Journal of Plant Sciences
2016; 4(5): 126-131
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20160405.16
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Overcoming Dormancy in Seeds of *Dialium guianense* (Aubl.) Sandwitch (Fabaceae – Caesalpinioideae)

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To cite this article:

Pedro Henrique Oliveira Simões, Denmora Gomes de Araújo, Marcos André Piedade Gama, Luiz Fernandes Silva Dionisio, Emanoel Rodrigues Caldas, Denise Siqueira Pereira, Glauco André dos Santos Nogueira, Cândido Ferreira de Oliveira Neto, Juscelino Gonçalves Palheta, Josilene do Carmo Mescouto de Sousa, Lenilson Ferreira Palheta. Overcoming Dormancy in Seeds of *Dialium guianense* (Aubl.) Sandwitch (Fabaceae – Caesalpinioideae). *Journal of Plant Sciences.* Vol. 4, No. 5, 2016, pp. 126-131. doi: 10.11648/j.jps.20160405.16

Received: September 27, 2016; **Accepted**: October 5, 2016; **Published**: October 27, 2016

Abstract: This study has aimed to test different methods of overcoming dormancy, accelerating and standardizing the initial seedling settling and determining the pre-germination treatment that is more effective for the species *Dialium guianense* (Aubl.) Sandwitch. The seeds were submitted to the following treatments: Control (T1) – only asepsis of seeds was done by immersion in sodium hypochlorite (2 %) for 3 minutes; immersion in sulfuric acid (98 % p.a.) for 5, 10, 15 and 20 minutes (T2, T3, T4 and T5, respectively); mechanical chisel plow (T6) – asepsis of seeds by immersion in sodium hypochlorite (2%) for 3 minutes and seed surface abrasion by 80 sandpaper for 15 seconds, always at the edge of the seeds (to avoid damaging the embryo) and then immersed in water for 24 hours. The *Dialium guianense* seeds have cutaneous dormancy and treatments of chemical chisel plow by means of immersion of the seeds in concentrated sulfuric acid (98%) for 15 and 20 minutes were more effective in overcoming seed dormancy, providing highest values of emergence and Emergence Speed Index (ESI) of seedlings, as well as the highest average of dry matter allocation in the shoot and primary root.

Keywords: Emergency, Chemical Scarification, Tree Species

1. Introduction

The species *Dialium guianense* (Aubl.) Sandwitch [Leguminosae (Caesalpinioideae)] is a tree that is native of South and Central America [1]. It is sized as a tree, with great economic and social potential and is efficient in cycling soil nutrients, thus it can be recommended for mixed reforestation with preservationists or ecological purposes [2]. The wood of this species is used for various purposes and the fruits are mainly consumed by their fleshy and edible mesocarp [2]. There are reports that the leaves and roots of *D. guianense* are used as antimalarial agents and as a food supplement for women during pregnancy, probably because of the micronutrient density of these plant parts [3].

The *Dialium guianense* seeds have cutaneous dormancy, coffee color, a glassy endosperm surrounding the cotyledon and radicle and their germination is epigeal [4]. Seed dormancy can be defined as the temporary inability to germinate under favorable environmental conditions and it can be regulated by factors such as water content, temperature, soil, light and oxygen concentrations, and internal factors such as plant hormones [5]. The various treatments used to overcome this kind of dormancy are based on the principle of dissolving the waxy cuticular layer or forming grooves/holes in the seeds integument [6].

In the seeds with impermeability, there is the presence of hard integument that is impermeable to water and gases, which perhaps can physically restrict the embryo growth [7]. According to [8], under natural conditions the impermeability gradually decreases so that a certain ratio of seeds germinates at each period of time. For the production of forest species seedlings, particularly of some legume species, it can be considered a problem because of the difficulty to break the seed integument and provide a uniform germination [9].

Among the treatments successfully used to overcome the cutaneous dormancy of tree species stand out: mechanical chisel plow (seed abrasion on rough surfaces) [10-11], chemical chisel plow (using acids) [12-14], besides other processes, such as temperature shocks and immersion in hot water [15-16]. The application and effectiveness of these treatments depend on the degree of dormancy, which varies among different species origins and sampling years [7-6].

This study has aimed to test different methods of overcoming dormancy, accelerating and standardizing the initial seedling settling and determining the pre-germination treatment that is more effective for the species *Dialium guianense* (Aubl.) Sandwitch.

2. Materials and Methods

The *Dialium guianense* seeds used in the experiment come from matrices located in the Ilha de Germoplasma, (concrete gravity) Tucuruí Dam, in the Brazilian state of Pará, geographically positioned 3º51'58.3" S and 49º38'25.8" W. After collection, the seeds were transported to the Laboratório de Tecnologia de Sementes do Instituto de Ciências Agrárias (ICA; Seed Technology Laboratory of the Institute of Agricultural Sciences) of Universidade Federal Rural da Amazônia (UFRA), in Belém, state of Pará, Brazil.

Six tests were performed: Control (T1) – only asepsis of seeds was done by immersion in sodium hypochlorite (2 %) for 3 minutes; immersion in sulfuric acid (98 % p.a.) for 5, 10, 15 and 20 minutes (T2, T3, T4 and T5, respectively); mechanical chisel plow (T6) – asepsis of seeds by immersion in sodium hypochlorite (2%) for 3 minutes and seed surface abrasion by # 80 sandpaper for 15 seconds, always at the edge of the seeds (to avoid damaging the embryo) and then immersed in water for 24 hours. For all treatments, 120 seeds were used in a completely randomized design with four replications of 30 seeds for each treatment.

For the chemical chisel plow experiment with sulfuric acid, the seeds were separated for each exposure time, which were separately placed in a beaker, adding sulfuric acid to cover the seeds. After the immersion time specified for each treatment, the seeds were removed from the beaker, placed in a metal strainer and washed in running water for about 10 minutes to remove acid and subsequently dried in absorbent paper. After administration of the dormancy breaking treatments, sowing in a sterilized substrate took place by autoclaving at 120 °C [17], consisting in sand and sawdust in the ratio 1:1 (v/v), depth of 1 cm, spacing of 2x2 cm, arranged in plastic trays within a greenhouse. Irrigation was done daily with a manual plastic sprinkler, aiming to maintain the maximum amount of water that the substrate used can hold. The counting of emerged seedlings occurred daily for 15 days.

The seeds water content was determined using the oven method at 105 ± 3 °C for 24 hours [17]. The parameters used to evaluate the seeds were: emergence percentage (E%) and Emergence Speed Index (ESI) [18]. The seedling emergence counts were performed daily until the 15th day after sowing. They seedlings which had cotyledons totally above the substrate surface were considered as having emerged.

The shoot length (SL) was also determined, taking into account the distance from the collar to the end of the plant apical bud, and the root system length (RSL), starting from the collar to the end of the main root. The dry weight of primary roots, stems and leaves was determined using material from seedlings emerging at the end of each treatment. After cutting the organs with a scalpel, they were placed in properly identified kraft paper bags and taken to an oven with forced ventilation at the temperature of 80 °C for 24 hours. Then they were individually weighed in an analytical balance, with the result expressed in grams (average).

The values generated were assessed through statistical analysis for the normal distribution using the Kolmogorov-Smirnov test. The data were transformed into an arcsen $\sqrt{x/100}$ and into the $\sqrt{x+0{,}5}$ when the parameter values were equal to zero. Next, an analysis of variance was used for the results, and the means were compared through the Tukey test ($p < 0.05$) using the Statistical Analysis System (SAS) software [19].

3. Results and Discussion

At the beginning of the experiment, the seed water content was 12.3%, which is within the range of 5 to 20% described by [20]. As the percentages found in most plant species. Similar values were found in other legume species such as *Parkia velutina* Bernoist, 11.3 % [21], *Parkia discolor* Spruce *ex* Benth., 9.8 % [22] and *Parkia multijuga,* 11.0% [23].

In line with the humidity value, similar to the data found for the other species mentioned, *Dialium guianense* presented emergence values above 83% for the most effective treatments, indicating that this percentage may be related to good quality seeds. Significant difference was noticed among the soaking time of seeds in sulfuric acid, chisel plow with sandpaper and the control to the percentage of emergence of seeds (Figure 1).

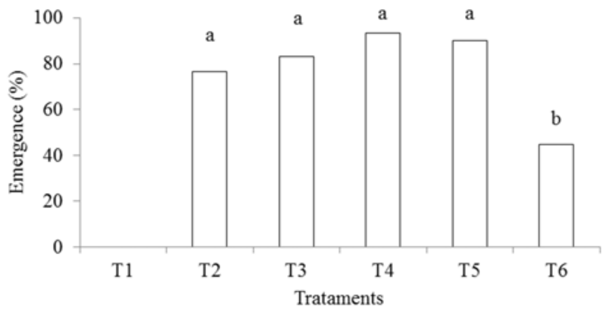

Figure 1. *Average percentage value emergency Dialium guianense seeds treated in six different treatments. (T1) - Control; Immersion in sulfuric acid for 5, 10, 15 and 20 minutes (T2, T3, T4 and T5, respectively); (T6) - Mechanical scarification. Means followed by the same letter in the column do not differ by Tukey test (p> 0.05).*

The highest percentage of seedlings emergence occurred when the seeds were submitted to immersion treatment in sulfuric acid. Immersion treatments for 15 and 20 minutes accounted for 93.33% and 90% of seedling emergence, respectively, being statistically equal to each other. As for the seeds that have been scarified without the use of sulfuric acid, they had the lowest emergence values, and the control and chisel plow with sandpaper treatments were 0.83% and 45% of seedling emergence, respectively.

However, [6] evaluating dormancy breaking of seeds of *Mimosa setosa* Benth. (Leguminosaea), have found that chemical chisel plow with sulfuric acid for one to three minutes have allowed a larger number of germinated seeds in a shorter time. Similar results using H_2SO_4 were found in seeds of *Sideroxylon obtusifolium* [14].

It was found that treatments with sulfuric acid in the present study have determined the softening and cracking in the integument, favoring its permeability and facilitating the overcoming of seeds dormancy. These results, obtained with chemical chisel plow, show that this species has integument impermeability to water ingress, which is one of the most common causes of dormancy in legumes [20], and also that chisel plow with sandpaper and subsequent immersion in water (T6) was insufficient to adequately increase the permeability of the integument and thus promote the absorption of water in the seed and early germination process. In seeds impermeable to water, the action of sulfuric acid in weakening the integument can be a result of removing the cuticle and consequent exposure of macro sclereids layers [24].

Similar results were obtained in other species, such as *Parkia platycephala* Benth. [8], where chemical chisel plow with H_2SO_4 between 15 and 45 minutes was efficient to overcome the dormancy imposed by the integument, reaching values near 100% of seedling emergence, and for the species *Parkia panurensis* and *Parkia velutina* [23], where chemical chisel plow with H_2SO_4 for 20 and 30 minutes is effective in overcoming dormancy of these two species. In turn, studying the species *Libidibia ferrea* var. *leiostachya* (Benth.) L. P. [25], have found that, for stored seeds, soaking for 10 minutes in sulfuric acid was enough to overcome dormancy. However, for freshly harvested seeds, the time required to overcome cutaneous dormancy is larger and soaking for 20 to 30 minutes has favored the germination percentage. According to [26], families such as Fabaceae, Cannaceae, Convolvulaceae, Malvaceae and Chenopodiaceae, for example, have a tissue called osteosclereids (a type of sclereids) on their seed coat which prevents the entry of water and delays germination.

It can be seen in Figure 2 that the treatments with H_2SO_4 can be used in overcoming the impermeability of the integument of the *Dialium guianense* seeds and the emergence increase by means of the application of this method is associated with the exposure time increase of the seeds to the acid, with the emergence percentage means between treatments of 5, 10, 15 and 20 minutes (T2, T3, T4 and T5, respectively) showing no statistical difference between them.

In seeds with impermeable integument, treatments done with the use of sulfuric acid have proved successful in overcoming this type of dormancy as reported by [20], and seen in seeds of *Parkia gigantocarpa* Ducke [9] *Senna silvestris* (Vell.) H. S. Irwin & Barneby [27], *Lithraea molleoides* (Vell.) Engl. [28], *Ipomoea indivisa* and *Ipomoea purpurea* [29].

However, despite the lack of statistical difference among treatments with the use of the acid, increase of the exposure time of the seeds to the acid over 15 minutes can damage the embryo structures, because sulfuric acid can promote the permeability of the seeds in a way that affects their viability when reaching areas below the seed coat and causing damage to the embryo. This fact is related to the integument resistance, which is a different characteristic for each species, and may be influenced by the maturation stage of the fruit and seed storage time. Thus, this feature will determine the ideal time to use the acid as a chisel plow method. As seen in Figure 2, after 15 minutes of immersion there was a decline of emergence (%), indicating that the acid may be causing damage to the seed embryo and thus affecting its viability.

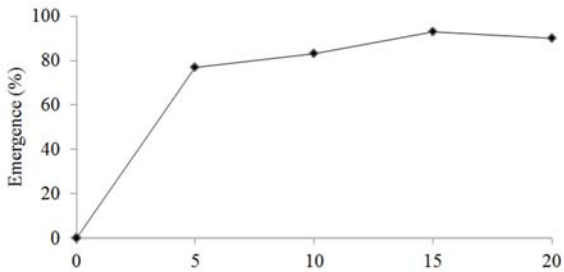

Figure 2. *Relationship between the percentage of emergence of Dialuim guianense seedlings and immersion time in sulfuric acid.*

Regarding the immersion time in H_2SO_4, in this study, immersion for 15 minutes has reached 93.33% of seedling emergence of *D. guianenses*. [8], when working with *Parkia platycephala*, have reached emergence values next to 100%

in immersion periods in H_2SO_4 between 15 and 45 minutes. As for [27], they have obtained emergence values above 80% with immersion times of 0.5, 3 and 5 minutes for the species *Senna silvestris* (Vell.) H. S. Irwin & Barneby. Thus, it is observed that the success in treatments with H_2SO_4 is related to the exposure time of seeds to acid and species, demonstrating that this technique can be harmful if used without considering the specifics of the species, especially the resistance of its integument.

As for the vigor determined by the Emergence Speed Index (ESI) (Figure 3), it was observed that the highest value was obtained with the chemical chisel plow treatment with immersion in concentrated sulfuric acid for 15 minutes (T3). The mechanical chisel plow by means of seed surface abrasion has corroded the integument, allowing the absorption of water and starting the germination process; however, this treatment showed inferior results compared to seeds that had undergone chisel plow with H_2SO_4, indicating that abrasion by sandpaper, although providing good emergence, provided emerging seeds with less vigor. The seeds that had undergone chisel plow showed greater impairment of the embryo development, something that is connected to the action of H_2SO_4, which acts over the whole area of the integument, while mechanical chisel plow was over only one area.

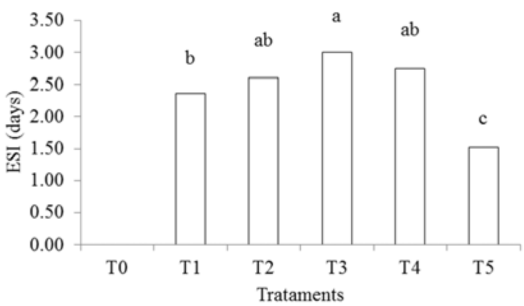

Figure 3. *Mean emergency speed index values (ESI) of Dialium. guianense seeds treated in six different treatments. (T0) - Control; immersion in sulfuric acid (98%) for 5, 10, 15 and 20 minutes (T1, T2, T3 and T4, respectively); (T5) - Mechanical scarification; Means followed by the same letter in the column do not differ by Tukey test (p> 0.05).*

Similar results were found with species Parkia platycephala [8]; Parkia multijuga, P. velutina and P. panurensis [23]; Lithraea molleoides [28]; Ipomoea indivisa, I. grandifolia and I. purpúrea [29]. For treatments without using acids, [22] have tested different types of lopping, pyrograph, sole iron and sanding, and demonstrated that the proximal and distal/proximal sanding were among the most effective for producing high vigor seedlings of the species Parkia discolor.

As for the length of shoot and root crown, there was no statistical difference among treatments, with the exception of seeds without chisel plow (T0), which had the lowest results for these two variables (Figure 4). The biggest shoot length averages were found with chemical chisel plow with H2SO4 for 5, 10, 15 and 20 minutes of immersion (T1, T2, T3 and T4, respectively). These results are probably due to a lower consumption of seed reserves during the germination process, since it occurred rapidly and uniformly; therefore, these reserves were intended for growth of the resultant seedling [8]. Similar results were found by [30], where the preconditioning of Albizia pedicellaris (DC.) L. Rico seeds in concentrated sulfuric acid was efficient, as it increased the seedlings height. In contrast to the results of this work with D. guianenses, [31], have observed a reduction in the growth of seedlings of Pithecellobium dulce (Roxb.) Benth. from chisel plow with H2SO4 for 5 and 10 minutes, over treatment with manual chisel plow (sandpaper).

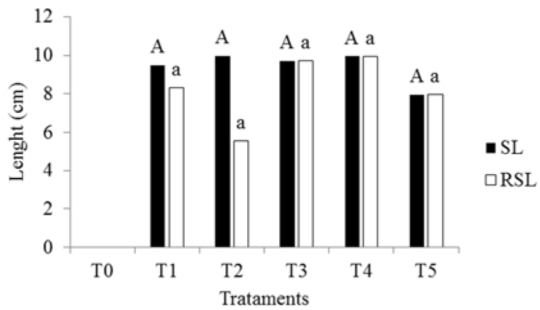

Figure 4. *Shoot length average values (SL) and length of the root system (RSL) of Dialium guianese seedlings treated in six different treatments. (T0) - Control; immersion in sulfuric acid (98%) for 5, 10, 15 and 20 minutes (T1, T2, T3 and T4, respectively); (T5) - Mechanical scarification; Means followed by the same capital letter (for the variable SL) or lower (for variable RSL) do not differ statistically by the Tukey test (p> 0.05).*

The experiment with *Dialium guianense* has shown that there was an increase in the shoot length (SL) and the primary root length (PRL), both in treatments with immersion in H_2SO_4 as in the mechanical chisel plow with sandpaper soaking in water for 24 hours, compared to control treatment, demonstrating that these methods were effective in breaking dormancy of the species and promoting greater uniformity and vigor in their development.

The dry mass allocation (g) in the shoot (SDM) and in the primary root (RDM) was also influenced by the treatments, with immersion in H_2SO_4 having the best results and statistically equal to each other for the SDM variable (Figure 5). For the RDM, the immersion treatments in acid for 15 and 20 minutes were more effective and statistically equal, providing heavier primary roots. The control treatment was inefficient in promoting increases in these variables. Thus, considering the two measures of great importance for the analysis of seedling vigor, the T3 and T4 treatments were the most effective for increasing shoot and primary root of D*ialium guianense* seedlings.

The other treatments had significantly lower values, not reaching half the dry weight of the two best treatments for RDM (Figure 5). Working with *Senna multijuga*, [32] have also found similar results, where the increase in sulfuric acid exposure time resulted in seedlings with larger collar diameter and a more developed root system. In seeds of *Parkia gigantocarpa*, according to results found by [9], acid chisel plow for a longer period of time improves the quality of primary roots, indicating that the greater immersion time in acid is most suitable for germination and development of the species, producing seedlings that are more likely to settle in the environment in the shortest time.

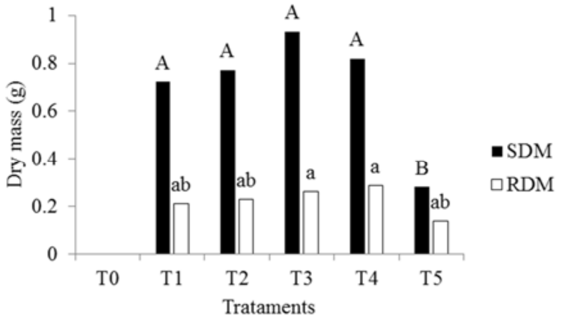

Figure 5. *Average values of shoot dry mass (SDM) and the primary root (RDM) of Dialium Guianese seeds treated in six different treatments. (T0) - Control; immersion in sulfuric acid (98%) for 5, 10, 15 and 20 minutes (T1, T2, T3 and T4, respectively); (T5) - Mechanical scarification; Means followed by the same capital letter (for MSPA variable) or lower (for variable MSR) did not differ statistically by the Tukey test (p> 0.05).*

4. Conclusions

The *Dialium guianense* seeds have cutaneous dormancy and treatments of chemical chisel plow by means of immersion of the seeds in concentrated sulfuric acid (98%) for 15 and 20 minutes were more effective in overcoming seed dormancy, providing highest values of emergence and Emergence Speed Index (ESI) of seedlings, as well as the highest average of dry matter allocation in the shoot and primary root.

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