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# BREAKING SEED DORMANCY IN *Sesbania punicea* (Cav.) Benth

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**ABSTRACT** - *Sesbania punicea* is a native plant of Brazil, has ornamental features inflorescences arranged in bunches of orange coloring. Its main form of propagation is through seeds, but these present integumentary dormancies, for which there is still no method of overcoming established. The objective of this study was to evaluate methods of breaking dormancy in *S. punicea* seeds for seedling production. The treatments used were control - no treatment, hand scarification with sandpaper grade 80 for 40 sec.; hand scarification with sandpaper grade 80 for 60 sec.; thermal treatment by soaking in water from 80ºC to room temperature and chemical treatment - stirring in 0.5 L solution of 12% sodium hypochlorite (NaClO), 3 mL of hydrochloric acid (HCl) and 20 g of commercial sodium hydroxide (NaOH) dissolved in 1 L of water, for 45 min.).After the treatments, the seeds were wrapped in rolls of autoclaved germitest paper and moistened with distilled water 2.5 times the weight of the sheet. Then, the rolls were placed in plastic bags, stored in the upright position, and incubated at 25ºC, in a 16 h photoperiod, for 14 days. A completely randomized design with four replications of 25 seeds was used. The germination percentage, average germination time, germination speed index, percentage of normal seedling formation, shoot length, root system length and percentage of hard seeds were evaluated. There was a difference for all the analyzes evaluated, except for the length of the aerial part. The dormancy breaking method through mechanical scarification using sandpaper for 60 sec., enables the propagation and formation of *S. punicea* seedlings.
**Keywords:** Fabaceae, native plant, sexual propagation.

# *SUPERAÇÃO DE DORMÊNCIA EM SEMENTES DE Sesbaniapunicea(Cav.) Benth*

**RESUMO -** *Sesbania punicea* é uma planta nativa do Brasil, tem como características ornamentais inflorescências organizadas em racemos de coloração alaranjada. Sua principal forma de propagação é por meio de sementes, contudo estas apresentam dormência tegumentar, porém ainda não há método de superação estabelecido. O objetivo deste trabalho foi avaliar métodos de superação de dormência em sementes de *S. punicea* para a produção de mudas. Os tratamentos utilizados foram controle - sem tratamento, escarificação manual com lixa grau 80 por 40 seg.; escarificação manual com lixa grau 80 por 60 seg.; tratamento térmico por imersão em água de 80ºC até a temperatura ambiente e tratamento químico - agitação em 0,5 L de solução de hipoclorito de sódio a 12% (NaClO), 3 mL de ácido clorídrico (HCl) e 20 g de hidróxido de sódio comercial (NaOH) dissolvido em 1 L de água, por 45 min.). Após os tratamentos, as sementes foram acondicionadas em rolos de papel germitest autoclavado e umedecidos com água destilada 2,5 vezes o peso da folha. Em seguida, os rolos foram colocados em sacos plásticos, armazenados na posição vertical e incubados a 25ºC, em fotoperíodo de 16 h, por 14 dias. Utilizou-se delineamento inteiramente casualizado, com quatro repetições de 25 sementes. Foram avaliados a porcentagem germinação, tempo médio de germinação, índice de velocidade de germinação, porcentagem de formação de plântulas normais, comprimento da parte aérea, comprimento do sistema radicular e porcentagem de sementes duras. Houve diferença para todas as análises avaliadas com exceção do comprimento da parte aérea. O método de quebra de dormência por escarificação mecânica com lixa por 60 segundos, possibilita a propagação e formação de mudas de *S. punicea*.
**Palavras-chave:** Fabaceae, planta nativa, propagação sexuada.

## INTRODUCTION

Among the species native to South America, *Sesbania punicea* (cav.) Benth, in the family Fabaceae, stands out for itsornamental qualities, particularly for the shape of the crown and the shape and orange-red color of the inflorescences. The species can be used for hedging or single planting. It is a 2-4 m high tree and grows in sandy and swampy soils (STUMPF et al., 2016). In Brazil, it is commonly known as "*fedegoso-da-praia*", or "*acácia-das-flores-vermelhas*". The species is distributed throughout the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro and MatoGrosso do Sul. It has been commercialized in the international market as an ornamental plant, but is very little known in Brazil (QUEIROZ, 2020). The ornamental potential of the species has been searched, but few studies have reported onpropagation by seed, which is essential for production and utilization as ornamental.

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Several genera of the family Fabaceae with ornamental function as *Bauhinia*, *Caesalpinia*, *Calliandra*, *Cassia*, *Senna* and *Sesbania* have seeds with dormancy (JAYASURIYA et al., 2013). Seed dormancy is considered to prevent germination, even when environmental conditions, including water, temperature, light and gases, are favorable. It consists of an adaptive mechanism to distribute germination over time so that germination occurs under favorable environmental conditions for seedling survival and plant maturation (BASKIN; BASKIN, 2014). However, the absence of uniformity of germination is one of the negative aspects of seed dormancyas it prevents the provision of a sufficient quantity of plants for commercial production.

Dormancy has been attributed to the impermeability of the integument to water and gases, mechanical resistance of the integument, rudimentary embryo, physiological immaturity, presence of inhibitory substances and combination of two or more of these characteristics (BASKIN; BASKIN, 2014; MARCOS FILHO, 2015). Seeds of *Sesbania rostrate* Bremek. & Oberm., *S. exasperate* Kunth, *S. tetraptera* Hochst. ex Baker, *S. sesban* (L.) Merr. and *S. virgata* (Cav.) Poir. have physical dormancy caused by impermeability to water, in which the integument prevents the absorption of water, being necessary its rupture for the imbibition and the beginning of the germination process (VEASEY et al., 2000).

The breaking of physical dormancy involves disruption or dislodgement of water-gap structures, which act as environmental signal detectors for germination. Once the closed water gap opens, a seed can imbibe water rapidly and germinate under a wide range of conditions (TIWARI et al., 2016). Due to the low germination percentages observed and the presence of physical dormancy in other species of the genus, the objective of this work was to evaluate methods for breaking dormancy in *S. punicea* seeds in order to achieve seedling production.

## MATERIAL AND METHODS

Brown-color fruits of *S. punicea* were collected from 20 mother trees *in situ*, in Barra do Ribeiro - RS, Brazil (30°21'27"S 51°20'32"W), on February 22, 2016. One specimen of the species was registered in the Herbarium of the Biosciences Institute with identification number ICN185165.

The seeds were removed by hand and stored in refrigerator at 4 to 6ºC, in glass bottles for two months. The water content of 9.5% was determined according to the methodology proposed by Brasil (2009), using four repetitions of 1g each. Before the treatments to break dormancy had been initiated, the seeds were disinfected with solution of 2% sodium hypochlorite (a. i.), for 5 min, and triple washed in distilled water, for 1 min.

Breaking dormancy treatments were as follows: control - no treatment, hand scarification with sand paper grade 80 for 40 sec.; hand scarification with sandpaper grade 80 for 60 sec.; thermal treatment by soaking in water from 80ºC to room temperature and chemical treatment - stirring in 0.5 L solution of 12% sodium hypochlorite ($NaClO$), 3 mL of hydrochloric acid ($HCl$) and 20 g of commercial sodium hydroxide ($NaOH$) dissolved in 1 L of water, for 45 min.).

After the treatments, the seeds were wrapped in rolls of autoclaved germitest paper and moistened with distilled water 2.5 times the weight of the sheet (BRASIL, 2009). Then, the rolls were placed in plastic bags, stored in the upright position, and incubated at 25ºC, in a 16 h photoperiod, for 14 days.

The experiment was arranged in a completely randomized design, with four replications of 25 seeds, totaling 100 seeds per treatment. Data were analyzed by variance analysis (ANOVA), and means were compared by the Tukey's test (5% probability). The statistics software CoStat (CoStat, 2022) was used to test data normality, and SigmaPlot 11.0 (SIGMAPLOT, 2022) was used for mean comparisons.

The percentage of physiological germination (radicle protrusion) (% G) was calculated, at every 2 days, from the first germinated seed. The mean germination time (MGT) (SILVA; NAKAGAWA, 1995) and the germination speed index (GSI) (SANTANA; RANAL, 2004) were evaluated. Normal seedlings (% NS) were those that presented all essential structures well developed, complete, proportional and healthy (BRASIL, 2009). The length of shoots (SL) and roots (RL) were measured with a millimeter ruler. At the end of the experimental period, the percentage of hardseededness (% HS) was also calculated. Hardseededness were considered to be those that remained without absorbing water until the end of the experiment, maintaining the appearance of seeds recently placed in the substrate (not swollen) (BRASIL, 2009).

When the assumptions of normality were not met, data of variable GSI were transformed to$\sqrt{x + 0.1}$, normal seedlings to$\sqrt{x + 5}$, and abnormal seedlings to$\sqrt{x}$. After data transformation, MGT continued to fail to meet the assumption of normality, then, for this case, the non-parametric KrusKal Wallis test was used.

## RESULTS AND DISCUSSION

Significant differences were found for all variables analyzed, with the exception of shoot length (Table 1). The highest germination percentage of seeds of *S. punicea* of 86% was found for the treatment using hand scarification with sandpaper for 60 s, followed by hand scarification with sandpaper for 40 s with 59%.

Similar results were obtained for other species of the Fabaceae family as *Pithecellobium dulce* (Roxb.) Benth. (PEREIRA et al., 2015), *Astragalus cicer* L. (STATWICK, 2016), *Albizia pedicellaris* (DC.) L. Rico (FREIRE et al., 2016), *Bituminaria basaltica* Miniss., C. Brullo, Brullo, Giusso & Sciandr. and *B. bituminosa* (L.) C.H.Stirt. (CARRUGGIO et al., 2020) in which superior results were observed when using mechanical scarification treatment. The opposite was observed for *Sesbania sesban*, in which the use of scarification with sandpaper was the worst result among the treatments tested, with leaching in running water having the highest percentage of germinated seeds (LANGAT; MAINA, 2018).

Physical dormancy is caused by one or more water impermeable layers of palisade cells in the seed (TIWARI et al., 2016). Soaking seeds in hot water provides a suitable temperature range for enzymes that catalyze reactions that break down the seed coat, while mechanical and acid scarification physically destroys seed coat hence allowing absorption of water and gases for germination to occur (LANGAT; MAINA, 2018).

**TABLE 1 -** Average germination values (%G), mean germination time (MGT) (days), germination speed index (GSI), normal seedlings (%NS), shoot length (SL) (cm), root length (RL) (cm) and hardseededness (HS) in treatments for breaking dormancy of *Sesbania punicea* (Cav.) Benth.

| Treatments[1] | CON | HS 40 | HS 60 | THS | CHS | p-valor | CV (%) |
|---|---|---|---|---|---|---|---|
| %G | 6 d* | 59 b | 86 a | 37 c | 25 c | >0,01 | 16.12 |
| MGT (days) | 7.25 ab | 4.25 a | 3.75 a | 10.5 b | 5.75 ab | >0.01 | 40.57 |
| GSI | 0.304 d | 4.71 b | 7.41 a | 0.95 cd | 1.37 c | >0.01 | 16.53 |
| %NS | 3 d | 45 b | 73 a | 8 cd | 19 c | >0.01 | 12.84 |
| SL (cm) | 3.38 | 6.10 | 4.98 | 3.63 | 7.08 | $0.05^{ns}$ | 35.79 |
| RL (cm) | 2 b | 7 a | 7 a | 4 b | 7 a | >0.02 | 32.15 |
| HS(%) | 94 d | 41 b | 14 a | 63 c | 75 c | >0.01 | 12.25 |

[1]CON = control, HS 40 = hand scarification with sandpaper (grade 80) for 40 sec., HS 60 = hang scarification with sandpaper (grade 80) for 60 sec, THS = thermal at 80ºC to room temperature, CHS = chemical: 12% NaClO, 3 mL of HCl and 20 g of NaOH (T5), ns = not significant. *Means followed by different letters on the line differ from each other by the Tukey test, at the level of 5% probability of error.

Although all the treatments used in this study are indicated for breaking seed dormancy with impermeability of the seed coat to water (MARCOS FILHO, 2015), the use of mechanical scarification with sandpaper for a longer time (60 s) was more effective than the use of thermal and chemical scarification, which may have also been influenced by the time of immersion of the seeds in these treatments. This was observed for *Lotus corniculatus* L., *Medicago lupulina* L., *Melilotus albus* Medik., *Melilotus officinalis* L., *Vicia sativa* L., *Trifolium campestre* Schreb. (MONDONI et al., 2013), *Albizia pedicellaris* (FREIRE et al., 2016), *Bituminaria basaltica* and *B. bituminosa* (CARRUGGIO et al., 2020), where in the effectiveness of chemical treatment with $H_2SO^4$ increased significantly with increasing immersion time.

Thermal scarification in boiling water, allowed to cool gradually to room temperature for different immersion times was effective in promoting *B. bituminosa* germination, however, this one treatment did not increase in *B. basaltica* over 50% (CARRUGGIO et al., 2020).

For *Senna multijuga* (Rich.) H.S.Irwin & Barneby (Fabaceae) the use of water with an initial temperature of 80°C or 100°C was an efficient method to break dormancy of seeds (RODRIGUESJUNIOR et al., 2014), contrary to what was observed in *Astraga luscicer* (STATWICK, 2016) and *Pithecellobium dulce* (PEREIRA et al., 2015)in which seeds from the hot water scarification treatment 95°C and 100°C, respectively, no germinated. Similar result was found for *Melilotus albus*, *M. officinalis* and *Vicia sativa* (Fabaceae), where germination was lower that 10% after all the treatments using hot water (MONDONI et al., 2013).

Events in nature, such as high and alternating temperatures, are known to break physical dormancy effectively in some species, allowing water to enter through a gap. However, seed storage at different alternating temperatures does not promote differences in the percentage of germination of *Cassia leptophylla* Vogel or *Senna macranthera* (DC. ex Collad.) H.S. Irwin & Barneby (Fabaceae) regardless of the upper and lower limit of temperatures. However, seed exposure in a humid environment substrate at 50°C for 4 h broke the seed hardness, and after 7 days the treated seeds had significantly higher mass than the controls (DE PAULA et al., 2012). This was observed for *Lotus corniculatus* L., *Medicago lupulina* and *Trifolium campestre* Schreb. (Fabaceae) that both the maintenance period and the water temperature influence the response of each species (MONDONI et al., 2013).

Thus, according to the data presented in the present work, the use of thermal and mechanical scarification was not ineffective for breaking dormancy of the seeds of *S. punicea*, and it is possible that adjustments in the seed residence times in these treatments may result in superior results. Comparison the scarification, sandpaper for 40 sec., 60 sec. and chemical treatment showed no significant differences for mean germination time (MGT) from the control. Differences was only foundin for sandpapertreatments for thermal treatment. Results like were verify for *Robinia pseudoacacia* L., *Acacia dealbata* Link, *Cytisus scoparius* (L.) Link, *C. multiflorus* (L'He´r.) Sweetand *Ulex europaeus* L. (Fabaceae) in which the use of mechanical scarification promoted superior results for the variables related to seed germination speed in relation to thermal treatments (PEDROL et al., 2018).

The GSI (germination speed index) followed the same behavior as MGT, with superior results for treatments using mechanical scarification. This result is similar of *Senna cana* (Nees & Mart.) H.S. Irwin & Barneby (Fabaceae) in which seeds scarified with sandpaper, was higher GSI than the thermal treatment imbibition in water and imbibition in hot water at 80°C (MEDEIROS et al., 2019). The same was observed for

*Albizia pedicellaris* in which treatments using mechanical scarification were superior to treatments using chemical and thermal scarification for GSI (FREIRE et al., 2016).

However, for *Senna multijuga* (Fabaceae) the use of water with an initial temperature of 80°C or 100°C was an efficient method to break dormancy of seeds and did not cause damage to the embryo, thus enabling a high percentage of normal seedlings and GSI values significantly higher than those of the control. The percentage of seeds that remained hard following the evaluation period was 90, 10 and 2% for the control treatment, and immersion in water at 80°C and 100°C, respectively (RODRIGUES JUNIOR et al, 2014).

The assessment of normal seedlings is important to determine the effective size of the plant population, which can be obtained for the production of seedlings. The highest rates of normal seedlings were found for sand paper scarification. The other treatments, with lower rates of normal seedlings, were due to the inefficiency breaking dormancy that resulted in low germination, and therefore, in general less formation of seedlings.

In this study, no difference was found between treatments for shoot length. The seedlings showed an average growth of up to 5.0 cm. This result differed from that found for *Pithecellobium dulce* (Fabaceae), in which seed dormancy breaking by sandpaper scarification showedhigher seedling growth (PEREIRA et al., 2015). The results found in this study also differ from those found for *Melilotus albus* (Fabaceae) in which the development of the aerial part was less when using acid scarification (MONDONI et al., 2013).

Root length was greater in the treatments with scarification with sandpaper for 40 sec., 60 sec., and chemical treatment. Each species has a specific behavior regarding the growth of seedlings, both for shoots and roots. Unlike the results found in this work, no difference in root length was found for *Pithecellobium dulce*, regardless of the method for breaking seed dormancy (PEREIRA et al., 2015).

The highest rate of hardseededness was found in the control (94%), followed by the chemical (75%) and thermal (63%) treatments. Similarly results also were observed for seeds of *Desmanthus virgatus* (L.) Willd. (Fabaceae) in which non-scarified seeds had values greater than 95% of hardseededness (RICHARD et al., 2018). Similar to the results of this study, *Chloroleucon foliolosum* (Benth.) G.P. Lewis (Fabaceae) had the highest rate of hardseededness in the control treatment, when compared with the chemical treatment with sulfuric acid and the mechanical treatments with sandpaper (SILVAet al., 2014). Hardseededness are the seeds that remain without absorbing water for a longer period than normal (BRASIL, 2009). Species, year of collection and storage had significant effects on imbibition as observed for species of *Caragana* (Fabaceae), in which the percentage of impermeable seeds of C. *roborovskyi* Kom. and *C. spinosa* (L.) DC significantly decreased after dry storage at room conditionsfor 6 months, regardless of harvest year (CHEN et al., 2019). Despite the high degree of germination found (86%) studies testing other methods can be applied together with the storage methods of these seeds, further enhancing this genetic material.

## CONCLUSION

The dormancy breaking method through mechanical scarification using sandpaper for 60 sec., enables the propagation and formation of *S. punicea* seedlings.

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