

Short communication

## 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<sub>2</sub>, MgSO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub> 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.

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### 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  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$ . However, the relative percentages of  $\text{Ca}^{2+}$  and  $\text{K}^+$  decreased when salinity rose, leading to an imbalance in favour of the most toxic cations, such as  $\text{Na}^+$  and  $\text{Mg}^{2+}$ . The same authors showed that the highest correlation coefficients for ions were obtained between  $\text{Cl}^-$  and  $\text{Na}^+$  and between  $\text{Cl}^-$  and  $\text{Mg}^{2+}$ .

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 ( $\text{NaCl}$ ,  $\text{MgCl}_2$ ,  $\text{MgSO}_4$  and  $\text{Na}_2\text{SO}_4$ ) (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  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) 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 =  $(\sum n_i \times d_i)/N$ , 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  $\text{MgCl}_2$ , 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  $\text{MgCl}_2$  (Table 1), but at concentrations higher than 2%, the same salt increased the mean time to germination of *A. macrostachyum* (Table 1).

$\text{NaCl}$  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%  $\text{NaCl}$  and much more so at 3%, the concentration at which mean time to germination increased significantly (Table 1).

None of the  $\text{MgSO}_4$  concentrations used had a significant effect on the germination of *A. macrostachyum* compared with the control (Table 1). At a concentration of 2%  $\text{MgSO}_4$ , 90% of

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