Pre-germination treatments on *Ochetophila trinervis*, a native Andean tree with potential use for restoration

Germinación de semillas de *Ochetophila trinervis*, árbol nativo de los Andes Centrales con uso potencial de restauración

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Scientific note

Abstract

In a climate change scenario, global forest loss had a direct impact on the hydrological cycle, making the protection of soil and water resources a central issue. In the Central Andes of western Argentina, information on Chacay (*Ochetophila trinervis*) mountain forests is scarce. This tree thrives along river and stream banks, fixes atmospheric nitrogen, and grows in impoverished soils. The seeds of *O. trinervis* are characterized by physical or physiological dormancy, and germination requires technique application. The main goal was to evaluate the effect of mechanical and chemical scarification, cold stratification, and hot water immersion on the final germination percentage, germination speed index, and the mean germination time of *O. trinervis* seeds. Our results show that mechanical and chemical scarification are the treatments that best inhibit seed dormancy in this species. Mechanical scarification with sandpaper is the treatment that offers a balance between effective results and an easy-to-apply technique. Sulfuric acid (SA) treatment is also efficient in breaking dormancy, but we recommend applying it under extreme careful laboratory conditions.

Keywords
Rhamnaceae • watershed restoration • pre-germinative treatments • seed dormancy

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Resumen

La pérdida global de bosques en un escenario de cambio climático tiene un impacto directo en el ciclo hidrológico, por lo cual, la protección del suelo y los recursos hídricos adquieren una relevancia crucial. En los Andes Centrales del Oeste de Argentina, hay escasa información sobre los bosques de montaña, incluido el Chacay (*Ochetophila trinervis*), el cual se establece en bordes de ríos y arroyos, fija nitrógeno atmosférico y crece en suelos empobrecidos. Las semillas de *O. trinervis* presentan dormición física o fisiológica y la germinación es promovida mediante la aplicación de técnicas previas. El objetivo principal de nuestro estudio es evaluar el porcentaje final de germinación, el índice de velocidad de germinación y el tiempo medio de germinación en cuatro tratamientos (escarificación mecánica, escarificación química con ácido sulfúrico, estratificación fría, inmersión en agua caliente) y su control. Nuestros resultados muestran que la escarificación mecánica y química son los tratamientos que mejor inhiben la dormición de las semillas de esta especie. La escarificación mecánica con papel de lija es un tratamiento efectivo que ofrece un equilibrio entre los resultados obtenidos y una técnica sencilla de aplicación. Por otro lado, el uso de ácido sulfúrico presentó resultados óptimos de germinación, sin embargo, su aplicación debe realizarse en condiciones de laboratorio y con extremo cuidado.

Palabras clave

Rhamnaceae • restauración de cuencas • tratamientos pre-germinativos • dormición de semillas

Introduction

The global forest loss between 1990 and 2015 (3%) had a direct impact on the hydrological cycle, soil resources and provision of ecosystem services (6, 8). In the context of climate change and environmental hazards, the protection of soil and water resources becomes a central issue (13). In Argentina, land-use changes have caused annual deforestation rates of values between 200,000 ha (14) and 300,000 ha (6), which led to the sanction of the Ley de Bosques in 2007. As a result, it is necessary to develop guidelines for forest protection, focusing on distribution, conservation, and ecological restoration of the degraded areas (27).

In Mendoza province, Central-West Argentina, the information about the current distribution and conservation of mountain forests, which includes Maitén (*Maytenus boaria*), Luma (*Escallonia myrtoidea*), and Chacay (*Ochetophila trinervis*), is scarce (3). These woodlands play a critical role in river landscape conservation (15). Therefore, further studies are necessary to develop and implement restoration projects to reverse river use modifications and transformations related to village settlements.

Ochetophila trinervis (Gillies ex Hook. & Arn.) Poepp. ex Miers (Rhamnaceae) is a native South American tree of the Andes of Chile and western Argentina (22), found from 31° and 48° S, and between an altitude of 1,400 and 2,300 m (20). This species thrives along river and stream banks in the mountains (22), fixes atmospheric nitrogen and grows in impoverished soils (18). The study of the potential distribution of *O. trinervis* indicates that watercourses and related local conditions present optimal habitat suitability (24). However, there is evidence that these forests show geographical range retraction: historical sites such as the Chacay stream in the Uspallata valley exhibits signs of use and degradation (11).

Ecological features of *O. trinervis* highlighted this species as a suitable option for restoration projects due to its ability to stabilize watersheds and prevent flooding. Information on the environmental requirements for germination and on the eventual presence of dormancy in the seeds of focal species is a fundamental input for restoration tasks. This actinorhizal tree has dry fruits known as tricocos and small, hard seed shells (20, 22). Germination tests conducted in northern Patagonia reached 65% in previously stratified seeds (17).

Dormancy is a stage when a seed is unable to germinate under specific environmental conditions (2). This adaptive strategy allows germination to occur when suitable conditions are within the range of requirements for radicle emergence. Different species from arid environments present physical dormancy (5, 25, 26), including some species in the Rhamnaceae family (1), characterized by the presence of water-impermeable layers in the seeds (2). Dormancy interruption techniques attempt to simulate the ecological process that promotes germination, such as cold or winter conditions, mechanical abrasion from stream transport and acid softening in the digestive tract of animals during granivorous dispersal, among others. Physiological dormancy is regulated hormonally and requires cold stratification, whereas scarification is applied to break physical dormancy (1, 2).

Objective

Our objective was to evaluate the effect of mechanical and chemical scarification, cold stratification, and hot water immersion on the final germination percentage, germination speed index, and the mean germination time of *O. trinervis* seeds. We hypothesized that this species seeds present physiological or physical dormancy.

Materials and methods

Seed collection area

The fruit was collected in the early autumn of 2018 and 2019 on the eastern slope of Cordon del Plata, Central Andes of Mendoza, Argentina (32°59'45.12" S, 69° 15'58.85" W). In this area, the weather is semi-arid and local variability in the temperature and rainfall influences the distribution and abundance of vegetation (12), which adapts to climatic conditions of dryness and cold (4).

Experimental design

Fruit collection was carried out manually in five different forest patches located at least 250 m apart to ensure genetic diversity, including 30 individuals with healthy fruit in the surroundings of the Blanco River. Seeds were removed using a threshing device and stored at room temperature (18 ± 4°C) until evaluation. Broken and insect-damaged seeds were discarded by visual observation. Before each assay, the seeds were sterilized by immersion in commercial hypochlorite diluted at 10% for ten minutes and then washed three times in sterile water. Petri dishes of 9 cm diameter were used for the germination trials, with a cotton-wool layer over a filter paper disk. The experiment lasted 16 days, with four replicates of 25 seeds per treatment.

The following randomized treatments were defined: M= Mechanical scarification with sandpaper for 20 s and water immersion for 24 h; SA= Sulfuric acid scarification for 10 min; T5 °C= Cold stratification at 5°C for 15 days (16); T80 °C= 12 hours immersion with an initial temperature at 80°C; and Control (tC)=seeds without any treatment. The Petri dishes with the seeds were placed in a chamber (O.R.L. S.A Hornos Eléctricos) with temperature control at 25°C (±2°C) (16). Germination was defined and recorded as radical emergence to 1 mm. The sprouted seeds were counted daily for 16 days. Then, three parameters were estimated: final germination percentage, germination speed index, and mean germination time. Final germination percentage (%) was calculated as (n/N) × 100; where n is the number of germinated seeds, and N is the total number of seeds (6). Germination speed index (seeds day^{-1}) was estimated using the Maguire index (9) = $\Sigma_{(ni/ti)}$, and mean germination time (day^{-1}) was measured as = $\Sigma_{ni \times ti} / \Sigma ni$; where *ni* is the daily number of germinated seeds, and *ti* is the number of days spent on each count.

Data Analysis

Final germination percentage (%) was calculated as (n/N) × 100; where n is the number of germinated seeds, and N is the total number of seeds (7). Germination speed index (seeds day^{-1}) was estimated using the Maguire index (10) = $\Sigma_{(ni/ti)}$, and mean germination time (day^{-1}) was measured as = $\Sigma\, ni \times ti / \Sigma ni$; where *ni* is the daily number of germinated seeds, and *ti* is the number of days spent on each count.

Final germination percentage data were analyzed with generalized lineal models. Poisson distribution was used with log link function and the five levels treatment factor was considered as the fixed effect. Post hoc tests were performed using Tukey's HSD. All data were calculated with the GermCalc function from SeedCalc package (21) for R software (16).

Germination speed index and mean germination time data were normalized using a log10 transformation and analyzed with ANOVA, and LSD Fisher's test ($p < 0.05$) was used for means comparison. All figures present the original values of these three parameters.

Results

The applied treatments have significant effects on final germination percentages (LRT=292.58; Prob>Chi2 =2.2e-16), germination speed index (F value=24.74; p-value<0.0001), and mean germination time (F value=3.115; p-value=0.04). Final germination percentage values increased with M and SA treatments, exceeding the 50% germination value. These treatments presented the highest values of daily germination (figure 1a). The T80 °C treatment presented intermediate values of final germination percentage (22%±2.6). The T5 °C treatment reached a low germination percentage (13%±3), the values achieved were similar to the control treatment (8%±2.8) (figure 1b).

Each letter indicates significant differences ($p < 0.05$, Tukey test).
Cada letra indica diferencias significativas ($p < 0,05$, prueba de Tukey).

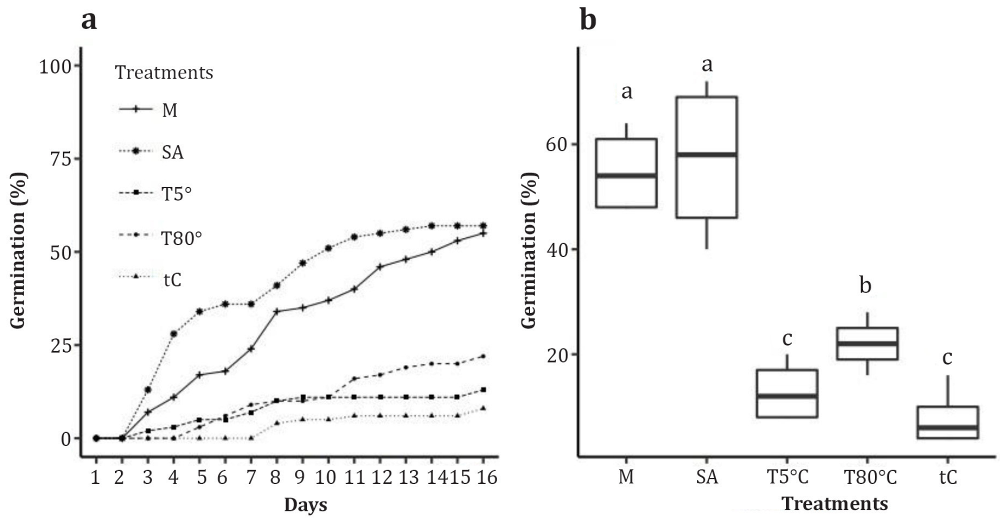
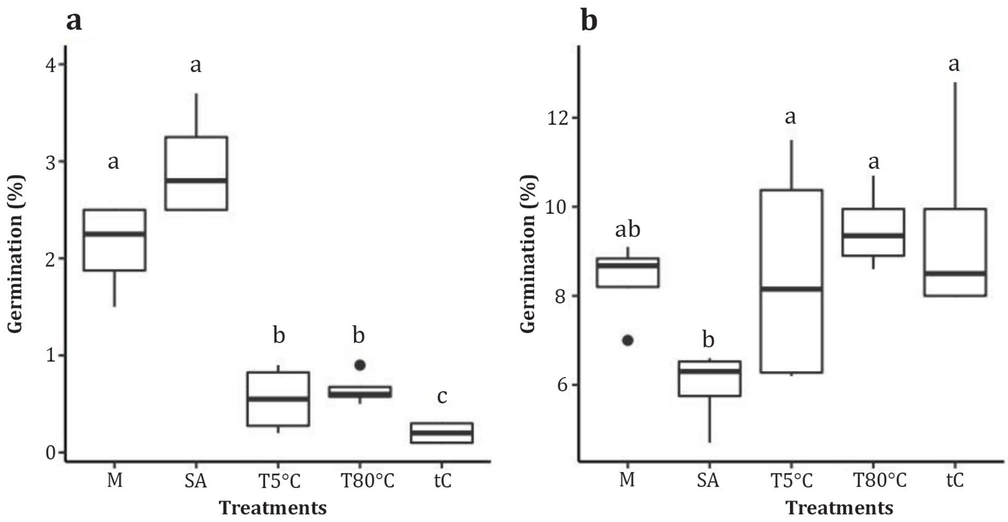


Figure 1 (a). Cumulative germination percentage and **(b)** final germination percentage (FGP) of *Ochetophila trinervis* seeds under different treatments (mechanical (M), sulfuric acid (SA), temperature at 5°C and 80°C (T5°C and T80°C, respectively) and control (tC).

Figura 1 (a). Porcentaje de germinación acumulada y **(b)** porcentaje final de germinación (PFG) de semillas de *Ochetophila trinervis* bajo diferentes tratamientos (mecánico (M), ácido sulfúrico (SA), temperatura a 5°C y 80°C (T5°C y T80°C, respectivamente) y control (tC).

M and SA treatments showed the highest values for the germination speed index, with a rate between 2.1 and 3 seeds per day (figure 2a, page 81). The temperature treatments (T5°C and T80°C) presented intermediate values for the germination speed index, with 0.6 and 0.7 germinated seeds per day, respectively. The SA treatment was the one that reached the shortest mean germination time (6 days ±0.2). Although these results are different from those obtained in the temperature treatments (T5°C and T80°C) and control (tC), they do not differ significantly from the values obtained in the M treatment (figure 2b, page 81).



Each letter indicates significant differences ($p < 0.05$, Fisher-LSD).

Cada letra indica diferencias significativas ($p < 0,05$, Fisher-LSD).

Figure 2(a). Germination speed index (GSI) and **(b)** mean germination time (MGT) of *Ochetophila trinervis* seeds under different pre-germination treatments (mechanical (M), sulfuric acid (SA), temperature at 5°C and 80°C (T5 °C and T80 °C, respectively) and control (tC).

Figura 2 (a). Índice de velocidad de germinación (IVG) y **(b)** tiempo medio de germinación (TMG) de las semillas de *Ochetophila trinervis* bajo diferentes tratamientos pre-germinativos (mecánico (M), ácido sulfúrico (SA), temperatura a 5°C y 80°C (T5 °C y T80 °C, respectivamente) y control (tC).

Discussion and conclusions

Our findings support the hypothesis that the seeds of *O. trinervis* show physical dormancy because either mechanical (M) or chemical scarification (SA) techniques achieved the highest germination values. The hot water immersion treatment (T80°C) performs poorly, similar to trials on other Rhamnaceae species from Australia (23). Regarding the stratified procedure (T5°C), our results show the lowest germination values, similar to the control treatment (tC). According to this outcome, seed dispersal is likely to occur through transport by streams or by ingestion by wildlife.

The cold stratification treatment (T5°C) seems viable for northwest Patagonia (17), where lower temperatures than in our study area can induce a winter pause and *O. trinervis* seeds do not need scarification to achieve a high germination rate. This observation differs from our results and the procedures applied to *Discaria tomatou*, a Rhamnaceae species from New Zealand, where scarification techniques also promote higher germination rates (9). The differences between each experiment could be explained by climate contrast between the study sites, the seed dispersal mechanisms and would imply a high interspecific variability.

In this study, we seek to adjust a simple and effective technique for the germination of *O. trinervis*, a tree with forestation potential and watershed restoration capacity. Our results indicate that mechanical scarification (M) might be a practical option for seedling germination in the Central Andes of Western Argentina. Sulfuric acid (SA) treatment is also efficient in breaking dormancy, but we recommend applying it under extreme careful laboratory conditions. Further study should focus on seed viability and other environmental factors that potentially regulate the germination process, such as temperature, humidity and light exposure.

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