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Pre-germination treatments on *Senna arnottiana* and *Senna kurtzii*: key contributions for restoration initiatives in Payunia, Patagonia, Argentina

Tratamientos pre-germinativos en *Senna arnottiana* y *Senna kurtzii*: aportes claves para la restauración en Payunia, Patagonia, Argentina

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Abstract. In arid and semi-arid ecosystems, increasing and irreversible degradation in areas of high conservation value has prompted the search for various solutions, such as rehabilitation and ecological restoration. To implement these strategies, it is essential to solve critical issues affecting the establishment of plants in the field and the production of seedlings in nursery gardens. In the present work, the following pre-germination treatments were evaluated for the species *Senna arnottiana* and *S. kurtzii*, endemic to the Patagonian steppe (Payunia): (1) Immersion in water at 80 °C for 5 minutes; (2) Immersion in water at 80 °C for 10 minutes; (3) Immersion in water at 100 °C for 5 minutes; (4) Immersion in water at 100 °C for 10 minutes; (5) Acid scarification with sulfuric acid for 30 minutes; (6) Acid scarification with sulfuric acid for 60 minutes; (7) Soaking in water at 80 °C until cooled for 24 hours, and (8) Control (no treatment). For both species, the highest, fastest, and most homogeneous germination was obtained with acid scarification, i.e., 70% germination for *S. arnottiana* and 95% germination for *S. kurtzii*. Although this method requires the use of a laboratory and has a high economic cost for local residents, the high germination percentages obtained make it highly recommended for the production of large quantities of seedlings.

Keywords: Protected Area; Fabaceae; Immersion; Scarification; Soaking.

Resumen. En ecosistemas áridos y semiáridos la degradación creciente e irreversible en áreas de alto valor de conservación ha impulsado la búsqueda de diversas soluciones, tales como la rehabilitación y restauración ecológica. Para esta actividad es fundamental resolver los puntos críticos que afectan el establecimiento de plantas en campo y la producción de plantines en vivero. En el presente trabajo se evaluaron para las especies *Senna arnottiana* y *S. kurtzii*, endémicas de la estepa patagónica (Payunia), los tratamientos pre-germinativos: (1) Inmersión en agua a 80 °C por 5 minutos; (2) Inmersión en agua a 80 °C por 10 minutos; (3) Inmersión en agua a 100 °C por 5 minutos; (4) Inmersión en agua a 100 °C por 10 minutos; (5) Escarificación ácida con ácido sulfúrico por 30 minutos; (6) Escarificación ácida con ácido sulfúrico por 60 minutos; (7) Remojo en agua 80 °C hasta enfriamiento por 24 horas, y (8) Control sin ningún tratamiento. En ambas especies se obtuvo la más alta, rápida y homogénea germinación con escarificación ácida, 70% para *S. kurtzii* y 95% para *S. arnottiana.* Aunque este método requiere del uso de laboratorio y tiene alto costo económico para pobladores locales, los altos porcentajes obtenidos lo hacen altamente recomendable para producciones de grandes cantidades de plántulas.

Palabras clave: Área Protegida; Fabaceae; Inmersión; Escarificación; Remojo.

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INTRODUCTION

The arid and semi-arid Patagonia has been classified by the IUCN as a biodiversity hotspot due to its high plant species richness and conservation value, as it possesses 23% of the vascular plant species of Argentina and a high percentage of endemism (Villamil, 1999; Beeskow et al., 2005). Yet, this region is affected by high levels of desertification (Mazzoni & Vazquez, 2010). Moreover, in arid and semi-arid environments, favorable conditions for the establishment of plants occur irregularly and with a low frequency in undisturbed areas. However, after a disturbance, the restoration of the original biodiversity may take decades or centuries without intervention (Brainbridge, 2007; Abella, 2010). Therefore, ecological restoration as a scientific discipline that proposes the recovery of damaged, destroyed or degraded ecosystems (SER, 2004) turns out to be an alternative to reverse desertification in Patagonia (Pérez et al., 2011). Different frameworks have been developed to ensure successful outcomes in the ecological restoration of arid regions, such as applying systems models (James et al., 2013). In this model, both the demographic stages of the species, the likelihood of transition between them, and management strategies are considered important. In particular, the probability of transition from seed sown-seed bank to germinated seed and emerged seedling will increase with seed coating treatments.

In arid ecosystems, the mechanisms of seed dormancy are complex and prevalent (Baskin & Baskin, 2001; Kos et al., 2012) because they block germination under unfavorable conditions and allow it when the chances of survival of young seedlings are greater (Raven & Johnson, 2002). In particular, a large number of species of the Fabaceae family possesses seeds with physical dormancy due to the presence of a waterproof layer (Baskin et al., 2000; Baskin & Baskin, 2004). This layer, in addition to preventing germination, acts as protection against fluctuations in temperature, humidity, and microbial attack (Mohamed-Yasseen et al., 1994). In nature, seed dormancy can be removed by the passage of seeds through the digestive tract of animals, abrasion by soil particles, fire, microorganism digestion, and temperature fluctuation, among others (Jones, 1999; Baskin & Baskin, 2001). For instance, Baskin & Baskin (2000) showed that there is mounting evidence about the breaking of physical latency by soil microbial action and/or abrasion by soil particles. In the laboratory, treatments such as acid scarification (Turner & Dixon, 2009), mechanical scarification (Ghantous & Sandler, 2012), and exposure to high temperatures (Auld & O'Connell, 1991) are successful in imitating natural conditions and removing the latency in seeds with physical dormancy.

In this context, the present work aimed at characterizing the seeds of *Senna arnottiana* and *S. kurtzii* to evaluate thermal and chemical pre-germination treatments to break their latency.

MATERIALS AND METHODS

Study area. The seeds of *Senna arnottiana* and *S. kurtzii* were collected from ripe fruits during 2011 in the Provincial Reserve of Multiple Uses Auca Mahuida, located to the Northwest of the Province of Neuquén, Argentina (37°42'S; 68°38'W). This reserve belongs to the Payunia Phytogeographic District of the Patagonian Steppe (Cabrera, 1976). It is characterized by the presence of low shrubs (with genera such as *Chuquiraga*, *Colliguaya*, *Mulinum*, *Senecio*, *Adesmia*, and *Senna*) and grasses (with genera such as *Stipa*, *Poa*, and *Festuca*) (Movia, 1982). This district is habitat to 37.7% of the plant families of Argentina, and 4.9% of the taxa are endemic and exclusive to the area (Prina et al., 2003). The Payunia District presents a temperate arid to desert climate, with average temperatures of 14-16 °C and an average annual precipitation between 100 and 200 mm (Morello et al., 2012). From the geomorphological point of view, it corresponds to a region with extra-Andean Patagonian features, with relief in the form of staggered plateaus originated by deposits of basaltic flows (Martínez Carretero, 2004). Despite being an area with low population density, overgrazing by goats as well as mining and oil activities cause disturbances that affect the conservation of the native flora (Fiori & Zalba, 2003).

Species description. The genus *Senna* (Fabaceae, Caesalpinoideae) is represented in Argentina by 32 species, and its distribution extends from the northernmost provinces of Misiones, Salta, and Jujuy to northern Patagonia, in the provinces of Neuquén and Río Negro (Bianco & Kraus, 1997; Arambarri, 2002). Specifically, *Senna arnottiana* (Gillies ex Hook.) H.S. Irwin & Barneby inhabits the provinces of Mendoza, Neuquén, and Río Negro, between 800 and 2800 m.a.s.l in the Payunia District (Zuloaga et al., 2008). It is a shrub of 0.15 to 0.4 m high; it presents clusters with 2 to 5 flowers. Its legumes measure 5-9.5 x 1.3-2 cm and are slightly flattened, with 4-7 seeds (Correa, 1984). In turn, *S. kurtzii* (Harms) H.S. Irwin & Barneby inhabits the provinces of Mendoza and Neuquén, also in the Payunia District, between 200 and 2000 m.a.s.l (Zuloaga et al., 2008). It is a shrub of 0.3 to 0.65 m of height with many branches. It has clusters with 1 or 2 flowers, and its legumes measure 7-10 x 0.8-1.1 cm with 15 to 20 seeds (Correa, 1984).

Material collection and laboratory testing. Mature fruits were harvested from, at least, 30 plants following the suggested protocols for restoration projects (Bainbridge, 2007). The seeds were manually extracted, observed under a magnifying glass to discard those damaged or attacked by insects, and stored until used in a cold room at 8 °C in the "Banco del Árido" germplasm bank (Rodríguez Araujo et al., 2015). Seeds of each species were characterized by weighing 5 groups of 100 seeds, and 10 seeds of each species were measured in their major and minor diameter with a digital caliper. To evaluate the germination process, eight treatments with three replicates of 25 seeds each were conducted: (1) Immersion in water at 80 ˚C for 5 minutes; (2) Immersion in water at 80 ˚C for 10 minutes; (3) Immersion in water at 100 ˚C for 5 minutes; (4) Immersion in water at 100 ˚C for 10 minutes; (5) Acid scarification with sulfuric acid for 30 minutes; (6) Acid scarification with sulfuric acid for 60 minutes; (7) Soaking in water at 80 ˚C until cooled for 24 hours, and (8) Control (no treatment). After treatments, the seeds were placed in 9-cm-diameter Petri dishes with moistened filter paper. These dishes were placed in a germination chamber under controlled conditions of temperature (minimum 10 ± 1 ˚C, maximum 20 ± 1 ˚C) and photoperiod (12 hours of light/12 hours of darkness). Germination (emergence of the radicle) was recorded every other day for a period of 40 days, and the germinated seeds were removed from the box.

Due to lack of normality, the data were analyzed with a Kruskal-Wallis test, and posteriori contrasts were performed. The software InfoStat (2007™) was used.

RESULTS

The average weight of 100 seeds was 13.80 (± 0.23) g and 1.86 (± 0.06) g for *Senna arnottiana* and *S. kurtzii*, respectively. For *S. arnottiana*, the average major diameter was 0.82 (± 0.04) cm, and the average minor diameter was 0.69 (± 0.03) cm, whereas for *S. kurtzii*, the average major diameter was 0.52 (± 0.03) cm, and the average minor diameter was 0.31 (± 0.02) cm.

Regarding the germination results, the percentages obtained with hot water immersion and soaking treatments were low (less than 15%) for both species. In contrast, high germination percentages (greater than 60%) were obtained with sulfuric acid (Fig. 1). For *S. arnottiana*, the germination percentage was significantly different and greater with acid scarification during 60 minutes, whereas for *S. kurtzii*, there were no differences between acid scarification for 30 and 60 minutes of immersion.

In the treatments that presented the highest germination percentages, the radicle emergence in seeds of *S. kurtzii* began 2 days after the beginning of the experiment. After 5 days, the percentage of germination reached a 95%-asymptote. For *S. arnottiana*, in the treatment with the greatest germination percentage, the emergence of the radicle began at the 7th day, and a 70%-asymptote was reached at the 40th day from the beginning of the experiment (Fig. 2).

DISCUSSION

The germination of the genus *Senna* was studied worldwide with different pre-germination treatments (Teketay, 1996a; Faria et al., 2012; Pereira et al., 2014). In most species, sulfuric acid was the treatment that yielded the best results. The success of this treatment in Fabaceae is due to the effect of acids, which oxidize and degrade sectors of the integument (Baskin et al., 2000). This process causes the formation of a gap that allows the absorption of water and gas exchange (Baskin & Jerry, 2005). The morphology, anatomy, and loca-

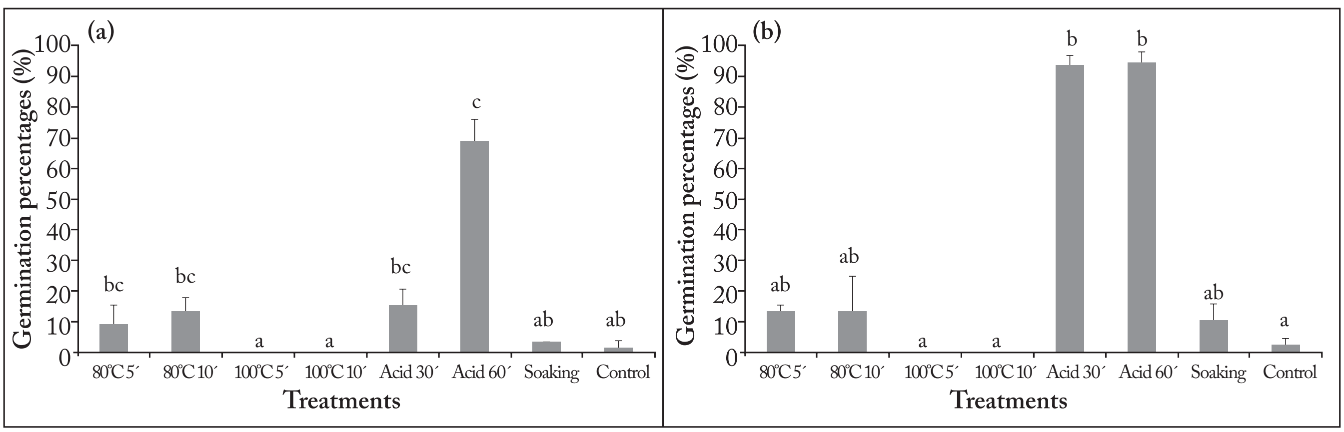

Fig. 1. Germination percentages (average ± SD) of seeds of (a) *Senna arnottiana* and (b) *S. kurtzii* based on the following treatments: 1. Immersion in water at 80 °C for 5 minutes (80 °C 5'); 2. Immersion in water at 80 °C for 10 minutes (80 °C 10'); 3. Immersion in water at 100 °C for 5 minutes (100 °C 5'); 4. Immersion in water at 100 °C for 10 minutes (100 °C 10'); 5. Acid scarification with sulfuric acid for 30 minutes (Acid 30'); 6. Acid scarification with sulfuric acid for 60 minutes (Acid 60'); 7. Soaking in water at 80 °C until cooled for 24 hours; 8. Control (no treatment). Different letters represent significant differences among treatments ($P<0.05$).

Fig. 1. Porcentaje de germinación (media ± DE) de semillas de (a) *Senna arnottiana* y (b) *S. kurtzii* para los tratamientos: 1. Inmersión en agua a 80 °C por 5 minutos. 2. Inmersión en agua a 80 °C por 10 minutos. 3. Inmersión en agua a 100 °C por 5 minutos. 4. Inmersión en agua a 100 °C por 10 minutos. 5. Escarificación ácida con ácido sulfúrico por 30 minutos. 6. Escarificación ácida con ácido sulfúrico por 60 minutos. 7. Remojo en agua 80 °C hasta que se enfría por 24 horas. 8. Control sin ningún tratamiento. Letras diferentes indican diferencias estadísticas significativas entre tratamientos ($P\leq0,05$).

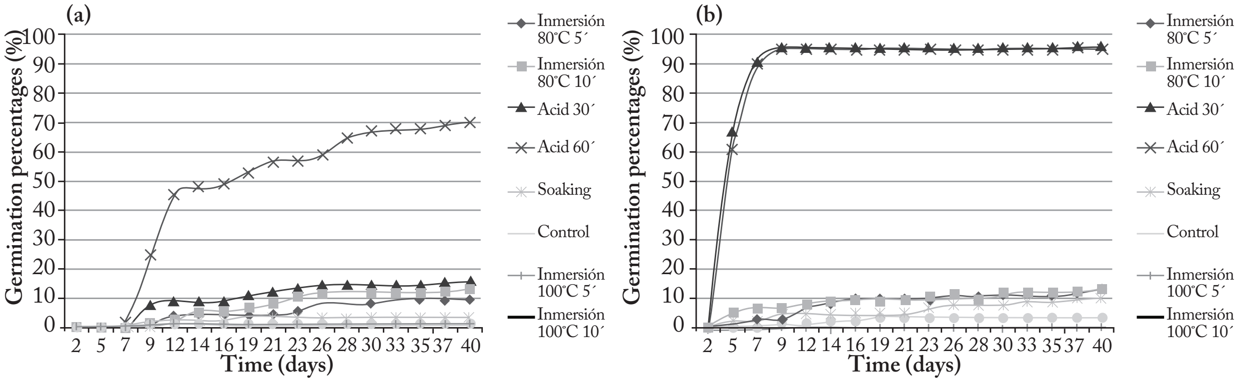

Fig. 2. Cumulative germination curves for (a) *Senna arnottiana* and (b) *S. kurtzii* based on the following treatments: 1. Immersion in water at 80 °C for 5 minutes (80 °C 5´); 2. Immersion in water at 80 °C for 10 minutes (80 °C 10´); 3. Immersion in water at 100 °C for 5 minutes (100 °C 5´); 4. Immersion in water at 100 °C for 10 minutes (100 °C 10´); 5. Acid scarification with sulfuric acid for 30 minutes (Acid 30´); 6. Acid scarification with sulfuric acid for 60 minutes (Acid 60´); 7. Soaking in water at 80 °C until cooled for 24 hours; 8. Control (no treatment). Different letters represent significant differences among treatments (P<0.05).

Fig. 2. Curva de germinación acumulada para (a) *Senna arnottiana* y (b) *S. kurtzii* para los tratamientos: 1. Inmersión en agua a 80 °C por 5 minutos. 2. Inmersión en agua a 80 °C por 10 minutos. 3. Inmersión en agua a 100 °C por 5 minutos. 4. Inmersión en agua a 100 °C por 10 minutos. 5. Escarificación ácida con ácido sulfúrico por 30 minutos. 6. Escarificación ácida con ácido sulfúrico por 60 minutos. 7. Remojo en agua 80 °C hasta que se enfría por 24 horas. 8. Control sin ningún tratamiento. Letras diferentes indican diferencias estadísticas significativas entre tratamientos (P≤0.05).

tion of this gap may vary among species, genera, and families (Gama - Arachchige et al., 2013). Therefore, the response to pre-germination treatments is specific in terms of exposure times and concentrations, and there is no single treatment equally effective for all species (Teketay, 1996a; 1996b).

In restoration projects, Brainbridge (2007) highlights the importance of evaluating pre-germination treatments with hot water to avoid damaging the seeds and to take advantage of their low cost. Other authors have obtained positive results in germination with exposure to hot water (Hu et al., 2009; Erickson, 2015), but in *S. arnottiana* and *S. kurtzii*, the treatments with hot water at 100 °C resulted in no germination at all. Although the germination percentages of the treatments at 80 °C were significantly different from the control, they were low. In another study with wet-cold stratification, the same species also showed low germination results (3.33 ± 5.77% for *S. arnottiana* and 10 ± 3.33% for *S. kurtzii*) (Masini, 2011). These results would demonstrate that, for these species, the use of moisture or liquid water, even at different temperatures, would not be a suitable technique to break the dormancy of large quantities of seeds and, consequently, would not be useful for the practice of ecological restoration. In contrast, the acid treatment is highly effective. Our results showed that the immersion time in sulfuric acid directly affected the effectiveness of the treatment. Likewise, the time necessary to induce germination was specific for each species. For *S. kurtzii*, 30 minutes of immersion resulted in the maximum germination percentage (95%), whereas for *S. arnottiana*, 60 minutes resulted in a germination of 70%. For the latter species, we propose that longer immersion times could potentially increase this percentage.

The high effectiveness of acid treatments allows the implementation of projects that require a mass production of these species in nurseries, particularly for *S. arnottiana*, whose capacity to establish plantations on severely degraded sites was tested (González & Pérez, 2017). In addition, our results allow us to advance the research about other demographic stages of these species and their management for successful restoration outcomes.

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