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OPTIMUM GERMINATIVE CONDITIONS OF A MULTIPURPOSE SHRUB FROM PATAGONIA: *PROSOPIS ALPATACO* (FABACEAE)

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Abstract. Boeri, P.; M. Cedrés Gazo; M. Failla; D. Barrio; D. Dalzotto & S. Sharry. 2019. Optimum germinative conditions of a multipurpose shrub from Patagonia: *Prosopis alpataco* (Fabaceae). *Darwiniana*, nueva serie 7(2): 199-207.

Several factors limit the native plants production. The aim of this study was to optimize the germination of *Prosopis alpataco* f. *alpataco* from northeastern Patagonia, Argentina. This is a key species of the Monte, one of the most threatened South American ecoregions. Seeds were incubated in chamber at 25 - 45 °C in darkness for 7 days, evaluating eight germinative parameters. Chemical scarification with H₂SO₄ (2, 5, 10, 20, 30, 40, 50 and 60 min) and mechanical scarification (by partial and total cutting of the seminal cover edge) were tested. The best results (100 % germinative capacity reached in one day) were recorded at 30 °C (optimum germination temperature) in seeds scarified with H₂SO₄ (during 30 min) and with total cutting of the seminal cover edge. The use of H₂SO₄ is recommended for mass propagation and the total cutting of the seminal cover edge for small-scale production. It can be concluded that *P. alpataco* f. *alpataco* from northeastern Patagonia had a higher germinative response after an exposure to a range of relatively simple chemical or mechanical treatments. The techniques achieved here provide valuable information for conservation and production of plants of this species. This work represents the first optimum germinative conditions of *P. alpataco* f. *alpataco* from northeastern Patagonia. Its results open new lines for future research of other austral *Prosopis* species.

Keywords. Dormancy; optimum germination temperature; scarification; semi-arid zones.

Resumen. Boeri, P.; M. Cedrés Gazo; M. Failla; D. Barrio; D. Dalzotto & S. Sharry. 2019. Condiciones óptimas de germinación para un arbusto multipropósito de la Patagonia: *Prosopis alpataco* (Fabaceae). *Darwiniana*, nueva serie 7(2): 199-207.

Varios factores limitan la producción de las plantas nativas. El objetivo de este estudio fue optimizar la germinación de *Prosopis alpataco* f. *alpataco* del noreste de la Patagonia Argentina. Esta es una especie clave del Monte, una de las ecorregiones sudamericanas más amenazadas. Las semillas se incubaron en cámara durante 7 días, a 25-45 °C en oscuridad. Fueron evaluados ocho parámetros germinativos. Se testearon pretratamientos de escarificación química con H₂SO₄ (2, 5, 10, 20, 30, 40, 50 y 60 min) y de escarificación mecánica (mediante corte parcial y total del borde de la cubierta seminal). Los mejores resultados (100 % de la capacidad germinativa alcanzada en un día) se registraron a 30 °C (temperatura óptima de germinación), en semillas escarificadas con H₂SO₄ (durante 30 min) y con el corte total del borde de la cubierta seminal. Se recomienda el uso de H₂SO₄ para la propagación masiva, y el corte total del borde de la cubierta seminal para la producción a pequeña escala. Se puede concluir que *P. alpataco* f. *alpataco* tiene una respuesta germinativa relativamente

alta después de su exposición a una serie de tratamientos químicos o mecánicos simples. Las técnicas logradas aquí proporcionan información valiosa para planes de conservación y producción de esta especie. Este trabajo representa el primer reporte sobre las condiciones óptimas de germinación para *P. alpataco* f. *alpataco* del noreste de la Patagonia. Estos resultados abren nuevas líneas para futuras investigaciones de otras especies australes de *Prosopis*.

Palabras clave. Dormancia; escarificación; regiones semiáridas; temperatura óptima de germinación.

INTRODUCTION

Prosopis alpataco Phil. (Fabaceae, Mimosoideae) is a multipurpose shrub (Vega Riveros et al., 2011; Henciya et al., 2017) typical of Argentinian arid and semi-arid regions (Roig, 1987; Vega Riveros et al., 2011). It is a key species of the Monte, a semi-desert scrubland characterized by shrub steppes and xerophytes forests along 2,000 km from Patagonia to northwestern Argentina (Cabrera, 1971). It is considered one of the most threatened ecoregions of South America, mainly due to the advance of the agricultural frontier. In northeastern Patagonia, the annual deforestation rate of Monte flora has been estimated at ten times higher than the average rate of global tropical rainforest loss (Balmford et al., 2003; Pezzola et al., 2004).

Numerous factors tend to limit the production of native plants. Among the internal factors, physical dormancy is a survival mechanism for several species of arid and semi-arid regions that prevent or delay germination (Baskin & Baskin, 2014). Concerning the external factors, it is relevant to study the optimum germination temperature for each species. It is the temperature at which the seeds reach the highest germinative rate in the shortest time (Mayer & Poljakoff-Mayber, 1982). In the case of *P. alpataco*, preliminary studies on the germinative aspects were limited to the central and austral Monte regions (Villagra, 1995; Galíndez et al., 2016; Rodríguez Araujo et al., 2017). Some authors reported physical dormancy imposed by the seminal tegument for this species (Villagra, 1995) while other authors denied it (Galíndez et al., 2016). There are various methods to break dormancy which must be evaluated in each species to understand its reproduction (Baskin & Baskin, 2014). Previous studies reported that the germination of this species was improved with the application of different treatments of chemical and mechanical scarification (Villagra, 1995, 1998; Rodríguez Araujo et al., 2017).

In addition, there is evidence of variation in germination and latency patterns that suggest the environmental influence of climate on global biomes (Baskin & Baskin, 2014). Some studies postulate the existence of functional germination groups along environmental gradients that interact in relatively similar types of vegetation (Fernández Pascual et al., 2013). Furthermore, the state of latency seems to be influenced by aridity, altitude and latitude (Cochrane et al., 2014). Burrows et al., (2009) found significant differences in seed size, mass, absorption and germination rate of leguminous seeds from different provenances.

Despite its ecological and economical value, *P. alpataco* is still one of the least studied species of *Prosopis* (Vega Riveros et al., 2011). The aim of this study was to optimize the conditions germination of *P. alpataco* var. *alpataco* seeds from northeastern Patagonia.

MATERIALS AND METHODS

Study area

The study area included four sampling sites located in Adolfo Alsina department, Río Negro province, Argentina (40° 48' S - 63° 14' W, 10-20 m.a.s.l.); in the Monte Phytogeographic Province (Cabrera, 1971) - Monte Oriental Floristic Unit (Oyarzabal et al., 2018). This zone has a semi-arid climate sub-humid to dry with annual rainfall of 200-350 mm concentrated in autumn-spring with an average temperature of 15 °C (Bran et al., 2000).

Collection, conditioning, and storage of seeds

During April 2012 and April 2013, seeds with uniform size and healthy aspect were harvested using a random procedure from 30 mother plants. To control possible insect infections seeds were placed at -20 °C for 15 days (Mazzuferi & Conles, 2005). Considering that storage time did not affect

the germinability of *P. alpataco* after 2-years (Rodríguez Araujo et al., 2017), the seeds were stored in paper envelopes under controlled conditions of humidity, light and temperature (0 °C) until its final use 2-14 months later in the experiments. Prior to scarification and optimum temperature assays, seeds were disinfected by immersion in 70 % v/v ethanol for 10 min, followed by 7 min in NaClO (48g active chlorine/L) diluted to 20 % v/v for 20 min. Subsequently, they were washed three times with sterile distilled water. Seeds were placed in sterile Petri dishes prepared with a filter paper disk saturated with distilled water. Finally, they were placed in a germination chamber at 30 °C, in darkness and the number of germinated seeds was recorded daily, for 7 days.

Pregerminative scarification treatments

Usually, germination was defined as the appearance of radicals at 0.5 mm (Bewley, 1997). However, when the appearance of the root is not the first event that occurs, germinated seeds are those that under favorable environmental conditions can provide viable seedlings (Pérez, 2003). In a40, a50 and a60 treatments the appearance of radicals was not observed, but since they can give viable seedlings, we decided to include these treatments in the evaluation of the germination parameters.

Ten pre-germination treatments were tested. Chemical scarification was carried out by immersion in concentrated H₂SO₄ during: 2 min (a2), 5 min (a5), 10 min (a10), 20 min (a20), 30 min (a30), 40 min (a40), 50 min (a50) and 60 min (a60). Then seeds were rinsed repeatedly with distilled water. Mechanical scarification treatments were performed by cutting the seminal cover edge with pliers: partial cut (PC) and total cut (TC).

To analyze the dynamics of germination during day 1, the GC was evaluated each hour in seeds exposed to all scarification treatments described before at optimum temperature germination detected. Additionally, the root length of scarified seeds (treatments a10, a20, a30, PC and TC) was evaluated during day 1 and 2.

Evaluation of Root Length

Root length of seedlings obtained from PC, TC, a10, a20 and a30 treatments were measured in centimeters on the first and second day of root emergence.

This procedure was repeated three times, with 25 seeds for each treatment and an average was registered. The treatments a40, a50 and a60 were excluded because in these cases the radicular emergence did not occur during this period.

Optimum Germination Temperature

To evaluate the optimum germination temperature scarified seeds by total cutting of the seminal cover edge (see below TC treatment) were germinated, for 7 days, at 25 °C, 30 °C, 35 °C, 40 °C and 45 °C, temperature range reported as optimal for *P. alpataco* (Villagra, 1995), for 7 days.

Germinative parameters

The germinative parameters evaluated were: Germinative capacity (GC): percentage of total germination at the end of the trial (Pece et al., 2010); Maximum germination value (MGV): maximum ratio between each accumulative daily germination and the number of days elapsed up to that value (Piedrahita Cardona, 1998); Germinative energy (GE): percentage of daily cumulative germination at the highest germination rate (González et al., 2008); Energy period (EP): number of days to reach the highest GE (Pece et al., 2010); Mean germination time (MGT): number of days used in germination $MGT = ((X_1 d_1) + (X_2 d_2) + \dots + (X_7 d_7)) / X_7$ which measures the speed and dispersion of the germinative process (Ranal & García de Santana, 2006); Germination rate or Maguire's Index (GR): number of seeds germinated per day, $GR = G_1/N_1 + G_2/N_2 + \dots + G_n/N_n$ (Maguire, 1962); Average daily germination (ADG): ratio between the final GC percentage and the number of days elapsed up to that value (Gómez Restrepo, 2004). MGV and GE were considered as indicators of seeds vigour (Pece et al., 2010).

Statistical Analysis

The assays followed a completely randomized experimental design with three replications of 25 seeds per treatment and its respective control, GC and MGT were transformed to Arc sen√x to fulfill the assumption of normality. Germination parameters and root length were performed with ANOVA and Tukey's test per each days and hours. The tables and figures show untransformed data. InfoStat software (Di Rienzo et al., 2016) was used and the significance level was set at 0.05.

RESULTS AND DISCUSSION

Scarification treatments effects

All tested scarification treatments increased significantly the germination (Table 1). When seeds exposure time to H_2SO_4 exceeded 30 min, the seed cover softened leaving the seed partially detached and with part of the cotyledons exposed. In these treatments the radical emergence was not observed but are those capable of given a viable seedling, we decided to include a40 a50 a60 treatments in the evaluation of germinative parameters (Boeri, 2017). Figure 1 shows the influence of scarification treatments on accumulated daily germination during the first two days. The maximum ADG values were observed at TC and maximum chemical scarification times (a30, a40, a50 and a60), during day 1 (Table 1). It should be noted that most of the scarification treatments showed 100 % germination at the end of the trial with significant differences observed during day 1 (Figure 1). The results show that germination starts earlier in the TC, a20 and a30 treatments (Figure 2). This could be due to water uptake kinetics seeds and the stationary phase of these treatments was practically nonexistent, coinciding with Villagra (1995).

Chemical and mechanical scarification were efficient methods to break the physical dormancy, coincidentally with all previous studies (Villagra, 1995; 1998; Rodríguez Araujo et al., 2017). In this sense, Villagra (1995) reported that mechanically scarified seeds do not present stationary phase, or this is very short after imbibition, reaching 100 % germination in temperatures between 15 °C and 40 °C (Villagra, 1995). About chemical scarified effects on *P. alpataco*, Rodríguez Araujo et al. (2017) reported similar GC values of control and scarified seeds with H_2SO_4 (5 min) as detected here, but at a lower temperature (20 °C). These results were similar to those obtained in other studies on Fabaceae, in which H_2SO_4 immersion and other treatments contributed to increase GC (Lv et al., 2010; Zare et al., 2011). Particularly, this was also observed for other *Prosopis* species from arid region with similar final germination percentage than those observed here, but the time to reach it was longer (Zare et al., 2011). These discrepancies could be due to differences in the morphology, chemical composition, thickness and / or permeability of the seminal tegument of each species in each region or environmental conditions.

Table 1. Germinative parameters (mean and standard deviation) of *Prosopis alpataco* seeds exposed to different scarification treatments. Chemical scarification with concentrated H_2SO_4 : 2 min (a2), 5 min (a5), 10 min (a10), 20 min (a20), 30 min (a30), 40 min (a40), 50 min (a50) and 60 min (a60). Mechanical scarification by cutting the seminal cover edge: partial cut (PC) and total cut test (TC). Control seeds (K). GC: Germinative capacity, MGv: Maximum germination value, GE: Germinative energy, EP: Energy period, MGT: Mean germination time, GR: Germination rate or Maguire's index, ADG: Average daily germination. Different letters indicate significant differences at $p < 0.05$ according Tukey's test.

Treatment	GC (%)	MGV (seeds/days)	GE (%)	EP (days)	MGT (days)	GR	ADG
a2	32 ± 14.24 ^b	4 ± 7.12 ^a	32 ± 14.23 ^a	2 ± 0 ^b	1.98 ± 0.041 ^c	4.13 ± 2.18 ^a	16 ± 7.12 ^{ab}
a5	100 ± 0 ^c	12.5 ± 0 ^b	100 ± 0 ^b	2 ± 0 ^b	1.99 ± 0.02 ^c	12.63 ± 0.50 ^b	50 ± 0 ^{bc}
a10	100 ± 0 ^c	20.13 ± 0 ^{cd}	97 ± 0 ^b	1.25 ± 0.5 ^a	1.26 ± 0.34 ^{ab}	21.75 ± 8.50 ^{cd}	62.5 ± 25 ^{cd}
a20	100 ± 0 ^c	18.75 ± 0 ^{bcd}	100 ± 0 ^b	1.5 ± 0.58 ^{ab}	1.32 ± 0.38 ^{ab}	21 ± 9.56 ^{cd}	75 ± 28.87 ^{cde}
a30	100 ± 0 ^c	25 ± 0 ^d	100 ± 0 ^b	1 ± 0 ^a	1 ± 0 ^a	25 ± 0 ^d	100 ± 0 ^e
a40	100 ± 0 ^c	24.5 ± 0 ^d	98 ± 0 ^b	1 ± 0 ^a	1.02 ± 0.04 ^a	24.75 ± 1.00 ^d	98 ± 4 ^c
a50	100 ± 0 ^c	24.25 ± 0 ^d	97 ± 0 ^b	1 ± 0 ^a	1.03 ± 0.03 ^a	24.63 ± 0.96 ^d	97 ± 3.83 ^c
a60	100 ± 0 ^c	24.75 ± 0 ^d	99 ± 0 ^b	1 ± 0 ^a	1.01 ± 0.02 ^a	24.88 ± 0.50 ^d	99 ± 2 ^c
PC	100 ± 0 ^c	13.75 ± 0 ^{bc}	80 ± 0 ^b	1.5 ± 0 ^{ab}	1.46 ± 0.07 ^b	19.25 ± 1.91 ^c	50 ± 0 ^{bc}
TC	100 ± 0 ^c	24.75 ± 0 ^d	99 ± 0 ^b	1 ± 0 ^a	1.01 ± 0.02 ^a	24.88 ± 0.51 ^d	87.5 ± 25 ^{de}
K	16 ± 3.27 ^a	2 ± 7.12 ^a	16 ± 3.27 ^a	2 ± 0 ^b	2 ± 0 ^c	2 ± 0.41 ^a	8 ± 1.63 ^a

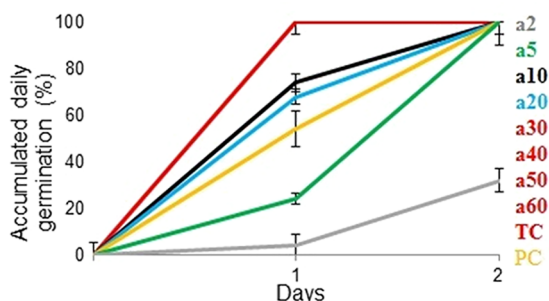


Fig. 1. Accumulated daily germination (mean and standard deviation) of *Prosopis alpataco* seeds exposed to different scarification treatments. Chemical scarification with concentrated H_2SO_4 : a2, 2 min; a5, 5 min; a10, 10 min; a20, 20 min; a30, 30 min. Mechanical scarification by cutting the seminal cover edge: PC, partial cut; TC, total cut. Treatments a30, a40, a50, a60 and TC are plotted grouped because they did not present significant differences according to Tukey's test. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/817/1169>

It should be noted that previous studies showed that, even for the same species, the germinative parameters can vary according to the provenance and / or the collection season (Baskin et al., 2004; Burrows et al., 2009). Also, as far as the fate of future generations of some species is concerned, this would depend on the influences of maternal and environmental factors, particularly when the seeds are still on the mother plant and mostly during the final stage of seed maturation (Guterman, 1994).

Root length

Root length is a morphological parameter used to assess the quality of plants and seedlings. This parameter indicates the possibility of the seedling to explore the soil to capture water and nutrients. The radical system is also essential for the anchoring and the establishment of field plants (Sáenz et al., 2010). Table 2 presents the effects of different scarification treatments on the root length. On day 1, at a30 root growth was higher (greater root development), followed by a20 and TC, which did not show significant differences between them. On day 2 this phenomenon occurred between TC and a30 treatments. As mentioned previously, in these treatments the germination starts earlier and have higher values of accumulated daily germination (Figure 2).

Table 2. Root length (mean and standard deviation) of *Prosopis alpataco* seeds exposed to different scarification treatments. Chemical scarification with concentrated H_2SO_4 : a10, 10 min; a20, 20 min; a30, 30 min. Mechanical scarification by cutting the seminal cover edge: PC, partial cut; TC, total cut. Different letters indicate significant differences at $p < 0.05$ according Tukey's test.

Treatment	Root length (mean) (mm)	
	Day 1	Day 2
a10	49 ± 0.28 ^{ab}	207 ± 0.03 ^{bc}
a20	77 ± 0.03 ^{bc}	187 ± 0.04 ^b
a30	107 ± 0.10 ^c	225 ± 0.31 ^c
PC	32 ± 0.10 ^a	149 ± 0.01 ^a
TC	76 ± 0.08 ^{bc}	236 ± 0.38 ^c
P-value	0.0013	0.0001

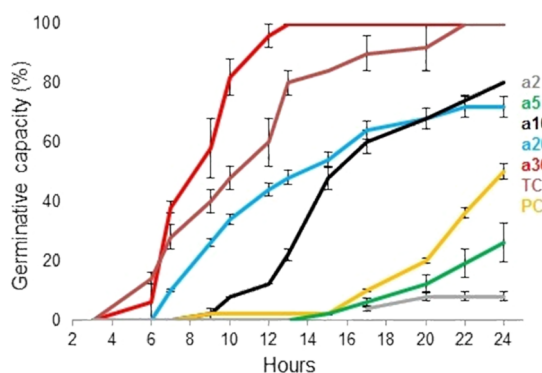


Fig. 2. Dynamics of germination in hours (germinative capacity mean and standard deviation) of *Prosopis alpataco* seeds exposed to different scarification treatments. Chemical scarification with concentrated H_2SO_4 : a2, 2 min; a5, 5 min; a10, 10 min; a20, 20 min; a30, 30 min. Mechanical scarification by cutting the seminal cover edge: PC, partial cut; TC, total cut. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/817/1169>

In this sense, some authors claim the importance of these treatments to obtain better vigor estimations for plantlets used in genetic improvement programs (Vargas, 1996). It has been proven that plantlets with better vigor have acceptable characteristics for foliar area, dry weight and root length (Martínez Solís et al., 2010). In arid conditions, the development of a good radical system represents an advantage for survival, given the rapid drying of the soil surface (Álvarez-Holguín et al., 2017).

Optimum germination temperature

Table 3 and Figure 3 shows the influence of temperature on seed germination. The optimum germination temperature was 30 °C. This condition was the only one where 100 % germination was obtained during day 1, showing the highest values of MG, GE and GR and the lowest MGT (Table 3). Although this value was achieved at different temperatures, significant differences were observed with respect to the germinative parameters related to the speed of the germination process. This was the case of MGT and GR, both considered relevant aspects in defining the optimum germination temperature. Germination in wide range of temperatures was previously related for seeds with a water-impermeable seed, or fruit coat, and have physical dormancy (physical dormancy “sensu” Baskin & Baskin, 1998) such as *Vachellia* (= *Acacia*) *aroma* (Gillies ex Hook. & Arn.) Seigler & Ebinger and *Vachellia* (= *Acacia*) *caven* (Molina) Seigler & Ebinger (Funes & Venier, 2006), *Prosopis alba* Griseb. (Catalán & Balzarini, 1992) and *P. flexuosa* DC. (Campos & Ojeda, 1997; Cony & Trione, 1996). This was also expressed by Baskin & Baskin (1998) and Baskin et al. (2000, 2004) in relation to the seeds that have physical dormancy, and which germinate under broad conditions of light and temperature once they are scarified naturally or artificially. When the testing temperature was higher than 40 °C the seed cover softened favoring the appearance of fungi contamination generating reversible or irreversible damage to the seedlings, depending on fungal colonization (Boeri, 2017).

During day 1 accumulated daily germination presented significant differences ($p < 0.02$) at the temperatures evaluated (Figure 3). The optimum germinative temperature of this study differed from previously reported (Villagra, 1995). It appears that *P. alpataco* would be capable of establishing itself at a lower temperature in the northeast Patagonia than in the Central Monte. There, Villagra (1995) reported an optimum germination temperature of 35 °C and seeds showed their maximum root growth between 25-35 °C. It has been proposed that its high optimum germinative temperature could be interpreted as a thermal adjustment of the species at the time of greatest water availability, typical of its distribution area (Villagra, 1995). There were also reported differences in other Argentinian species of *Prosopis* sympatric to *P. alpataco*.

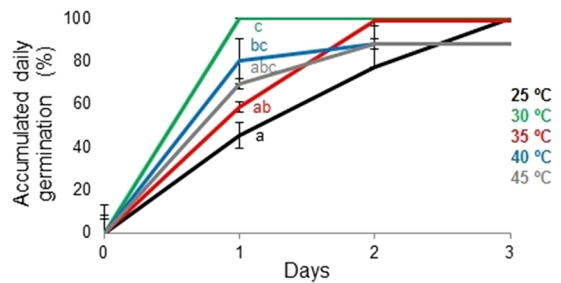


Fig. 3. Accumulated daily germination (mean and standard deviation) of *Prosopis alpataco* seeds exposed to different temperature. Different letters indicate significant differences at $p < 0.05$ according Tukey's test. Color version at <http://www.ojs.darwin.edu.ar/index.php/darwiniana/article/view/817/1169>

Table 3. Germinative parameters (mean and standard deviation) of *Prosopis alpataco* seed exposed to different temperature at the end of the trail. GC: Germinative capacity, MG: Maximum germination value, GE: Germinative energy, MGT: Mean germination time, GR: Germination rate or Maguire's index. Different letters indicate significant differences at $p < 0.05$ according Tukey's test.

Temperature	GC (%)	MGV (seeds/days)	GE (%)	MGT (days)	GR
25 °C	100 ± 0 ^a	11.5 ± 1.83 ^a	45.3 ± 12.56 ^a	1.77 ± 0.18 ^c	27.08 ± 3.90 ^a
30 °C	100 ± 0 ^a	25 ± 0 ^b	100 ± 0 ^c	1 ± 0 ^{ab}	45.83 ± 0 ^d
35 °C	98.7 ± 2.31 ^a	14.67 ± 0.76 ^{ab}	58.67 ± 6.11 ^{ab}	1.41 ± 0.08 ^{bc}	35.22 ± 1.51 ^b
40 °C	88 ± 10.58 ^a	20 ± 2.1 ^b	80 ± 8 ^{bc}	1.09 ± 0.16 ^a	38.89 ± 1.48 ^c
45 °C	88 ± 10.58 ^a	17.33 ± 3.51 ^{ab}	69.3 ± 22.03 ^{abc}	1.21 ± 0.23 ^{ab}	36.67 ± 6.72 ^{bc}
P-value	0.097	0.0013	0.008	0.0005	0.0001

In the case of *P. flexuosa*, its optimum germination temperature reported from the Central Monte area (Cabrera, 1971) was near to 20-25 °C, being values lower than those registered for other species that inhabit the North Monte as *P. chilensis* (Molina) Stuntz emend. Burkart and *P. argentina* Burkart (Villagra, 1995; Cony & Trione, 1996). It has been suggested that there is a relationship between the germination temperature of *Prosopis* species and their geographical distribution (Villagra et al., 2010). Considering this, the temperature range evaluated and the optimum germinative temperature detected here generates valuable bioecological information for *P. alpataco* in its austral distribution limit.

CONCLUSIONS

It is worth noting that this work evaluated the highest diversity of germinative parameters studied throughout Argentina for this species. Although some germinative studies for *P. alpataco* from other region were available, this work represents the first optimized germinative conditions to produce this species in northeastern Patagonia, on two productive scales. The acid scarification for large-scale production and the total cut of the edge of the seminal cover for the small-scale were considered. These methodologies allow obtaining seedlings in nurseries and native plants of the arid and semi-arid regions of Patagonia, which are required in conservation programs. Therefore, this should open new lines for future research and applications of other southern *Prosopis* species.

Based on the reported results to produce *P. alpataco* in incubation chambers at 30 °C, different scarification methods can be recommended according to the scale of work: the use of H₂SO₄ (30 min) would be ideal to be applied in large-scale production and the total mechanical cutting of the seminal cover edge for both experimental and small-scale.

It can be concluded that *P. alpataco* var. *alpataco* from northeastern Patagonia had a higher germinative response after an exposure to a range of relatively simple chemical and mechanical treatments. The techniques achieved here represent valuable information both for plans of environmental remediation and production at small and large-scale, as well as for germplasm banks and conservation programs of this multipurpose species.

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