritimus* were obtained from the seed bank of "Oficina Técnica de la Devesa de La Albufera", collected in August 2004 in the Natural Park of Albufera, Valencia (Spain). Seeds were hand-sieved and air dried thoroughly and stored at room temperature for six months. Previous to germination tests, seeds were sterilised by 5 min treatment with 70% ethanol, followed by 5 min with 30% bleach containing 0.05% Triton X-100, and then thoroughly washed in distilled water.

Germination conditions

Germination was carried out in a germination chamber ASL Ibercex, with a 12 h photoperiod, and a photon flux of approximately 30 μmol photons m^{-2} s^{-1}, provided by cool white fluorescent lamps (Sylvania). The temperature was kept at 25ºC in the light and 15ºC in the dark, a regime similar to the natural conditions during seed germination of the species of interest in the sampling area.

Germination tests in the presence of salt

Four replicas of 25 seeds per treatment were placed onto two layers of filter paper and cotton in standard Petri dishes. The filter paper was moistened with 8 mL of distilled water (for the controls) or aqueous solutions of 100, 200, 300, 400 or 500 mM NaCl, the Petri dishes were sealed with parafilm to avoid evaporation during the experiments, and the plates were incubated as described above. Upon radicle emergence, seeds were considered as germinated and were removed from the Petri dish. The number of germinated seeds was counted every two days. Germination capacity (GC) was expressed as the percentage of germinated seeds. Germination rate (GR) was calculated according to the Timson velocity index (Timson, 1965), as modified by Khan & Ungar (1984), a reliable : Σ G/t, where G is the percentage of seeds germinated after two days intervals, and t is the total time of germination. The assay was finished after 30 d, since previous tests indicated that most seeds germinated within the first 20 d.

Germination recovery

All seeds from the previous tests that did not germinate after one month at different salt concentrations, were taken from the plates and washed in distilled water. The seeds were then placed in fresh Petri dishes with filter paper moistened with distilled water and kept for additional 30 d in the germination chamber, to determine GC and GR in water, as described above.

Plant growth in the presence of salt

Plant growth was studied using young plants obtained by seed germination and kept in a greenhouse with controlled minimal and maximal temperatures of 15 and 25ºC, respectively, and under natural light conditions. Seeds were sown in seed trays containing a mixture of peat, coconut fibre and sand (3:2:1). After two months, plants were transferred to individual plastic pots of 12 cm diameter with the same substrate, and grown for one additional month before starting the salt treatments (100, 200, 300, 400 or 500 mM NaCl), which were carried out during three months, from May to July 2005. For this, 150 mL of salt solutions (or distilled water for the control treatment) were added weekly to the pots. This volume was enough to maintain the moisture of the substrate throughout the experiment. After 90 days of salt treatments, the plants were harvested, weighed on a precision balance, and then frozen and stored at -70ºC. For *J. acutus*, 20 plants were taken for each treatment, whereas in *J. maritimus*, due to the higher mortality of the plants, the number varied from eight to 15 plants per treatment.

Determination of Na^+, K^+, Ca^{2+} and Mg^{2+}

The levels of these cations were measured in leaves of the same plants used for the growth experiments. Cations were separated by HPLC in a IC-Pak cation exchange

column coupled to a Waters 432 conductivity detector, and sodium, potassium, calcium and magnesium contents were determined from the areas of their elution peaks, by comparison with the corresponding standards, as previously described (Vicente et al., 2004). Three samples (0.2 g of fresh material), from three independent plants per treatment were used for each measurement.

Statistical analysis

Data were analysed using SPSS, version 15. Requirements of ANOVA were checked by normality plots and by testing the homogeneity of variance of residual means. Several tests were applied, and the Levene test was selected as the most reliable. Prior to analysis of variance, the percentage data were normalized by an arcsine transformation, while weight measurements and ion content data were subjected to a logarithmic transformation. Significance of differences among treatments was tested by applying one-way ANOVA. When the ANOVA null hypothesis was rejected, post-hoc comparisons were performed using the Tuckey test. A correlation of germination percentages and NaCl concentrations was established by applying linear regression.

Results

Seed germination under saline conditions.

Seeds of *Juncus acutus* showed a high viability in the absence of salt. After a lag period of about one week, most seeds germinated in water, reaching 95% by the end of the second week. Seeds of *J. maritimus* had similar germination behaviour, but only 48% germinated under the same conditions (Table 1).

Germination was inhibited by salt in both species, in a concentration-dependent manner. In the presence of NaCl, at a concentration of 100 mM, the percentage of germinated seeds was slightly reduced, but at 200 mM NaCl less than half of the seeds germinated, as compared to the corresponding controls. Germination percentages dropped below 10% at 300 mM NaCl, while 400 and 500 mM NaCl completely inhibited germination in both species (Figs. 1A, 1B). One-way ANOVA showed that the differences among treatments were significant ($F = 80.05$, $p < 0.05$ in *J. acutus* and $F = 32.45$, $p < 0.05$ in *J. maritimus*).

Germination rates also decreased with increasing salt concentrations for both species, and the differences among treatments were significant ($F = 41.30$, $p < 0.05$ in *J. acutus* and $F = 25.88$, $p < 0.05$ in *J. maritimus*). Table 1 shows Timson index values and the results of post-hoc Tuckey tests.

Germination recovery

Upon completion of the recovery period, germination in *J. acutus* ranged from 68.75% in seeds previously treated with 100 mM NaCl to more than 90% in seeds treated with 200, 300, 400 or 500 mM NaCl ($F = 12.50$, $p < 0.05$). The differences observed in *J. maritimus* were more accentuated ($F = 16.85$, $p < 0.05$): germination in distilled water increased from about 11%, for the seeds that had not germinated in the presence of 100 mM NaCl, to more than 53% for those from the 500 mM NaCl plates (Table 2). Based on the post-hoc Tuckey tests (Table 2), we can conclude that salt pre-treatments did not affect germination of *J. acutus* seeds in water, but clearly enhanced germination of *J. maritimus* seeds, in a concentration-dependent manner (Figs. 1C, 1D).

Germination rates closely paralleled final germination percentages for the different salt treatments. In *J. acutus*, the Timson germination velocity index was very similar for the batches of seeds pre-incubated with 200 to 500 mM NaCl, and somewhat lower for those collected from the 100 mM NaCl plates. In *J. maritimus*, on the other hand, the Timson index clearly increased with increasing salinity in the previous germination tests, up to four-fold when comparing seeds maintained for 30 days in the presence of 500 mM NaCl with those from the 100 mM plates (Table 2).

Plant growth in the presence of salt

Salt stress affected growth, expressed as fresh weight of the plants, in both species (Fig. 2). The maximum mean weight was observed in control plants, and the lowest corresponded to plants grown in the presence of 500 mM NaCl. One-way ANOVA showed that differences among treatments were significant ($F = 9.08$, $p < 0.05$).

Determination of Na^+, K^+, Ca^{2+} and Mg^{2+} contents

For cation measurements, leaf material was collected from some of the plants treated for 90 days with different NaCl concentrations, from 100 to 500 mM, and control plants grown in the absence of salt; only green, healthy-looking leaves were selected for these experiments. In the case of *J. maritimus*, most leaves of the plants grown in the presence of 500 mM NaCl were senescent and there was not enough material to perform these measurements; therefore, these samples were omitted from the analysis. The obtained values were expressed as µmol per gram fresh weight and are shown in Table 3.

Plants of both species accumulated sodium ions in the leaves upon salt treatments. In *J. acutus*, the mean Na^+ levels increased about 20-fold from the control to the 500 mM NaCl treatment ($F = 276$, $p < 0.05$). In *J. maritimus* the variation was not so high: the maximal concentration of Na^+, observed in the 300 mM salt treatment, was less than 10-fold higher than that registered in the control, but differences among treatments were also significant ($F = 69.75$, $p < 0.05$). Values of K^+ content were relatively low and varied little with increasing salt concentrations, in both species (data not shown). Regarding divalent cations, Ca^{2+} levels showed a significant increment with increasing external salinity in *J. acutus* ($F = 27.12$, $p < 0.05$) but not in *J. maritimus* ($F = 1.68$, $p = 0.22$). Similarly, changes in Mg^{2+} content were also not significant for *J. maritimus* ($F = 2.82$, $p = 0.07$), whereas *J. acutus* plants grown in the presence of 500 mM NaCl accumulated twice as much Mg^{2+} as the control plants ($F = 23.80$, $p < 0.05$). It is important to note that, in control plants grown in the absence of salt, the concentration of Mg^{2+} and Ca^{2+} is higher (three to four fold) in *J. maritimus* than in *J. acutus*.

Discussion

The results presented here indicate that seed germination of the two *Juncus* species studied is optimal under non-saline conditions, reduced by moderate salt concentrations, and completely inhibited above 300 mM NaCl, a salinity level that adult plants survive in their natural habitats, albeit reducing their growth rate. A similar behaviour has been shown for most halophytes, although there is a large variability in the level of salt that completely inhibits germination (Woodell, 1985; Flowers et al., 1986; Ungar, 1995, 1996; Gulzar & Khan, 2001; Vicente et al., 2004). Salt tolerance is developmentally

regulated and the responses to high salinity may be quite different at different developmental stages (Lauchli & Epstein, 1990; Johnson et al., 1992). Perennial halophytes, in particular, seem to be far more sensitive to salt during seed germination than for vegetative growth and the more tolerant the adult, the greater the difference between seed/seedling and adult responses (Partridge & Wilson 1987a).

Our data generally agree with previous reports on seed germination of the same species, although a direct comparison of the results cannot be made since the germination tests were performed under different conditions. Thus, for *Juncus maritimus*, Clarke & Hannon (1970) also reported a 50% inhibition of germination by 200 mM NaCl, while Woodell (1985) found that a high percentage of seeds (84%) germinated in water but only about one third of them in the presence of 300 mM NaCl. *Juncus acutus*, on the other hand, has been the subject of several recent studies. In this species, Vicente et al. (2007) reported a stronger inhibition of germination by salt than that observed by us (95% germination in the control and 15% in the presence of 1% NaCl, equivalent to about 170 mM). It should be noted, however, that their experiments were performed at a temperature regime of 30/20ºC, higher than the 25/15ºC used in our case. No significant differences were observed at these two alternating temperature regimes, or at a fixed temperature between 15ºC and 30ºC, in the absence of salt (Martínez-Sánchez et al., 2006). However, an interaction between salinity and temperature on seed germination was detected in several species by Greenwood & MacFarlane (2006), who found better germination under salt stress conditions at lower temperatures. In *J. acutus*, for instance, these latter authors reported inhibition of germination in the presence of 260 mM NaCl of ca. 20% and 60%, under temperature regimes of 25/10º C and 30/15ºC, respectively.

Despite the relatively high percentages of germinated seeds observed under moderate saline conditions in *Juncus acutus* and *J. maritimus*, there are other species in the genus more tolerant to salt stress at the germination stage. Seeds of *Juncus subulatus* germinated as the control up to 175 mM NaCl, with a 50% inhibition observed at 350 mM NaCl (Espinar et al. 2005, 2006). In *J. kraussi*, 50% germination was also registered at approximately 350 mM NaCl (Greenwood & MacFarlane, 2006; Naidoo & Kift, 2006).

The recovery of germination after removal of salt stress is common among halophytes. At high osmotic potentials of the soils in their environment, seeds of many halophytes enter in dormancy, but germination capacity is usually recovered when the stress conditions are alleviated (see, for example, Khan & Ungar, 1997; Khan et al. 2000; Pujol et al., 2000; Gulzar & Khan, 2001). Quantitatively, the effects on germination of previous seed exposure to salt are very variable. In some cases, germination percentages are higher after a pre-treatment with salt (Barbour, 1970; Ungar, 1978; Woodell, 1985; Keiffer & Ungar, 1997), in other species germination is not affected (Pujol et al., 2000; Vicente et al., 2004; Naidoo & Kift, 2006), and sometimes there is a decrease in germination frequencies with increasing salinity (Woodell, 1985; Ungar, 1991; Gulzar & Khan, 2001). In *J. acutus*, seeds maintained their ability to germinate in water after one month exposure to NaCl, and the percentages of germinated seeds were similar, and very high for all treatments, including controls. A similar behaviour, regarding sensitivity of germination to salt and recovery of germination capacity after salt pre-treatments, has been reported for *J. kraussii* (Naidoo & Kift, 2006). In the case of *J. maritimus*, we noted that seeds exposed to high salinity conditions (500 mM NaCl) germinated even better than control seeds

maintained in water, and that the effect of NaCl on germination frequencies was concentration-dependent, which has also been reported by Woodell (1985) for the same species. Similar results were obtained for the rate of germination, which was more or less constant in *J. acutus*, but increased in *J. maritimus* with increasing salinity, surpassing the rate in control samples. Our data suggest that optimum germination of *J. maritimus* requires the seeds to be previously subjected to high salinity conditions, whereas *J. acutus* is more indifferent to the soil salinity, in agreement with the ecology of the two species, as *J. maritimus* is more tolerant to salt stress than *J. acutus*.

Concerning vegetative plant growth in the presence of salt, both species, *J. maritimus* and *J. acutus*, survived high concentrations of NaCl (400 - 500 mM), but optimum growth was registered in control treatments, in the absence of salt. Rozema (1976) obtained similar results in *J. maritimus*, although other studies suggested that a low NaCl concentration (100 mM) could stimulate growth of this species (Clarke & Hannon, 1970; Partridge & Wilson, 1987b). In the highly tolerant species *J. krausii*, maximal growth was also observed in the absence of salt (Naidoo & Kift, 2006). This seems to be the common behaviour in most halophytic species (Flowers et al., 1986; Vicente et al., 2004), especially in monocotyledonous halophytes, which differ in some respects from salt-adapted dicotyledonous taxa (Rozema, 1991): their growth rate is not stimulated by salt and is often lower than in dicotyledonous plants at increased salinity; they have a markedly lower internal sodium to potassium ratio and a lower water content.

Salt tolerance of plants largely depends on their ability to compartmentalize toxic ions in the vacuoles, thus avoiding the inhibition by salt of key enzymatic activities and metabolic processes in the cytosol or the nucleus (Flowers et al., 1986;

Serrano & Gaxiola, 1994; Forment et al., 2002). In the two species studied, increasing external NaCl concentrations induced a clear accumulation of Na^+ in the leaves of the plants, reaching a 20-fold increase over the control in *J. acutus* plants treated with 500 mM NaCl. This is a fundamental response of halophytes to salt stress; the glycophytes, on the contrary, have the tendency to exclude sodium ions from the roots (Flowers et al., 1986).

Variations in the contents of the divalent cations analysed (Ca^{2+} and Mg^{2+}) were not significant for *J. maritimus*. However, in *J. acutus*, both cations accumulated in leaves in response to increasing external NaCl concentrations. The accumulation of Ca^{2+} and Mg^{2+}, even though it is small (about two-fold in plants watered with 500 mM NaCl, as compared to control plants), may be physiologically meaningful. Ca^{2+} has a well-known protective role in conditions of salt stress (Rengel, 1992; Bressan et al., 1998; Gul & Khan, 2008), whereas inhibition by Na^+ of different enzymatic activities is due to displacement of the Mg^{2+} cofactor from the active centre (e.g., Albert et al., 2000), suggesting that a higher (but still non-toxic) intracellular Mg^{2+} concentration could also confer halotolerance. Thus, the progressive increment of Ca^{2+} and Mg^{2+} levels with increasing external NaCl concentration in *J. acutus*, could be considered as a salt-inducible defence mechanism conferring some degree of salt tolerance to the plants. In *J. maritimus*, on the other hand, there is no accumulation of these cations in response to salt. However, the levels of Ca^{2+} and Mg^{2+} in the leaves of *J. maritimus*, in the absence of salt, are already three to four-fold higher than in *J. acutus*. Following the previous reasoning, one can speculate that the presence of relatively higher concentrations of calcium and magnesium in *J. maritimus* could represent a constitutive mechanism of tolerance to salt stress. It would be interesting to determine Ca^{2+} and Mg^{2+} levels, and

their variation in response to salt, in other related species with different degrees of salt tolerance, to check whether this putative mechanism can be generalised.

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Table 1. Seed germination in the presence of increasing NaCl concentrations. For each species, values shown are percentages of germinated seeds (means ± S.D., n = 4) in the "Germination" column, and Timson velocity index (means ± S.D., n = 4), in the "Germination rate" column. Within each column, values followed by a different lower case letter are significantly different from each other (Tuckey test; $P < 0.05$, n = 4).

	Germination		Germination rate	
NaCl (mM)	*J. acutus*	*J. maritimus*	*J. acutus*	*J. maritimus*
0	95.00 ± 3.83 c	48.00 ± 8.64 c	35.93 ± 1.86 d	17.18 ± 3.36 d
100	84.00 ± 10.83 c	40.00 ± 8.64 c	29.53 ± 3.99 d	13.75 ± 2.82 d
200	40.00 ± 4.62 b	24.00 ± 16.97 b	10.75 ± 1.63 c	7.03 ± 5.35 c
300	5.00 ± 6.00 a	4.00 ± 5.66 b	1.33 ± 1.72 b	1.20 ± 1.70 b
400	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
500	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

Table 2. Recovery of germination in distilled water after 30 d in the presence of NaCl, at the indicated concentrations. For each species, values shown are percentages of germinated seeds (means ± S.D., n = 4) in the "Germination" column, and Timson velocity index (means ± S.D., n = 4), in the "Germination rate" column. Within each column, values followed by a different lower case letter are significantly different from each other (Tuckey test; $P < 0.05$, n = 4).

	Germination		Germination rate	
NaCl (mM)	*J. acutus*	*J. maritimus*	*J. acutus*	*J. maritimus*
100	68.75 ± 12.50 a	11.11 ± 9.07 a	29.80 ± 5.40 a	4.63 ± 3.93 a
200	91.07 ± 3.57 b	11.76 ± 0.00 a	39.43 ± 1.55 b	5.05 ± 0.10 a
300	98.53 ± 2.94 b	28.95 ± 5.26 b	42.63 ± 1.22 b	12.10 ± 2.20 b
400	95.65 ± 3.55 b	31.52 ± 4.16 c	41.40 ± 1.51 b	13.28 ± 1.75 b
500	97.73 ± 4.55 b	52.27 ± 4.55 d	42.25 ± 1.91 b	22.10 ± 2.20 c

Table 3. Sodium, calcium and magnesium levels (µmol g^{-1} Fresh Wt), in leaves of *Juncus acutus* and *J. maritimus* plants treated for 90 d with NaCl, at the indicated concentrations, as determined by cation-exchange HPLC. The values shown are the means (± S.D.) of samples from 3 independent plants per treatment. Within each column, values followed by different lower case letters are significantly different on the basis of Tuckey test ($P < 0.05$). Values marked by an asterisk (*) within a column are not significantly different, according to one way ANOVA. n.d.: not determined

NaCl (mM)	Na^+		Mg^{2+}		Ca^{2+}	
	J. acutus	*J. maritimus*	*J. acutus*	*J. maritimus*	*J. acutus*	*J. maritimus*
0	9.30 ± 0.73 a	14.29 ± 2.39 a	14.07 ± 2.16 a	44.98 ± 12.65*	8.64 ± 1.00 a	32.17 ± 1.84*
100	32.07 ± 6.91 b	50.64 ± 14.17 b	13.64 ± 2.08 a	36.41 ± 2.02*	7.61 ± 1.79 a	32.02 ± 3.08*
200	82.01 ± 3.50 c	52.71 ± 10.82 b	19.33 ± 1.59 b	32.02 ± 3.61*	11.63 ± 0.42 b	29.57 ± 0.14*
300	84.65 ± 4.50 c	116.29 ± 11.72 c	19.61 ± 2.74 b	39.97 ± 1.44*	13.59 ± 1.82 b	30.03 ± 0.94*
400	148.91 ± 11.33 d	75.96 ± 3.33 c	31.14 ± 4.75 c	32.26 ± 0.50*	17.47 ± 1.50 c	30.12 ± 0.29*
500	194.01 ± 23.43 d	n.d.	31.89 ± 2.73 c	n.d.	19.50 ± 1.91 c	n.d.

LEGENDS TO THE FIGURES

Fig. 1. Linear regressions for germination (A and B) and recovery of germination (C and D) trends in *Juncus acutus* (A and C) and *J. maritimus* (B and D).

Fig. 2. Effects of salinity on plant growth. Fresh weight (± S.D.) of *Juncus acutus* (white bars) and *J. maritimus* (black bars) plants grown in the presence of the indicated NaCl concentrations (N = 20 for *J. acutus*; N varies from 8 to 15 in *J. maritimus*). Plants were subjected to salt treatemnts for 90 days. Different latin letters for *J. maritimus* and greek letters for *J.acutus* represent significant differences according to Tuckey's post-hoc test ($\alpha = 0.05$).

Figure 1

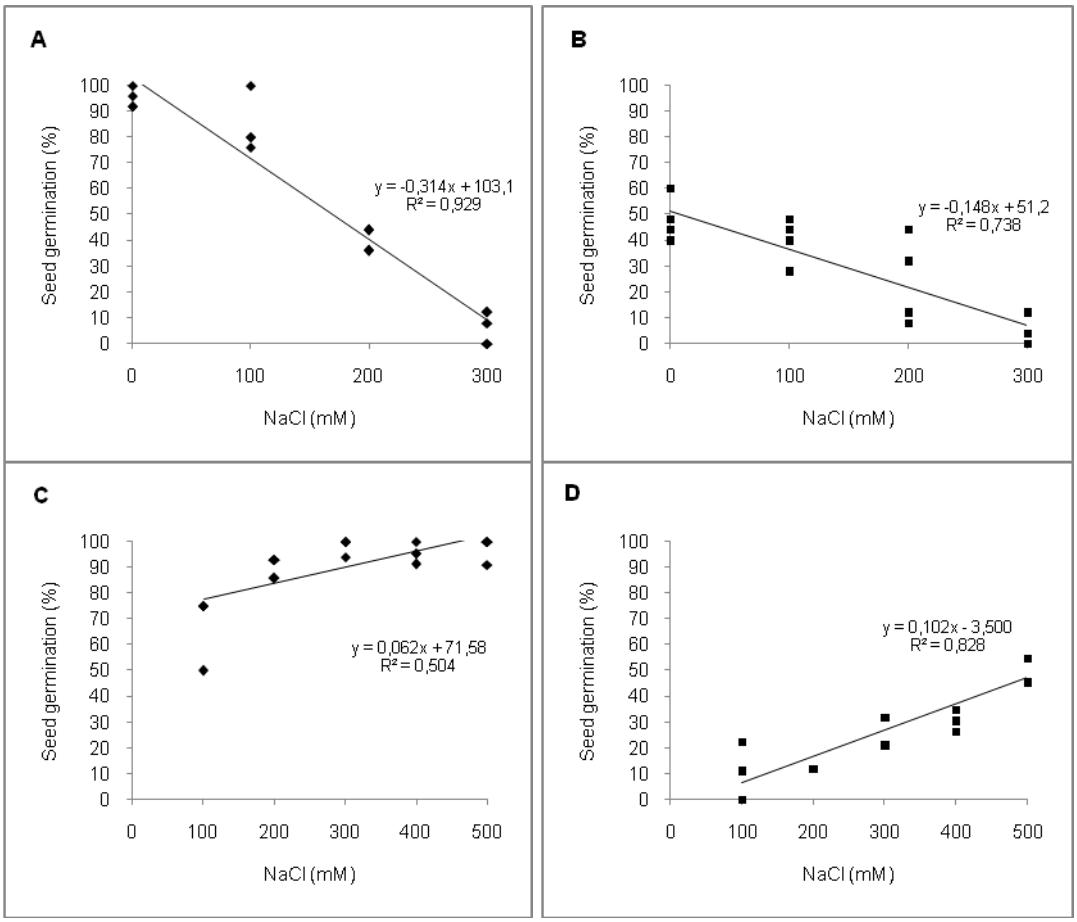
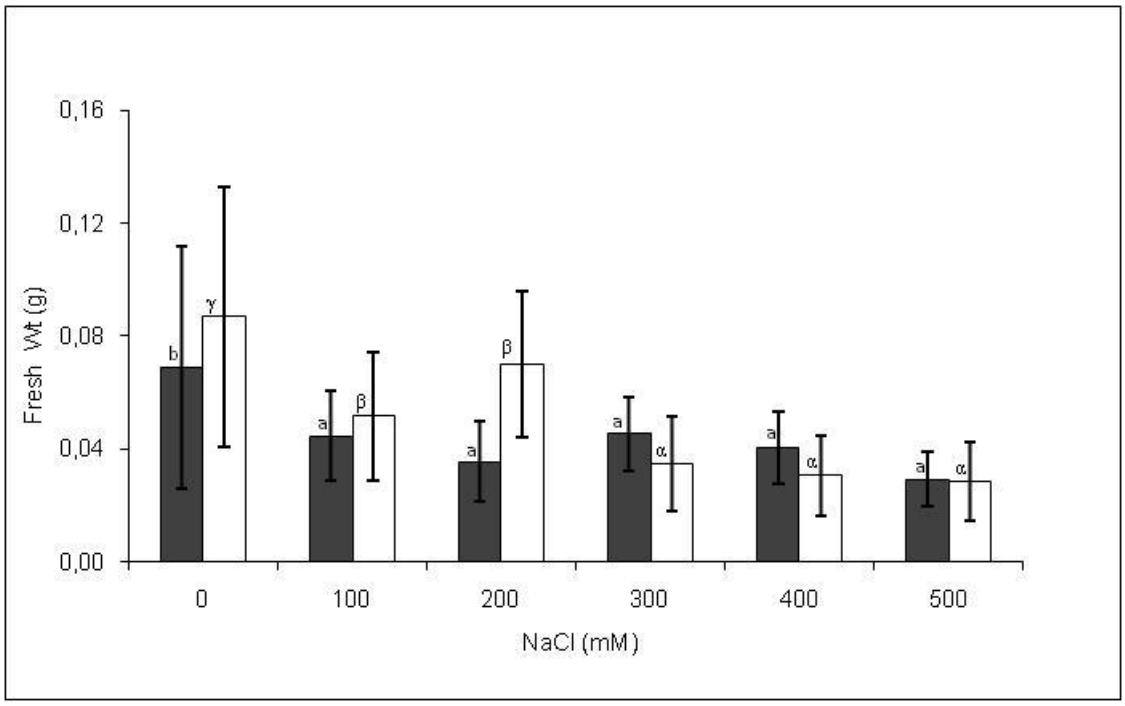

A
Seed germination (%)
NaCl (mM)
y = -0,314x + 103,1
R² = 0,929
B
Seed germination (%)
NaCl (mM)
y = -0,148x + 51,2
R² = 0,738
C
Seed germination (%)
NaCl (mM)
y = 0,062x + 71,58
R² = 0,504
D
Seed germination (%)
NaCl (mM)
y = 0,102x - 3,500
R² = 0,828

Figure 2


0,16
0,12
0,08
0,04
0,00
Fresh Wt (g)
b
γ
a
β
a
β
a
α
a
α
a
α
0
100
200
300
400
500
NaCl (mM)

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