Effects of various salts on the germination of three perennial salt marsh species

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Abstract

We studied the germination responses of *Arthrocnemum macrostachyum*, *Juncus acutus* and *Schoenus nigricans* to saline stress caused by different salt types. The germination percentage and mean time to germination data were obtained by incubating seeds for 30 d in 1, 2, 3, 4 or 5% saline solutions of NaCl, $MgCl_2$, $MgSO_4$ and Na_2SO_4 at 30/20 °C and with a 12 h photoperiod. *A. macrostachyum* was the most tolerant species to salinity during the germination (65% in 2% NaCl). *S. nigricans* showed the lowest germination (none germinated in salt and only 26% in distilled water). *J. acutus* showed intermediate behaviour between the two above species, its germination being inhibited by high salt concentrations. The sulphates had less inhibitory effect than the equivalent chloride concentrations.

Keywords: Seed ecology; *Arthrocnemum macrostachyum*; *Juncus acutus*; *Schoenus nigricans*; Salinity

1. Introduction

Arthrocnemum macrostachyum (Moric.) Moris., *Juncus acutus* L. and *Schoenus nigricans* L. are typical species in the salt marshes of the Mediterranean region, although the last two species also appear in western and northern Europe. In SE Spain the three species tend to appear in coastal and inland salt marshes on different type of soils with variable moisture and salinity levels (Álvarez-Rogel et al., 2000).

Although there are references available on the germination of *A. macrostachyum*, which is known to be affected by salinity (Pujol et al., 2000; Rubio-Casal et al., 2002; Herranz et al., 2004), no information exists concerning the seed germination of *J. acutus* and *S. nigricans* in saline conditions. Recently Martínez-Sánchez et al. (2006) reported the effects of photoperiod and temperature on the germination of *J. acutus* and *S. nigricans* seeds in non-saline conditions, the former showed a wide range of ecological tolerance with regards to temperature and light conditions and the latter species manifested seed dormancy.

Several studies have indicated that an increase in salinity stress induces both a reduction in the percentage of seeds germinated and a delay in the initiation of the germination (Ungar, 1982; Phillipupillai and Ungar, 1984; Khan and Ungar, 1996; Keiffer and Ungar, 1997). Moreover, high salinity can also cause a complete inhibition of the germination at concentrations beyond the tolerance limits of the species (Ungar, 1991).

Many authors have used NaCl solutions to study salinity tolerance in the germination of halophyte species (Khan and Ungar, 1996; Keiffer and Ungar, 1997; Khan et al., 2000; Gulzar and Khan, 2001; Khan and Gulzar, 2003; Herranz et al., 2004), but little information exists concerning the effect of other salts on the seed germination (Pujol et al., 2000; Ramoliya and Pandey, 2002).

According to Ungar (1987), saline soils tend to show higher salinity and have more negative water potentials in the summer

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than in the other seasons. Álvarez-Rogel et al. (2000, 2006) related the increase in soil salinity in summer to a higher content in Cl^-, Na^+, SO_4^{2-}, Ca^{2+}, Mg^{2+} and K^+. However, the relative percentages of Ca^{2+} and K^+ decreased when salinity rose, leading to an imbalance in favour of the most toxic cations, such as Na^+ and Mg^{2+}. The same authors showed that the highest correlation coefficients for ions were obtained between Cl^- and Na^+ and between Cl^- and Mg^{2+}.

Because soil salinity has traditionally been considered one of the most important physical factors in the plant zonation of salt marshes (Egan and Ungar, 2000) and due to the absence of germination data in literature for two of the three species studied and only partially tested in *A. macrostachyum*, the aim of the present work was to determine the salinity tolerance of the three species during germination.

2. Materials and methods

2.1. Study species

A. macrostachyum (Chenopodiaceae) is a perennial halophytic shrub typical of Mediterranean salt marshes, can endure sporadic floods and frequently occurs in the coastal and inland salt marshes of SE Spain (Álvarez-Rogel et al., 2000; Pujol et al., 2000). Both *J. acutus* and *S. nigricans* are densely caespitose plants. *J. acutus* (Juncaceae) lives on maritime sands and rarely on damp or saline inland soils through the Mediterranean region and western Europe, northwards to Ireland. *S. nigricans* (Cyperaceae) colonizes maritime sands or acid peat and grows throughout Europe, including Scotland.

Seeds were obtained from the "Arenales y Salinas de San Pedro del Pinatar" Regional Park (Murcia, SE Spain, N37°46′–37°52′ W0°44′–0°48′), where the three species colonizes different microtopographical sites. This area has a semiarid Mediterranean climate characterised by irregular rainfall events and a harsh dry summer period. Annual rainfall is around 340 mm and mean annual evapotranspiration 1019 mm. Mean annual temperature is 17 °C. August is the warmest month with an average temperature of 24.9 and 42 °C maximum. The coldest month is January, with an average temperature of 10.6 °C and minimum always above 0 °C (Martínez-Sánchez et al., 2006).

Seeds were isolated from fruits and stored in the dark in paper bags at room temperature (18–22 °C), until the germination experiments began 4 months later.

2.2. Effects of salinity on germination

Four 25-seed replicates of each species were placed on filter paper in 9 cm tight-fitting Petri dishes and submerged in 4 mL of each solution. Solutions of the most common salts in the salt marshes of the area (NaCl, $MgCl_2$, $MgSO_4$ and Na_2SO_4) (Álvarez-Rogel et al., 2000, 2006, 2007) were used at concentrations of 1, 2, 3, 4 or 5%. Distilled water was used as control. The dishes were placed in growth chambers and maintained at 30/20 °C with a 12 h photoperiod (400–700 nm, 35 μmol photons $m^{-2}\,s^{-1}$) for 30 d. This temperature/light regime has been described as optimal for germination in these species by Martínez-Sánchez et al. (2006). Seeds were counted at 2-d intervals and were considered to have germinated when the radicle emerged. These germinated seeds were removed from the Petri dishes. The water level was adjusted at 2-d intervals with distilled water to avoid changes in salinity due to evaporation. At the end of the germination period, the germination percentage and the mean time to germination under salinity were calculated. The latter was determined according to the following formula (Brenchley and Probert, 1998): mean time to germination = $(\sum_i n_i \times d_i)/N$, where n is the number of seeds germinated at day i, d the incubation period in days and N is the total number of seeds germinated in the treatment.

2.3. Statistical analysis

A multivariate ANOVA was used to evaluate the effects of salinity on seed germination. Data were analysed using SPSS 11.5 for Windows (SPSS Inc., 1999). When significant main effects existed, differences were tested by a multiple comparison Tukey test at 95% confidence. Germination data were arcsine transformed before statistical analysis to ensure homogeneity of variance.

3. Results

3.1. Effects of salinity on germination

Significant differences were obtained for the three factors considered (species, salt and concentration) and their interactions regarding seed germination ($P < 0.05$). The mean time to germination was also significantly affected ($P < 0.05$) by all the factors and interactions except that between species and salt.

In the control treatment, *S. nigricans* showed the lowest germination percentage and longest time to germination (Table 1). When the seeds were incubated with $MgCl_2$, concentrations higher than 2% gradually reduced the germination of *A. macrostachyum* (relative to the control) until it was totally inhibited at 5%. However, the 1% concentration was already sufficient to significantly reduce the germination of *J. acutus*, while higher concentrations inhibited its germination totally (Table 1). The seeds of *S. nigricans* did not germinate at all in the presence of $MgCl_2$ (Table 1), but at concentrations higher than 2%, the same salt increased the mean time to germination of *A. macrostachyum* (Table 1).

NaCl concentrations of 1% and above sharply reduced the germination of *J. acutus* compared to the control and completely inhibited the germination of *S. nigricans* (Table 1). The percentage of germination of *A. macrostachyum* seeds was reduced by 2% NaCl and much more so at 3%, the concentration at which mean time to germination increased significantly (Table 1).

None of the $MgSO_4$ concentrations used had a significant effect on the germination of *A. macrostachyum* compared with the control (Table 1). At a concentration of 2% $MgSO_4$, 90% of

Table 1
Germination percentage and mean time to germination of *Arthrocnemum macrostachyum*, *Juncus acutus* and *Schoenus nigricans* seeds in saline solutions of $MgCl_2$, NaCl, $MgSO_4$ and Na_2SO_4

Salt	Salinity (%)	Germination in saline solutions (%)			Mean time to germination		
		A. macrostachyum	*J. acutus*	*S. nigricans*	*A. macrostachyum*	*J. acutus*	*S. nigricans*
$MgCl_2$	0	84 ± 8.6cB	95 ± 3.8cB	26 ± 13.2bA	8.6 ± 0.8abA	9.8 ± 0.1aA	22.2 ± 1.8B
	1	82 ± 6.9cC	45 ± 18bB	0 ± 0aA	7.7 ± 0.4aA	12.1 ± 0.6bB	[a]
	2	72 ± 11.7cB	0 ± 0aA	0 ± 0aA	11 ± 0.7bc	[a]	[a]
	3	40 ± 12.6bB	0 ± 0aA	0 ± 0aA	13.5 ± 1.2c	[a]	[a]
	4	8 ± 6.5aB	0 ± 0aA	0 ± 0aA	16.6 ± 2.3d	[a]	[a]
	5	0 ± 0aA	0 ± 0aA	0 ± 0aA	[a]	[a]	[a]
NaCl	0	84 ± 8.64cB	95 ± 3.8cB	26 ± 13.2bA	8.6 ± 0.8aA	9.8 ± 0.1aA	22.2 ± 1.8B
	1	83 ± 6.0cB	15 ± 9.4bA	0 ± 0aA	7.6 ± 0.2aA	9.2 ± 0.9aB	[a]
	2	65 ± 8.2bB	0 ± 0aA	0 ± 0aA	11.1 ± 1a	[a]	[a]
	3	6 ± 5.1aA	1 ± 2aA	0 ± 0aA	15.8 ± 3.7b	[a]	[a]
	4	0 ± 0aA	0 ± 0aA	0 ± 0aA	[a]	[a]	[a]
	5	1 ± 2aA	0 ± 0aA	0 ± 0aA	[a]	[a]	[a]
$MgSO_4$	0	84 ± 8.64aB	95 ± 3.8cB	26 ± 13.2bA	8.6 ± 0.8bA	9.8 ± 0.1aA	22.2 ± 1.8aB
	1	79 ± 6aB	98 ± 2.3cC	23 ± 3.8bA	6.4 ± 0.2aA	7.8 ± 0.5aB	21.2 ± 0.7aC
	2	83 ± 12.8aB	93 ± 6.8cB	1 ± 2aA	6.4 ± 0.3aA	9.8 ± 1.2abB	[a]
	3	74 ± 13.6aB	64 ± 16.3bB	0 ± 0aA	6.8 ± 0.1aA	11.5 ± 0.1abB	[a]
	4	77 ± 11.4aC	29 ± 11.4aB	0 ± 0aA	8.2 ± 0.4bA	13 ± 4.6bA	[a]
	5	71 ± 2aB	8 ± 6.5aA	0 ± 0aA	10.1 ± 0.6cA	11.3 ± 1.5abA	[a]
Na_2SO_4	0	84 ± 8.64cB	95 ± 3.8cB	26 ± 13.2bA	8.6 ± 0.8abA	9.8 ± 0.1aA	22.2 ± 1.8aB
	1	78 ± 9.5bcB	93.3 ± 6.1cC	4 ± 5.6aA	7.5 ± 0.3aA	8.6 ± 0.2aB	22 ± 0aC
	2	76 ± 8.6bcC	39 ± 17.3bB	0 ± 0aA	8.4 ± 1.1aA	12.6 ± 1.1bB	[a]
	3	65 ± 5.0abB	1 ± 2aA	0 ± 0aA	9.1 ± 0.2ab	[a]	[a]
	4	62 ± 2.3abB	0 ± 0aA	0 ± 0aA	10.3 ± 1.1bc	[a]	[a]
	5	43 ± 3.8aB	0 ± 0aA	0 ± 0aA	11.1 ± 0.3c	[a]	[a]

Values are mean ± S.D. ($n = 4$). Means within a column that have a different small letter are significantly different from each other, and means within a row that have different capital letter are significantly different from each other (Tukey test; $P < 0.05$).
[a] Mean time to germination values could not be estimated because germination percentage was ≤1%. Therefore, these treatments were excluded from ANOVA.

J. acutus seeds germinated, but germination progressively decreased at higher concentration than 2% (Table 1). Concentrations of 3% or below provided germination percentages in *J. acutus* similar or above those obtained in *A. macrostachyum*. Solutions above 2% inhibited germination in *S. nigricans* (Table 1). With regards to the mean time to germination, concentrations up to 3% $MgSO_4$ shortened the time in seeds of *A. macrostachyum* and only increased it at 4% and above (Table 1).

When the seeds were exposed to Na_2SO_4, concentrations of 3% and above reduced germination in *A. macrostachyum* although the percentage never fell below 40%. On the other hand, concentrations as low as 2% Na_2SO_4 in *J. acutus* and 1% in *S. nigricans* inhibited germination. As the concentration of Na_2SO_4 increased, the mean time to germination gradually lengthened in both *A. macrostachyum* and *J. acutus* (Table 1).

4. Discussion

As showed in results *A. macrostachyum* was the most salt-tolerant species. Although maximum germination was obtained under non-saline conditions (control treatment), its seeds had the ability to germinate at 3% NaCl, although at 2%, germination significantly decreased and was drastically inhibited at 4% NaCl. Taking into account the results obtained in the same species by other authors (Pujol et al., 2000, 2001; Herranz et al., 2004), these findings were not unexpected. *S. nigricans*, on the other hand, was unable to germinate in the presence of any concentration of NaCl and it could only do so in fresh water or low sulphate concentrations. *J. acutus* showed an intermediate type of behaviour, germinating at 1% NaCl, although with a decreased percentage (80%) compared with the control. This reduction in the percentage of seeds germinating induced by an increase of salinity stress has been described by numerous authors (Breen et al., 1977; Ungar, 1982), as has the complete inhibition of the germination at salinities beyond the tolerance limits of the species (Ungar, 1991).

As the results demonstrate, the salt-tolerance of the three species studied in the germination phase differed substantially, especially in the case of *A. macrostachyum*. Ungar (1991) mentioned the fact that different halophytes vary greatly in their ability to germinate under hypersaline conditions.

Seeds of perennial halophytes, such as *Arthrocnemum indicum* (Willd.) Moq. and *Salicornia rubra* Nels. are able to germinate at 1000 mM (5.8%) NaCl (Khan and Gul, 1998; Khan et al., 2000), whereas *Atriplex prostrata* Boucher ex DC., *Hordeum jubatum* L. or *Salicornia europaea* L. cannot tolerate values higher than 342 mM (2%) (Keiffer and Ungar, 1997), while intermediate values of tolerance were registered in *Haloxylon recurvum* Bunge ex Boiss., *Suaeda fruticosa* Forssk.

and *Sarcocornia fruticosa* (L.) A.J. Scott germinating at 500 mM (2.9%) NaCl, respectively (Khan and Ungar, 1996, 1998; Redondo et al., 2004).

When sulphates were used as salt, the germination of *S. nigricans* continued to be inhibited, except at 1% $MgSO_4$, while *J. acutus* germinated better than in NaCl. This differential behaviour of seeds according to the salt type is presumably due to the fact that the same concentration of salt generates different osmotic potentials and the osmotic effect may well have a greater influence on germination than specific ion toxicity, as has been suggested by several authors in other halophytes (Ungar, 1996; Pujol et al., 2000).

Many halophytes show maximal germination in freshwater conditions, differing from the responses of less salt-tolerant glycophytes only in that they can usually germinate at higher levels of salinity (Ungar, 1974). In our case, *J. acutus* and *S. nigricans* only germinated in distilled water or at very low salt concentrations, and the germination of *A. macrostachyum* gradually decreased with increasing salt concentrations, preferring to germinate in freshwater or at very low salt concentrations.

The dormancy of *S. nigricans* seeds, as mentioned by Martínez-Sánchez et al. (2006), is confirmed in this study. However, of the three species studied, *S. nigricans* is the one that germinates least, so we think that ungerminated seeds should be recruited from the soil seed bank to establish populations following fluctuations along the salinity gradient.

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