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Nota Técnica

Overcoming dormancy in *Tachigali micropetala* (Ducke) Zarucchi & Pipoly (Fabaceae – Caesalpinioideae) seeds

Superação de dormência em sementes de *Tachigali micropetala* (Ducke) Zarucchi & Pipoly (Fabaceae – Caesalpinioideae)

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ABSTRACT

The use of forest species for the production of seedlings has been intensified due to the need for recovery of deforested areas and biomass production. However, the domestication of native forest species is restricted due to a lack of studies related to the ecology, physiology, and morphology of its seeds. The species *Tachigali micropetala* (Ducke) Zarucchi & Pipoly, which belongs to the family Fabaceae, presents tegument dormancy that hinders the entry of water and consequently the germination. The objective of this work was to select the best treatments for breaking dormancy in *T. micropetala* seeds. The treatments were: control (without pre-germinative treatment), cut in the integument, scarification in sandpaper, immersion in sulfuric acid for 5, 10, 15, and 20 minutes and immersion in water at 80°C for 20 minutes. The sowing was done in germitest paper and the statistical design used was the completely randomized with eight treatments, five replicates of 25 seeds. For the sanity test, the seeds were submitted to two types of treatments: asepsis with 1.5% of sodium hypochlorite and control (seeds without asepsis). Each treatment contained 10 replicates, with 20 seeds each. The results obtained show that the treatments in acid for 15 and 10 minutes and mechanical scarification with sandpaper presented 90.4, 85.6 and 83.2% of germination, respectively, being these the most efficient for overcoming dormancy in *T. micropetala* seeds.

Keyword: Forest species; Scarification; Germination; Biometry

RESUMO

O uso de espécies florestais na produção de mudas tem sido intensificado devido à necessidade de recuperação de áreas desmatadas e produção de biomassa. No entanto, a domesticação de espécies florestais nativas é restrita devido à falta de estudos relacionados à ecologia, fisiologia e morfologia de suas sementes. A espécie *Tachigali micropetala* (Ducke) Zarucchi & Pipoly, pertencente à família Fabaceae, apresenta dormência de tegumento que dificulta a entrada de água e, consequentemente, a germinação. O objetivo deste trabalho foi selecionar os melhores tratamentos para a quebra da dormência em sementes de *T. micropetala*. Os tratamentos foram: controle (sem tratamento pré-germinativo), desponte, escarificação em lixa, imersão em ácido sulfúrico por 5, 10, 15 e 20 minutos e imersão em água a 80°C por 20 minutos. A semeadura foi realizada em papel germiteste e o delineamento estatístico utilizado foi o inteiramente casualizado com oito tratamentos, cinco repetições de 25 sementes. Para o teste de sanidade, as sementes foram submetidas a dois tipos de tratamentos: assepsia com 1,5% de hipoclorito de sódio e controle (sementes sem assepsia). Cada tratamento continha 10 repetições, com 20 sementes cada. Os resultados obtidos mostram que os tratamentos em ácido por 15 e 10 minutos e a escarificação mecânica com lixa apresentaram 90,4%, 85,6% e 83,2% de germinação, respectivamente, sendo estes os mais eficientes para superar a dormência em sementes de *T. micropetala*.

Palavras-chave: Espécie Florestal; Escarificação; Germinação; Biometria

1 INTRODUCTION

Tachigali micropetala (Ducke) Zarucchi & Pipoly, classified in the Fabaceae family, subfamily Caesalpinoideae, has an arboreal habit, flowers with a strong scent, green chalice, pistils and yellow stamens. The genus *Tachigali* was established by Aubl. (1775), which covers 84 species and six varieties (THE PLANT LIST, 2014). In Brazil, the genus *Tachigali* is represented by 58 species, being 26 endemic species, occurring in the phytogeographical areas of Amazonia, Caatinga, Cerrado and Mata Atlântica. According to Cruz *et al.* (2020), individuals of this genus have rapid growth, high biomass and leaf debris, allowing a rapid formation of litter, being this species an alternative for reforestation, recovery of degraded areas and production of renewable energy.

In 2021, 1,655,782 hectares were deforested in Brazil, with most of this amount in the Amazon (977,733) and Cerrado (500,537) biomes (MAPBIOMAS, 2022). Therefore, there is a global demand for restoration of ecosystems, encouraged by the integration of the ecological restoration with the international policies oriented

to biodiversity (TOLVANEN; ARONSON 2015). Actions related to conservation and restoration have traditionally been directed to tropical forests (OVERBECK *et al*., 2013; PALMA; LAURANCE, 2015). According to Fernandes *et al*. (2018), for the reforestation of devastated and urban areas the discernment of the reproductive biology, the germination and propagation of the native species are preponderant, so that the forest restoration can be made rationally. As a primary species, with fast growth and contributing to nitrogen fixation in the soil, *T. micropetala* is a viable option for ecological restoration (HUAMANTUPA-CHUQUIMACO *et al*., 2016).

However, the species' seed has dormancy, common in other representatives of the genus *Tachigali*, as well as in the Fabaceae family (ABREU *et al*., 2017; GEISLER *et al*., 2017). Dormancy is a resource that plants use to perpetuate their species, by preventing viable seeds from germinating, contributing to their permanence in nature and reducing the risk of extinction (PENFIELD, 2017). Despite the ecological advantages for the species, dormancy is not economically interesting, since it delays the initial establishment of seedlings and causes unevenness in the squad (BENTSINK *et al*., 2018). In *Tachigali*, this dormancy is attributed to the restriction of water diffusion in the embryo by the seed tegument, configuring itself as a physical type of dormancy, following the Fabaceae family individuals (PILON *et al*., 2012).

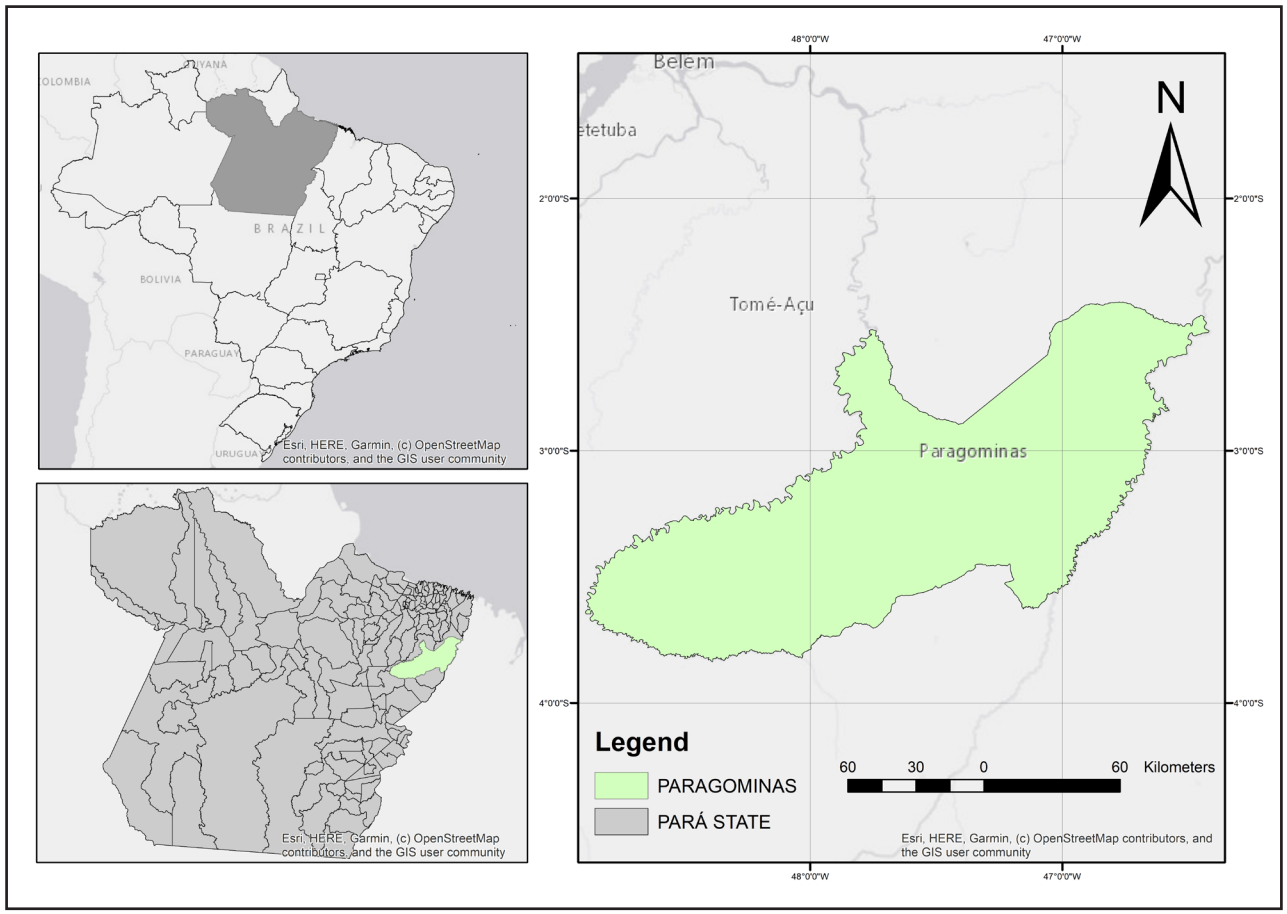
To overcome dormancy, there are methods of laboratory analysis carried out under controlled conditions of some or all external factors, which have been studied and developed to allow more regular, rapid and complete seed germination. These conditions, considered optimal, are standardized so that the results of the germination tests can be reproduced and compared, within limits tolerated by the Seed Analysis Rule (BRASIL, 2009). Among these methods, the most used are the topping of the proximal region of the radicle, the mechanical scarification, through sandpaper; chemical scarification, with concentrated sulphuric acid; and soaking the seed in hot water. All of these methods aim to break the physical barrier composed of the integument, thus allowing water and oxygen to enter the embryo and, consequently, germination (ABREU *et al*., 2017).

It is worth mentioning that, depending on the species, there is great variability in the effectiveness of dormancy breaking methods. Therefore, it is essential to assess which method is most effective in each species, to contribute to its commercial aptitude through the uniformity of seedling lots. Thus, the objective of this essay was to evaluate and select the best treatments for dormancy overcoming in *Tachigali micropetala* (Ducke) Zarucchi & Pipoly seeds.

2 MATERIAL AND METHODS

The present study was carried out in the Seed Laboratory of the Agricultural Sciences Institute (ICA), at the Federal Rural University of Amazonia (UFRA), Campus Belém. The mature seeds of *Tachigali micropetala* used in the trial came from matrices located in a forest reserve belonging to the company Mineração Paragominas SA of the Hydro group, in the municipality of Paragominas, Pará, latitude 02°59'45"S and longitude 47°21'10"W, in 2016 (Figure 1).

Figure 1 – Map of the region in which the *Tachigali micropetala* seeds were collected



Source: Authors (2020)

For biometric evaluations, 200 seeds from five matrices were used, in which it was measured: length, width, and thickness with the 0.01 mm precision digital calliper. For the length measurement, the portion between the basal and the apical portion of the seed was considered and the width and thickness were measured in the intermediate part of the seed. The analysis of seed biometrics was determined using frequency distribution and utilizing univariate statistics that comprised position measures (mean, standard deviation variance, and minimum and maximum values) and dispersion measures (standard deviation and variation coefficients).

For the dormancy overcome, five tests were performed: Control (T1): the seeds did not receive any pre-germinative treatment; Cut in the integument (T2): a small cut was performed on the seeds with scissors in the region opposite the embryonic axis; Mechanical scarification (T3): the seeds were scarified with sandpaper No. 80 in the region opposite the embryonic axis; Chemical scarification: The seeds were emersed in concentrated sulfuric acid for 5 (T4), 10 (T5), 15 (T6) and 20 (T7) minutes. The seeds were placed in glass containers containing 20 ml of acid. During the treatment period, the stirring was done with a glass stick to standardize the activity of the solution. After the times of each treatment, the seeds were removed with the aid of a metallic sieve and immediately put in running water for 10 minutes, to eliminate acid residues, and placed on absorbent paper to remove water excess; Water at 80°C (T8): previously sanitized seeds were immersed in water heated to 80°C for 20 minutes.

Seeding was performed on germitest paper moistened with 2.5 times the paperweight. The seeds were kept in a BOD (Biochemical Oxygen Demand) germination chamber with a photoperiod of 12-12 hours (light/dark) and temperature at 28°C, remaining in these conditions until the end of the experiment. The experimental design was completely randomized, containing eight treatments, with 5 replicates of 25 seeds, totalling 40 experimental units.

The seeds germination was evaluated daily, from the third day after sowing, and was observed daily until the 23th day. Seedlings with protrusion of the

primary root equal to or greater than 2.0 mm were considered as germinated. The parameters evaluated were percentage of germination (%G), germination speed index and average germination time (MAGUIRE, 1962). The results of the germination percentage were transformed using the sine-arc equation $(\%G/100)^{1/2}$, to approximate the normal distribution.

At the end of the germination test, the normal seedlings of each replicate were measured from root to shoot using a graduated ruler (cm), and the results were expressed in cm/seedling. The same seedlings from the previous evaluation were placed in kraft paper bags and dried in a controlled oven at 65°C until reaching constant weight (48 hours). After that period, the samples were weighed in an analytical balance with an accuracy of 0.001 g. The results were expressed in g/seedling.

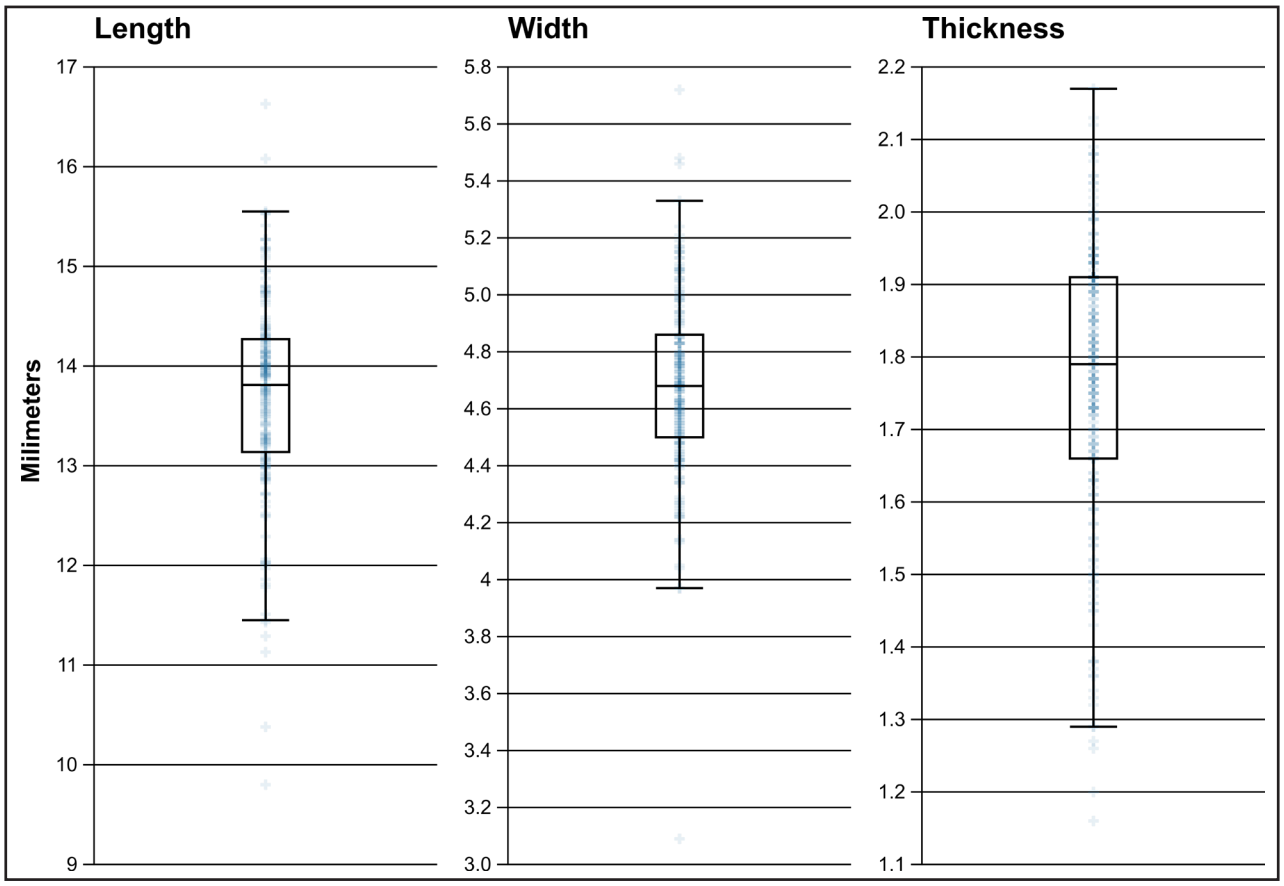
The results expressed in percentages were transformed into arc sen (%G/100), but the interpretation was presented with the means of the original data. The data obtained for the evaluated parameters were submitted to an analysis of variance, followed by a test for mean grouping (Scott-Knott) all at 5% of probability employing the statistical program ASSISTAT version 7.7 (2016).

3 RESULTS AND DISCUSSION

The seeds of *Tachigali micropetala* analysed in this article had, on average, a length of 13.07 mm, a width of 4.68 mm, and a thickness of 1.77 mm (Figure 2). Leão *et al*. (2018), analysing *Tachigali guianensis* seeds, observed close averages (10.69 mm, 5.70 mm, and 1.57 mm, for length, width, and thickness, respectively). Abreu *et al*. (2017) also registered similar values when measuring *Tachigali vulgaris* seeds. Therefore, a certain pattern is observed in the morphometry of seeds of the genus *Tachigali*.

The length, width, and thickness of *Tachigali micropetala* seeds vary from 9.8 to 14.67 mm; 3.09 to 4.96 mm and 1.16 to 3.74 mm, respectively. In general, most seeds have a length between 13.7 to 14.18 mm, width from 4.59 to 4.77 mm, and thickness from 1.67 to 2.18 mm (Figure 2). The standard deviation value of these parameters is relatively high, indicating a high sample heterogeneity.

Figure 2 – Length, width and thickness of *Tachigali micropetala* seeds, in milimeters



Source: The Authors (2020)

The individual variability observed within the species itself is due to environmental factors during seed development (SILVA *et al.*, 2014). The influence of the environment on seed development is mainly reflected by variations in size, weight, physiological potential, and health (ARAÚJO NETO *et al*., 2014). However, the rate of seed development is relatively stable in different environments, since adjustments in the number of seeds produced by the plant or by the plant community can maintain a relatively constant supply of assimilated seeds.

The seeds water content was around 10.7% at the experiment installation time. All variables analyzed were highly significant ($p < 0.01$), according to the F test. For treatments T2 (cut in the integument), T5 (acid immersion for 10 minutes)

and T6 (acid immersion for 15 minutes), germination started on the third day after sowing. On the fourth day, the seeds submitted to treatments T3 (scarification) and T7 (acid immersion for 20 minutes) started to germinate. Lastly, on the tenth and twelfth days, respectively, the seeds without any treatment (T1) and the ones that were immersed in hot water (T8) germinated.

Based on the obtained data, it was verified that the treatments with concentrated sulfuric acid for 15 and 10 minutes and mechanical scarification with sandpaper had germination percentages of 90.4%, 85.6% and 83.2%, respectively, being the most efficient for overcoming dormancy in *Tachigali micropetala* seeds (Table 1). On the other hand, the seeds that received the treatment in hot water at 80°C and those that did not receive pre-germination (control) presented a low percentage of germination, with 42.4% and 7.2%, respectively (Table 1). Similar results were obtained in a study carried out by Pereira *et al*. (2015), in which the seeds of *Tachigali myrmecophila* submitted to sulfuric acid at 20 minutes and mechanical scarification, obtained the best results to break dormancy, with percentages of germination above 80%. Pilon *et al*. (2012) obtained satisfactory results with scaling in sandpaper and sulfuric acid to overcome dormancy in *Tachigali vulgaris* seeds, with germination percentages of 84% and 71%, respectively.

According to the germination rate data, the worst indexes were obtained with T8 and the control treatment, which comprised the lower group, according to the means test. The other treatments were superior to T1 and T8, despite being statistically similar to each other. There is a direct relation between germination rate, seed vigour and uniform emergence potential of seedlings (ZUCARELI *et al.,* 2017). From this principle, it can be stated that the treatments T3 to T6 influenced the greater vigour and physiological potential of the *Tachigali micropetala* seeds, which consequently present greater seedling germination and uniformity.

Table 1 – Percentage of germination, rate of germination and mean germination time, of *Tachigali micropetala* seeds submitted to treatments to overcoming dormancy

Treatments	Germination Percentage (%)	Germination rate	Average germination time (days)
Control (T1)	7.20 d	0.25 b	16.38 a
Cut in the integument (T2)	60.80 b	1.60 a	7.46 b
Mechanical scarification (T3)	83.20 a	2.18 a	7.97 b
Sulfuric acid for 5 minutes (T4)	68.00 b	1.54 a	8.37 b
Sulfuric acid for 10 minutes (T5)	85.60 a	1.86 a	7.78 b
Sulfuric acid for 15 minutes (T6)	90.40 a	2.78 a	7.40 b
Sulfuric acid for 20 minutes (T7)	72.80 b	2.04 a	6.59 b
Water heated to 80°C (T8)	42.40 c	0.55 b	12.65 a
CV (%)	14.02	23.24	17.64

Source: Authors (2020)

In where: Averages followed by the same letter do not statistically differ from each other, according to the Scott-Knott Test, at a 5% probability level.

The highest values of mean germination time were obtained with the control and immersion treatments in the water at 80°C, which confirms the inefficiency of these treatments and the need to overcome dormancy in the seeds of *Tachigali micropetala*. The other treatments showed no statistical difference. According to Costa *et al*. (2012) seeds of *Adenanthera pavonina* L., when submitted to treatment with sulfuric acid in 10 minutes, obtained the lowest values of mean germination time. Pereira *et al*. (2015) found that immersion in acid for 20 minutes and scarification on sandpaper favours the germination process in less than 3 days in *Tachigali myrmecophila* seeds, being these efficient to overcome dormancy by emitting a larger number of seedlings in a smaller time. Araújo Neto *et al*. (2012), obtained the lowest germination times with the chemical scarification with sulfuric acid for 5, 10 and 20 minutes, thus promoting the reduction in the meantime and the increase in the mean emergency speed.

The seeds that did not receive pre-germination treatments had low germinability and vigour due to the impermeability of the tegument which hinders the imbibition, process necessary for germination. Untreated seeds do not lose

germination power, but the germination process without any treatments would be slow and uneven, which is undesirable for seedling production (PILON *et al.*, 2012). Therefore, overcoming dormancy increases the percentage and speed of emergence, resulting in a uniform stand in the early development process.

For shoot length and primary root length, treatments T3, T4 , T5, T6, T7 and T8 showed the highest values (Table 2). Pereira *et al*. (2015) observed close results with seeds of *Tachigali myrmecophila*, where the scarification in sandpaper provided a higher average length of the aerial part and the main root, with efficient results for the chemical scarification of 10 and 20 minutes as well. According to Nascimento *et al*. (2009), sandpaper scarification triggered larger average lengths of the main root and shoot seedlings of *Parkia platycephala*. Although no statistical difference was observed between the best treatments, the highest nominal values were observed for treatment T6, with sulfuric acid for 15 minutes, with values considerably higher than the others were.

Table 2 – Mean values of the shoot and main root length, and shoot and root dry matter in *Tachigali micropetala* seedlings under influence of dormancy overcoming methods

Treatments	Shoot length (mm)	Main root length (mm)	Shoot dry matter (g)	Root dry matter (g)
Control (T1)	0.25 b	0.18 b	0.01 b	0.01 b
Cut in the integument (T2)	1.17 b	1.05 b	0.18 a	0.09 a
Mechanical scarification (T3)	2.48 a	1.97 a	0.17 a	0.09 a
Sulfuric acid for 5 minutes (T4)	2.28 a	1.94 a	0.13 a	0.08 a
Sulfuric acid for 10 minutes (T5)	2.77 a	2.30 a	0.19 a	0.09 a
Sulfuric acid for 15 minutes (T6)	3.39 a	3.00 a	0.22 a	0.10 a
Sulfuric acid for 20 minutes (T7)	2.93 a	2.67 a	0.17 a	0.14 a
Water heated to 80°C (T8)	2.93 a	2.23 a	0.07 b	0.04 b
CV (%)	21.15	24.22	23.61	25.92

Source: Authors (2020)

In where: Averages followed by the same letter do not statistically differ from each other, according to the Scott-Knott Test, at a 5% probability level.

Regarding the average shoot dry matter and root dry matter, the similarity was observed in six of the eight treatments administered (Table 2). The values measured for T1 and T8, two unique treatments statistically different from the others, were considerably lower. Both treatments were less efficient on dormancy breaking and had lowered germination percentage values, influencing both shoot dry matter and root dry matter values. Thus, these treatments are contraindicated for the species.

Nascimento *et al*. (2009) found similar results, where sanding scarification resulted in a higher dry mass of *Parkia platycephala* seedlings, and higher averages in immersion treatments in sulfuric acid for 30 and 45 minutes. According to Pedó *et al*. (2018), there is a direct relation between average dry matter weights and seedlings vigour. Therefore, T3 to T7 treatments promoted greater vigour potential and seedling uniformity.

This knowledge is extremely important to start the *Tachigali micropetala* domestication process, opening the range of possibilities for its use. It is worth mentioning the great potential of the species to recover degraded areas, as it is a legume, with N fixation capacity, in addition to being arboreal in size, with large biomass production and rapid growth (CRUZ *et al*., 2020). In an Amazonian context, this potential is accentuated by being a native species. According to the new forest code (BRASIL, 2012), rural properties in the Amazon must have 80% of the native vegetation preserved, with the need to recompose or compensate the legal reserve, if the property is not meeting the established amount. The offer of a native species such as *Tachigali micropetala* adapted to local conditions and of rapid growth, in use together with other species, will allow a greater adhesion to the established conformities, by the producers; and a greater amount of recovered areas, benefiting the environment.

4 CONCLUSION

Treatments with chemical scarification by immersion in concentrated sulfuric acid for 10 and 15 minutes and abrasion in sandpaper nº 80 are the most efficient treatments to overcome the dormancy in *Tachigali micropetala* seeds. Although the results observed with treatments via chemical scarification are among the best, the difficulty of obtaining the acid, handling the acid and the need for personnel training must be considered. This study will help the establishment of propagation protocols for *T. micropetala*, being the first step to use the species in a commercial scale or reforestations. Ultimately, to recommend *T. micropela* for those purposes, the next step is to study if the species is able to withstand disturbed environments with impoverished soils and full sun.

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