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CORRELATION OF BIOMETRIC CHARACTERISTICS OF FRUITS AND SEEDS WITH THE VIGOR OF *Agonandra brasiliensis* SEEDLINGS IN NORTHERN AMAZONIA

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Abstract

The goal of this study was to determine and correlate the main morphometric characteristics of fruits, seeds, and seedling vigor of *Agonandra brasiliensis*, in Roraima. The design was completely randomized with three treatments, consisting of six replications with 20 seeds. The treatments established were three classes of seeds: T1 - small seeds (S = 1.50-1.69 g); T2 - medium seeds (M = 1.70-1.89 g) and T3 - large seeds (L = 1.90-2.09 g). The variables evaluated were: length; diameter; the fresh mass of fruits and seeds; percentage, speed (in index) and average time of emergence, length of roots and aerial part; number of leaves; fresh, dry and total seedling mass; seedling lignification index; root/shoot ratio and dry root/shoot ratio and correlations between all studied variables. The fresh mass of the fruit of *Agonandra brasiliensis* has a high positive correlation with the mass of seeds. Small seeds of *Agonandra brasiliensis* exhibit a higher percentage of emergence in the conditions of the northern Amazon.

Keywords: Morphobiometry. Opiliaceae. Pau-Marfim. Santalales.

1. Introduction

The *Agonandra brasiliensis* Miers ex Benth. & Hook. f., a species popularly known as *cerveja-de-pobre, pau-marfim, quina-de-veado*, is a species belonging to the family Opiliaceae (Smiderle et al. 2020). This species occurs in the Cerrado and Cerradão in the states of Roraima, Amazonas, Acre, Rondônia, Bahia, Ceará, Espírito Santo, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Piauí, São Paulo, also in the District Federal (Souza et al. 2020a). The fruit is classified as obovoid berry, which presents oil seeds and edible by wild animals (Ramalho et al. 2017).

In the seedling production sector, interest in the propagation of native forest species is growing (Smiderle et al. 2017). Thus, many species in the North region lack basic information on fruits and seeds, as little is known about their potential for exploitation in the conditions of Roraima (Nascimento et al. 2019).

The seeds of forest species need to have sanitary quality and vigor, as they are important factors in the success of germination and provide quality in a batch of seeds, in order to avoid losses through deterioration and decomposition or even to generate abnormal seedlings or with lesions that prevent them from growing vigorous (Smiderle et al. 2018).

In the search for information involving morphophysiological descriptions of fruits, seeds, seedling vigor (Menegatti et al. 2019), as well as their phenotypic correlations, which allow the quantitative assessment of the relevance of one character in relation to the other (Souza et al. 2016) is of paramount

importance in the identification of species, being able to better assist in the interpretation of seedling emergency tests, as it analyzes a wide simultaneous set of characters.

The biometry of fruits and seeds is important for the area of plant systematics and, consequently, for the development of environmental restoration works, contributing to discoveries of genetic variability within populations of the same species and the relationship with environmental factors (Souza et al. 2017). In this context, it is necessary to conduct research on biometric and morphological characteristics of fruits and seeds of native species in the northern Amazon, such as *Agonandra brasiliensis* aiming at maximum seedling emergence and vigor, so that this information can be made available to the scientific community, foresters, nurseries and seedling producers. Considering the above, the objective was to determine and correlate morphometric characteristics of fruits, seeds, and seedling vigor of *Agonandra brasiliensis* Miers ex Benth. & Hook.f., in Roraima.

2. Material and Methods

Study location

The work was carried out in the Seed Analysis Laboratory (LAS) and in the seedling nursery of Embrapa Roraima's forestry sector from January to August 2019. The species used in the present research was *Agonandra brasiliensis* Miers ex Benth. & Hook. The fruits were collected manually from trees located in the Serra de Nova Olinda, municipality of Boa Vista (RR), in April 2019, at coordinates 2° 56’ 58.99” N, 60° 44’ 48.30” W. Subsequently the fruits were taken to the Seed Analysis Laboratory (LAS), Embrapa-RR, where they were washed in running water, discarding the damaged fruits.

Characterization of the fruits and seeds of *Agonandra brasiliensis*

The evaluations were carried out in the laboratory and the seeds were characterized according to the methodology described in the Seed Analysis Rules (Brasil 2009). The collected fruits were selected by removing fruits with mechanical damage, deteriorated, allowing them to obtain a uniform lot.

For biometric characterization of the fruits, 225 fruits were selected randomly from the batch of 2500 fruits, and subsequently, the measurements were performed. Then the pulp was removed from the fruits, with the aid of a sieve and washing under running water. The seeds were dried in the shade, where they remained for 24 hours.

For the biometric characterization of fruits and seeds (Figure 1), the length (mm) and diameter (mm) measurements were recorded, measured in the middle portion, using a digital caliper with 0.01 mm precision. Besides, the fresh weight (g) of the fruits was determined using a precision scale (0.001 g).

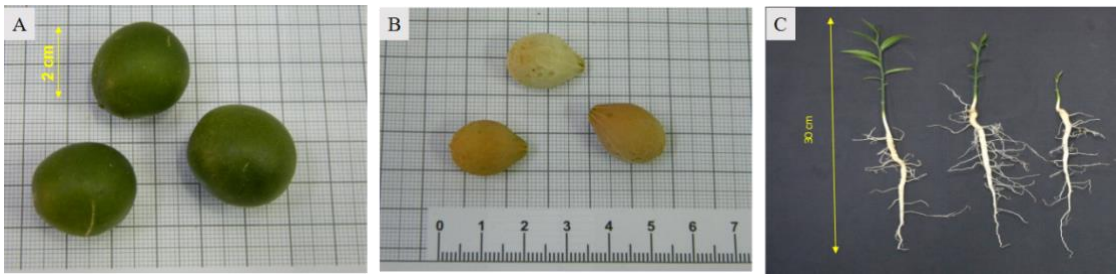

Figure 1. A – fruits; B – seeds; C – seedlings of *Agonandra brasiliensis*, Boa Vista (RR).

Emergency and vigor test of *Agonandra brasiliensis* seedlings

The implantation site was in a covered area under a temperature of 25 ± 1 ºC, in an environment without direct sunlight. For the emergency test, tubes placed in trays with 54 tubes containing 100% medium sand as substrate was used. The seeds were sown with 1.0 cm of the substrate above the seed and without performing any pre-germinative treatment. A completely randomized design with three treatments was used, consisting of six replications with 20 seeds, making up 120 seeds. The treatments established came from the three central biometric classes obtained: T1 - small seeds (S = 1.50-1.69 g); T2 - medium seeds (M

= 1.70-1.89 g) and T3 - large seeds (L = 1.90-2.09 g). The substrate humidity was maintained by daily manual irrigation according to the visual verification of the wet or dry substrate.

The emergency test evaluations were performed by daily seedling count until stabilization was verified (Labouriau 1983). An emerged seed was considered to be the one that presented the emission of the aerial part with at least 5 mm above the level of the substrate. The emergency values were expressed as a percentage. The experiment was completed when all seeds emerged or were deteriorated according to the rules for seed analysis (Brasil 2009). From the data obtained in the emergency test, the speed of emergency index - SEI (Maguire 1962) and the mean emergency time - MET (Labouriau and Agudo 1987) were calculated.

Seedling production of *Agonandra brasiliensis*

At 100 days after sowing, the number of leaves (NL), shoot length (SL, cm) and root (SR, cm) (Figure 1 C), and total fresh weight (TFW, g plant^{-1}) were evaluated. To determine the dry mass, the seedlings were divided into roots and aerial parts, by cutting at the height of the seedling collection. Both parts were placed separately in identified Kraft paper bags and taken to oven drying at 65°C until a constant mass was obtained.

After this period, the samples were weighed on an analytical balance with an accuracy of 0.001 g, obtaining the shoot dry weight (SDW, g plant^{-1}), root dry weight (RDW, g plant^{-1}) and total dry weight (TDW, g plant^{-1}). From the data, the dry matter ratio of the aerial part and dry mass of the root (SDW/RDW) and the relation of seedling height and root length (H/RL) and lignification index (Reyes et al. 2014).

Data analysis

The values obtained for the fruit and seed variables were analyzed by calculating the frequency distribution and calculating the maximum and minimum values, mean, standard deviation, and coefficient of variation. Pearson's Correlation (r) was performed for all variables, where the values can vary from -1 to +1 and the proximity of these values indicates a stronger correlation, and the deviation indicates a weak correlation. Positive values mean direct proportional relations and negative values inversely proportional. Values close to zero indicate little or no correlation.

To verify the assumptions of the analysis of variance (ANOVA), the data related to seedlings were first checked for: a) normality with the Shapiro-Wilk test ($p > 0.05$), and b) homoscedasticity by the Bartlett test ($p > 0.05$). Once these assumptions were met, the data were subjected to analysis of variance and, with significant differences between the data, the means were compared by Tukey's posthoc test ($p < 0.05$). All statistical analyzes were performed using software R version 3.6.1 (R Core Team 2019).

3. Results and Discussion

Morphometric characterization of fruit and seeds n

The average values of length, diameter, and fresh weight of the fruits and seeds of *Agonandra brasiliensis* are presented in Table 1, where the variability of the dimensions of the fruits of *Agonandra brasiliensis* is observed. These variations may be due to genetic variability or phenotypic plasticity or even represent a variety of the species under study.

Table 1. Means of length (mm), diameter (mm) and fresh weight (g) of fruits and seeds of *Agonandra brasiliensis* obtained in Boa Vista – Roraima.

	Fruits			Seeds		
	Length	Diameter	Mass	Length	Diameter	Mass
MIN	20.09	17.60	4.39	15.47	11.19	1.02
MAX	25.52	23.04	8.80	21.00	18.89	2.36
MED	22.63	20.59	6.61	18.74	13.69	1.80
*SD	1.12	1.08	0.92	0.84	0.75	0.20
CV	4.94	5.25	13.87	4.47	5.49	11.17

*SD = Standard Deviation; CV = Coefficient of Variation for N = 225.

Based on the results obtained in this research, the biometric data of the fruits and seeds of *Agonandra brasiliensis* presented indicate that the sampling was taken from the population with precision, since the values of the standard deviation, for all the evaluated characteristics, were small; therefore, the highest standard deviation values indicate that there is a greater sample variance for the fresh weight of fruits and seeds in relation to the other characteristics evaluated. Finally, the values of the variation coefficients (Table 1) refer to the smaller variation in the length of the fruits and seeds in relation to the value of the other biometric characteristics determined.

In addition, the results of the analysis of the fruits (Figure 2A) showed that 71% had a length ranging from 21.50 to 23.74 mm, the density distribution of the length of the fruits also varied, ranging from 20.09 to 25.52 mm, with an average of 22.63 mm. A result already evidenced by Moares et al. (2018) which proposes that populations of *Agonandra brasiliensis* trees in the state of Acre provide low heterogeneity in the biometric characteristics of fruits.

On the other hand, the diameter of the fruits (Figure 2C) ranged from 17.60 to 23.04 mm which showed homogeneity for most fruits showing a density of 20.59 to 21.00 mm. Such results, obtained in the present work, reinforce the results published by Marquete (2005), having verified that the diameter of the fruits of *Agonandra brasiliensis* presented frequency varying between 20 mm to 22 mm. Figure 2E shows the distribution of the fruit mass of *Agonandra brasiliensis*, which ranged from 4.39 to 8.80 g, with a greater frequency (51 fruits) showing an average mass of 6.61 g. Also, the data showed normal distribution and with 95% confidence in the t-test, the averages were between 6.48 to 6.72.

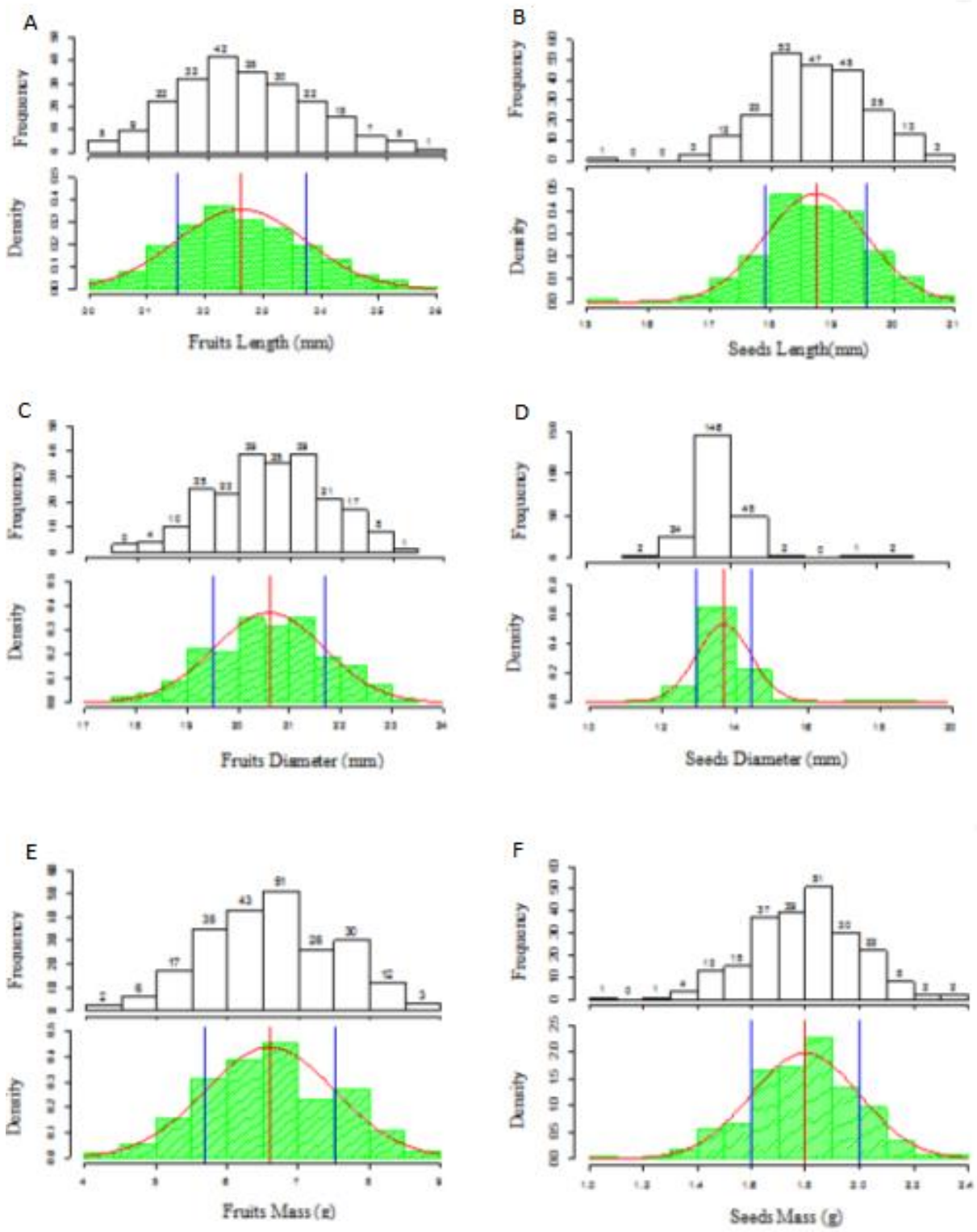

Figure 2. Distribution of frequency and density for: A – fruits length (mm); B – seeds length (mm), C – fruits diameter (mm); D – seeds diameter (mm); E – fruits mass (g); and F – seeds mass (g), respectively from *Agonandra brasiliensis*, Boa Vista – RR.

Note that the seeds of *Agonandra brasiliensis* (Figure 2B, D, and F), in terms of length, occupy 82% of the total fruit, while for the diameter it is approximately 86%. In turn, the seed mass showed a filling within the fruit of approximately 90%. Possibly, the remaining value of approximately 10% is equivalent to the fleshy pulp and the skin of the fruit of *Agonandra brasiliensis*.

In addition, the results showed that the seeds of *Agonandra brasiliensis* have lengths ranging from 15.47 to 21.00 mm, while the seed diameters varied from 11.19 to 18.89 mm and the seed masses showed a variation of 1.02 to 2.36 g. A similar answer was also obtained by Moares et al. (2018), having verified that the seeds have, on average, 20.39 mm in length and 14.65 mm in diameter, with a dry weight of seeds equal

to 2.20 g. This fact was also verified by Mekedece et al. (2011), where the seeds of *Agonandra brasiliensis* had an average of 16.99 mm in length and 13.73 mm in diameter. This information shows the peculiarities of this species with greater credibility and its low heterogeneity, which makes them attractive for the composition of selection programs.

The knowledge of the correlations existing between the characteristics of the fruits and the seeds allows us to know the behavior of one variable through the analysis of another. In this case, a positive Pearson correlation was verified when the morphological variables of fruits and seeds of *Agonandra brasiliensis* were correlated (Figure 3). It is worth noting that there was no correlation between fruits and seedlings, as well as between seeds and seedlings (Figure 3).

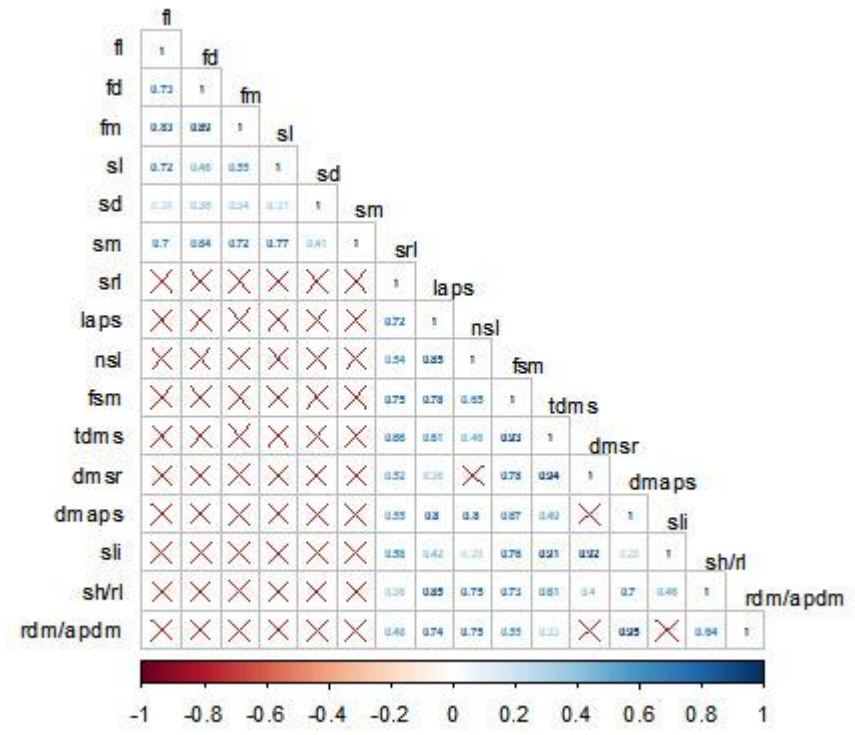

Figure 3. Pearson's correlation (r) between fcmp - fruit length; fdm - fruit diameter; fms - fruit mass; scmp - seed length; sdm - seed diameter; sm - mass of the seed; pcr - seedling root length; pcpa - length of aerial part of the seedling; pnf - number of seedling leaves; pmf - fresh seedling mass; pmst - total dry mass of the seedling; pmsr - dry mass of seedling root; mspa - dry mass of aerial part of the seedling; plig - seedling lignification index; palcr - seedling height and root length ratio; pmsrpa - ratio of root dry matter to aerial part dry matter. Positive (blue) values are directly proportional and negative (red) inversely. Values overlaid with an "x" were not significant at p = 0.01.

A strong and positive Pearson correlation was found between the dry mass of the fruit and the diameter of the fruit (0.89), that is, these variables are proportional to each other. It is worth highlighting the moderate and positive correlation (Figure 3) between the seed mass and the length (0.77) and the dry mass of the fruit and the seed mass (0.72) and relatively low and positive between the seed diameter and the seed mass (0.41). Moares et al. (2018), in his study with seeds of *Agonandra brasiliensis,* found a strong and positive correlation between seed width and diameter (0.84), indicating that these variables are quite related. Such fact, evidence the respective uniformity of the dimensions of the seeds determining the characters determined genetically for the species.

According to Santos (2010), the correlation is considered high when it has a correlation coefficient of $0.8 \leq r < 1.0$ and moderate when the correlation coefficient varies from $0.5 \leq r < 0.7$. Based on the results obtained in the present research, it can be inferred that the larger the diameter of the fruit, the greater the seed mass.

In general, the correlation between the morphological variables of fruits, seeds, and initial seedling growth of *Agonandra brasiliensis* evidenced in this work can be described as useful to assist in the selection of new

genotypes for use in silviculture. All this information, when used together, allows us to suggest the improvement of the traditional system of production of native seedlings in the northern region of Brazil, based on the morphological characteristics of the fruit and seeds.

Seedling emergence of *Agonandra brasiliensis*

The seed emergence process is one of the most important steps for seedling establishment (Souza et al. 2020b). For example, the faster the seed emerges, the less time it remains under adverse conditions, increasing the possibilities for seedling establishment (Nascimento et al. 2019). In turn, the seedling emergence in the present study started at 52 days after sowing and extended for another 48 days (total of 100 days), with an average emergence time of 63 days (Figure 4).

Additionally, seeds of *Agonandra brasiliensis*, classified as a small class, exhibited expressiveness in the continuity of the emergency process in relation to the other classes after 70 DAS, highlighting the maximum potential for emergence at 100 DAS (97%), while large seeds expressed 91% emergency at 100 DAS (Figure 4).

In addition, middle-class seeds showed higher emergence values up to 70 DAS (Figure 4), and soon after this period, there was stabilization in the emergency, remaining with values similar to those of large seeds for approximately 10 days. In view of this, it is important to highlight that after this period, both classes exhibited continuity in the emergency process, reaching a maximum value of 91% at 100 DAT.

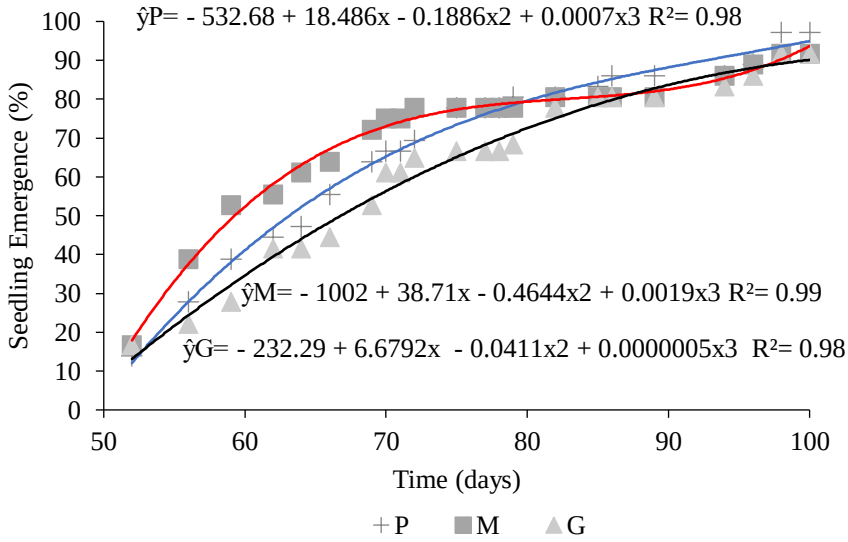

Figure 4. The emergence of *Agonandra brasiliensis* seedlings from three biometric classes (small, medium, and large) depending on days after sowing, Boa Vista - RR, at 100 DAS.

Considering the diagnosis made by Smiderle et al. (2018) seeds of native species (*Pochota fendleri*) from the northern Amazon were directly proportional to their morphological characteristics, with small seeds showing greater vigor and emergence of seedlings. This fact was also verified by Oliveira et al. (2016), working with *Acacia mangium* in the state of Roraima, in which they obtained a greater emergence of seedlings (93%) from seeds classified as small. Pagliarini et al. (2014) found that *Hymenaea courbaril* seeds classified as medium and large stood out in promoting seedling emergence when compared to small seeds.

Possibly, the smaller seed size accelerated the imbibition process, due to the smallerneed for water to reach seed turgor, to reach Phase faster III of this process, related to the elongation of the radicle and the formation of new tissues. Consequently, this made it possible for the roots to absorb nutrients and producephotoassimilated by the leaves, causing greater development of seedlings.

In practical terms, the results obtained in this study show the absence of a positive correlation between the size of the seed and the morphological characteristics of initial seedling growth (Figure 4) evaluated. This fact can be attributed to the existence of low heterogeneity, that is, the vigor of seeds is not a limiting characteristic for the initial growth of seedlings of the species under study, as shown in Table 2

where it was possible to verify that there was no significant difference between the factors: seed size and initial seedling growth, for all evaluated growth variables.

Table 2. Mean values of root length (RL, cm); shoot length (SL, cm); number of seedling leaves (NL); root dry mass (RDM, g plant^{-1}); shoot dry mass (SDM, g plant^{-1}); total dry mass (TDM, g plant^{-1}); index lignification seedling (INDLIG); ratio between seedling height and root length (H/RL); shoot and root dry matters ratio (SDM/RDM) of *Agonandra brasiliensis* at 100 DAS.

Variables	Seed sizes			Average	CV (%)
	Small	Medium	Large		
RL	11.25^{a**}	11.35^{a}	10.60^{a}	11.07	21.22
SL	15.16^{a}	15.68^{a}	14.24^{a}	15.03	20.71
NL	6.44^{a}	7.16^{a}	6.75^{a}	6.79	23.77
TDM	0.52^{a}	0.46^{a}	0.49^{a}	0.50	16.73
RDM	0.43^{a}	0.35^{a}	0.40^{a}	0.40	16.25
SDM	0.09^{a}	0.10^{a}	0.08^{a}	0.10	26.29
INDLIG	24.57^{a}	20.96^{a}	23.31^{a}	22.95	13.24
H/RL	1.32^{a}	1.22^{a}	1.25^{a}	1.27	18.77
SDM/RDM	0.24^{a}	0.28^{a}	0.21^{a}	0.25	22.96

** Means followed by the same lowercase letter on the line do not differ at 5% probability, using the Tukey test.

4. Conclusions

The fresh mass of the fruit of *Agonandra brasiliensis* has a high positive correlation with the mass of seeds.

Small seeds of *Agonandra brasiliensis* exhibit a higher percentage of emergence in the conditions of the northern Amazon.

The three classes of small, medium, and large seeds can be used in the production of *Agonandra brasiliensis* seedlings in Roraima.

Authors' Contributions: MONTENEGRO, R.A.: conception and design, acquisition of data, analysis and interpretation of data, drafting the article; SMIDERLE, O.J.: acquisition of data, analysis and interpretation of data, drafting the article; SOUZA, A.G.: conception and design, analysis and interpretation of data, drafting the article. All authors have read and approved the final version of the manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Ethics Approval: Not applicable.

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