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# Influence of abiotic factors on the germination of an endemic sea lavender: First steps to shape local assemblage

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ABSTRACT

Different Mediterranean sea lavender species, especially endemisms, appear within specific soil conditions led by vegetative trait syndromes. Nonetheless, seed traits contribute to functional structuring of plant communities, shaping the first stages of plant fitness and niche competition in stressful habitats. In this framework, we assess abiotic factors related to germination and establishment of *Limonium tobarrense*, an inland saltmarsh endemism located at the southeastern of the Iberian Peninsula, to shed light about the assembly processes in its early life stage. Experiments were performed to determine the effects of different salinities (0%, 0.5%, 1%, 1.5% and 2% NaCl) on seed germination under three combinations of day/night temperature (30 °C/20 °C, 25 °C/20 °C and 25 °C/15 °C) with a photoperiod of 12 h in light and 12 h in dark. Final germination percentage (FGP) and mean time-to-germinate (MTG) were studied after 30 days. The best FGP values were obtained in freshwater and low salinity conditions (1% and 1.5% NaCl). Moreover, similar MTG were observed at different salinities, but MTG was significantly high at lower temperature (i.e., 25 °C/15 °C). Finally, no differences between light conditions were observed. Our results suggest that salinity determines the germination and establishment of *L. tobarrense*, germinating successfully in freshwater and low salinity. In addition, a wide range of thermoperiod for its successful germination was found, although its interaction with the salinity was not significant. In consequence, *L. tobarrense* would germinate after the autumn rainfalls, when the salts are lixiviated in soil, and the medium temperature are favourable for the best seed germination conditions. Thus, specific environmental conditions would allow the own regeneration and adult niche of this endemism.

## 1. Introduction

Disentangling the community assembly rules, defined as ecological processes which select the local community composition, is relevant for explaining biodiversity patterns and for predicting future changes over the ongoing global change (Götzenberger et al., 2012; Newbold, 2018). Trait-based approaches provide fundamental tools to understand these ecological processes (McGill et al., 2006), being the functional traits (i.e., morphological, physiological, phenological or behaviour features affecting on growth, reproduction and survival) essential in the community assembly (Violle et al., 2007; Pillar et al., 2021). According to ecological theory, a plant species cannot be part of the community unless its seed traits are optimised to local assembly processes (Grubb, 1977; Poschlod et al., 2013). Even if the local environmental conditions are suitable for the adult niche (i.e., adult plant growth and survival), a long-term persistence of species is related to the regeneration niche (i.e., its capacity to produce viable seeds which can successfully disperse, persist, germinate and produce viable seedlings) (Grubb, 1977; Rosbakh et al., 2022). Thus, seed traits contribute to functional structuring of the plant communities along environmental gradients (Rosbakh et al., 2022), being related to fitness and niche differences among species and contributing to species coexistence in competitive habitats (Kraft et al., 2015; Poschlod et al., 2013). Concretely, the seeds of some halophytes might evade times of elevated salinity by entering dormancy (e.g., species of the family Amaranthaceae), while other ones are immensely tolerant to salinity and employ physiological mechanisms (e.g., ion compartmentalisation and osmotic adjustment) (Seal and Dantas, 2021). Nonetheless, these strategies are plastic and can differ among and within species, as well as depending on the habitat and its conditions.

Mediterranean saltmarshes have high saline soils (>4 mS/cm) under

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arid or semiarid conditions (Chapman, 1974). In these stressful habitats, only halophytes (i.e., plant species which are able to complete their life cycle in a NaCl concentration of at least 200 mM) can survive through the soil gradients (Flowers and Colmer, 2008; Teege et al., 2011). Moreno et al. (2018a) established that the plant assemblage in Mediterranean saltmarshes was markedly influenced by the salinity gradient, founding two main plant zones: succulent and non-succulent zones. Different sea lavender species (*Limonium* Mill.) appears within non-succulent zones (i.e., the lowest saline zones) with a specific edaphic distribution within it, mainly gathered by morphotypes defined by vegetative trait syndromes (i.e., presence and number of sterile branches and presence/absence of leaves at the anthesis) (Moreno et al., 2018a).

The genus *Limonium* (sea lavenders) is the most species-rich genus of the Plumbaginaceae, including c. 400 taxa (Erben, 1993). This genus has a wide variety of endemisms along the Mediterranean Basin (Erben, 1993), being recently identified several sea lavender species in the last decade (Valli and Artelari, 2015; Moreno et al., 2016, 2018b; Bogdanović et al., 2022, among others). These endemisms usually appear in local restricted areas and studies about their traits-based ecological patterns are scarce or absent from the current literature. An example corresponds to the species *Limonium tobarrense* J.Moreno et al., an endangered endemic sea lavender from the province of Albacete (SE Spain) which only appears in an only known population (i.e., c. 300–400 individuals in <0.5 ha) (Terrones et al., 2016). This species appears in a specific area within the non-succulent zone of the Saltmarsh of Cordovilla (SE Spain), but there are no studies which shed light about its traits-based ecology.

Previous studies have shown the great influence of salinity and temperature on the germination of sea lavender species (Redondo-Gómez et al., 2008; Zia and Khan, 2008; Giménez-Luque et al., 2013; Delgado Fernández et al., 2015, 2016; Fos et al., 2021; Moreno et al., 2022), suggesting that seed traits may be relevant to the plant assemblage community in Mediterranean saltmarshes. Salinity and temperature affect the germination of *Limonium* species defining different germination syndromes (e.g., rapid germination velocity when the soil salinity decreases or physiological dormancy) which favour the adaptation of different species under favourable conditions and, consequently, shaping the community assembly (Moreno et al., 2022). Nonetheless, in some plant species, the absence of light has played a crucial role on germination patterns, inhibiting the seed germination either completely (Benvenuti et al., 2004), or partially (Zia and Khan, 2004), or, even, showing no effects (Wei et al., 2008). Curiously, the absence of light had been only considered in a few previous germination assays of sea lavender species (Zia and Khan, 2002, 2004, 2008; Yildiz et al., 2008). Furthermore, the interaction of absence of light and temperature have been also reported previously as a controlling factor for dormancy where seed germination was inhibited at some temperature regimes more in the absence of light (Vleeshouwers et al., 1995; Baskin and Baskin, 1998, 2004).

Recently, Moreno et al. (2022) studied the germination of different co-occurring *Limonium* species (i.e., *L. admirabile* Terrones et al., *L. cossonianum* Kuntze, *L. delicatulum* Kuntze, *L. tobarrense* and *L. supinum* (Girard) Pignatti), growing all of them in the Saltmarsh of Cordovilla (SE Spain). Overall, these results showed that the sea lavender species germinated rapidly in freshwater, while the mean time-to-germinate (MTG) in *L. tobarrense* was significantly longer with a second increase in germination on the eighth day. Besides, *L. tobarrense* seeds showed a peculiar behaviour with a slight increase in the percentage of viability at low salinities. Based on the last evidence, it is considered necessary to develop an in-depth study focused exclusively on *L. tobarrense* and its optimal germination conditions, disentangling the trait-based ecology of it.

In this context, we consider relevant to study environmental factors which allow the germination and establishment of *L. tobarrense* in its particular habitat, as a first step to establish the assembly processes of this endemism. Thus, the main goals of this study are to assess: (i) the influence of salinity, temperature and its interaction; and (ii) the absence of light, temperature and its interaction on germination patterns.

## 2. Material and methods

### 2.1. Study site

The current study was performed in the Saltmarsh of Cordovilla (38° 32′ 15″ N, 1° 36′ 40'' W), included within the special area of conservation (SAC) "Saltmarshes of Cordovilla and Agramón and the lagoon of Alboraj" in the southeast of the Iberian Peninsula (Province of Albacete, Spain). This habitat is located between the municipalities of Tobarra and Hellín (i.e., southeast of the province of Albacete), and it corresponds to one of the best-preserved inland salt marshes in Spain (Dirección General de Política Forestal y Espacios Naturales, 2015). The Saltmarsh of Cordovilla is characterized by halophilic and halonitrophilous vegetation, with endemic and unique plant species of this region (Dirección General de Política Forestal y Espacios Naturales, 2015). The annual mean temperature and precipitation is 15.1 °C and 358 mm, respectively (Dirección General de Política Forestal y Espacios Naturales, 2015), and hence, this area is part of the semiarid Mesomediterranean bioclimatic belt (Rivas-Martínez, 2007). This study site was selected because it includes the unique Iberian populations of *L. tobarrense* (Moreno et al., 2016), where the optimal conditions for the development of this endemism occurs.

### 2.2. Germination experiments

Inflorescences with seeds were collected from twenty well-separated individuals within the Cordovilla population (c. 50 individuals) during September 2020 and stored in paper bags at room temperature. Seeds were manually extracted from fruits of each individual of *L. tobarrense* and sterilized immediately before starting the experiments by immersion in 10% sodium hypochlorite solution for 10 min and washed using sterilized water (Redondo-Gómez et al., 2008). The germination essays were carried out with different treatments of temperature, light and salinity. For each treatment, four sets of 25 seeds were selected (using a total of 100 seeds for each treatment) and placed in 9-cm Petri dishes on a filter paper disk and submerged in 3 ml of the appropriate solution. Petri dishes were sealed with Parafilm to avoid evaporation during the experiment. These Petri dishes were maintained in a germination chamber with controlled photoperiod and temperature during the experiment. Seed germination was checked daily for 30 days and germinated seeds were counted and removed. Seeds were considered germinated once the radicle had elongated.

For temperature treatments, three combinations of day/night temperature (30 °C/20 °C, 25 °C/20 °C and 25 °C/15 °C) were performed, all three with a photoperiod of 12 h in light and 12 h in dark. These temperatures were selected to include the existing seasonal temperature between summer and winter in the study area, according to data from the closest meteorological station from Hellín (Rivas-Martínez and Rivas-Sáenz, 1996–2020). For each temperature treatment, five NaCl solutions (0%, 0.5%, 1%, 1.5% and 2%) were used to cover in detail the range of salinity in which *L. tobarrense* is able to germinate (Moreno et al., 2022). An additional treatment in which the 0% NaCl solution was used for each temperature analysed was added to evaluate whether the presence of light influences germination; in this case, light was excluded by using aluminium foil.

After the 30 days, two variables of germination were determined following Delgado-Fernández et al. (2016): (i) final germination percentage at 30 days (FGP), and (ii) mean time-to-germinate (MTG). MTG was calculated by $\Sigma\ (n_i \times d_i)/N$; where $n_i$ is the number of seeds germinated in day $i$; $d_i$ is the incubation period in days, and N is the total number of seeds germinated in the treatment (Delgado-Fernández et al., 2016).

### 2.3. Data analyses

Statistical analyses were carried out using R software version 4.1.1 (R Core Team, 2021). The effect of the three studied factors (salinity, temperature and light) on the two recorded variables (FGP and MTG) was established with different nested linear models created with the 'lm' function. The temperature and light factors were treated as fixed factors with three and two levels, respectively, while the salinity factor was treated as a continuous variable. Since light exclusion treatment was only done in 0% salinity treatment, and hence not all combinations between salinity and light treatments were performed, two different analyses were made for each response variable.

To study the effect of salinity, temperature and its interaction, all samples (except for the samples of the light exclusion treatment) were included in the analyses. Two different analyses were built for each studied response variable: (i) FGP and (ii) MTG. The effect of salinity was assessed using a regression to test the global effect of salinity on FGP and MTG throughout the studied salinity gradient. However, in the case of FGP, a non-linear quadratic regression was used because the relationship between FGP and salinity was clearly not linear. The constructed models included three (for MTG) and four (for FGP) factors as predictors: (i) the first degree component of salinity (i.e., 0%, 0.5%, 1%, 1.5% and 2%), (ii) the second degree component of salinity (only for FGP), (iii) temperature (i.e., 30 °C/20 °C, 25 °C/20 °C and 25 °C/15 °C), and (iv) the interaction between salinity and temperature.

To study the effect of light, temperature and its interaction, different analyses were done for each response variable. In the different constructed models, three factors were included as predictors: (i) light (i.e., light vs darkness), (ii) temperature (i.e., 30 °C/20 °C, 25 °C/20 °C and 25 °C/15 °C), and (iii) its interaction.

The significance of each considered predictor in each model was tested using analysis of variance (ANOVA) between the different nested models with the 'anova' function (R Core Team, 2021). For the temperature factor, significant results ($P \leq 0.05$) were subjected to post-hoc analyses by Tukey's tests using the 'glht' function of the 'multcomp' package (Hothorn et al., 2008). For each analysis, a final model was constructed only including the significant predictors, and the residuals of this model were plotted and visually inspected for normality and homoscedasticity.

## 3. Results

Salinity had a very marked effect on the germination of *L. tobarrense* (Fig. 1). The highest values of FGP were found in freshwater conditions and a fast FGP decrement was observed in the low salinity ranges (from 0 to 0.5% and 1%) (Fig. 2A). The germination values at 1% and 1.5% saline conditions were quite similar, dropping the values of FGP almost zero at 2% of salinity (Fig. 2A). At higher salinities (i.e., 1%, 1.5% and 2%), the decrease of FGP was slower when salinity increased (Fig. 2A). Consequently, the salinity has a non-linear behaviour on FGP, and hence, the ANOVA results show a significant effect on the first and second degree of the quadratic regression ($P < 0.001$, Table 1). However, no significant differences were found for FGP between the different temperature treatments or its interaction with salinity (Table 1 and Fig. 1). Therefore, FGP patterns were clearly explained by salinity changes ($R^2 = 0.841$) (Table 2).

Overall, similar MTG were observed at different salinities (Fig. 2B). According to the temperature, significant differences in MTG were observed between the three different studied temperatures ($P < 0.001$, Table 1). MTG was significantly high at the lower temperature (i.e., 25 °C/15 °C) in comparison to the other studied temperatures (Tukey's test $P < 0.001$), being 7.3 ± 1.5 days higher at 25 °C/15 °C (Table 2). Besides, MTG at 30 °C/20 °C treatment did not significantly differ to the 25 °C/20 °C treatment (Tukey's test $P = 0.772$). However, although

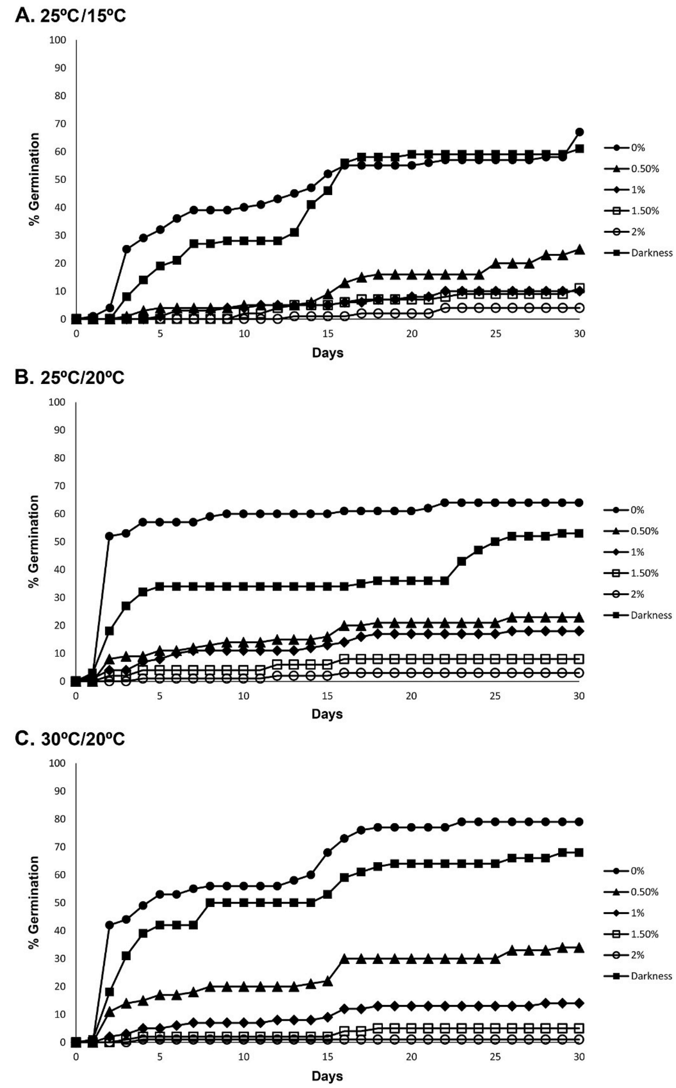


**Fig. 1.** Germination percentage of *Limonium tobarrense* throughout the 30 days of the experiment at different temperatures in different salinity and darkness treatments: (A) 25 °C/15 °C, (B) 25 °C/20 °C and (C) 30 °C/20 °C.

MTG variation was significantly explained by temperature, its patterns indicated that there is a very high variation in MTG not explained by any of the predictors ($R^2 = 0.335$; Table 2).

Finally, in the light absence experiment, dark conditions showed a tendency of a lower FGP and higher MTG compared to the light conditions (Fig. 3), although these patterns were non-significant (Table 3). The interactions between light and temperature were non-significant for any of the FGP and MTG variables (Table 3). Similarly to the analyses with salinity and temperature, significant differences in MTG were also observed between the studied temperatures ($P = 0.029$, Table 3), although the *P*-values were higher given the lowest number of samples in the light/temperature analysis.

## 4. Discussion

### 4.1. Salinity

Despite *L. tobarrense* is a halophytic species, an optimal germination was obtained in the absence of salinity. Similar results can be observed for different Mediterranean sea lavender species (e.g. Giménez-Luque et al., 2013; Melendo and Giménez-Luque, 2018; Fos et al., 2021), and also for other halophytes (Gul et al., 2013). Furthermore, Moreno et al.

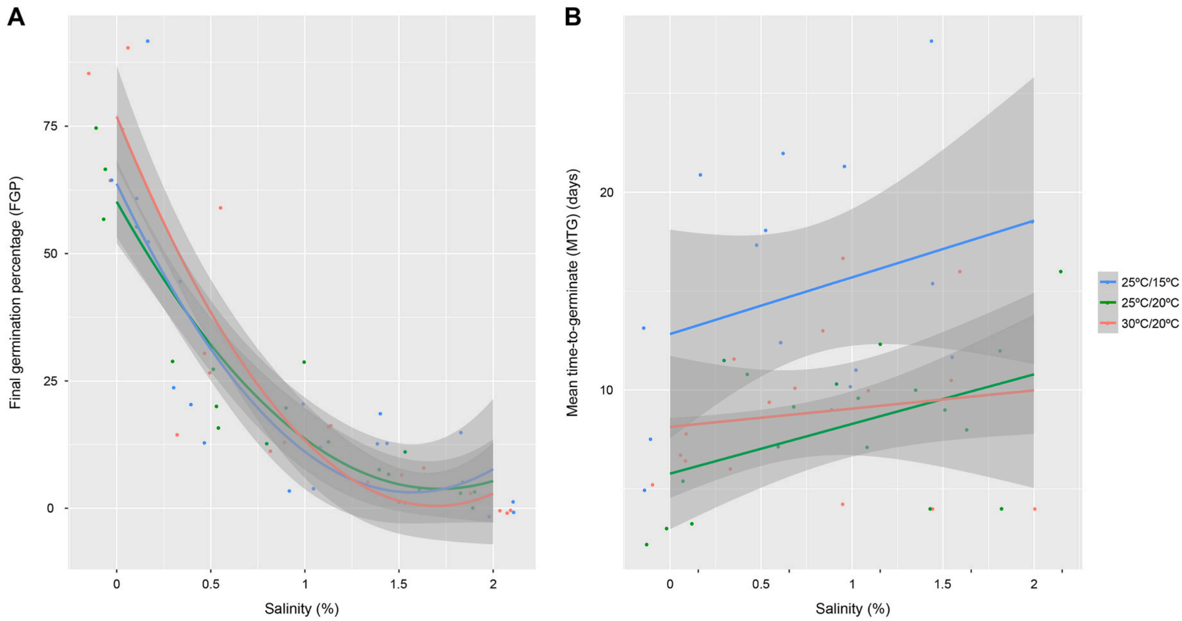


**Fig. 2.** (A) Final germination percentage (FGP) and (B) mean time-to-germinate (MTG) of *Limonium tobarrense* after 30 days of experiment at different temperatures (30 °C/20 °C, 25 °C/20 °C and 25 °C/15 °C) in different salinity treatments. Points were slightly scattered to avoid overlapping.

**Table 1**
Results of the analyses of variance (ANOVA) of the final germination percentage (FGP) and the mean time-to-germinate (MTG) in the first and the second degree components of salinity, temperature, and the interaction between salinity and temperature. Abbreviations: df, degrees of freedom.

| | Salinity (1st degree) | | | Salinity (2nd degree) | | | Temperature | | | Salinity x Temperature | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | F | P | df | F | P | df | F | P | df | F | P |
| **FGP** | **1** | **55.623** | **< 0.001** | **1** | **55.623** | **< 0.001** | 2 | 0.703 | 0.500 | 2 | 2.791 | 0.070 |
| **MTG** | 1 | 3.147 | 0.083 | – | – | – | **2** | **12.130** | **< 0.001** | 2 | 0.314 | 0.732 |

**Table 2**
Coefficients of significant models of regressions of salinity and temperature.

| Model | Response variable | Predictors | Estimate | Std. Error | $R^2$ adj |
|---|---|---|---|---|---|
| FGP ~ Intercept + Salinity + Salinity$^2$ | FGP | Intercept | 66.914 | 2.837 | 0.841 |
| | | Salinity (1st degree) | −77.657 | 6.721 | |
| | | Salinity (2nd degree) | 23.429 | 3.223 | |
| MTG ~ Intercept + Salinity + Temperature | MTG | Intercept | 6.107 | 1.401 | 0.335 |
| | | Salinity (1st degree) | 2.159 | 0.991 | |
| | | Temperature (25 °C/15 °C) | 7.28 | 1.546 | |
| | | Temperature (30 °C/20 °C) | 1.06 | 1.546 | |

(2022) reported that *L. tobarrense* germinates rapidly under freshwater conditions, showing the same germination pattern observed in closely-related sea lavenders in sympatry (i.e., *L. caesium*, *L. supinum*, *L. admirabile*, *L. cossonianum* and *L. delicatulum*). In this study, we have found that *L. tobarrense* shows a non-linear behaviour in the salinity interval in which it can successfully germinate, i.e., the germination decreased when the salinity increased, but this trend changed at higher salinity concentrations. A similar behaviour can be observed for other *Limonium* species, such as *L. admirabile*, *L. cossonianum* and *L. delicatulum* [as found in Giménez-Luque et al. (2013) and Moreno et al. (2022)]. Nonetheless, other *Limonium* species seem to have a constant linear behaviour, such as *L. caesium* and *L. supinum* (Melendo and Giménez-Luque, 2018; Moreno et al., 2022), in which the germination percentage decreased more or less constantly with the salinity increment. Indeed, Moreno et al. (2022) already reported that there were two germination syndromes between *Limonium* species depending on the width of the range of the salinity in which they can germinate: syndrome 1, successful germination under non-saline conditions but with a decrement of FGP at 1% of salinity and above, and an almost complete recovery percentage of potential germination after the high salinity exposure (up to 6%); and syndrome 2, germination at a wider salinity range, but the germination recovery is not complete after a saline shock. In this way, the FGP of *L. tobarrense* decreases at higher salinities but it continues germinating over time, showing as the salinity has a non-linear behaviour. This germination pattern under saline stress could be attributed to lowering of soil water potential and/or ionic toxicity (Katembe et al., 1998; Bajji et al., 2002), but this effect was also ascribed to oxidative stress induced by salinity (Amor et al., 2005; Demiral and Turkan, 2005).

Overall, *L. tobarrense* showed the lowest values of MTG in freshwater, suggesting this endemism could germinate more easily after the fall of autumn rains, when the soil salinity is reduced. This could be supported by the successful germination of *L. tobarrense* under low concentrations of salinity (i.e., 0.5% and 1%). According to the climatic maps of Spain (1981–2010) (AEMET, 2018), the average rainfall during the fall in the study area is 100–200 mm after the summer drought period (i.e., average rainfall 25–50 mm during the summer), so the salts could be lixiviated on the soils providing favourable germination conditions. This germination strategy could be also found in *L. cossonianum* since a high salinity increased its MTG and blocked seed germination (Giménez-Luque et al., 2013).

Within the saline habitat, other halophytic species, co-occurring with *L. tobarrense,* can also resist a wide range of variations in salinity. For

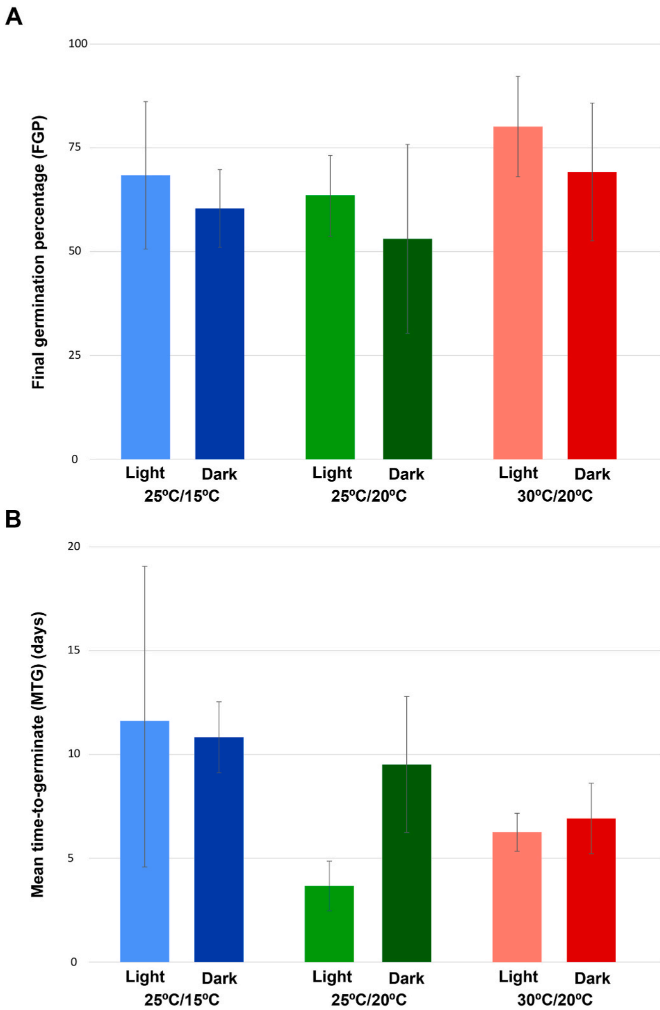


**Fig. 3.** (A) Final germination percentage (FGP) and (B) mean time-to-germinate (MTG) of *Limonium tobarrense* after 30 days of experiment at different temperatures (25 °C/15 °C, 25 °C/20 °C and 30 °C/20 °C) in different light treatments. Bars indicate standard errors.

instance, the succulent species *Arthrocaulon macrostachyum* (Moric.) Piirainen & G.Kadereit (named as *Arthrocnemum macrostachyum* (Moric.) K.Koch) (Amaranthaceae) germinated successfully at < 1% of NaCl (0–10 dS/m), losing germination progressively to c. 1–2% (20–40 dS/m) (Khan et al., 2006), but there was an absence of germination at 5% and 10% of salinity (Rubio-Casal et al., 2003). In the case of *Salicornia fruticosa* (L.) L. (Amaranthaceae), this succulent species germinated better at freshwater (85% FGP) than at 2% of salinity (61% FGP) (Redondo et al., 2004). Compared to these examples, the FGP of *L. tobarrense* was practically the same at 1% and 1.5% of salinity (despite this represents an increment of 50% of salinity) and the values of FGP almost zero at 2% of salinity. Conversely to *L. tobarrense*, the germination of these two succulent species might follow a linear pattern related to salinity, with a decrease of the germination percentage that was proportional to the salinity increment. Nonetheless, there would be a general tendency towards germinating when the salinity stress was reduced to ensure the success of the maintenance of seed viability and their ability to germinate readily (Gul et al., 2013; Yuan et al., 2019).

Different patterns of germination of halophytes in sympatry could also be caused by plant phenology. The typical flowering period of *L. tobarrense* takes from August to October and the fructification period is during September–November (pers. obs.). In contrast, *A. macrostachyum* usually flowers during April–June and fruits during July–October, while *S. fruticosa* flowers and fruits during October–December (Valdés et al., 1987). The seeds of these three co-occurring halophytes within the Saltmarsh of Cordovilla may be established according to their seasonal behavior and their different tolerance to salinity (see above). Specific seasonal germination niche might result in response to dormancy and germination requirements (i.e., climatic conditions and salinity gradient), shaping the plant species distribution within saltmarshes. Therefore, germination varies along the salinity gradients, appearing in seasonally favourable gaps and, consequently, might define the vegetation zonation.

### 4.2. Temperature

Similarly to other halophytes, seed germination of *L. tobarrense* resists a wide range of temperature variations when the saline stress is reduced. Certain co-occurring species within the Saltmarsh of Cordovilla can also germinate successfully under different thermoperiods under the absence of salinity (e.g., Redondo et al., 2004; Khan et al., 2006; Giménez-Luque et al., 2013). The succulent species *A. macrostachyum* and *S. fruticosa* both showed the best germination results at 30 °C/20 °C and 25 °C/5 °C, respectively (Redondo et al., 2004; Khan et al., 2006), whereas the maximum of germination of *L. cossonianum* was recorded at 20 °C/10 °C (Giménez-Luque et al., 2013). The existence of a wide range of thermoperiods could have favoured the process of the seed germination under non-stressful conditions (i.e. absence of salinity) along the saltmarshes, but temperature fluctuations might also affect to the final efficiency of the seed germination for some halophytes. Thus, Nisar et al. (2019) reported for *A. macrostachyum* better germination percentages at 25 °C/15 °C (c. 80%) than at 30 °C/20 °C and 35 °C/25 °C (c. 60%) under non-saline treatment, though the seed germination percentages remained quite high. The studied moderate temperatures influenced on the seed germination behaviour at 25 °C/15 °C, had a strong effect on the MTG though the FGP was quite similar for any temperature. Similar results were also denoted by Zia and Khan (2004), as the slowest seed germination of the species *L. stocksii* (Boiss.) Kuntze was at 20 °C/10 °C. These results would support that *L. tobarrense* seeds could be able to germinate more easily after the fall of autumn rains (i.e. after decreasing the soil salinity), but even the capacity of germination is kept with different temperature conditions [i.e., 10.0–22.5 °C during fall in the Saltmarsh of Cordovilla, according to AEMET (2018)]. Therefore, halophytes can support different temperatures for seed germination, not being as significantly relevant as salinity, and they could germinate successfully throughout the year in their natural habitats, shaping the plant distribution in saltmarshes.

Conversely to our data, the salinity-temperature interaction was significant for a few scarce previous reports on other *Limonium* species, such as *L. tabernense* Erben (Delgado-Fernández et al., 2016). The optimum germination percentage under non-saline condition were therefore obtained at the highest temperature regime (35 °C/25 °C), while this

**Table 3**
Results of the analysis of variance (ANOVA) of the final germination percentage (FGP) and the mean time-to-germinate (MTG) in the light and temperature treatments and its interaction. Abbreviations: df, degrees of freedom.

| | Light | | | Temperature | | | Light x Temperature | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | F | P | df | F | P | df | F | P |
| **FGP** | 1 | 2.219 | 0.154 | 2 | 1.956 | 0.170 | 2 | 0.071 | 0.932 |
| **MTG** | 1 | 1.783 | 0.198 | **2** | **4.325** | **0.029** | 1 | 1.901 | 0.178 |

germination value was superior under the combination higher salinity condition (e.g. 100 mM NaCl) plus a lower temperature regime at 20 °C/10 °C. The existence of different ecological patterns between *L. tobarrense* and *L. tabernense* would be the potential explanation of the observed discrepancy about salinity-temperature interaction. These two species thrive in different environmental conditions, salt marshes and gypsiferous soils, respectively. The differences about temperature-salinity interaction would be suitably adapted to their particular habitats.

### *4.3. Light*

The germination percentage of *L. tobarrense* was similar in presence/absence of light at three studied temperatures. Similarly, Zia and Khan (2004) obtained that seed germination of *L. stocksii* was not affected by darkness under any temperature regime, except for the lowest studied range of temperatures 20 °C/10 °C (germination percentage was reduced to 50%). Conversely, other halophytes were reported as light sensitive species [e.g. *Halocnemum strobilaceum* (Pall.) M. Bieb. (Qu et al., 2008), *A. macrostachyum* (Nisar et al., 2019) or *S. fruticosa* (Redondo et al., 2004)]. Nisar et al. (2019) revealed an absolute light requirement for the *A. macrostachyum* seed germination (i.e. positive photoblastic response), failing completely to germinate under darkness. Similarly, the absence of light decreased the germination percentage of *S. fruticosa* in freshwater (Redondo et al., 2004), but the germination was favoured for higher values of salinity (2%, 4% and 6%). These two succulent species typically grow on the highest saline soils of salt-marshes characterized by a scarce diversity of plant species and a remarkable dominance of these two succulent species (Moreno et al., 2018a), forming, generally, well-isolated patches with open soils among them. This ecological particularity would ensure seed germination on/near the soil surface successfully when favourable conditions for seedling arrives. Conversely, *L. tobarrense*, usually grows close to (and even under) other shrubs and grasses, and the soil is clearly covered by this vegetation. Consequently, this ecological pattern could explain that the presence/absence of light did not directly affect the germination of this species. These different patterns of seed germination in the absence of light of co-occurring halophytes could define specific assembly rules within the saltmarsh, shaping the local community composition. However, more specific studies about the effect of the light/dark treatments in germination patterns of Mediterranean sea lavender species should be performed to support our findings.

### *4.4. Local assemblage and conservation*

On a local scale, species assemblage is mainly affected by light quality, soil physics and chemistry through their impact on specific germination demands (Poschlod et al., 2013). Thus, the specific germination patterns obtained from *L. tobarrense* [i.e., highest values of FGP under the lack of salinity and a fast FGP decrement with low salinity conditions (0.5% and 1%) and a MTG significantly high at the lower studied temperature (25 °C/15 °C)] would be considered relevant, since they may define the local assembly rules and shape the population distribution within the saline ecosystem. Nonetheless, further *in situ* experiments would be necessary to validate the behavioural patterns of this species in the field.

Poschlod et al. (2013) and Rosbakh et al. (2022) emphasized the importance of germination traits in community functional structure. Moreno et al. (2018a) described three *Limonium* morphotypes (A, B and C; according to different vegetative and reproductive features) across saltmarsh zones. Morphotype B would mainly grow in low saline soils within *Lygeum spartum* steppes, while morphotype C would prefer higher salinity under dominant *Limonium* patches. In the studied area, *L. tobarrense* (morphotype B) coexists with *L. admirabile*, *L. cossonianum*, and *L. delicatulum* (all morphotypes C) under similar environmental conditions (pers. obs.). Therefore, the observed coexistence would suggest the existence of shared seed traits that shapes the plant community. Castillo et al. (2021) and Moreno et al. (2022) noted a common germination pattern among these species under non-saline conditions, declining above 1% salinity, but differing FGP-salinity relationships (linear for B, non-linear for C). Thus, *L. tobarrense* would share a regeneration niche with *Limonium* morphotypes C, indicating a feasible potential seed viability and dispersal. Our findings would support that further in-depth research on *in situ* germination requirements is crucial for understanding local assembly rules.

Finally, the obtained results may provide useful information for the conservation of *L. tobarrense*, an endemic Critically Endangered taxon within the special area of conservation (SAC) “Saltmarshes of Cordovilla and Agramón and the lagoon of Alboraj” (Moreno et al., 2016). Currently, many remarkable threats have been identified for this protected area (e.g., grazing, rabbit proliferation, wild board damages, and fires), which impact on the conservation of the SAC and the species living there (Moreno et al., 2023), being necessary measures to enhance the conservation. The endemicity of *L. tobarrense* requires both *in situ* and *ex situ* conservation strategies for population preservation. Our findings would aid in establishing effective *ex situ* protocols by leveraging knowledge of seed germination and species reproductive behavior. Thus, the obtained results would offer valuable insights for designing germination protocols and *ex situ* conservation measures related to seed germination and halo-priming. Moreover, this information could also be useful to minimize seed usage, crucial given the low number of *L. tobarrense* individuals and their limited seed availability.

## 5. Conclusions

The salinity is a relevant environmental factor which determines the germination and potential seeding establishment of *L. tobarrense* in Saltmarsh of Cordovilla. The germination traits of this endemism led the local assembly along the salinity gradient, germinating successfully in freshwater and low salinity. In addition, *L. tobarrense* showed a wide range of thermoperiod successful for its germination, but the interaction with the salinity was not significant. Besides, *L. tobarrense* is not light sensitive and the presence/absence of light would not affect its germination, suggesting it may grow in close vegetation zones and non-competing with the succulent species which are light sensitive. Therefore, these environmental factors would allow *L. tobarrense* to have its own regeneration and adult niche, competing successfully with the remaining co-occurring halophytes.

## CRediT authorship contribution statement

**Joaquín Moreno:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Alejandro Terrones:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ana Juan:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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