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Research Note

Germination traits of *Prosopis alba* from different provenances

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Abstract

Seed germination traits may vary among populations and this knowledge may have important implications in conservation and restoration programmes. Here, we evaluated seed mass, germination capacity at different temperatures under light and dark conditions, and germination rate of *Prosopis alba* seeds of four provenances from Argentina (Salta, Formosa, Chaco and Santiago del Estero). Seeds from the four provenances exhibited low germination percentages at the lowest temperature (10/5°C), whereas at the remaining temperature regimes (20/10°C, 25/15°C and 35/20°C), seeds from all the provenances had high germination percentages, especially Salta and Formosa seeds, which showed the highest values. In addition, Salta seeds exhibited the highest germination rate and seed mass.

Experimental and discussion

Seed germination requirements can vary in response to small differences in environmental conditions (Abe and Matsunaga, 2011) and these variations across a species' distribution range might enable adaptation to environmental conditions, maximising seedling survival in each location (Bischoff *et al.*, 2006). Several studies have specifically demonstrated that there is variation in germination requirements within a species growing in different sites (Honěk and Martinková, 1996; Khurana and Singh, 2001; Harrison *et al.*, 2014). Knowing inter-population differences in seed germination is key for planning conservation areas and for potential use of the species in restoration programmes.

Light and temperature are among the most important factors regulating germination processes (Baskin and Baskin 1998). In particular, temperature instead of light has been identified as the main regulator of germination in several species from the Argentine Chaco region, most of which exhibit high germination percentages at 25/15°C (Funes *et al.*, 2009). Hence, populations that have developed under different climatic conditions might exhibit different optimum ranges and germination rates (Keller and Kollman, 1999).

Identifying these characteristics is important for determining the capacity of a population to respond under different environmental conditions.

Seed mass could vary among populations and is another important factor influencing the establishment potential of a species (Leishman *et al.*, 2000). In general, bigger seeds have higher germination rates and higher possibilities of producing more vigorous seedlings (Galíndez *et al.*, 2009).

In Argentina, *Prosopis alba* Griseb. is one of the most useful tree species in the native forest of the Chaco region (Burkart, 1976) due to its high quality of firewood for charcoal and timber posts and furniture, among many uses. In recent years, *P. alba* timber has been increasingly used due to the clearing of the native forest. To date, four morphotypes of *P. alba* have been identified in the Chaco region of Argentina, which are distributed in different areas of the region and differ mainly in their leaf traits (Verga *et al.,* 2009). However, there is no information about possible differences in regenerative characters among these morphotypes. The aim of this work was to determine the differential response in the germination traits of *P. alba* seeds of four different provenances to increase the knowledge about inter-population variability in germination traits which could be extremely useful in restoration strategies in the semi-arid environments of the Chaco region.

Seeds of four morphotypes of *P. alba* were provided by the National *Prosopis* Germplasm Bank of Córdoba, Argentina. The morphotypes originated from four natural populations in the Chaco phytogeographic province of northern Argentina (Cabrera, 1976): 1) *P. alba* "santiagueño" morphotype from Chañar Bajada, northwestern Santiago del Estero, hereafter "Santiago" provenance; 2) *P. alba* "salteño norte" morphotype, northern Salta, hereafter "Salta" provenance; 3) *P. alba* "chaqueño" morphotype, Isla Cuba, western Formosa, hereafter "Formosa" provenance; and 4) *P. alba* "chaqueño sur" morphotype, from Villa Ángela, Chaco, hereafter "Chaco" provenance. The climatic parameters of the geographic areas where each population grows were obtained from WorldClim Global Climate GIS (www.worldclim.org; Hijmans *et al.*, 2005; table 1).

Table 1. Geographical locations, historical mean temperature and precipitation, and mean seed mass ± standard deviation for the four *Prosopis alba* populations in the semi-arid Argentine Chaco. In seed mass, different letters indicate statistically significant differences ($P \leq 0.05$).

Provenance	Coordinates	Mean annual temperature (°C)	Mean annual precipitation (mm)	Min. temp. (°C)	Max. temp. (°C)	Mean temp. (wettest season) (°C)	Seed mass (g) mean ± s.e.
Santiago	26° 14' 59" S 63° 47' 33" W	21.5	658	7.2	34.4	26.9	0.026 ± 0.004 c
Chaco	27° 73' 46" S 60° 59' 95" W	21.8	1101	8.3	36	27.3	0.032 ± 0.005 b
Salta	22° 12' 1"S 63° 40' 33" W	21.9	1054	8.7	32.7	25.3	0.035 ± 0.004 a
Formosa	24° 15' 58" S 61° 54' 0"W	22.8	678	9.5	35.1	26.6	0.025 ± 0.004 c

The germination capacity of the seeds was evaluated using four temperature regimes 35/20°C, 25/15°C, 20/10°C and 10/5°C, which are the most usual temperature conditions in the Argentine Chaco region (Funes *et al.*, 2009). The different temperature regimes were evaluated both under light/dark (12/12 hours) and permanent dark conditions. For each treatment, seeds were distributed into five replicates of 25 seeds each (a total of 160 Petri dishes = five replicates × four provenances × four temperature conditions × two light conditions). Seeds were scarified with sand paper and placed in the Petri dishes with one layer of filter paper soaked in distilled water. They were incubated in four germination chambers equipped with fluorescent tubes of 20W cool white light with a radiation density (400-700 nm) of approximately 38 μmol m^{-2} s^{-1} for 30 days. The treatment under permanent dark conditions consisted of covering the Petri dishes with aluminum paper. The number of germinated seeds was counted every two days, except for the treatment under dark conditions, which was evaluated for germination percentage at the end of the experiment. Emergence of a 2 mm-long radicle was used as the criterion for germination. Germination rate was calculated for each seed provenance as Mean Time to Germination (MTG) using the formula (Daws *et al.*, 2002):

$$\mathrm{MTG} = \sum (\mathrm{n} \times \mathrm{d}) / \mathrm{N}$$

where n = number of seeds germinated between count intervals, d = incubation period in days at the time of counting and N = total number of seeds germinated during the treatment. Mean mass of 100 seeds of each provenance was determined using a precision balance (0.001 g). Percentage of germinated seeds and MTG were analysed using generalised linear models (GLM). Seed germination percentage was arcsine-transformed and a GLM with a Gaussian error structure correcting for the heteroscedasticity in the factor provenance was run, using the function varIdent in Infostat (Di Rienzo *et al.*, 2013). First, a model including light conditions (light/dark and permanent dark), provenance, temperature and their interaction as fixed factors was run. Since light was not a significant factor, another model that did not include light was fitted and was selected. The fixed factors used for MTG were provenance, temperature and their interaction. For MTG, a Gaussian error structure was used and a GLM correcting the heteroscedasticity for the factor temperature was run using the function varIdent in Infostat. MTG was not calculated for 10/5°C because no germination was observed in most of the replicates. For seed mass, an ANOVA test was performed with provenance as factor. In all the analyses, a *Di Rienzo, Guzmán and Casanoves* (DCG) *a posteriori* test was performed. A $P \leq 0.05$ was established as the criterion for rejecting the null hypothesis.

The fixed factor referring to light conditions (light/dark and permanent dark) did not show a significant effect on germination percentage ($F = 0.02$, $P = 0.8926$) and was removed from the statistical model. The final model showed a significant effect of provenance, temperature and their interaction on germination percentage ($F = 194.72$, $P < 0.0001$; $F = 1744.99$, $P < 0.0001$; and $F = 18.99$, $P = 0.0004$, respectively). Seeds from the four provenances showed very low germination percentages at the lowest temperature (10/5°C). In the remaining evaluated temperature regimes, the seeds from Salta and Formosa exhibited the highest germination percentages, followed by Chaco seeds, with intermediate values, and Santiago seeds, which yielded the lowest germination percentages (figure 1a). Provenance and temperature, and their interaction had significant effects on MTG ($F = 16.4$, $P < 0.0001$; $F = 3.2$, $P = 0.0497$; and $F = 5.25$, $P = 0.0003$, respectively). Results of the *a posteriori* test indicated that Salta seeds at 20/10°C and 25/15°C had the lowest MTG values, whereas Salta seeds at 35/20°C, Chaco and Formosa seeds at all the temperature regimes, and Santiago seeds at 25/15°C and 35/20°C had intermediate MTG. Finally, Santiago seeds showed the highest MTG values at 20/10°C (figure 1b).

Mean seed mass showed significant differences among provenances ($F = 105.51$, $P < 0.0001$), with the highest values corresponding to Salta seeds and the lowest ones to Formosa and Santiago seeds (table 1).

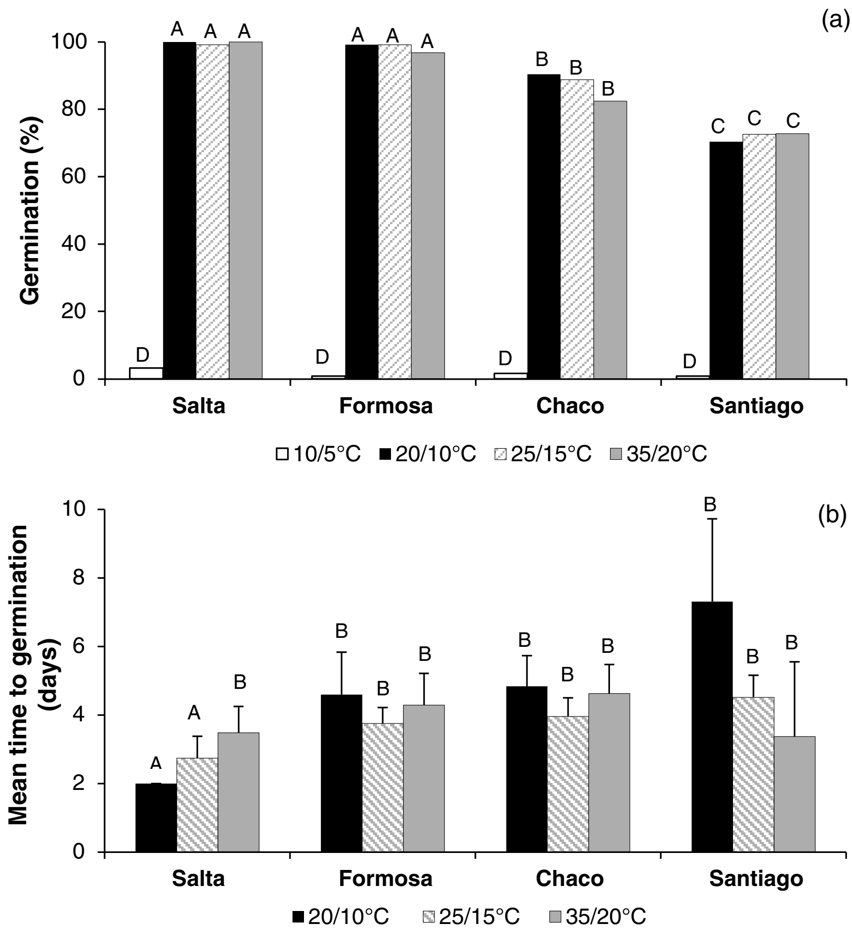

Figure 1. (a) Germination and (b) mean time to germination ± standard deviation of seeds of *Prosopis alba* from four locations within the Argentine Chaco region. Seeds were placed to germinate at the temperature regimes indicated (12/12 hours).

Some plant characters may not be uniform across populations of a species that has a wide latitudinal distribution (Abe and Matsunaga, 2011). The results obtained here for *P. alba* show that temperature, instead of light, was the most important factor for seed germination, as reported for other species (Keller and Kollman 1999; Funes *et al.* 2009). Seeds from all provenances showed very low germination percentages at the lowest temperature (10/5°C). Therefore, these populations would have few chances of establishment in areas where cold temperatures (below 5°C) prevail. The high germination percentages recorded at the highest temperatures for the four populations would coincide with the austral spring-summer period, when precipitation is concentrated, being optimum for natural regeneration of the species. Moreover, germination did not vary among provenances in the different light conditions (light/dark and permanent dark), as indicated for several species of the Argentine arid Chaco (Funes *et al.*, 2009).

It has been suggested that geographically different plant populations exhibit climatic adaptations for germination (Keller and Kollman, 1999). However, no clear relationships were observed in the studied populations involving germination percentages and available temperature or precipitation records for the seed provenance areas (table 1). Furthermore, seeds of Salta provenance exhibited the highest germination rate and seed mass. Under natural conditions, rapid germination and seedling emergence might be a survival strategy enabling rapid resource exploitation for plant establishment (Leishman *et al.*, 2000). At the adult stage, Salta provenance seedlings were found to exhibit rapid growth in different geographical areas of the Argentine Chaco region compared with the remaining provenances (López Lauenstein *et al.*, unpublished data). These previous records, along with the present results in terms of regenerative characters, suggested that seedlings from this provenance might be more vigorous. Accordingly, *P. alba* from northern Salta might be considered the most suitable population for restoration purposes.

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