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Germination studies in *Juncus acutus* L. (Juncaceae), *Schoenus nigricans* L. (Cyperaceae) and *Arthrocnemum macrostachyum* (Moric.) Moris (Chenopodiaceae) for salt-marshes restoration

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INTRODUCTION

Fig. 1. Halophytic plant commnunity with *Arthrocnemum macrostachyum* growing in deeper areas of saline depression and *Juncus acutus* and *Shoenus nigricans* in upper soils

Since the 1970's, salt marshes have been reduced in area by a great antropogenic pressure derived from agriculture, and more recently, from demand for tourist urban facilities. Therefore, restoration and preservation of plant communities in salt marshes are needed. Among the perennial species that appear typically in maritime sands, on the Mediterranean region and western Europe *Juncus acutus* L. (Juncaceae), *Schoenus nigricans* L. (Cyperaceae) and *Arthrocnemum macrostachyum* (Moric.) Moris (Chenopodiaceae) frequently occur (Álvarez-Rogel *et al*., 2000; Vicente *et al*., 2007). On other hand soil salinity has traditionally been considered one of the most important physical factors in plant zonation of salt marshes (Fig.1). Álvarez-Rogel *et al*. (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 between Cl^- and Na^+ and between Cl^- and Mg^{2+}.

The use of the above mentioned species in restoration programs require to know the germination responses to light and temperature and their salinity tolerance during germination.

GERMINATION RESPONSE

In order to know the effects of photoperiod and temperature conditions on germination, lots of seeds of *Juncus acutus* and *S. nigricans* were obtained from the "Arenales y Salinas de San Pedro del Pinatar" Regional Park (Murcia SE Spain, N 37º 46'-37º 52' W 0º 44'-0º 48') and put on germination. The germination response of *A. macrostachyum* was not studied due to the existence of scientific information available about this. This area is a semiarid region, with typically 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 and mean annual temperature is 17ºC.

Seeds were placed in growth chambers in two light conditions (12 h photoperiod at 400 - 700 nmol, 35 nmol photon m^{-2} s^{-1}, and total darkness) and seven temperature regimes: five constant temperatures of 10, 15, 20, 25, or 30ºC and two alternating temperatures of 15:25 or 20:30ºC dark:light cycle, and then final germination percentage and mean time to germination (MTG) were determined.

Final germination in *J. acutus* was significantly affected by temperature, light and their interaction, obtaining germination percentages higher than 95% at some light and temperature conditions. The germination of *S. nigricans* was not dependent by light but was affected by temperature. The final germination of this species did not exceed the

average value of 37% at any light condition and temperature under study. The low germination percentages obtained from *S. nigricans* suggests it belongs to a species with seed dormancy (Martínez-Sánchez *et al*., 2006).

None of the two species is totally dependent on light to germinate. The results might indicate that neither species is specialized in colonizing exclusively bare soils, but that they could also germinate in areas where plant cover is high (Martínez-Sánchez *et al*., 2006).

SALINITY TOLERANCE

In order to determine the salinity tolerance, 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*., 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 30:20ºC with a 12 h photoperiod, for 30 days. This temperature/light regime has been reported to be optimal for germination in these species (Martínez-Sánchez *et al*., 2006). At the end of the germination period, the germination percentage and the mean time to germination (MTG) under salinity were calculated.

The results showed that *A. macrostachyum* was the most salt tolerant species (Vicente *et al*., 2007, 2009). Although maximum germination was obtained under non-saline conditions (84%), its seeds had the ability to germinate at 3% NaCl, although at 2%, germination significantly decreased (64%) and was drastically inhibited at 4% NaCl. On the other hand, none of the $MgSO_4$ concentrations used had a significant effect on the germination of *A. macrostachyum*, while higher concentrations of $MgCl_2$ and $NaSO_4$ solutions decreased progressively its germination. However, *S. nigricans* was unable to germinate in the presence of any chloride concentrations and it could only do so in fresh water (26%) or low sulphate concentrations. *Juncus acutus* showed an intermediate type of behaviour, germinating at 1% both NaCl and $MgCl_2$, although with a decreased percentage (15% and 45%, respectively) compared with the control (95%). When sulphates were used as salt, *J. acutus* germinated better than in chlorides (Vicente *et al*., 2007, 2009).

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

As these results demonstrate, the salt tolerance of the three species studied in the germination phase differed substantially, especially in the case of *A. macrostachyum*, varying greatly in their ability to germinate under hypersaline conditions (Vicente *et al*., 2007).

GERMINATION RECOVERY

Germination recovery of seed lots incubated in 1, 2, 3, 4, or 5% saline solutions of NaCl, $MgCl_2$, $MgSO_4$, and Na_2SO_4 for 30 days was studied by transferring ungerminated seeds to distilled water.

Seed germination of *A. macrostachyum* was only inhibited at the highest salinity (4% NaCl, 5% $MgCl_2$) but these ungerminated seeds showed a high germination recovery in distilled water (81% and 83%, respectively) (Vicente *et al*., 2009). Seed germination of *S. nigricans* was totally inhibited by salts and only 26% of them germinated in the control treatment. However, the germination recovery of this species could be stimulated by high salt concentrations (germination of ungerminated seeds incubated at 5% Na_2SO_4 reached 66% in distilled water). *Juncus acutus* germinated well without salt (95% in the control treatment) but high salt concentrations inhibited them from germination (Vicente *et al*., 2007, 2009). However, ungerminated seeds were not damaged by salt, showing a high level of recovery (100% in all salt types and concentrations). The chlorides were more inhibitory to germination than sulphates at equivalent concentrations.

The germination-related characteristics of *J. acutus* and *S. nigricans* mean that both species may be considered halophytes although neither of them can tolerate such high salinity levels as *A. macrostachyum*. *Juncus acutus* and *A. macrostachyum* produce nondormant seeds, whereas a high proportion of *S. nigricans* seeds are dormant (Martínez-Sánchez *et al*., 2006; Vicente *et al*., 2007, 2009).

Based on the results obtained, and following the classification Boorman, 1968 (Vicente *et al*., 2009), *J. acutus* and *S. nigricans* can be classified as halophytes type 2 (Dune hollows or low lying areas that are occasionally inundated. Germination is strongly inhibited even in very low salinities. Recovery: germination is usually equal to that in freshwater or only slightly reduced), since their germination is strongly inhibited even in very low salinities but after recovery in distilled water germination is equal to that in freshwater. *Arthrocnemum macrostachyum* fits halophytes type 3 (Marsh habitat that is subject to frequent long-lasting periods of inundation. Seeds germinate to high

percentages even at high salinities and show high viability rates after exposure to any salinity. Recovery: germination is enhanced after pretreatment in saline solutions), since their seeds can germinate in high salinities (Vicente *et al*., 2009). These results are in accordance with the conceptual model proposed by Álvarez-Rogel *et al*. (2006) for monitoring hydrological and saline gradients in coastal dune salt marshes of southeast Spain (Vicente *et al*., 2007; 2009).

Of the three species studied, *S. nigricans* is the one that germinates to the lowest percentage. Thus, perhaps dormant seeds should be used from the soil seed bank to re-establish populations following fluctuations of the salinity gradient. In fact, our results demonstrate that exposure to salt can break dormancy in seeds of *S. nigricans* (Vicente *et al*., 2009).

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