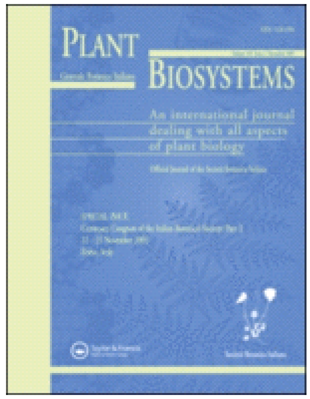

Effect of salinity, temperature and hypersaline conditions on the seed germination in Limonium mansanetianum an endemic and threatened Mediterranean species

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Effect of salinity, temperature and hypersaline conditions on the seed germination in *Limonium mansanetianum* an endemic and threatened Mediterranean species

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Abstract

Limonium mansanetianum is catalogued as critical threatened species (CR) and it is included in Valencian Catalogue of Threatened Plant Species. *L. mansanetianum* is a gypsicolous species, which only lives in a restricted area to south-centre of Valencia province (Spain). The species is a low-branched woody shrub with summer flowering. The influence of incubation temperature (10º, 15º, 20º and 25º/20º C) and salinity (0% to 3.0% NaCl) on seed germination of *L. mansanetianum* was studied. Best seed germination was obtained in a distilled water controls. Seed germination decreased with an increase in salinity and few seeds germinated at 2.5% and 3.0% NaCl. Optimal temperature regime for germination was 15ºC where germination in 0.5% and 1.0% NaCl was not affected. Recovery and hypersaline conditions experiments showed that *L. mansanetianum* seeds displayed a greater tolerance to high salinity and temperature stress before germination.

Introduction

Limonium Mill. is the only genus of *Plumbaginaceae* with a cosmopolitan distribution (Koutroumpa et al. 2018) and by far the most species-rich genus in the family with 633 species (Hassler 2019). The genus has a main diversification centre in the western Mediterranean region, where 70% of the total number of species are endemic (Koutroumpa et al. 2018). *Limonium* species are mostly perennial herbs and shrubs growing in coastal areas, salt marshes, lagoons, meadows, steppes and continental inland deserts and they are characterized as facultative halophytes (Koutroumpa et al. 2018). In the Spanish peninsular territory and Balearic Islands, this genus is represented by 113 species (Hassler 2019) and at least 87 of them are endemic (Erben 1993). Eighty-two *Limonium* species are included in the 2010 Red List of Spanish Vascular Flora (Bañares et al. 2010). In the Valencian Community, the genus is represented by around 30 species (Crespo and Lledó 1998; Mateo and Crespo 2014), at least 19 of them being endemic (Serra et al. 2000) and seven of them being threatened (Aguilella et al. 2010).

One of the most threatened endemic species of *Limonium* of the Valencian territory is *Limonium mansanetianum* M.B. Crespo & Lledó. This species is a low-branched woody shrub with summer flowering that has been catalogued as critical threatened species (CR) according 2001 IUCN criteria (Moreno 2008; Bañares et al. 2010) and it has been included in Valencian Catalogue of Threatened Plant Species (Aguilella et al. 2010). The species only lives in a restricted area to south-centre of Valencia province (Spain) and the area of occupancy is around 1 km^2, delimited by one main population with almost all individuals and three new populations placed on highly anthropized habitats that are difficult to managed (Navarro et al. 2010, Ferrer-Gallego and Laguna 2011). The main population is currently protected as a plant micro-reserve (Laguna et al. 2004). According to Ferrer-Gallego and Laguna (2011), *L. mansanetianum* is a species which only lives on inland gypsic outcrops, in the European Union's habitat 1520

"Iberian gypsum steppes (*Gypsophiletalia*)" but showing also preferences to 1510 "Mediterranean salt steppes (*Limonietalia*)". Both types of vegetation are considered as priority habitats for conservation tasks and land protection. Other dominant species in the Valencian gypsum steppes are *Ononis tridentate* L., *Gypsophila struthium* Loefl., *Helianthemum squamatum* (L.) Pers, *Moricandia moricandioides* (Boiss.) Heywood and *Lygeum spartum* Loefl. ex. L.

Halophytes are distributed on a variety of saline habitats which include inland or coastal salt-marshes, dunes, deserts, sabkha, beach and gypsum soils and sea cliffs throughout the world (Adam 1990; Gul et al. 2013). They are adapted to survive and complete their biological cycle under saline levels of least 1.2% NaCl (Flowers and Colmer 2008; Flowers and Colmer 2015). The populations of halophytes are subjected to high mortality risks because of high-salinity stress or other associated abiotic factors (Ungar 1991). Perennial halophytes vary in their ability to tolerate salinity and three strategies to salinity tolerance have been described: (1) the ability to germinate at high salinity levels, (2) the ability to tolerate high salinity without losing viability of seeds while stored in the soil and (3) the ability to complete the life cycle at high salinity (Khan and Gul 2002).

Germination and seedling establishment are the most critical phases in the biological cycle of seed plants as only those that overcome the first phase can survive in a given territory (Donohue et al. 2010). Seeds of halophytes often show optimal germination in freshwater, similar to glycophytes, but differ in their ability to germinate at higher salinity (Ungar 1995; Gul et al. 2013). Halophytes show variable degrees of salinity tolerance during germination, and in part this variation could be due to a number of factors such as temperature, moisture stress and light (Noe and Zedler 2000; Baskin JM and Baskin CC 2001). Germination of many halophytes occurs at times when there is an optimal combination of daylight, temperature regime and salinity (Naidoo and Naicker 1992; Gutterman et al. 1995). The increase in temperatures and alteration in rainfall regimes due to global climate change constitutes a potential threat in saline habitats and/or to the species that undergo osmotic stress.

Understanding reproductive biology traits, seed germination behaviour and the influence of the environmental factors on germination is an important aspect in the conservation and management of endemic or threatened plant species (Evans et al. 2003; Heywood and Iriondo 2003). Given the biogeographical and ecological interest of the most threatened endemic species in a European diversity scenario, we have conducted a study to determine the best requirements for seed germination of *L. mansanetianum*. The effects of different saline solutions and temperature regimes on the germination success and velocity and responses to the recovery of *L. mansanetianum* seeds were studied to determine the effect of each factor and the interaction between them. The effect of previous hypersaline conditions on seed germination was also studied. The aim of this study was to determine the optimal conditions for germination of this species for future restoration and proper management to conserve the current populations.

Materials and methods

Plant material

Seed were collected from the main population of *Limonium mansanetianum* in Villanueva de Castellón (Valencia, Spain) in autumn of 2009. The estimated size of the main populations is around 37,300 individuals (Navarro et al. 2010). Seeds were manually separated from inflorescence and healthy seeds selected (36-38% of the inflorescence), placed in paper envelopes, and dry–stored in a refrigerator at 4ºC until their use.

Germination test

Germination tests were performed in spring of 2010. The seeds were sterilized with 0.5% sodium hypochloride (NaClO) solution for two minutes and then washed several times with distilled water. Germination seeds were carried out using 9 cm diameter Petri-dishes on two layers of filter paper Whatman nº 1 moistened to saturation with distilled water or test solution. Four replicates of 25 seeds each were used for each treatment. Seeds were considered to be germinated at the emergence of the radicle.

To determine the effect of temperature, seeds were germinated in incubators (MLR-350, Sanyo, Japan) at four temperatures regimes of 10ºC, 15ºC, 20ºC and 20/25ºC with 12-h light period (25 µmol photons m^{-2} s^{-1}, 400-700 nm with cool white fluorescent lamps). For each temperature seeds were germinated at six salt concentrations (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (w/v) NaCl). Percent germination was recorded on every day for 30 days. Ungerminated seeds previously incubated from 1.5 to 3.0% NaCl were transferred to distilled water after 30 days to study the recovery of germination, which was also recorded for 10 days.

Those seeds infected by fungi or bacteria were removed and not considered for the calculations. At the end of the experiment, non-germinated seeds were dissected in order to determinate their viability and only seeds having a complete embryo were considered as full seeds. The percentage of cumulative seed germination (G) for each replicate was calculated at the end of the experiment as: $\%G = 100 \times (GS/FS)$, were *GS* is the number of germinated seeds, and *FS* is the number of full seeds. Rate of germination was estimated by using T_{50} determined as the number of days elapsed from initial until germination of 50% of total germinated seeds according to Thanos and Doussi (1995).

Hypersaline solutions

To determine the effect of hypersaline solutions, four replicates of 25 seeds were incubated during two months at 4% and 8% NaCl (w/v) at 4ºC in darkness, and then seeds were transferred to distilled water and germinated at two temperatures regimes of 10ºC and 15ºC as described before for germination test.

Statistical analysis

All percent G values were arcsine square root transformed before analysis to normalize the variance. Statistical analysis of the percentage of cumulative seed germination (G) and T_{50} were performed using Statgraphics plus 5.1 for Windows program. A Tukey's multiple comparison test was used to determine significant differences between temperatures and salinity for all the parameter means ($P<0.05$).

Results

Effect of salinity and temperature on germination success and velocity

Maximum germination of *L. mansanetianum* seeds after 30 days of incubation were obtained in non-saline control at all temperature regimes (Table 1). Seed germination in the non-saline solution was not affected by temperature treatment (Table 1, capital letters). Seed germination in 0.5% saline solution was also not significantly different from the control at all temperatures (Table 1, small letters). Seed germination at different salinity levels resulted in a gradual decrease in final germination percentages (Table 1, small letters). Best seed germination under saline solution was observed at 15ºC treatment (Table 1, small letters). Seed germination in saline solution at 20ºC and 25º/20ºC temperature regime was similar to germination at 15ºC in saline solution from 2.0% to 3.0% NaCl (Table 1, capital letters). Seed germination in saline solution at 10ºC was lower than germination at 15ºC in all saline solutions (Table 1, capital letters).

Germination velocity of *L. mansanetianum* seeds was significantly affected by incubation at 10ºC in all saline solutions (Table 2, capital letters). Germination velocity was not affected by seed incubation in 0.5% NaCl at all tested temperatures. (Table 2, small letters). *L. mansanetianum* seed incubation at higher doses of NaCl (1.0% to 3.0%) significantly increased T_{50} in all temperatures (Table 2).

Germination after seed incubation at saline solutions

The figure 1 shows the germination response of *L. mansanetianum* seeds when transferred to distilled water after incubation in saline solutions (from 1.5 to 3.0% NaCl) that inhibited strongly germination. Non-germinated seeds from previous NaCl incubations recovered to equal or very little levels that control seeds (Table 1). The germination percentages were not affected by previous NaCl doses or temperature treatment (Fig. 1). Germination velocity of non-germinated seeds increased respect to germinated seed incubated in saline solution (no shown).

Effect of hypersaline solutions on germination

Table 3 shows the final germination percentage and velocity germination of *L. mansanetianum* seeds when transferred to distilled water after incubation in hypersaline solutions. The final germination percentages were not affected by previous incubation at 4% NaCl at both temperatures (Table 3, lowercase letters) and no significant difference was observed among optimal temperature and 10ºC (Table 3). Previous incubation in 8% NaCl solution significantly reduced the germination percentage by 30% at both temperatures (Table 3, lowercase letters).

The germination rate of *L. mansanetianum* seeds increased by previous incubation at hypersaline solutions when seeds were germinated at optimal temperature (Table 3, lowercase letters). T_{50} was not affected when seeds were germinated at 10ºC and previously incubated at 4% and 8% NaCl during 2 months (Table 3).

Discussion

The present study indicates that seeds of *L. mansanetianum* showed a higher percentage of germination under non-saline conditions and germination in non-saline solution is not affected by temperature regimes (Table 1). High germination rate of *L. mansanetianum* seeds in non-saline solution and the short lapse between seed collection and germination experiments suggested the absence of innate dormancy in this species (Table 1). Similar germination capability was observed in different western Mediterranean species of *Limonium*, such as *L. magallufianum* L. Llorens and *L. gibertii* (Sennen) Sennen (Galmés et al. 2006), *L. emarginatum* (Willd.) O. Kuntze (Redondo-Gómez et al. 2008), *L. cossonianum* O. Kuntze (Giménez-Luque et al. 2013), *L. insigne* (Coss.) O. Kuntze (Delgado-Fernández et al. 2015), *L. tabernense* M. Erben (Delgado-Fernández et al. 2016), *L. avei* (De Not.) Brullo & Erben (Santo et al. 2017), *L. narbonense* Miller, *L. girardianum* (Guss.) Fourr. and *L. santapolense* Erben (Al Hassan et al. 2018; Monllor et al. 2018), *L. lobatum* (L. fil.) Chaz. (Kleemann and Gill 2018) and *L. supinum* (Girard) Pignatti (Melendo and Giménez-Luque 2018), as well as non-western Mediterranean species *L. binervosum* (G. E. Sm.) Salmon (Woodell 1985), *L. axillare* (Forsk.) O. Kuntze (Mahmoud et al. 1983), *L. stockii* (Boiss.) O. Kuntze (Zia and Khan 2004; Hameed et al. 2014), *L. iconicum* (Boiss. & Heldr.) O. Kuntze and *L. lilacinum* (Boiss. & Bal.) Wagenitz (Yildiz et al. 2008), and *L. bicolor* (Bunge) Kuntze (Liu et al. 2009). In contrast, low germination percentage of seeds associated to physiological dormancy was observed in *L. bellidifolium* (Gouan) Dumort. (23% germination), *L. humile* Miller (27%) and *L. vulgare* Miller (28%) (Boorman 1968), *L. nashii* Small (Shumway and Bertness 1992), *L. virgatum* (Willd.) Fourr. (40%) (Al Hassan et al. 2018) as well as in different *Limonium* species of the Sicilian flora (Airò et al. 2004).

Salinity is a main environmental stress that can limit the growth and development of plants. Our results are according to several studies which reveal that optimal germination of most halophytes occurs in fresh water (Ungar 1995; Gul et al 2013). As previously reported for other halophytic species, the best germination of *L. mansanetianum* seeds was obtained in distilled water control and the increase in salinity progressively inhibited germination (Table 1). The halophytic species vary greatly in their response to salinity as quantified by the germination percentage (Ungar 1995, Gul et al. 2013). Maximum salt tolerance for seed germination has been reported for *Suaeda aralocaspica* (Bunge) Freitag & Schütze (8.8% NaCl, Wang et al. 2008), *Limonium vulgare* (8.8% NaCl, Woodell 1985), *Haloxylon persicum* (Bunge) (7.6% NaCl, Tobe et al. 2000), *Sarcocornia perennis* Miller (7.6% NaCl, Redondo et al. 2004), *Haloxylon ammodendron* (C. A. Mey.) Bge ex Fenzl (7.6% NaCl, Huang et al. 2003), and *Spartina alterniflora* Loisel. (6% NaCl, Mooring et al. 1971). Germination was about 10% in the seeds of 37 halophytes species in strongly saline solution above 3.5% NaCl (Gul et al. 2013). Additionally, seeds of more 50 salt-tolerance halophytes can germinate at salinity levels around or above that of seawater which varies from 2.7-3.5% NaCl while few of them have low salt tolerance (Gul et al. 2013). *L. mansanetianum* seeds can germinate about 10% in strongly saline solutions (3.0% NaCl, Table 1) and its salt salt tolerance is similar to most halophytes (Gul et al. 2013).

L. mansanetianum is a high salt-tolerance halophyte, as are *L. vulgare* and *L. bellidifolium* (Boorman 1968), the fact that their seeds can germinate at seawater salinity levels (Table 1). A moderate salt tolerance has been described in *L. humile* (Boorman 1968), *L. binervosum* (Woodell 1985), *L. stockii* (Zia and Khan 2004; Hameed et al. 2014), *L. lilacinum* and *L. iconicum* (Yildiz et al. 2008), *L. cossonianum* (Giménez-Luque et al. 2013), *L. insigne* (Delgado-Fernández et al. 2015), *L. narbonense* and *L. virgatum,* (Al Hassan et al. 2018) and *L. lobatum* (Kleemann and Gill 2018). In contrast, a low salt tolerance in seed germination has been described in *L. axillare* (Mahmoud et al. 1983), *L. emarginatum* (Redondo-Gómez et al.

2008*), L. tabernense* (Delgado-Fernández et al. 2016), *L. girardianum* and *L. santapolense* (Al Hassan et al. 2018).

Temperature and salinity interact to affect the germination of halophytes (Khan et al. 2001). The negative effect of high salinity is further aggravated by either an increase or decrease in temperature although the sensibility to changes in temperature varied with species (Khan and Rizvi 1994). Seed germination of *L. mansanetianum* was not affected by temperature in non-saline solution (Table 1) as it happens in *L. cossonianum* (Giménez-Luque et al. 2013), *L. tabernense* (Delgado-Fernández et al. 2016), *L. girardianum,* and *L. santapolense* (Monllor et al. 2018) however a lower germination has been obtained with a decrease in temperature in the case of *L. virgatum* and *L. narbonense* (Monllor et al. 2018). The optimal temperature for seed germination of *L. mansanetianum* in saline solutions was 15ºC and an increase in temperature regimes (20º and 25º/20º) did not significantly affected the germination (Table 1). Others authors found that a temperature increase regarding the optimal condition (20/10ºC) cause a pronounced decrease in seed germination in saline solution at temperature regimes of 30/20º and 35/25º (Giménez-Luque et al. 2013; Delgado-Fernández et al. 2016; Melendo and Giménez-Luque 2018). In contrast, a decrease at temperature regime reduced final germination of *L. mansanetianum* seeds in all saline solutions (Table 1). Seed germination of *L. mansanetianum* in natural conditions appears to take place after spring rains when environmental temperatures are around 13 to 19ºC. Recruitment of new specimens *L. mansanetianum* has been observed in translocation experiences (Ferrer-Gallego et al. 2009).

Very rapid germination has been observed in *L. mansanetianum* seeds under non-saline conditions (Table 2 and Fig. 1), as in other halophytes and in other *Limonium* species (Giménez-Luque et al. 2013; Delgado-Fernández et al. 2016; Al Hassan et al. 2018). Using the short period of water availability after rainfall for rapid and massive germination should be an efficient strategy to ensure the success of seedling establishment. Another advantage of this response pattern of seed germination would be to minimize intraspecific competition in the critical step of seedling establishment as is the case for *Trifolium repens* L. (Roger et al. 1995). In contrast with germination percentage, T_{50} was significantly different at 1.0% NaCl for optimal temperature. It also was significantly different at 10ºC for non-saline control (Table 2). This suggested that the rate of germination of *L. mansanetianum* seeds was more sensitive to salinity and temperature than the final germination percentage. Similar results have been observed in different glycophytes (West and Taylor 1981; Dudeck and Peacock 1985) and in other species of *Limonium* genus such as *L. axillare* (Mahmoud et al. 1983), *L. stocksii* (Zia and Khan 2004; Hameed et al. 2014), *L. cossonianum* (Giménez-Luque et al. 2013), *L. tarbenense* (Delgado-Fernández et al. 2016), *L. girardianum* and *L. santapolense* (Al Hassan et al. 2018).

Seeds of halophytes must remain viable in high salinity levels and germinate when salinity decreases and providing a viable seed bank could be a selective advantage for plants growing in saline habitat (Khan and Ungar 1997). The term "recovery of germination" is used to evaluate the ability of seeds subjected to hypersaline conditions to germinate when transferred to fresh water (Ungar 1991). Boorman (1968) and Woodel (1985) have established a classification of halophytes based on the germinative response to salinity and their recovery after hypersaline exposure. In types 1 and 2, the germination was inhibited at low salinity doses, but a partial germination recovery is observed in type 1 halophytes. In contrast, in type 2 halophytes a complete germination recovery is observed when ungerminated seeds were transferred to fresh water. In type 3, the germination was stimulated by salinity exposition. *L. mansanetianum* seeds recovered completely when transferred to non-saline solution after a 30 d from various salinity treatments and temperature regimes (Fig. 1) According to this classification *L. mansanetianum* can be considered as type 2. Similar response have been observed in recovery tests in *L. axillare* (Mahmoud et al. 1983), *L. tabernense* (Delgado-Fernández et al. 2016), *L. supinum* (Melendo and Giménez-Luque 2018), *L. girardianum* and *L. narbonense* (Al Hassan et al. 2018). Other

Limonium species, such as, *L. bellidifolium, L. humile* and *L. vulgare* (Boorman 1968), *L. santapolense* and *L. virgatum* (Al Hassan et al. 2018) were considered as type 3 since seed exposure to salinity can increase the seed germination. Seeds previously incubated at 4ºC in hypersaline solutions during 2 months exhibited a similar germination rate (4% NaCl) or greater than 65% (8% NaCl) compared to seeds not exposed to hypersaline conditions (Table 3). These results shown that seeds of this species remain viable after a long exposure to salinity and temperature stresses, as well as they can germinated when salinity is reduced by rains. Similar results have been observed in other *Limonium* species (Mahmoud et al. 1983; Zia and Khan 2004) and other halophytes (Khan and Ungar 1997; Gul et al. 2013). The ability of halophyte seeds to survive in hypersaline solutions and germinate when salinity is reduced provides them multiple opportunities for the establishment of cohorts in unpredictable saline environments. Regarding also the evidences from conservation translocations performed in the Valencian area, the plantation experiences with the inland sea lavender *L. mansanetianum* (Ferrer-Gallego et al. 2009) and the coastal *L. perplexum* L. Sáez & J.A. Roselló (Laguna et al. 2016) have been successful, yielding a regular recruitment of new specimens born in the translocated populations.

In conclusion, *L. mansanetianum* seeds showed an elevated germination in non-saline solution as occurs with other halophytes. Temperature and salinity affected the germination of *L. mansanetianum* seeds. The optimal temperature range suggests that the main recruitment should happen after spring rains. *L. mansanetianum* can be considered as a moderately high salt-tolerant halophyte with the ability to maintain a threshold of germination at high salinity. However, the main trait of its seed ecology is the salinity tolerance during storage in the soil seed bank. This trait represents a successful reproductive strategy in the episodic and unpredictable conditions associated to its natural habitat.

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Table 1. Effect of temperature (10ºC, 15ºC, 20ºC and 20/25ºC) and salinity incubation (0%-3%) on the germination of *Limonium mansanetianum* seeds.

% NaCl	Temperature (ºC)			
	10º	15º	20º	25/20º
0.0	97.0±2.0 a, A	100.0±0.0 a, A	96.1±2.5 a, A	99.0±1.0 a, A
0.5	93.1±1.5 a, AB	100.0±0.0 a, C	88.7±1.7 ab, A	95.7±2.5 a, BC
1.0	79.1±4.9 b, AB	90.8±2.0 a, B	79.4±4.6 b, AB	65.2±12.6 b, A
1.5	17.2±4.7 c, A	41.8±6.6 b, B	37.7±5.0 c, B	21.8±1.4 c, A
2.0	3.5±1.1 d, A	26.2±9.5 c, B	19.7±3.5 d, B	18.4±2.6 c, B
2.5	4.0±2.8 d, A	12.0±3.5 c, AB	9.6±1.6 d, A	20.1±4.7 c, B
3.0	0.0±0.0	12.1±5.1 c, B	13.1±4.6 d, B	19.1±4.6 c, B

Final germination percentages (mean values ± standard error) after 30 days.

Different letters denote statistically significant differences by a Tukey's multiple comparison test ($P < 0.05$) within final germination and % NaCl range (lowercase letters) or within final germination and incubation temperature range (capital letters)

Table 2. Effect of temperature (10ºC, 15ºC, 20ºC and 20/25ºC) and saline incubation (0%-3% Na Cl) on germination velocity of *Limonium mansanetianum* seeds.

% NaCl	Temperature (ºC)			
	10º	15º	20º	25/20º
0.0	6.0±0.5 a, B	2.4±0.1 a, A	1.7±0.1 a, A	2.0±0.1 a, A
0.5	9.7±0.5 a, B	4.4±0.2 a, A	5.5±0.4 a, A	4.7±0.6 a, A
1.0	22.1±1.2 b, B	10.1±1.8 b, A	13.2±4.1 b, A	13.7±4.6 b, A
1.5	22.0±2.9 b, B	17.1±3.8 b, AB	13.0±3.4 b, A	12.1±4.2 b, A
2.0	24.2±2.7 b, B	13.5±2.7 b, A	17.1±3.8 b, AB	12.4±1.1 b, A
2.5	30.1±1.4 c, B	13.0±3.5 b, A	10.8±2.2 b, A	12.2±2.8 b, A
3.0	-	16.1±6.9 b A	23.0±4.1 c A	23.9±5.9 c, A

T_{50} (mean values ± standard error).

Different letters denote statistically significant differences by a Tukey's multiple comparison test ($P < 0.05$) within T_{50} and % NaCl range (lowercase letters) or within T_{50} and incubation temperature range (capital letters)

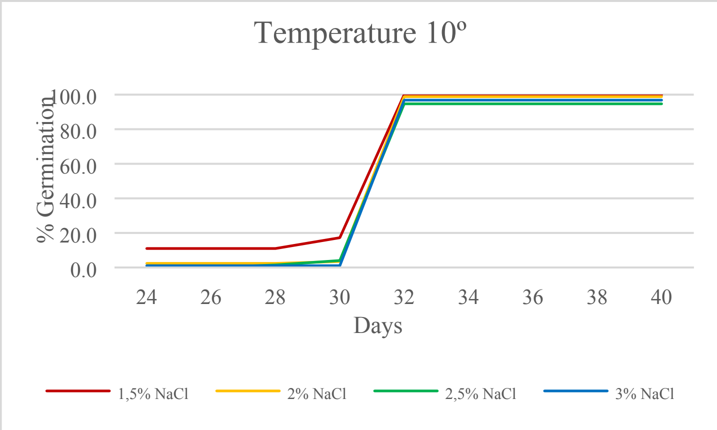
Table 3. Effect of previous seed incubation in hypersaline solutions (4%-8% NaCl during two months) and temperature (10ºC and 15ºC) on germination of *Limonium mansanetianum* seeds.

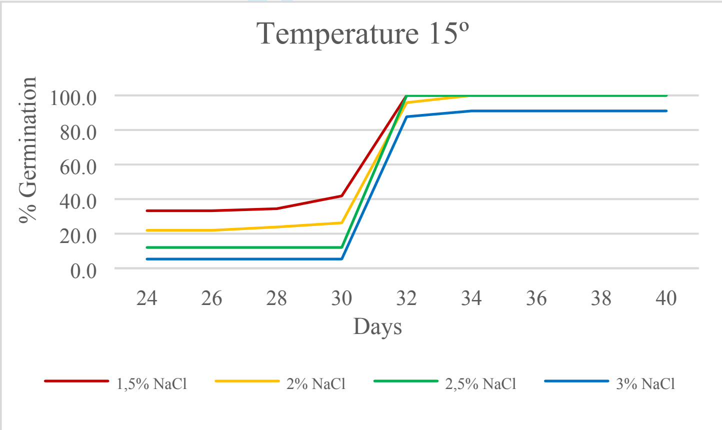
Previous seed incubation on NaCl solutions	% Germination		T_{50}	
	10º	15º	10º	15º
0.0%	97.0±2.0 a, A	100.0±0.0 a, A	6.0±0.5 a, A	2.4±0.1 a, B
4.0%	87.5±3.2 a, A	92.5±4.3 a, A	6.9±0.1 a, A	3.8±0.1 b, B
8.0%	66.3±5.2 b, A	66.3±1.3 b, A	7.0±0.2 a, A	3.7±0.1. b, B

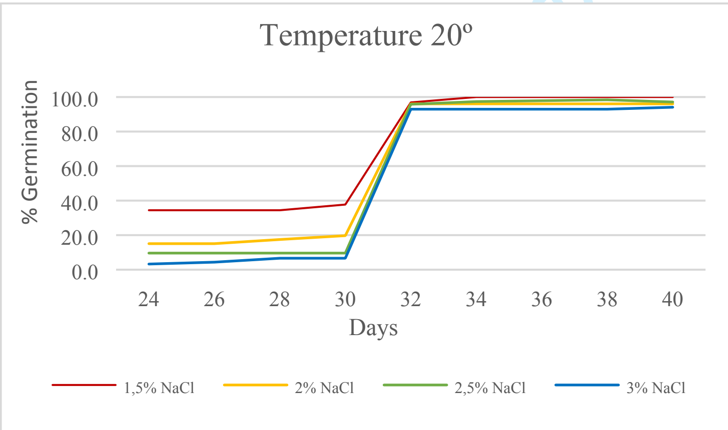
Final germination percentages and T_{50} (mean values ± standard error) after 30 days.

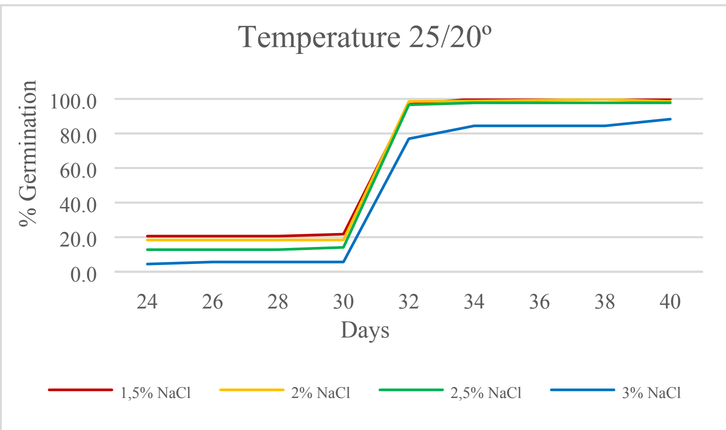
Different letters denote statistically significant differences by a Tukey's multiple comparison test ($P < 0.05$) within final germination or T_{50} and % NaCl range (lowercase letters) or within final germination or T_{50} and incubation temperature range (capital letters)

Figure 1. Cumulative germination percentage of *Limonium mansanetianum* seeds after trasferred to distilled water (30 days) from 1.5 to 3.0% NaCl at 10ºC, 15ºC, 20ºC and 20/25ºC.


Temperature 10º
% Germination
100.0
80.0
60.0
40.0
20.0
0.0
24 26 28 30 32 34 36 38 40
Days
1,5% NaCl
2% NaCl
2,5% NaCl
3% NaCl


Temperature 15º
% Germination
100.0
80.0
60.0
40.0
20.0
0.0
24 26 28 30 32 34 36 38 40
Days
1,5% NaCl
2% NaCl
2,5% NaCl
3% NaCl


Temperature 20º
% Germination
100.0
80.0
60.0
40.0
20.0
0.0
24 26 28 30 32 34 36 38 40
Days
1,5% NaCl
2% NaCl
2,5% NaCl
3% NaCl


Temperature 25/20º
% Germination
100.0
80.0
60.0
40.0
20.0
0.0
24 26 28 30 32 34 36 38 40
Days
1,5% NaCl
2% NaCl
2,5% NaCl
3% NaCl