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Biometric characterization, post-seminal development and overcoming seed dormancy of *Albizia polycephala* (Benth.) Killip ex Record

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Abstract

Albizia polycephala (Benth) Killip, popularly known as angico-branco, belongs to the Fabaceae - Mimosoideae family, is a pioneer species used for environmental restoration, ornamentation and urban afforestation. Biometric characterization is an important tool to provide information on the type of germination, in addition to describing the morphology of the seed, it stimulates studies inherent to taxonomy, ecology and seed technology. For the production of seedlings, the most used form of propagation of this species is through seeds. Therefore, with the establishment of protocols to overcome dormancy of forest species, the producer will be able to choose the most efficient method and also the one that best adapts to his reality, both commercial and technological, providing an increase in the number of species destined to compose the models for the recovery of degraded areas to be implemented. Based on this, the objective of this work was to carry out the biometric characterization of the seeds, describe the post-seminal stages and verify the best way to promote the germination of *A. polycephala* using pre-germination treatments to overcome dormancy. The work was carried out at the Laboratory of Phytotechnics of the Campus of Engineering and Agricultural Sciences (CECA) of the Federal University of Alagoas (UFAL). The differentiation and development of *A. polycephala* seedlings was measured. In the dormancy breaking trial, the following treatments were used: i) control (intact seeds); (ii) chemical scarification by immersing the seeds in sulfuric acid for 5 min, followed by washing in running water; iii) cut (cut) on the side opposite the micropyle; iv) immersion in hot water (80°C) and cooling for 24 h; v) immersion in distilled water (room temperature) for 24 h; and vi) immersion in distilled water (room temperature) for 48 h. The seeds were incubated in a germination chamber at a constant temperature of 30°C. The design used was completely randomized, with 4 replications of 25 seeds per treatment, and the means were compared by Tukey's test at 5% probability. At the time of installation of the experiment, the seeds had a water content of 12.4%, with an average of 10.3 mm in length, 7.42 mm in width and 2.46 mm in thickness. Germination is epigeous and seedlings are phanerocotyledon. The physical rupture of the seed coat from the bud treatment contributed to the increase in permeability to water and gases, thus benefiting the germination process. Thus, topping can be recommended to overcome *A. polycephala* seed dormancy.

Keywords: Angico-branco, Fabaceae - Mimosoideae, Viability.
Abbreviations: BOD_Biochemical Oxigen Demand; G_germination; PC_first count of normal seedlings; IVG_Germination Speed Index.

Introduction

Albizia polycephala (Benth) Killip, popularly known as angico-branco, belongs to the family Fabaceae – Mimosoideae, is a semideciduous tree, heliophyte, with individuals that reach a height between 8 and 25 m and with a trunk of 40 to 60 cm in diameter at adulthood. Endemic in the Caatinga, Atlantic Forest and Cerrado in 15 Brazilian states. The fruit of the angico-branco is dehiscent, flat, cream color when ripe, containing three to seven hard yellowish seeds (Santos, 2015). It is a pioneer species used for environmental restoration,
urban ornamentation and afforestation (Satori, 2018).

Observations of floral parts and vegetative organs are sometimes not enough to resolve taxonomic doubts, so the study of seedling morphology is of paramount importance in matters related to taxonomy, phylogeny and plant ecology (Gomes, 2015). The increase in research on plant morphology makes available essential information to improve plant production, such as helping the planning and the type of seed processing to be used (SANTIAGO et al., 2021).

Biometric and morphological studies of seeds enable a knowledge information about the germination process, which may provide the identification of problems arising from dormancy related to morphology, such as the presence of substances that prevent the permeability of the tegument, hindering the entry of gases and water (Rocha, 2018). Thus, the need for research aimed at describing morphological data of seedlings has been highlighted nowadays. The analysis of post-seminal development encompasses important aspects to assess the variability genetics within and between species, also helping in the definitions between this variability and the edaphoclimatic factors, contributing with the programs of genetic improvement (Melo Júnior et al., 2018).

Germination is a process that involves several steps of metabolic processes and a series of chemical reactions that have their own requirements in terms of temperature, as they depend on the activity of specific enzymatic systems (Marcos Filho, 2015). For Carvalho and Nakagawa (2012), there are factors that affect germination, being these intrinsic and extrinsic, among which are humidity, temperature, substrate, light and oxygen. However, the set is essential for that the process takes place normally and the absence of one of these factors may prevent the seed germination.

For the production of seedlings of forest species, the most used form of Propagation is through seeds. Since the seeds of angico-branco dormancy and the impermeability of the tegument to water is the most common cause of dormancy in legume species (Mendonça, 2020). The germination impediment established by dormancy is a beneficial strategy adopted by nature, which distributes the germination capacity over time, reduces the competition between the seedlings and the mother plant, increasing the probability of survival of the species. The presence of dormancy, as well as its germination, varies between species, being influenced by their genotype, and may also be influenced by the environment. (Carvalho and Nakagawa, 2012). Under natural conditions dormancy can be overcome by temperatures alternating, action of microorganisms, heating of the soil and action of acids, when these are ingested by dispersing animals (Souza and Carrasco, 2021). Nonetheless, there are several treatments that can overcome dormancy, such as immersion in hot or cold water, immersion in acids, scarification or seed cutting, among others (Brazil, 2009). Research in this sense, on the most appropriate method for overcoming dormancy, has shown to be efficient, and it is necessary to take into account variables such as the feasibility of its implementation, time, labor, financial investment, among others. Therefore, with the establishment of protocols in the overcoming dormancy of seeds of forest species, the producer will be able to choose the most efficient method and also the one that best adapts to his reality, both commercial technological, providing an increase in the number of species destined to compose the models for the recovery of degraded areas to be implemented.

In recent years there has been an intense concern in the restoration of degraded, and the species of the genus Albizia have a great capacity for agroforestry use (Santos, 2015). Thus, it is important to develop effective techniques in order to increase production and encourage the management of seeds of these species, in order to make available data that characterizes their physical and physiological attributes.

Considering that A. polycephala is not described in the Rules for Seed Analysis (RAS) and is also not covered in the Instructions for the Analysis of Seeds of Forest Species, documents that guide seed analysis procedures in Brazilian laboratories, if necessary to obtain information to base future research and standardize laboratory tests for this species.

In this way, the objective of the work was to carry out the biometric characterization of the seeds, describe the post-seminal stages and verify the best way to promote the germination of *A. polycephala* using pre-germination treatments to overcome dormancy.

Results and Discussion

Morphometric analyzes

The seeds of *Albizia polycephala* (Benth) Killip presented, on average, 10.3 mm in length, 7.42 mm in width and 2.46 mm in thickness, with a variation amplitude of 7.25; 4.66 and 1.94 mm respectively (Table 1). At the time of harvest, the moisture content of the seeds was 12.4%. According to Melo et al. (2018) Biometric data of fruits and seeds are taxonomically problematic, due to the strong influence of changes in latitude, season and microclimate, but it has important biological significance and is related to the dispersion syndrome.

It can be observed, in the frequency histograms corresponding to the data on length, width and thickness of A. polycephala seeds (Figure 1), that 26% of the length is distributed between 11.13 and 12.13 mm; approximately 41% of the width is between 6.32 and 7.32 mm and about 32% of the thickness has been determined between 2.01 and 2.31 mm. Presenting small asymmetry (Figure 1). Freitas et al. (2015) report that native species exhibit variability in relation to biometric characteristics. This variation in the size of *A. polycephala* seeds is probably due to climatic factors and the genetic characteristics of the parent trees.

The median is usually one of the best measures of central tendency, when considering types of asymmetry (Ferreira, 2018), the mean is sometimes directed to abnormal values because it is influenced by extreme measures, and can be overestimated or underestimated. When working with batches of the same species, it is possible to verify the health and maturity status of the seeds using data provided by biometrics (Aimi et al., 2016).

In addition, within the biometric aspects, we sought to verify if the seeds contained damage. Damage caused by insects and some deformations were verified, but most of the seeds (95%) can be classified as without damage. Bellei et al. (2022) show that such data are important from the point of view of seed production, as forest species are usually characterized due to the high occurrence of losses by deformed seeds, predation by insects and attack by microorganisms.

Post seminal development

The embryo (Figure 2a) has a hypocotyl-radicular axis facing the hilar region of the seed and the cotyledons facing the opposite region. In figure 2b, it can be seen, in the external view of the seed, the presence of the U-shaped pleurogram present on both sides, characterized by a groove that can facilitate the absorption of gases and water by the seed. Melo et al. (2021) working with seeds of *Mimosa bimucronata* (DC) O. Kuntze, a species also belonging to the Fabaceae family, obtained morphological characteristics similar to the present work. Some features presented by *A. polycephala* seeds as the apparent pleurogram and the presence of impermeable tegument, characterizing physical dormancy, predominate in several species of the Fabaceae family, information highlighted by Moraes et al. (2018), working with *Albizia pedicellaris*.

On the fourth day after sowing, the root protrusion occurred, and the germination of *A. polycephala* seeds characterized as epigeous and phanerocotyledon (Figure 2b and c). In species with epigean and phanerocotyledonary germination, the growth of the epicotyl is very small during germination (Marcos Filho, 2015), a fact also analyzed in this research. This classification associates the morphology with the ecological strategies of the species, and the type of germination is related to its rapid establishment and this is a characteristic behavior of pioneer species (Melo et al., 2018).

The hypocotyl measures 2.5 cm and has a greenish-white color on the sixth day after sowing.The root has a glabrous, cylindrical, vigorous shape, measuring 1.6 cm with a whitish color, presenting a cream color without the presence of hairs in the cap region (Figure 2d). On the tenth day, the primary root changes from a whitish to brown color, without the presence of hairs being observed to the naked eye, the hypocotyl is more elongated, measuring about 5.3 cm (Figure 2f). At this stage, the tegument is still attached to the cotyledons, which has a light green color and slow, but uniform growth.

After this stage, the hypocotyl becomes more vigorous and the root begins to show fine absorbent hairs. On the 12th day after sowing, the integument of the cotyledons was detached (Figure 2g), with opposite phyllotaxis, with a greenish color, oval, wide, glabrous, with a green petiole, glabrous, green cotyledonary blade and obtuse apex (Figure 2f) , have an entire margin and a convex adaxial surface. Ten days after sowing, the presence of first leaflets, ranging from five to seven light green, rounded oval leaflets (Figure 2f). On the 15th day, the cotyledons are completely open and the apical bud becomes apparent with a green color, the epicotyl is glabrous, slightly curved, cylindrical, tender, thin and green. The plant collar, due to its different coloration, is easily distinguishable (Figure 2h). Bellei et al. (2022) report that post-seminal development is an important tool to detect genetic variability within a population of the same species.

The performance of the seeds, in function of the different treatments for overcoming dormancy, can be seen in Table 2. This describes the germination values, first count, germination speed index and average germination speed. There was a statistical difference for the variables analyzed ($p<0.05$).

Overcoming dormancy

Intact seeds showed 35% germination in the first count (PCG) (Table 2), indicating that part of the population seeds is released from the dormancy-free mother plant. Similar results were found by Melo et al. (2018) and Melo Júnior et al. (2018), studying seeds of *Mimosa bimucronata* and *Colubrina glandulosa*, respectively. Pinto (2013) reports that among different populations there may be variation in the amount of dormant and non-dormant seeds, depending on the environmental conditions of soil moisture and nutrition, the density of leaves covering the plants or the degree of disturbance in the environment of that population. According to Carvalho and Nakagawa (2012) and Marcos Filho (2015), the seeds are released from the mother plant with different degrees of dormancy, a phenomenon known as heteroblasty or polymorphism. Although presenting a certain percentage of seeds non-dormant, germination was low enough for the seeds to be considered dormant.

Based on the observed results, the presence of tegumentary dormancy can be confirmed, verified by the absence of germination of a good part of the seeds of the control in the PCG, in which there was no opening in the teguments for imbibition and germination promotion, contrary to the observed by Santos et al. (2015), also with *A. polycephala*, where the control showed germination higher than 80%, statistically equal to the best treatments used, which suggests the absence of tegumentary dormancy in the seeds used by them.

The sprout treatment in the region opposite the hilum induced the maximum percentage of seed germination (100%) (Table 2). This result corroborates the existence of tegumentary dormancy related to its impermeability to water and the efficiency of the topping in overcoming it. It is noteworthy that no research was found, using the blunt, for this species.

The physical rupture of the seed coat from the bud treatment contributed to the increase in permeability to water and gases, thus benefiting the germination process. The highest percentage of germination in treatments submitted to topping was also observed in seeds of *Cassia leptophylla* (Rocha et al., 2018), *Mimosa bimucronata* (Melo et al., 2018), *Colubrina glandulosa* (Melo Júnior et al., 2018), *Mimosa ophthalmocentra* (Freitas, 2016) and *Parkia discolor*, by Pereira and Ferreira (2010), who observed that the blunt treatment provided the highest germination percentages.

The germination speed is associated with the speed of water entry into the seed, and, consequently, the activation of metabolic processes. In this case, the topping promoted the wear of the seed coat, making the processes of imbibition and germination faster, demonstrated with the variables of germination speed index and average germination speed. Therefore, it may be recommended for conducting standard germination tests for *A. polycephala* seeds. Similar results were found by Benedito et al. (2017), who observed a significant increase in the emergence speed with the emergence of the tegument in seeds of *Mimosa tenuiflora* Willd.

The immersion treatment in concentrated sulfuric acid for 5 minutes also enabled rapid germination, however, statistically lower than the bud break (Tables 2 and 3). Cavalheiro, Pimenta and Torezan (2007) found that chemical scarification with sulfuric acid resulted in an increase in the percentage of germination, however, the average germination time was not altered.

Santos et al. (2014) state that despite the efficiency of chemical scarification, using sulfuric acid, for seeds of many species whose dormancy is caused by the impermeability of the tegument, the corrosive effect of this compound can promote irreversible injuries to the embryo, a fact not witnessed in this study job. Another negative factor to be considered for acid scarification in forest seeds is related to the release of sugars resulting from the degradation of cellulose, increasing the availability of substrate for colonization by fungi (Melo, 2017), if not observed in the present research.

Immersion treatments in distilled water at room temperature for 24 and 48 hours delayed the germination process (Table 2). Garcia, Moraes and Sousa (2009) reported that immersion treatments in hot water (100 ºC), with seeds remaining in water at room temperature for 8 and 16 hours, reduce the germination percentage, as they cause possible damage to the seed embryo.

Table 3 shows the results of mean time (Tm), uncertainty (I) and germination synchrony (z) of *A. Polycephala* seeds. Based on these, it was possible to confirm that the breakout treatment was significantly superior to the other

Table 1. Descriptive analysis of the length, width and thickness of the seeds of *Albizia polycephala* (Benth.) Killip ex Record.

Statistical Measures	Length (mm)	Width (mm)	Thickness (mm)
Median	10.30	7.42	2.46
Moda	11.00	7.32	2.21
Median	10.34	7.35	2.42
Minimum	7.13	5.32	1.71
Maximum	14.38	9.98	3.65
Desvio padrão	1.43	0.75	0.33
CV (%)	14	10	13

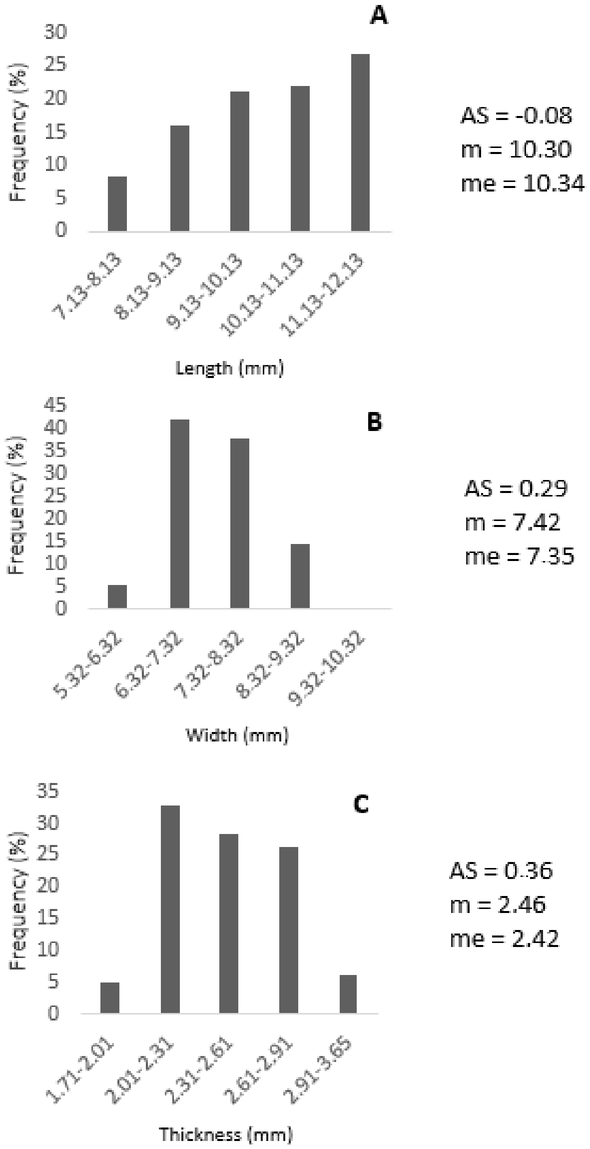

Fig 1. Distribution of relative frequencies of length (A), width (B) and thickness (C) of seeds of *Albizia polycephala* (Benth.) Killip ex Record. Subtitle: AS = Asymmetry Coefficient, m = mean (mm), m = median (mm).

Table 2. First germination count (PCG), germination (GER), index of germination speed (IVG) and average germination speed (VMG) of seeds of *Albizia polycephala* (Benth.) Killip ex Record, submitted to treatments to overcome dormancy.

Treatments	PCG (%)	GER (%)	IVG	VMG
Witness	35 c	50 c	3.701 c	0.076 e
H_2SO_4/5 minutes	62 a	68 b	4.111 b	0.171 b
Desponte	40 b	100 a	5.382 a	0.255 a
H_2O 80 ºC/24 hours	12 e	60 b	4.110 b	0.125 c
H_2O/24 hours	24 d	28 d	3.021 c	0.098 d
H_2O/48 hours	4 f	20 d	3.205 c	0.098 d
CV (%)	11.00	8.68	8.99	9.72

Means followed by the same lowercase letter in the column do not differ at 5% probability by Tukey's test.

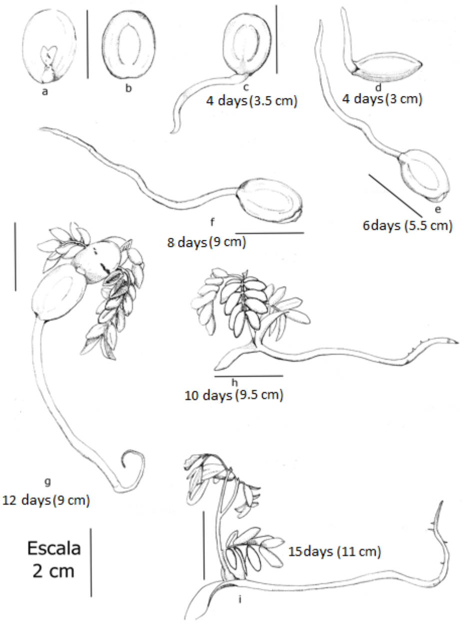

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Fig 2. Stages of post-seminal development of *Albizia polycephala* (Benth) Killip.

Table 3. Mean time (MT), uncertainty (I) and germination synchrony (Z) of seeds of *Albizia polycephala* (Benth.) Killip ex Record, submitted to treatments to overcome dormancy.

Treatments	TM (days)	I (bit)	Z
Witness	13.0 e	1.397 b	0.384 b
H_2SO_4/5 minutEs	5.8 b	1.610 c	0.277 c
Desponte	4.0 a	0.298 a	0.986 a
H_2O 80 ºC/24 hours	6.0 c	2.009 c	0.242 c
H_2O/24 hours	6.2 c	2.081 c	0.181 d
H_2O/48 hours	9.8 d	1.609 b	0.311 b
CV (%)	12.32	19.98	9.28

Means followed by the same lowercase letter in the column do not differ at 5% probability by Tukey's test.

Table 4. Length (COMP) and green mass (MV) of seedlings from seeds of *Albizia polycephala* (Benth.) Killip ex Record, submitted to treatments to overcome dormancy.

Treatments	COMP (cm)	MV (g)
Witness	5.90 c	0.080 c
H_2SO_4/5 minutes	6.70 b	0.099 b
Desponte	12.50 a	0.130 a
H_2O 80 ºC/24 hours	5.50 d	0.076 c
H_2O/24 hours	5.70 d	0.065 d
H_2O/48 hours	4.50 d	0.058 d
CV (%)	12.55	16.48

Means followed by the same lowercase letter in the column do not differ at 5% probability by Tukey's test.

treatments, differing statistically from each other. Similar results were found by Melo et al. (2017) in *Mimosa bimucronata* seeds, expressing the physiological homogeneity of the seeds at the time of germination. Santana et al. (2010) working with seeds of *Kielmeyera coriacea* found that they have a high degree of uncertainty, low synchrony and scattering in relation to the mean time, different from that found in the present study.

Based on the results of seedling length and green mass (Table 4), it was noticed that the seeds, when submitted to topping, originated larger seedlings and with a greater accumulation of green mass, statistically different from the other treatments. Araújo (2014) in a study with *Senegalia tenuifolia* seeds, noticed that the topping was the treatment that presented the highest values, in relation to the length of seedlings. However, Nascimento et al. (2009) working with seeds of *Parkia platycephala* did not find any statistical difference for the length of seedlings when the seeds were submitted to topping and scarification with acid. Regarding the green mass, it was observed that the most vigorous seedlings came from seeds submitted to the shoot-off treatment, statistically different from the other treatments.

Lambrecht et al. (2015) in a study with *Parapiptadenia rigida* used the green mass variable, demonstrating its importance in aiding research works.

Materials and Methods

Place of execution of the experiment

The work was carried out in the Laboratory of Phytotechnics belonging to the Campus of Engineering and Agrarian Sciences (CECA), from the Federal University of Alagoas(UFAL).

Experimental design

All statistical analyzes were performed using the SISVAR 5.6 program, from the Federal University of Lavras (Ferreira, 2014). The design used was completely randomized, when there was significance of the F test, the means were compared by Tukey's test at 5% probability.

Harvesting and processing of seeds

The fruits of *A. polycephala* were harvested in June 2021 with the aid of aerial scissors with an extension cable, from eight trees located in the rural area of the municipality of Recife-PE.

Biometric characterization of seeds and seedlings

The recording and monitoring of the seedlings' development and differentiation phases was performed daily, following the recommendations of Oliveira (1993), also identifying the type of germination. To record the development of the seedlings, four replications of 25 seeds were used, topped up, placed to germinate on two sheets of germitest paper placed in transparent plastic boxes of the Gerbox® type, moistened daily with distilled water and placed in a germination chamber of the Biochemical Oxygen type. Demand (B.O.D.) regulated at a constant temperature of 30 ºC.

The representations of the seed and seedlings were performed with the aid of an optical stereomicroscope. The nomenclature used in the description of the seedlings was based on Melo Júnior et al. (2018), they are: healthy root development, total expansion of the first leaf and appearance of the second leaf, for young plants the appearance of the third leaf.

Overcoming seed dormancy

The following treatments were tested: i) control (intact seeds); (ii) chemical scarification by immersing the seeds in sulfuric acid for 5 min, followed by washing in running water; iii) cut on the side opposite the micropyle; iv) immersion in water hot (80°C) and cooling for 24 h; v) immersion in distilled water (temperature environment) for 24 h; and vi) immersion in distilled water (room temperature) for 48 h.

After the pre-germination treatments, the seeds were subjected to asepsis, performed by immersing them in 70% alcohol for one minute and washing in distilled water (RIOS et al., 2016). Subsequently, sowing was carried out, using four replications of 25 seeds, on two sheets of germitest paper (sterilized), placed in Gerbox® type transparent plastic boxes (11.0 x 11.0 x 3.5 cm). After seed distribution, all treatments were incubated in a germination type B.O.D. temperature regulated to 30 °C.

The evaluation of the physiological quality of the seeds was carried out through the following tests and determinations:

Germination: The germinated seed counts were performed daily, during the period of fifteen days, being considered germinated the seeds that presented primary root with length ≥ 2 mm (Giachini et al., 2010).

First germination count: Performed simultaneously with the germination test germination, being the accumulated percentage of seeds germinated on the fourth day after sowing.

Germination speed index (IVG): performed simultaneously to the germination test germination, computing the germinated seeds daily until the stabilization of germination, and calculated by the formula proposed by Maguire (1962).

Average germination time: $t = (\Sigma niti)/\Sigma ni$ where: t = average incubation time; ni = number of seeds germinated per day; ti = incubation time (days) (LABOURIAU and VALADARES, 1976).

Average speed of germination: $V = 1/t$ where: V = average speed of germination; t = average germination time (Labouriau and Valadares, 1976).

Uncertainty (I) and germination synchrony (Z): were calculated by proposed formulas by Labouriau and Valadares (1976) and Santana and Ranal (2004) respectively, we used the software Germina Quant 1.0 (Marques et al, 2015) in the calculation of these variables.

Seedling length: at the end of the germination test, the seedlings of each repetition were used to evaluate the length (of the shoot and root), with the aid of a ruler graduated in centimeters, and the results were expressed in centimeters per seedling.

Green seedling mass: at the end of the germination test, the seedlings from each treatment were placed in paper bags and weighed on an analytical balance with a precision of 0.0001 g, the results being expressed in grams per seedling.

Conclusions

The seeds of *Albizia polycephala* (Benth) Killip are, on average, 10.3 mm long, 7.42 mm wide and 2.46 mm thick.

Germination is epigeous and seedlings are phanerocotyledonary.

The pruning treatment was efficient to overcome the dormancy of *A. polycephala* seeds.

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