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Biometry and vigor of seeds of *Myrciaria dubia* (Kunth) McVaugh

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Camu-camu has aroused the interest of various industries like natural preservatives, ice creams, juices, jellies, wines, natural dyes, but there is little technical information about the seeds. The objective of this work was to determine the biometric, physical and vigor characteristics of camu-camu seeds. Seeds originating from native populations of Roraima were used. The biometry was determined and the data were analyzed in Excel spreadsheet and calculated the mean, median, variance, standard deviation and seeds classified as small, medium and large, based on mass. The vigor was determined by electrical conductivity, seedling emergence, emergence velocity, plant height, stem diameter, in a completely causal design, with four replicates of 25 seeds. The average results for width, thickness, length, individual mass, volume, weight of one thousand seeds and number of seeds per kilogram showed large variability. The size of the seed has direct correlation with vigor, large seeds have greater vigor.

1. Introduction

The camu-camu, *Myrciaria dubia* (Kunth) McVaugh, is a small fruit tree of the Myrtaceae family, popularly known as araçá-dágua, azedinho, camocamo and caçari (Smiderle and Sousa, 2008). It occurs naturally in the Amazon region, growing naturally along the banks of rivers, streams, channels and lakes (Chagas *et al*., 2012; Souza *et al*., 2017). The fruit of the camu-camu is used in the preparation of juices, jellies, alcoholic drinks and ice creams, among others (Yuyama, 2011; Sousa *et al*., 2013). The main property of the camu-camu is the high vitamin C content, around 6,112 ± 137.5 ascorbic acid 100 g^{-1} of pulp, better than most cultivated plants (Yuyama, 2011). The skin of the camu-camu when fresh contains high levels of anthocyanin and ascorbic acid (Villanueva-Tiburcio *et al*., 2010). The interest aroused by the camu-camu is due to its bioactive compounds and biological activity of antioxidant action, which reduce lipid peroxidation, revert high levels of total cholesterol and triacylglycerols, and increase the levels of HDL-cholesterol (Gonçalves, 2012).

Seed characteristics are important in the study of any species, as they give an understanding of the dispersion and establishment of seedlings. The classification of seeds by size or weight is a strategy to be adopted in the standardisation and emergence of seedlings, and to obtain seedlings of similar size or greater vigour (Carvalho and Nakagawa, 2012). Therefore, the biometry of fruits and seeds have a diagnostic value in differentiating species within a genus, and can contribute to their recognition (Pereira and Ferreira, 2017).

In order to find the ideal size class for the multiplication of different plant species, biometry is used to determine physiological quality, but the results have differed considerably, even when the seeds are from the same species. Biruel *et al*. (2010) found that larger seeds of *Caesalpinia leiostachya* displayed a greater percentage and speed of germination; different results to those reported by Queiroga *et al*. (2011), who studied peanut seeds and found that smaller seeds had a higher germination percentage.

Seeds of different sizes showed the same high germination percentage, with small seeds being the most viable, and medium and large seeds yielding seedlings that were more vigorous (Vendramin and Carvalho, 2013; Smiderle *et al*., 2016).

This ambiguous situation may be one of the reasons for two or more seed sizes being produced by the same species. Variations in size were seen in seeds collected in an area of natural occurrence in the State of Roraima, Brazil. In view of the above, the aim of this work was to determine the biometry, physical characteristics and vigour of seeds of the camu-camu.

2. Materials and Methods

The experiment was carried out at the Seed Analysis Laboratory of Embrapa Roraima. The camu-camu fruit used in the experiment were harvested from native plants located on the banks of the Jatapú River (0° 41.072” N and 59° 18.046” W) at an altitude of 144 m. Here the plants develop in rocky soil in the district of Caroebe in the State of Roraima, and are distributed along the river in various populations and subpopulations.

After harvesting, the fruits were packed in plastic bags to prevent damage from crushing, and carefully transported to the laboratory, where they were selected for lack of damage, and then homogenised and sanitised. The mature selected fruit were then depulped and washed in running water, using a sieve for the complete removal of any pulp adhering to the seeds.

Physical characterisation of the seeds

The seeds were evaluated for their physical characteristics.

Seed biometry. The individual thickness, length and width of 600 seeds were measured using a calliper, with the results expressed in mm. The length was measured along the longitudinal axis of the seed; the width was measured perpendicular to the length, considering the broad face of the seed, which corresponds to the median line; and the thickness, by again measuring perpendicular to the length, but on the smaller seed face, corresponding to the median line including the two cotyledons. The following were then obtained:

Seed volume (mm^3): multiplying the three dimensions (width, length and thickness), without considering the actual shape of the seed.

Individual seed weight: obtained by weighing each seed.

Based on these measurements, the seeds were separated into three size classes (large, medium and small).

1000 seed weight (g). Six samples of 100 seeds were used for each size class. The samples were weighed on a 0.001 g precision balance and the weight determined by multiplying the result by 10.

Number of seeds per kilogram. After determining the thousand-seed weight (TSW), the number of seeds per kilogram was calculated using the “rule of three”.

Water content (%). Carried out using the standard oven method at 105±3°C for 24 hours. Four replications of 10 seeds were used, where each sample was wrapped in aluminium and placed in an oven. After 24 hours, the samples were removed from the oven and placed in a desiccator for cooling, and then weighed on a 0.001 g precision balance. The water content was calculated based on the difference between the wet and dry weights, applying the formula proposed in the Rules for Seed Analysis (MAPA, 2009), with the result expressed as a percentage.

The biometric data were analysed in an Excel spreadsheet. The mean, median, variance and standard deviation were all calculated.

Determining the vigour of small, medium and large seeds of the camu-camu

The experiment was carried out in a greenhouse at Embrapa Roraima, in Boa Vista. The experimental design was completely randomised (CRD), with three treatments comprising the size classes (small, medium and large), and four replications of 25 seeds. Seeds with a weight between 0.66 g and 0.84 g were considered small, those with a weight between 0.92 g and 1.20 g were considered medium, and those with a weight between 1.27 g and 1.60 g considered large. After classification, the following analyses were carried out:

Electric conductivity (EC). the seeds were weighed on a 0.0001 g precision balance and then immersed in 75 mL of water for 24 hours at a constant temperature of 25°C. The electrical conductivity was then measured using a digital conductivimeter. The results were expressed in µS cm^{-1} g^{-1} of seed.

Seedling emergence. The test was carried out following procedures described in the Rules for Seed Analysis (MAPA, 2009). The tests were conducted in trays containing 50% sand + 50% sawdust as substrate moistened with water up to 60% of its retention capacity, using four replications of 25 seeds distributed at a depth of 2.0 cm. The test was performed in a nursery of 50% Sombrite shade screen. To evaluate the non-germinated seeds, they were removed from the substrate, cut down the middle with pruning shears and identified for viability and senescence. The formula used for calculation was:

$$E = (N/A) \times 100:$$

Where E = percentage emergence; N = number of emerged seedlings; A = total number of seeds placed for emergence.

Speed of seedling emergence. Established by means of a daily count of emerged seedlings, the index being calculated from the expression:

$$SE = (E1/N1) + (E2/N2) + \ldots + (En/Nn), \text{ where:}$$

SE = speed of seedling emergence; E1 = number of seedlings emerged at the first count; N1 = number of days elapsed until the first count; E2 = number of seedlings emerged at the second count; N2 = number of days elapsed until the second count; n = last count.

Plant height. Evaluated four months after sowing with the aid of a graduated rule (cm). Measured from the cotyledon node to the end of the first pair of leaves in normal seedlings identified at the end of the test for seedling emergence.

Stem diameter. Evaluated four months after sowing using a digital calliper (mm). Determined at the insertion of the cotyledon.

The data for water content, electrical conductivity, seedling emergence, speed of emergence, plant height and stem diameter were submitted to the Lilliefors test for normality. They were then submitted to analysis of variance (ANOVA), using a completely randomised design with four replications, and the mean values compared by Tukey's test at 5% probability using the SISVAR software (Ferreira, 2014).

3. Results and Discussion

From the results, it was seen that there was a significant difference in the biometric variables of the analysed seeds, and it was possible to separate them into three classes according to size: small, medium and large. The data for variance, standard deviation and coefficient of variation, and the mean results for individual weight, width, thickness, length and volume of the camu-camu seeds are shown in Table 1.

The biometric data shown indicates that the studied population was accurately sampled, since the values for variance were low (<1) for each of the characteristics under study. The values for standard deviation shown in Table 1 indicate a low sample variation for each of the characteristics under evaluation.

The values for the coefficient of variation demonstrate the low variation in the variables, considering the mean value of the characteristics. However, variation can be seen when the seeds are sorted by size.

Table 1 - Biometric characteristics of camu-camu seeds from native populations in the State of Roraima, classified by size class

Class	Mean	Variance	Standard deviation	Coefficient of variation (%)
		Weight (g)		
Small	0.79	0.002	0.04	5.65
Medium	1.11	0.004	0.06	5.90
Large	1.45	0.008	0.08	6.12
		Width (mm)		
Small	12.04	0.541	0.73	6.11
Medium	12.90	0.147	0.38	2.97
Large	14.42	0.233	0.48	3.34
		Thickness (mm)		
Small	5.54	0.065	0.25	4.59
Medium	6.11	0.213	0.46	7.56
Large	6.74	0.329	0.57	8.51
		Length (mm)		
Small	15.54	0.620	0.79	5.07
Medium	17.42	0.206	0.45	2.61
Large	19.14	0.342	0.58	3.05
		Volume (mm^3)		
Small	1,035.77	6,144.378	78.38	7.57
Medium	1,371.98	6,104.830	78.13	5.69
Large	1,859.47	20,759.298	144.08	7.75

Similar results were found in camu-camu seeds from the banks of the Anauá River in Roraima, with a weight which ranged from 0.80 g to 1.46 g, and greater results than the seeds of populations from the Rio Urubu, which ranged from 0.56 to 0.78 g (Souza *et al*., 2017). According to Gonçalves *et al*. (2008), species with wide geographic distribution may present differences in their characteristics due to the effects of adaptation and to origin.

The variation in seed size may interfere with their physiological quality, still poorly researched in forest species (Oliveira *et al*., 2009; Smiderle *et al*., 2016). The three classes of seed size displayed significant differences for thousand-seed weight (TSW) and the number of seeds per kilo (NSK) with a low coefficient of variation, showing little variation within each size (Table 2).

The initial water content of the seeds in the different size classes at the time the experiment was set up was greater than 35%, with significant differences between the classes (Table 3), however it was lower than that reported by Yuyama *et al*. (2011), who obtained a value for moisture between 45 and 56%, with no effect on germination. According to Braga *et al*. (2012), hydration of the seeds can favour test performance, because seeds that are more humid, within certain limits, germinate more quickly.

The different classes of seed displayed variations in the variables under analysis. It was found that values for electrical conductivity depend on the size of the seed, where the large, small and medium seeds presented higher, lower and intermediate physiological quality respectively (Table 3). According to Vieira and Krzyzanowski (1999), the lower physiological potential in small seeds is probably due to the lower organisational intensity of the cell-membrane systems.

The data for electrical conductivity in the small seeds showed a negative correlation with the other variables under study, demonstrating that the highest value for electrical conductivity corresponded to reductions in the percentage and speed of seedling emergence. These results confirm those reported by Vinhal-Freitas *et al*. (2011), and demonstrate that tests of vigour differentiate between seed classes, indicating significant differences between the larger and smaller size classes, where larger seeds showed greater vigour with the smallest values for electrical conductivity.

With the large seeds, the lower value for electrical conductivity was due to a greater organisation of the cell components, since, despite field emergence not differing statistically from that of the small seeds, they showed greater speed of emergence in addition to viability, even when not germinated, as the small seeds that did not emerge had all died.

It is important to point out that despite the differences seen in electrical conductivity between the seed sizes, each seed class had low values for EC, and values for emergence that were higher than the mini-

Table 2 - Summary of the analysis of variance and comparison of the mean values for thousand-seed weight (TSW) and number of seeds per kilo (NSK) in camu-camu seeds from native populations in the State of Roraima, classified by size

Source of variation	DF	Mean square	
		TSW	NSK
Treatment	2	346,622.68*	435,142.53*
Replication	5	633.44	672.57
Error	10	1,610,651.55	1,318.23
CV (%)		4.16	3.35
Overall mean value		963.88	1,082.76
Class		Mean value	
Small		734.27g	1.362
Medium		943.67 g	1.061
Large		1,213.70 g	825

* significant at 5% probability by F-test.

Table 3 - Summary of the analysis of variance and comparison of mean values for water content (WC, %), electrical conductivity (EC, µS cm^{-1} g^{-1}), emergence (EMERG, %), speed of emergence, (SE, index), seedling height (HT S, cm) and stem diameter (DIAM S, mm) in camu-camu seeds classified by size

Source of variation	DF	Mean square					
		WC (%)	EC µS cm^{-1} g^{-1}	EMERG (%)	SE	HT S (cm)	DIAM S (mm)
Treatment	2	14.67*	1.83*	433.33*	0.021*	79.12*	0.599*
Replication	3	0.08	0.002	5.55	0.000008	0.131	0.0012
Error	6	0.29	0.001	5.55	0.000008	0.049	0.0008
Mean		37.17	3.27	88.33	0.056	15.61	2.13
CV (%)		1.46	1.11	2.67	5.17	1.42	1.36
Class					Mean values		
Small		35.35 c	3.95 c	80 b	0.01 c	11.30 c	1.71 c
Medium		36.99 b	3.22 b	100 a	0.03 b	15.34 b	2.20 b
Large		39.17 a	2.61 a	85 b	0.14 a	20.18 a	2.48 a

* significant at 5% probability by F-test. Mean values followed by the same letter do not differ by Tukey's test at 5% probability.

mum established by the Seed Standards (MAPA, 2009).

As for plant height and stem diameter, the larger seeds promoted the best results, followed by the medium and small seeds respectively. There was a positive correlation between seed size and plant height and stem diameter, i.e. larger seeds gave rise to larger, more vigorous plants. These results differ from those obtained by Souza *et al*. (2017), who reported that seeds from a population of the Anauá River considered small, displayed better results for these characteristics.

Wagner Junior *et al*. (2011) demonstrated that seed size has an effect on the emergence and initial development of jabuticaba seedlings (*Plinia cauliflora*), and that large seeds gave seedlings that were more vigorous. In açaí Silva *et al*. (2017) in organic substrate obtained higher values for plant height and stem diameter when large seeds were used, as well as. large seeds produce more vigorous plants independent of the substrate in *Euterpe oleracea.*

Alves *et al*. (2005) pointed out that in general, larger seeds are correlated with higher rates of initial seedling growth, which increases the probability of success during their establishment, since the rapid growth of roots and shoots allows the plant to take advantage of the nutrient and water reserves of the soil and carry out photosynthesis. Wagner Junior *et al*. (2011) stated that the germination process in many species is influenced by seed size. Consequently, within the same batch, small seeds present lower seedling emergence and less vigour than the medium and large seeds.

4. Conclusions

The physical characterisation of camu-camu seeds shows great variability in weight, width, thickness, length and volume.

The thousand-seed weight and the water content are influenced by size, with values increasing in direct proportion to the size of the seed, while the number of seeds per kilo decreases in inverse proportion.

Medium and large seeds give rise to plants that are more vigorous.

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