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Effects of scarification and nutrient mineral concentrations on the *in vitro* germination of *Senna macranthera* (Collad.) H. S. Irwin & Barneby seeds

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Abstract

Senna macranthera is a tree species used in the recovery of degraded ecosystems whose wood is also used in carpentry and for cooking. Extracts of its leaves and seeds have shown potential pharmacological activities. The seeds demonstrate dormancy, which affects seedling production and the commercial propagation of the trees. We evaluated different methods for overcoming seed dormancy and the effects of nutrient media concentrations and light on the *in vitro* germination of *S. macranthera*. Seeds were subjected to chemical scarification with H_2SO_4 or mechanical scarification using sandpaper. Non-scarified seeds were used as control. Seeds were inoculated into test tubes containing MS, MS ½, or WPM medium and kept in growth rooms under a 16L:8D photoperiod or in total darkness. Scarification treatments promoted greater germination percentages than controls under all germination conditions tested. Sixty days after inoculation, the seedlings germinated from scarified seeds had greater root and shoot lengths than those of the controls, regardless of the culture medium used. It can be concluded that the physical methods used for overcoming dormancy are necessary and effective in promoting *in vitro* germination and subsequent seedling growth.

Key words: Dormancy, *In vitro* germination, Scarification, *Senna macranthera*

Abbreviations: MS = Murashige and Skoog medium; MS½ = half concentrations of MS macro and micronutrients; H_2SO_4 = sulphuric acid; WPM = Lloyd & McCown medium; SGI = speed germination index; % G = germination percentage; APL = aerial part length; RPL = root system length

Introduction

Senna macranthera (Collad) H.S. Irwin & Barneby (Fabaceae – Caesalpinoideae) is a tree species commonly found in semi-deciduous altitudinal forests; it can grow up to 8 m in height with a trunk diameter of 20 to 30 cm, the leaves are composed of two pairs of opposite folios (Lorenzi, 1992). This species is widely utilized in both rural and urban landscaping and its wood can be used in carpentry and as firewood.

Species of the genus *Senna* have been found to produce a wide variety of bioactive compounds and more than 350 secondary metabolites are known from species growing in tropical and subtropical regions (Viegas et al., 2006). Extracts of *S. macranthera* bark contain compounds such as rubrofusarin, which shows significant biological activity (Pereira et al., 1995; Mata et al., 2003; Song et al., 2004; El-Halawany et al., 2007), and extracts of the seed endosperm have been shown to have anti-coagulant activity (Pires et al., 2001). Nogueira (2009) reported anti-bacterial, laxative, anti-inflammatory and anti-oxidant activities of ethanol extract fractions of *S. macranthera* leaves and this species demonstrates great economic potential as a source of tannins and galactomannans (Santarém and Aquila, 1995).

S. macranthera is a pioneer species indicated for recuperating degraded ecosystems (Lorenzi, 1992). Its utilization in forest recuperation projects has been limited, however, by a lack of information concerning its biology and ecology or the appropriate techniques for propagation and management (Ranieri et al., 2003).

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In spite of the potential usefulness of *S. macranthera* to humans, its cultivation has been limited by difficulties encountered in obtaining commercial stocks of seedlings due to its low seed germination rate. Seed dormancy has been observed in a large number of forest species, and is considered an adaptive mechanism under natural conditions (Bruno et al., 2001). Seed dormancy is one of the principal problems facing attempts to produce seedlings of native forest species and conserve their germplasm (Oliveira et al., 2003; Dôres, 2007). *S. macranthera* seeds demonstrate dormancy caused by the impermeability of the tegument (Filho et al., 1997; Santarém and Aquila, 1995), which impedes imbibition, but it can be overcome by chemical scarification using sulfuric acid or mechanical abrasion (Santarém and Aquila, 1995; Eschiapati and Perez, 1997).

Tissue culture has been successfully used to propagate and conserve many economically important plants species and maximize large-scale seedling production efforts – thus reducing the need for *in situ* harvesting of natural native forest resources (Bapat et al., 2008)

The present work evaluated the effects of scarification by sulfur acid or mechanical abrasion and exposure to different concentrations of culture media on overcoming dormancy of *S. macranthera* seeds and on their subsequent *in vitro* growth.

Materials and Methods

S. macranthera seeds were acquired through the Clube da Semente (www.clubedasemente.org) and remained stored in paper envelopes at room temperature until used.

The seeds were submitted to the following treatments to overcome dormancy: control (seeds not subjected to any scarification); chemical scarification (immersion in 98% concentrated sulfuric acid for 45 min.); mechanical manual scarification (abrasion on the side of the seed opposite the embryo, using sterilized 100 grit sandpaper).

The scarified and non-scarified seeds were disinfected by immersion in 70% ethyl alcohol for 1 min. and then 2.5% sodium hypochlorite for 10 minutes followed by rinsing in sterile distilled water. After disinfection, the seeds were inoculated into test tubes containing the following sterile culture media: standard MS medium (Murashige and Skooge, 1962); MS½ medium and 3% sucrose; and WPM medium (Lloyd & McCown, 1980) with 2% sucrose. The pHs of the three media types were just to 5.8 ± 0.1 before adding 6.0 g L^{-1} of agar and autoclaving at 121°C and 1.1 kg cm^{-2} for 20 minutes.

The seeds inoculated into the different media were then maintained at 25 ± 2°C under two different illumination regimes: 1) a 16L:8D photoperiod with 42 µmol m^{-2} s^{-1} of PAR furnished by fluorescent daylight-type lamps; 2) total darkness.

The experiments were conducted by testing all possible combinations of culture media, scarification methods, and illumination regimes, with five repetitions each; each trial involved 10 test tubes with one seed each.

Daily observations were made to accompany seed germination and to calculate the speed germination index (SGI) (Maguire, 1962) and germination percentage (%G). Seeds were considered to have germinated if they demonstrated development of their aerial portion. After the germination percentages had stabilized, measurements were made of the aerial part length (APL) and root system length (RPL). The data were submitted to analysis of variance at a 5% probability level and compared using the Duncan and Tukey tests, all run on the STATISITCA program for Windows.

Results and Discussion

S. macranthera seeds germinated well in both presence and absence of light and can therefore be considered photoblastically neutral (Table 1). The methods tested for overcoming dormancy were efficient under both light and dark conditions, always demonstrating greater %G than seen among the controls (Table 1).

Table 1. Percentage germination of *S. macranthera* seeds in different culture media under different illumination conditions.

Percentage germination (%G)						
Light				Dark		
Medium	Sulfur acid	Sandpaper	Control	Sulfuric acid	Sandpaper	Control
MS	68 ab A	58 b A	28 c A	56 b A	80 a A	18 c A
MS ½	38 b B	54 ab A	20 c A	62 a A	46 ab B	18 c A
WP	56 a AB	52 ab A	36 bc A	62 a A	62 a B	30 c A

Values followed by the same lowercase letter on the same line, or the same uppercase letters in the same columns, do not statistically differ at the 5% probability level by the Duncan test.

Table 2. Speed Germination Index of *S. macranthera* seeds in different culture media.

	Speed Germination Index (SGI)		
Medium	Sulfuric acid	Sandpaper	Control
MS	0.059 aA	0.041 bA	0.021 bA
MS ½	0.034 abA	0.046 aA	0.016 bA
WPM	0.055 aA	0.041 aA	0.033 aA

Values followed by the same lowercase letter on the same line, or the same uppercase letters in the same columns, do not statistically differ at the 5% probability level by the Duncan test.

Seeds that had been scarified with sulfuric acid and germinated under light conditions showed greater %G in the MS and WPM media. Mechanically scarified seeds demonstrated no significant differences in %G in any of the growth media, indicating that the different mineral salt concentrations did not greatly affect their germination. According to Eschiapati and Perez (1997), immersion in 98% sulfuric acid for 50 min. to overcome dormancy resulted in the highest germination rates of *S. macranthera* seeds when germinated on humidified filter paper. Chemical scarification of *S. macranthera* seeds with dark incubation resulted in germination percentages that did not statistically differ from the control; mechanical scarification with sandpaper with dark incubation promoted higher %G only in the MS media.

The analyses of the germination responses in the different culture media indicated that under conditions of illumination both mechanical or chemical scarification were efficient in promoting higher %G in relation to the control in the MS and WPM media (Table 1). Under dark conditions, mechanical scarification yielded the highest %G in the MS medium, although the MS ½ and WPM media both gave satisfactory results. Filho et al. (1997) and Santarém and Aquila (1995) examined the germination of *S. macranthera* seeds on filter paper and observed that overcoming dormancy using sandpaper or sulfuric acid for 12 and 15 minutes respectively was sufficient to overcome dormancy. These same methods were found to be deficient in overcoming dormancy in *Senna siamea* seeds, with the greatest %G been seen with diaspores sown onto humidified filter paper (Dutra et al., 2007).

Table 2 shows that the SGI values were not significantly influenced by the mechanical or chemical methods used for overcoming dormancy or the types of culture media used. Scarification with sulfuric acid gave the best results in the MS medium. This same result was observed by Eschiapati and Perez (1997) who scarified seeds by immersion in sulfuric acid for 50 min. and observed 90% germination within 2.17 days on filter paper. The greatest SGI values in *Mimosa caesalpiniaefolia* were also observed under these same conditions (Bruno et al., 2001). MS ½ favored high SGI with both types of scarification, while there was no significant difference between the scarification treatments when compared to the control in WPM medium (Table 2). Santarém and Aquila (1995) observed that, independent of the storage time of *S. macranthera* seeds, mechanical scarification resulted in germination rates above 80% among seeds sown onto filter paper.

When the lengths of the aerial parts of the seedlings were evaluated, the greatest growth was observed to have occurred among plants cultivated in the dark, without significant statistical differences between the two scarification treatments and the control (Table 3 and Figure 1). Both scarification techniques resulted in high APL values when the seeds were inoculated into MS or MS ½ media, but the results of these treatments and the control were inferior to those in the WPM medium.

According to Coelho et al. (2001), techniques that help overcome seed dormancy can result in high APL values due to the acceleration of the imbibition processes and consequent rapid seed germination. The greatest APL values in *Zizyphus joazeiro* (Rhmneae) were observed among seedlings derived from seeds scarified with sulfuric acid (Alves et al., 2006).

Table 3. Lengths of the aerial portions (cm) of *S. macranthera* seedlings grown in different culture media and under different light conditions.

	Length of the aerial portion (APL)					
	Light			Dark		
	Sulfuric acid	Sandpaper	Control	Sulfuric acid	Sandpaper	Control
MS	7.36 b A	6.15 b A	5.91 b A	16.73 a A	16.92 a A	14.07 b A
MS ½	6.82 ac A	7.81 ac A	5.16 b A	17.07 a A	18.73 a A	10.86 b A
WPM	6.23 b A	7.30 b A	5.48 b A	14.17 b A	13.29 b B	17.31 a A

Values followed by the same lowercase letter on the same line, or the same uppercase letters in the same columns, do not statistically differ at the 5% probability level by the Duncan test.

Table 4. Lengths of the radicles (cm) of *S. macranthra* seedlings grown in different culture media and under different light conditions.

Length of the radicle (RPL)						
	Light			Dark		
	Sulfuric acid	Sandpaper	Control	Sulfuric acid	Sandpaper	Control
MS	5.46 ae A	5.17 bcde A	4.94 bde A	6.94 a A	6.61 ac A	6.37 ad A
MS ½	6.45 ac A	6.43 ab A	4.15 bcde A	7.51 a A	5.21 ae B	3.48 be B
WPM	5.59 a A	5.03 a A	4.93 a A	6.02 a A	4.43 a B	4.41 a B

Values followed by the same lowercase letter on the same line, or the same uppercase letters in the same columns, do not statistically differ at the 5% probability level by the Duncan test.

In relation to the radicule length, no significant differences were noted between the different methods of scarification in the three different cultivation media under light conditions (Table 4). The same scarification techniques likewise did not significantly influence RPL values among seeds cultivated in the MS and WPM media (Table 4); in the MS ½, chemical scarification was observed to be more efficient in relation to the control but statistically equivalent to mechanical scarification.

Pterodon pubescens seeds germinated *in vitro* in MS medium showed the greatest APL, RPL, and %G values when their teguments were removed (Coelho et al., 2001), which corroborates our results. Martins and Nakagawa (2008) observed that mechanical scarification did not influence radicule development in *Stryphnodendron adstringens* seeds germinated on paper towels.

Data in the published literature indicates that overcoming seed dormancy by mechanical scarification can compromise the RPL of seedlings as the scarification is performed in the region on the opposite side of the seed from the radicle and the region where the embryo is located thus remains impermeable – which slows water infiltration into the embryo and maintains the physical barrier (the seed coat) to initial root growth (Martins and Nakagawa, 2008). The effectiveness of sulfuric acid in overcoming tegument-imposed dormancy is related to the removal of the cuticle and exposure of the macro-sclereids, thus allowing the uniform imbibition of the seed and its rapid germination – which can result in seedlings having more highly developed aerial and radicle organs (Santarém and Aquila, 1995).

Figure 1. *S. macranthera* seedlings germinated *in vitro*. (a) seedling cultivated in WPM medium in the light from seeds that did not receive any treatment for overcoming dormancy (control), (b) seedlings cultivated in the dark in WPM medium derived from seeds that had been mechanically abraded to overcome dormancy, (c) seedling cultivated under illumination conditions in WPM medium derived from seeds that had been abraded to overcome dormancy, (d) seedling cultivated in the dark in WPM medium derived from seeds that had been immersed in sulfuric acid to overcome dormancy.

Conclusions

Chemical and mechanical scarification were efficient in overcoming dormancy in *S. macranthera* seeds and favored their germination under both light and dark incubation conditions; nutrient mineral concentrations in the culture media influenced germination responses. The greatest germination percentages were observed in MS medium with chemical scarification under light incubation conditions, and with mechanical scarification and incubation in the dark. The different scarification treatments did not influence the SGI values in relation to the different culture media utilized. The greatest SGI value in MS medium was obtained with chemical scarification. The length of the aerial part of the seedlings was principally influenced by germination in the dark, and scarification of the seeds with sulfuric acid or sandpaper favored the development of the aerial portions of the seedlings cultivated in both the MS and MS 1/2 medium. Neither the scarification methods nor the culture media significantly influenced the length of the radicle among seedlings cultivated in the light; under dark conditions the radicles grew statistically longer in MS ½ medium.

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