



Influence of salinity and storage on germination of *Tamarix* taxa with contrasted ecological requirements



Alejandro Terrones*, Joaquín Moreno, Jonás C. Agulló, José L. Villar, Alicia Vicente, M. Ángeles Alonso, Ana Juan

Departamento de Ciencias Ambientales y Recursos Naturales/Instituto de la Biodiversidad CIBIO, Universidad de Alicante, Carretera de San Vicente s/n, 03690, San Vicente del Raspeig, Alicante, Spain

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ABSTRACT

Tamarix typically appears in arid and semiarid habitats that are characterised by a broad spectrum of soil salinity. Scarce data are available about the influence of salt on seed germination. The aim of this paper is to analyse the influence of salinity and the loss of viability over time on seed germination of three western Mediterranean autochthonous *Tamarix* species (*Tamarix africana*, *Tamarix boveana* and *Tamarix gallica*) plus the eastern Mediterranean allochthonous *Tamarix parviflora*. These species appear in non-saline to hypersaline ecosystems. Germination percentage and mean-time germination were calculated. In general, germination was higher at no salinity, whereas higher salinities delayed germination. Seed germination of *T. africana* dramatically dropped at 1% salinity (from 23.7% to 4.1%), whereas for *T. boveana* and *T. parviflora*, germination decreased at 6% salinity (from 95% to 42% and from 89% to 14%, respectively), though *T. boveana* still retained a high germination percentage. Seeds of *T. gallica* showed intermediate behaviour and no germination was recorded for 6% salinity. Seed germination is also influenced by the storage time. After 3 months, germination values notably decreased to almost zero (from 70.6% to 0% for *T. gallica* and from 89% to 8% for *T. parviflora*), except for *T. africana*. In the latter case, 1 month was enough to reduce germination values (from 23.7% to 2%). The observed germination differences match with their presence or absence in different semiarid damp environments and point at a dramatic loss of viability over time.

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1. Introduction

Tamarix L. comprises between 54 and 90 species of trees and shrubs that grow mainly in salt marshes, ravines and more or less saline rivers (Baum, 1978; Villar et al., 2012). This genus shows great clonal dispersal ability because of a remarkable capability of regrowth from its crown area, stems and roots (Brock, 1994). In addition, a *Tamarix* plant may produce several hundred thousand seeds in a single growing season (Merckel and Hopkins, 1957), which can be dispersed by wind or water (Brock, 1994). Both dispersal abilities have allowed some species to exhibit invasive behaviour in those continents where they are not native, such as America and Oceania (Baum, 1967; Merckel and Hopkins, 1957; Shafroth et al., 1995).

Previous studies have analysed the germination of certain

Tamarix species, mainly *Tamarix aucheriana* (Decne.) B.R. Baum (Zaman et al., 2006, 2009), *Tamarix gallica* L. (Merckel and Hopkins, 1957), *Tamarix ramosissima* Ledeb (Brock, 1994; Shafroth et al., 1995; Yan et al., 2011; Young et al., 2004). and *Tamarix pentandra* Pall. (Ungar, 1967). Nevertheless, *T. pentandra* and *T. ramosissima* refer to the same biological entity (Villar et al., 2014), and those American populations analysed by Merckel and Hopkins (1957), named as *T. gallica*, would actually correspond to *T. ramosissima* (Carter and Nippert, 2012). Consequently, current germination data of *Tamarix* are mainly available from two Irano-Turanian species to our knowledge. These studies have shown that *Tamarix* seeds can germinate in high saline conditions (Ungar, 1967; Yan et al., 2011; Zaman et al., 2006, 2009), though not all *Tamarix* species can grow under the same saline conditions (Baum, 1978). In addition, many authors stated that seed viability is rapidly lost in *Tamarix* (Brock, 1994; Yan et al., 2011; Akhiani, pers. comm.), though there have been few experimental analyses thus far (Merckel and Hopkins, 1957; Zaman et al., 2009). Nevertheless, seeds of

* Corresponding author.

E-mail address: alejandro.terrones@ua.es (A. Terrones).

halophytes, including *Tamarix*, vary considerably in their salt tolerance (Khan and Gul, 2006); therefore, it is interesting to study how salinity can influence seed germination on this genus. Moreover, none of these studies have tried to compare germination among different *Tamarix* species. Germination data are required to properly manage the invaded places and protected areas.

The southeast of the Iberian Peninsula is mostly characterised by a Thermomediterranean Semiarid bioclimatic belt (Rivas-Martínez, 2007), and its geography, lithology and topography favour the existence of seasonal streams, saline rivers and salt marshes. The potential vegetation of these natural environments is well constituted by the presence of different species of the genus *Tamarix* (Izco et al., 1984). Four *Tamarix* species are commonly reported as native: *Tamarix africana* Poir., *Tamarix boveana* Bunge, *Tamarix canariensis* Willd. and *T. gallica* (Cirujano, 1993; Izco et al., 1984). These species differ widely in their salt tolerances (from 4 dSm⁻¹ for *T. africana* to over 50 dSm⁻¹ for *T. boveana* (Gómez Mercado et al., 2012)), and therefore, some of them appear in different habitats. *T. africana* grows in non-saline and low-saline damp habitats, whereas *T. boveana* grows exclusively in hypersaline salt marshes. Both *T. canariensis* and *T. gallica* show a broad ecological spectrum, appearing in a great variety of damp habitats independent of their salinity. Moreover, other allochthonous *Tamarix* species, such as *Tamarix aphylla* (L.) H. Karst., *Tamarix parviflora* DC. and *T. ramosissima*, are also present around these Iberian territories as ornamentals (Cirujano, 1993). Among them, the eastern Mediterranean *T. parviflora* is the only species with the ability to establish spontaneous and even invasive populations (pers. obs.), and as a result, they would colonize certain habitats and compete with the autochthonous *Tamarix* species of the Iberian Peninsula.

To date, no comparative germination studies have been conducted on western Mediterranean *Tamarix* species. In the present study, three autochthonous species were selected, namely *T. africana*, *T. boveana* and *T. gallica*, to cover the broad range of salt tolerance in their natural habitats, plus the allochthonous *T. parviflora*. The main aim of this study was to analyse the effect of salinity on seed germination and the loss of seed viability over time on the above-mentioned four selected species to assess the effects of salinity on their adaptive mechanisms to colonise wetlands.

2. Material and methods

Plant material was collected from well-established and large natural populations of *Tamarix* in Alicante province (Spain). The species *T. boveana* and *T. parviflora* were collected from El Hondo salt marsh (38° 11' 10"N, 0° 47' 17"W) on 18 April 2014, where *T. boveana* had a large and dominant population. For *T. parviflora*, only five individuals were collected because their Spanish-introduced populations are limited. Finally, *T. gallica* and *T. africana* were collected from Monnegre river (38° 26' 22"N, 0° 27' 44"W) on 14 May 2014; their populations appeared intermixed along the river. Racemes with seeds were collected from 10 individuals well separated and stored in independent paper bags at room temperature.

Seeds from different individuals of each species were mixed in the same proportion to have representative pools for the experiments. Seeds were later selected from these pools. Four sets of 25 seeds for each species were placed on filter paper in 9-cm Petri dishes and submerged in 3 ml of the appropriate solution. Dishes were maintained in a climate-controlled cabinet, for 12 h in light at 25 °C and for 12 h in dark at 20 °C, according to Young et al. (2004). Seed germination was checked every day and germinated seeds were counted and removed. Seeds were considered germinated once the radicle had elongated.

Two different experiments were carried out. To assess the

influence of salinity on seed germination, seeds were germinated in five different solutions of NaCl. These solutions were prepared with concentrations of 0%, 1%, 2%, 4% and 6% (w/v) to cover the variability of salinity of natural salt marshes from southeast Spain (Álvarez-Rogel, 1997). To assess the loss of viability over time, germination with distilled water was repeated 1 and 3 months after the collection.

Four variables of germination were determined: (i) final germination percentage, (ii) number of days to first germination, (iii) number of days to final germination and (iv) mean time-to-germinate (MTG) (Redondo et al., 2004). Statistical analyses were carried out using R software version 3.1.0 (R Core Team, 2014). Pearson coefficients were calculated to assess correlation between different variables. Data were tested for normality and homogeneity of variance and then analysed using two-way analysis of variance. The number of days to first germination, number of days to final germination and MTG variables were transformed using 1/x function to analyse statistically the non-germinated plates. Significant test results were analysed post-hoc by Tukey's tests.

3. Results

Pearson coefficients between the four variables showed that all of them were significantly correlated with one another ($P < 0.001$). The percentage of germination was not highly correlated with any other variable ($r < 0.55$). However, MTG was strongly correlated with the number of days to first germination ($r = 0.95$) and to final germination ($r = 0.81$); therefore, these three variables should be analysed together as they describe the time of germination.

Differences for taxa and salinity treatments for the four variables were significant ($P < 0.05$), but interaction between these two factors was only significant with the percentage of germination ($P < 0.05$), since not all taxa behave similarly with increasing salinity concentrations, as it occurs with *T. boveana* and *T. parviflora* (see further comments). The highest germination percentages were always obtained at 0% treatment, though *T. africana* showed the lowest percentages ($P < 0.05$) (Tables S1 and S2). In addition, germination dramatically dropped at 1% and even stopped at 4% for *T. africana* ($P < 0.05$). On the contrary, germination values decreased to 0 at 6% salinity for *T. gallica* ($P < 0.05$) and significantly dropped for *T. boveana* and *T. parviflora* (Figs. 1 and 2A, Table S1), though *T. boveana* showed higher germination than *T. parviflora* ($P < 0.05$) (Table S2). The increase in salinity significantly delayed germination in the four species; hence, MTG increased with salinity ($P < 0.05$) (Table S1). The number of days to first germination increased with the highest salinity treatment for the four species ($P < 0.05$). For final germination, the number of days was independent of salinity ($P > 0.05$), except for *T. boveana* and *T. gallica*, both of which showed an increase with salinity ($P < 0.05$).

Differences for taxa and time of storage were significant for the studied variables ($P < 0.05$), but their interaction was only significant with the percentage of germination ($P < 0.05$). The highest values of germination percentage were obtained for 0-month seeds, but these values were only significantly different from 1-month seeds for *T. africana* and *T. parviflora* ($P < 0.05$) (Table S3). Germination significantly dropped and even stopped after 3 months of storage except for *T. africana*, which dropped after 1 month ($P < 0.05$) (Figs. 1 and 2B, Tables S3 and S4). MTG increased with storage time for *T. africana*, *T. gallica* and *T. parviflora* ($P < 0.05$), whereas the time of germination for *T. boveana* did not show any correlation ($P > 0.05$) (Table S3).

4. Discussion

This study shows that *Tamarix* taxa colonising contrasted

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