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Influences of salinity and light on germination of three *Sarcocornia* taxa with contrasted habitats

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Abstract

We analysed the responses of seeds of three closely related halophytic taxa of the genus *Sarcocornia* in contrasted habitats, *Sarcocornia perennis* (low marsh), *Sarcocornia fruticosa* (high marsh) and *S. perennis* × *fruticosa* (middle marsh), to different salinity concentrations (0, 2, 4 and 6%) and light regime (light/dark and dark). Germination reflected their position in the tidal gradient, showing higher percentages at hypersalinity those taxa colonising upper positions (c. 40%). *S. fruticosa* showed germination in darkness and it accelerated its germination after prolonged salinity exposure. *S. perennis* × *fruticosa* presented intermediate responses, sharing the capacity of germinating in hypersaline conditions with *S. fruticosa* and the absence of germination in darkness and the absence of acceleration of germination with *S. perennis*. Finally, we recorded common germination behaviours for the three taxa: short germination periods, reduction of final germination percentage, enforcing of seed dormancy and reduction of seed viability by increasing salinity.

Germination responses to salinity of the three *Sarcocornia* seem likely to influence their colonisation capacities down the tidal gradient and hence may be important in the maintenance of taxa zonation in salt marshes.

Keywords: Hybrid; Iberian Peninsula; Mediterranean salt marsh; *Sarcocornia fruticosa*; *Sarcocornia perennis*; Seed viability

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1. Introduction

The existence of spatio-temporal gradients of soil salinity and moisture has traditionally been considered one of the most important physical factors in the plant zonation of salt marshes (Chapman, 1974). Additionally, variation in light availability has been identified to play an important role; germination of various halophytes seeds are affected adversely by darkness, deep in the substrate or under high vegetation cover (Gul and Weber, 1999). Species that live in highly specific habitats, such as salt marshes, often produce seeds with highly specialised adaptations (Pickart, 1988; Navarro and Guitián, 2003).

A number of halophyte species co-occur in the coastal salt marshes of the Iberian Peninsula, though often partly separated in zones (Nieva et al., 1999). Two members of the perennial genus *Sarcocornia* are common and hybridise in the south-west of this Iberian Peninsula. *Sarcocornia perennis* (Mill.) A.J. Scott is an intertidal macrophyte that grows in regularly flooded and open low marshes (Castellanos et al., 1994), whilst *Sarcocornia fruticosa* (L.) A.J. Scott is normally found at high levels in the tidal gradient exposed to high soil salinities, high vegetation covers and less frequent flooding (Álvarez Rogel et al., 2000).The hybrid, *S. perennis* × *fruticosa*, dominates in intermediate habitats (Figueroa et al., 2003). As in other salt marsh plants, zonation of *Sarcocornia* may well be caused largely by differences in germination ecology, coupled to spatio-temporal variations in soil salinity and moisture (Egan and Ungar, 2000) or light availability (Clevering, 1995).We did germination experiments to asses the interactive effects of salinity and light for the two Iberian *Sarcocornia* species and their hybrid.

2. Material and methods

In mid-November 2002, ripe fruits were collected from *S. perennis* and the hybrid *S. perennis* × *fruticosa* that were growing in Odiel coastal salt marshes (37°15′N, 6°58′W; south-west Iberian Peninsula). Ripe fruits of *S. fruticosa* were collected in December 2002 from Piedras salt marshes (37°13′N, 7°9′W). Seeds were stored at ambient temperature (25 °C).

Both salt marshes are subject to Mediterranean climate and affected by oceanic influences. Winter is wet and with mild temperatures (mean temperature ca. 11 °C in January) and summer is long and dry (mean temperature is ca. 25 °C). Mean annual precipitation is 510 mm with an interannual variation coefficient of 31%. Sediment salinity ranges from 0.4% in the low marsh (Castillo et al., 2000) to 6% in high marshes, varying markedly through the year (Rubio-Casal et al., 2001).

Three experiments were conducted to analyse germination in response to salinity. Firstly, dark/light conditions were used to quantify germination when seeds lie on or in the substrate surface prior to germination. Secondly, the effects of drops in salinity on germination were studied in distilled water and dark/light after salinity pre-treatments. Finally, germination in darkness was analysed to explore when seeds are under dense canopies or deep in the sediment.

2.1. Seed germination and salinity

Four 25-seed replicates of each taxon were placed on filter paper in 5 cm Petri dishes and submerged in 5 ml solutions of 0, 2, 4 and 6% (w/v) sea salt. Salinity concentrations were chosen to cover variations though the tidal frame in Mediterranean salt marshes. Dishes were placed in a germinator (ASL Aparatos Científicos M-92004, Madrid, Spain), and subjected to a regime of 10 h of light (25 °C, 400–700 nm, 35 μmol photons m^{-2} s^{-1}) and 14 h of dark (5 °C) for 30 days (Keiffer and Ungar, 1997). This temperature regimen was chosen to replicate the autumn temperatures in Odiel and Piedras marshes, when these species germinate. The dishes were inspected daily and germinated seeds were counted and removed. Seed germination was accepted when the radical appeared. The water level was adjusted daily with distilled water to avoid changes in salinity due to evaporation (Mauchamp and Mésleard, 2001).

2.2. Recovery experiment

This experiment was carried out to determine whether high salinities inhibit or damage the seeds. Four 25-seed replicates of each taxon were maintained for 30 days in 2, 4 and 6% of salinity in dark at ambient temperature (25 °C). After continuous exposure to these pre-treatments, they were submerged in 5 ml of distilled water in new Petri dishes, and maintained at the same temperature and light conditions as the previous experiment for 30 days. Germinated seeds were counted and removed daily during this period.

Four characteristics of germination were determined: final germination percentage, number of days to first germination, number of days to final germination, and mean time-to-germinate (MTG). MTG was calculating using the equation:

$$\mathrm{MTG} = \frac{\sum_i n_i \times d_i}{N}$$

where n is the number of seeds germinated at day i, d the incubation period in days and N the total number of seeds germinated in the treatment (Brenchley and Probert, 1998).

2.3. Seed germination and light regime

Germination percentage of the three taxa was compared between light/dark conditions (first experiment) and darkness (pre-treatment of the second experiment). Four 25-seed replicates were maintained in darkness for 30 days and 0% of salinity to complete data series.

2.4. Viability test

The tetrazolium test was applied to seeds that had not germinated after the germination experiment to determine the viability of the embryo (MacKay, 1972). Seeds were kept in water during 16 h at a constant temperature of 25 °C. Seeds were then submerged in a 1% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride, pH 7, in darkness for 24 h at a constant temperature of 25 °C. Subsequently, seeds were dissected and the embryo was analysed through a magnifying glass (Bradbeer, 1998).

2.5. Statistical analysis

Statistical analysis was carried out using Statistica v. 6.0 (Statsoft Inc.). Pearson coefficients were calculated to assess correlation between different variables. Data were analysed by one- and two-way analysis of variance (*F*-test). Data were tested for normality with the Kolmogorov–Smirnov test and homogeneity of variance with the Brown–Forsythe test. The data were transformed using $\log_{10}$ and $1/x$ functions when homogeneity of variance was not reached. Significant test results were followed by Tukey test for identification of important contrasts (Day and Quinn, 1989). *U*-test was used when data violated normality and homogeneity assumption. Differences between seed germination experiments and recovery experiments in every taxa were compared by the Student's *t*-test.

3. Results

3.1. Seed germination and salinity

Final germination showed that differences between *Sarcocornia* taxa, salinity treatments and their interaction were significant (two-way ANOVA, $P < 0.001$). It was very high for the two lowest treatments (0 and 2%), but decreased in salinities higher than 2%. On the other hand, *S. perennis* × *fruticosa* tended to show higher germination than *S. perennis* and *S. fruticosa* in 4% of salinity and light/dark conditions (Table 1 and Fig. 1).

The number of days to first germination increased with salinity except for *S. fruticosa*, while the number of days to final germination was independent of the treatment. MTG increased with salinity for the hybrid (Table 1).

Table 1
Final germination (%), days to first germination, days to final germination and mean time-to-germinate (MTG) of three *Sarcocornia* taxa in four salinity treatments for 30 days

Taxon	Salinity treatment (%)	Germination characteristics			
		Final percentage	First germination (days)	Final germination (days)	MTG
S. perennis	0	88 ± 2.8 a	2.7 ± 0.5 a	9.7 ± 1.8	4.8 ± 0.7
	2	79 ± 8.1 a	3.7 ± 0.5 a	13.0 ± 3.1	7.4 ± 1.9
	4	16 ± 9.1 b	5.0 ± 1.1 ab	7.7 ± 0.3	6.7 ± 0.2
	6	3 ± 1.9 b	8.5 ± 0.5 b	9.0 ± 0.0	8.7 ± 0.2
S. perennis × *fruticosa*	0	89 ± 2.5 a	3.2 ± 0.2 a	14.2 ± 1.9	4.8 ± 0.3 a
	2	80 ± 3.3 a	3.7 ± 0.2 ab	11.5 ± 1.8	5.9 ± 0.5 a
	4	44 ± 9.4 b	5.2 ± 0.5 b	13.5 ± 2.2	7.8 ± 0.1 b
	6	6 ± 2.6 c	9.0 ± 0.6 c	9.0 ± 0.6	9.0 ± 0.6 b
S. fruticosa	0	85 ± 1.9 a	3.0 ± 0.0	11.5 ± 1.5	5.3 ± 0.2
	2	61 ± 6.6 b	4.2 ± 0.6	16.5 ± 0.5	8.4 ± 0.5
	4	10 ± 1.1 c	10.7 ± 2.6	13.7 ± 3.4	12.3 ± 3.0
	6	4 ± 2.3 c	7.5 ± 1.5	11.0 ± 2.0	9.4 ± 0.4

Values are mean ± S.E. ($n = 4$). Means within a taxon that have different letter are significantly different from each other (Tukey test; $P < 0.05$).

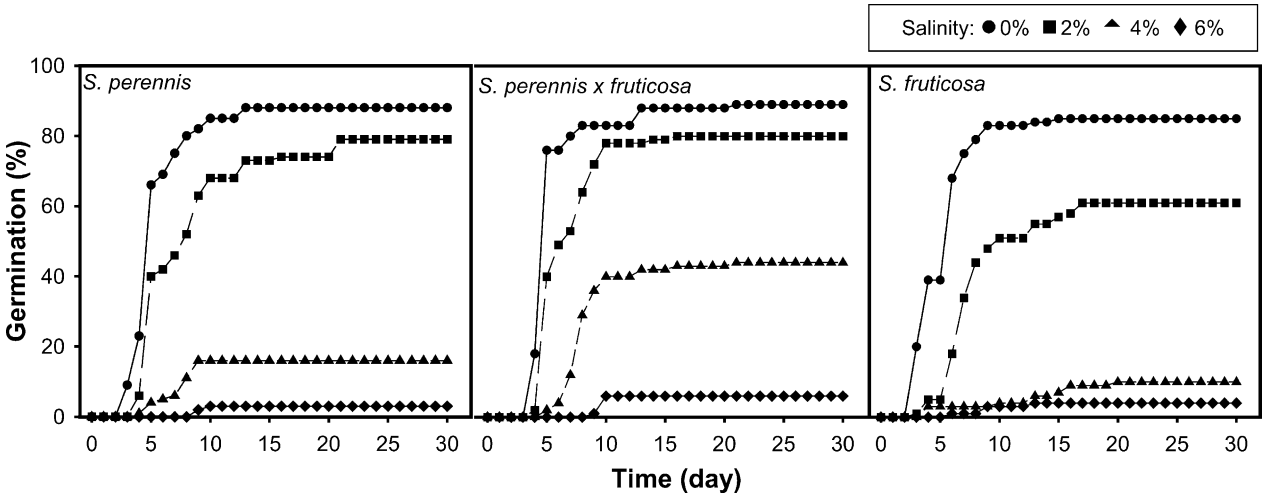

Fig. 1. Accumulative germination of three *Sarcocornia* taxa during 30 days in four salinity treatments ($n = 4$).

Table 2
Final germination (%), days to first germination, days to final germination and mean time-to-germinate (MTG) of three *Sarcocornia* taxa in distilled water after three salinity pre-treatments for 30 days (recovery experiment)

Taxon	Salinity treatment (%)	Germination characteristics			
		Final percentage	First germination (days)	Final germination (days)	MTG
S. perennis	2	83.0 ± 3.0	1.0 ± 0.0	6.0 ± 0.0	3.3 ± 0.1
	4	89.9 ± 5.2	1.5 ± 0.5	8.0 ± 0.9	3.7 ± 0.3
	6	82.9 ± 5.2	1.5 ± 0.5	6.2 ± 0.2	3.3 ± 0.1
S. perennis × *fruticosa*	2	80.4 ± 3.8	1.0 ± 0.0 a	9.0 ± 3.7	3.8 ± 0.4
	4	83.0 ± 1.0	2.0 ± 0.6 ab	5.5 ± 0.5	3.6 ± 0.1
	6	73.0 ± 3.0	3.0 ± 0.0 b	7.0 ± 2.1	3.7 ± 0.3
S. fruticosa	2	78.0 ± 6.0	1.5 ± 0.3	8.5 ± 2.6	3.1 ± 0.2 a
	4	74.0 ± 5.0	2.0 ± 0.0	5.0 ± 0.6	2.9 ± 0.0 ab
	6	83.0 ± 4.4	1.2 ± 0.2	3.7 ± 0.2	2.5 ± 0.1 b

Values are mean ± S.E. ($n = 4$). Means within a taxon that have different letter are significantly different from each other (Tukey test; $P < 0.05$).

3.2. Recovery experiment

Final germination was not affected by salinity pre-treatment. The number of days to first germination increased with salinity in the hybrid ($r = 0.82$, $P < 0.001$) and the number of days to final germination decreased in *S. fruticosa* ($r = -0.58$, $P < 0.05$) (Table 2).

MTG decreased with salinity pre-treatment only in *S. fruticosa* ($r = -0.78$, $P < 0.01$). MTG in *S. perennis* and the hybrid was significantly higher than in *S. fruticosa* for 4 and 6% pre-treatment (ANOVA, $P < 0.05$) (Table 2).

3.3. Germination and light regime

Final germination varied between *Sarcocornia* taxa, light regimes and their interaction (two-way ANOVA, $P < 0.0001$). *S. fruticosa* was the only taxon that germinated in darkness during salinity pre-treatments. Furthermore, it tended to show higher final germination in darkness than under alternating light/darkness in the presence of NaCl, however, significant differences were recorded only for 4% of salinity. Final germination in darkness was negatively correlated with salinity ($r = -0.78$, $P < 0.0001$), showing its maximum values at 2% (Table 3).

3.4. Viability test

The number of dormant and unviable seeds increased with salinity in the three taxa. In hypersalinity (6%), the hybrid showed the lowest percentage of seed dormancy (40 ± 3%) and the highest percentage of unviable seeds (54 ± 2%; Table 4).

Table 3
Final germination (%) for *S. fruticosa* under four salinity treatments in light/dark and dark for 30 days

	Germination (%)			
	0%[a]	2%[a]	4%[a]	6%[a]
Light/dark	85.0 ± 1.9	61.0 ± 6.6	10 ± 1.1	4 ± 2.3
Dark	56.0 ± 4.9	71.0 ± 3.0	40 ± 4.3	14 ± 4.8
Student's *t*-test	5.51**	−1.04 (n.s.)	−6.71***	−1.89 (n.s.)

Values are mean ± S.E. ($n = 4$); *t*-test compared light/dark versus dark treatments; n.s.: not significant.
[a] Salinity.
** $P < 0.01$.
*** $P < 0.001$.

Table 4
Dormant and unviable seeds (%) for three *Sarcocornia* taxa under four salinity treatments in light/dark for 30 days

Taxon	Salinity treatment (%)	Non-germinated seeds	
		Dormant (%)	Unviable (%)
S. perennis	0	0 ± 0.0 a	12 ± 1.6 a
	2	4 ± 1.6 a	17 ± 1.0 a
	4	54 ± 2.6 b	30 ± 1.1 b
	6	70 ± 2.0 c	27 ± 1.0 b
S. perennis × *fruticosa*	0	3 ± 1.0 a	7 ± 1.0 a
	2	7 ± 1.9 a	13 ± 1.0 a
	4	22 ± 1.1 b	34 ± 2.6 b
	6	40 ± 2.8 c	54 ± 2.0 c
S. fruticosa	0	3 ± 1.0 a	12 ± 1.6 a
	2	12 ± 0.0 b	27 ± 1.9 b
	4	66 ± 2.6 c	24 ± 1.6 b
	6	60 ± 2.3 c	36 ± 1.6 c

Values are mean ± S.E. ($n = 4$). Means within a taxon that have different letter are significantly different from each other (Tukey test; $P < 0.05$). The percentage of dormant, unviable and final germinated seeds (Table 1) add up to 100%.

4. Discussion

This study shows how three closely related *Sarcocornia* taxa colonising contrasted habitats had different germination patterns in response to salinity and light regime. However, also we recorded some features common to all three taxa which have previously been reported for other halophytes: short germination periods, reduction of final germination percentage, enforcing of seed dormancy and reduction of seed viability by increasing salinity.

Germination period (time between first and final germination) was short in every taxon, ranging between 2 and 12 days due to an increase in the number of days to first germination with increasing salinity while the number of days to final germination was constant. This germination strategy has been recorded previously in other halophytes (Rubio-Casal et al., 2002) and it is common in desert annuals (Gutterman, 1994). It would secure emergence

of a large number of seedlings during favourable conditions and hence also favour the development of monospecific stands. This may, however, also provoke density-dependent mortality due to intraspecific competition (Rubio-Casal et al., 2001).

A significant inhibition of germination with increasing salinity was recorded in all three *Sarcocornia* taxa. In all cases, final germination was very high in distilled water (between 85 and 89%). Similar results have been obtained for *Halocnemum strobilaceum* (Pujol et al., 2001), *Phragmites australis* (Mauchamp and Mésleard, 2001) and *Salicornia ramosissima* (Rubio-Casal et al., 2002). This suggests that the studied taxa do not necessarily have a physiological requirement for salt to germinate. Our values for *S. fruticosa* in distilled water (85%) were higher than recorded previously (50%; Pujol et al., 2000). Germination in 6% salinity was very low in every taxon (lower than 10%); it may be attributed to decreasing osmotic potential of the solution by salinity that would avoid seed hydration (Ramoliya and Pandey, 2002).

On the other hand, salinity enforced seed dormancy (Khan and Ungar, 1996; Pujol et al., 2000) and decreased seed viability (Keiffer and Ungar, 1997). This induced dormancy was broken mainly after salinity decreased in the three taxa. Seed viability decreased more in the hybrid than in either of its parents at higher salinities, which may limit its colonisation of upper zones in the tidal gradient.

With a Mediterranean climate, seeds remain dormant during summer drought and salinity decreases with first rainfalls allowing germination during autumn and winter (Chapman, 1974). Germination speed accelerated when seeds were removed from a saline solution only for *S. fruticosa*. This behaviour may confer on it ecological advantages in the high marshes where it grows. A quick establishment may allow this species to: (1) avoid having to endure extreme summer conditions as a seedling, which is a particularly sensitive stage in the plant's development (Ungar, 1991); and (2) occupy the space before other species, which would enhance its interspecific competitive capacity (Navarro and Guitián, 2003). On the other hand, *S. perennis* and *S. perennis* × *fruticosa* live in low and middle marshes, respectively, where salinity does not drop to very low values, as it does in high marshes during rainfall season, due to periodical tidal flooding. This is in accordance with the absence of germination speed activation by salinity in these two taxa.

S. fruticosa was the only taxon that germinated in darkness. This behaviour has been related to the control of germination by phytochromes (Probert et al., 1985). Furthermore, germination in darkness was higher than in light/darkness conditions at 4% of salinity, while the opposite response was found in distilled water. As we have pointed out previously, *S. fruticosa* colonises high marshes where salinity levels are usually high (Ranwell, 1972), vegetation cover rises to values close to 100% (Curcó et al., 2002) and soil fissures appear due to the contraction of clays during drought periods (Moreno et al., 1995). In this environment where seeds may easily be under canopies and/or deep into the soil, germination in darkness and hypersalinity would be necessary. On the other hand, *S. perennis* and the hybrid *Sarcocornia* did not germinate in darkness. This germination response may limit both species colonisation to open locations, such as among *Spartina maritima* tussocks in case of *S. perennis* (Castellanos et al., 1994) and gaps in *S. perennis* in the case of *S. perennis* × *fruticosa* (Figueroa et al., 2003).

Anoxia has been described to reduce germination in halophytes (Wijte and Gallagher, 1996). Seeds of *S. perennis* and its hybrid would not suffer low oxygen concentrations

during their germination, since they would germinate only on sediment surface (light/dark conditions). In contrast, germination of *S. fruticosa* in darkness could be constrained at lower levels in the tidal gradient where anoxia is high.

In general, *S. fruticosa*, a high marsh species, showed very different germination responses than *S. perennis*, a low marsh species. The former showed germination in darkness and it accelerated its germination after prolonged salinity exposure. *S. perennis* × *fruticosa*, a middle marsh hybrid taxon, presented intermediate responses, sharing the capacity of germinating at high percentage in hypersaline conditions with *S. fruticosa* and the absence of germination in darkness and the absence of the accelerated of germination with *S. perennis*. Germination responses to salinity of the three *Sarcocornia* seem likely to influence their colonisation capacities down the tidal gradient and hence may be important in the maintenance of taxa zonation in salt marshes.

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