Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v42i1.51183

ECOLOGY

Seed biometry: another functional trait in caatinga

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ABSTRACT. Seed biometrics is an excellent tool for understanding environmental filters, species potential and dispersal, successional stages of communities and the dynamics used by seeds to germinate, establish and develop, thus constituting a functional trait of the species. We aim to understand the relationship between aspects of seed biometrics and other phenological traits, in order to infer contributions about functional strategies in Caatinga. Between September 2017 and January 2019, 10 individuals of *Cenostigma pyramidale* (Tul.) LP Queiroz, *Commiphora lepthophloeos* (Mart.) JB Gillett, *Jatropha mollissima* (Pohl.) Baill., *Monteverdia rigida* (Mart.) Biral. *Pseudobombax marginatum* (A.ST.-Hil) A. Robyns, *Zizyphus joazeiro* Mart. were monitored for the selection of three seed collection matrices. From each matrix 100 seeds were collected, which after screening resulted in 35 intact seeds that were used to measure length, width, thickness and volume. Caatinga seeds are generally small, as a result of the environmental stresses of the Brazilian semiarid. The analyzed variables separated three groups in the PCA. We noticed that these groups evidenced the successional stages in the Caatinga. One group included *M. rigida* and *P. marginatum,* which are late secondary. *C. pyramidale* and *C. lepthophloeos* formed another group coincident with intermediate successional stages. *J. mollissima* and *Z. joazeiro* formed a group of pioneer species in anthropized environments. Another finding was that the area was in secondary succession. The pioneer species presented the largest seeds and this characteristic is associated with the need for nutrient reserve that allows the embryo to germinate and last for a longer period without the need for exogenous nutrients, an essential condition for the Caatinga. With the advancement of ecological succession and the establishment of a more consolidated plant community, competition for resources, the frequent dispersion vector type and the nature of the environmental stresses present, the seeds may be smaller.

Keywords: ecological succession; functional groups; plant ecophysiology; semiarid.

Received on November 28, 2019.
Accepted on May 11, 2020.

Introduction

Seed characteristics are an important functional trait because they reflect directly on how plants can overcome stress. Seed biometrics is a relevant tool to detect genetic variability within populations of the same species and the relationships between this variability and environmental factors, as well as in breeding programs (Gonçalves et al., 2013). The variability normally found results from the high phenotypic plasticity that plants encounter, being able to react differently to the same type of stress and may contribute to the development of works in the areas of restoration and conservation (Khurana & Singh, 2001; Long et al., 2015). Genetic and ecological factors are the ones that apply the largest effect over the size of the diaspore (Wiesbauer, Giehl, & Jarenkow, 2008; Steiner, Zuffo, Busch, Sousa, & Zoz, 2019). They range from height, life and fertility of the mother plant pollen to abiotic factors such as temperature, length of day, soil quality, presence of pathogens, type of pollinators and dispersers, among others (Leishman, Wright, Moles, & Westoby, 2000; Jakobsson & Eriksson, 2000; Westoby, Falster, Moles, Vesk, & Wright, 2002; Pizo, 2003; Gressler, Pizo, & Morellato, 2006; Aud & Ferraz, 2012; Tanveer, Tasneem, Khaliq, Javaid, & Chaudhry, 2013).

Peres and Fagg (2011) found that depending on the dispersing agent, seed size is the main determining factor for the success of establishing a new plant. In anemochoric dispersion, for example, the seeds must necessarily be small to facilitate their transport. Bird-dispersed seeds are also usually tiny and light. For mammals, however, diaspore size is not a limitation and dispersal is predominantly for large and heavy fruit species (Peres & Fagg, 2011; Santana, Baccaro, & Costa, 2016).

Seed biometrics is the trait that best indicates the resources that the seedling has to germinate, settle and develop in the environment (Leishmann et al., 2000; Goudel, Shibata, Coelho, & Miller, 2013), it is a predictor of germination and establishment performance. Larger seeds would have better germination and seedling development qualities than smaller seeds (Malavasi & Malavasi, 2001; Green & Juniper, 2004; Shahi, Vibhuti, Bargali, & Bargali, 2015), thus having a positive correlation between seed size and seedling survival. Biometrics can also reveal the successional stages of species in plant physiognomies (Aud & Ferraz, 2012). Pioneer species tend to produce smaller seeds, and secondary or late species have larger, rapidly germinating seeds whose higher nutritional storage capacity compensates for the low carbon assimilation and enable them to remain in their seedling phase over long periods (Carvalho & Nakagawa, 2000; Steiner et al., 2019).

In the Brazilian semiarid, environmental conditions are similar to those of other dry tropical forests, with large interspecific and seasonal physiological variations (Trovão, Fernandes, Andrade, & Dantas Neto, 2007; Albuquerque et al., 2012). As for physiognomy, Lima, Carvalho, Ribeiro, and Manfrin (2018) pointed out similarities with arid regions in northern Colombia, Venezuela and Central America. However, the situation is even more complicated in Caatinga, a dry tropical forest that occupies one of the largest land areas in the world and exists at the limit of water resources availability for forests (Miles et al., 2006; Santos et al., 2014).

Caatinga plant species develop under severe climatic and edaphic conditions. The intense water, light and thermal stresses during most of the year are probably filters that can define common response traits in species that do not necessarily have phylogenetic proximity (Díaz et al., 2007; Pérez-Camacho et al., 2012; Oliveira et al, 2014; Silva et al., 2014). By observing the vegetation structure, it is possible to verify that the woody species do not reach high heights, although they have large branches. The floral phenology of most species is restricted to a single reproductive period (Barbosa, Barbosa, & Lima, 2003).

Considering the aspects already addressed, it is noticeable that seeds play an important role in the dispersal and establishment of plant species in any vegetation. The environmental filters present in the Caatinga form conditions that make the establishment of new individuals even more difficult. Thus, the objective of this work is to understand the relationship between biometric characteristics of seeds of Caatinga woody species of economic and cultural value, and other functional traits of the plant, check if this relationship will be able to form groups and understand which variables were responsible for any groups formation. In order to infer contributions on functional strategies of the analyzed species, contributing to the information database of the Caatinga and to possible conservation plans.

Material and methods

The study was conducted between September 2017 and January 2019, in two Caatinga fragments located nearby, on Vereda Grande farms (7° 31,613′ S, 36° 2,991′ W), with an altitude of 514 m, and Pocinho (07º 29,929’ S, 35º 58,237’ W) with an altitude of 391 m, located in the municipality of Barra de Santana in the state of Paraíba. The analyzes were conducted at the Plant Ecophysiology Laboratory of the Caatinga - LEPCaa, UEPB Campus I.

The fragments are geographically inserted in the Cariri Oriental microregion, Borborema mesoregion (Alves, 2009). The vegetation of the region is predominantly steppe savannah - TP (Instituto Brasileiro de Geografia e Estatística [IBGE], 2004). Located on the dry diagonal (NE-SW) (Alves, 2009) the region has tropical savannah BSw-type climate, with winter dry season, according to the climate classification of Peel, Finlayson, and McMahon (2007). It has a high radiation index with an average temperature of around 25°C. Annual rainfall varies around 450 mm, with rainfall concentrated between March and July, being quite uncommon during the other months of the year (Silva et al., 2014).

The relief of the region is inserted in the southwest escarpment of Borborema’s plateau, with the highest levels exceeding 600 meters, in a wavy, strong wavy and mountainous relief in some areas (Paraiba, 2007). The soil of the study areas varies between vertisol, litholic neosol and luvisols (Marques, Nascimento, Araujo Filho, & Silva, 2014). These are shallow soils, with clay properties and varied fertility.

Six species with wide distribution in the Caatinga were selected. Ten individuals in reproductive stage have been monitored since September 2017 to ensure seed harvesting. The species were classified into two phenological groups based on leaves: Deciduous (DE) and Evergreen (EG); Based on wood density: High (A) and Low (B); and based on dispersal syndrome (Table 1).

Table 1. List of tree species included in this study. Scientific name, Common Name, Family, Leaf Phenology, Wood Density and Dispersion Syndrome (Autochoric, Zoo-zoochoric, Anemochoric).

Specie	Common Name	Family	Phenology	Density	Dispersion
Cenostigma pyramidale (Tul.) L. P. Queiroz	Catingueira	Fabaceae	DE	A	Aut
Commiphora lepthophloeos (Mart.) J. B. Gillett	Umburana	Burseraceae	DE	B	Zoo
Jatropha molissima (Pohl.) Baill.	Pinhão	Euphorbiaceae	DE	B	Aut
Monteverdia rigida (Mart.) Biral.	Bom nome	Celastraceae	SV	A	Zoo
Pseudobombax marginatum (A.ST.-Hil) A. Robyns	Imbiratanha	Malvaceae	DE	B	Ane
Zizyphus joazeiro Mart.	Juazeiro	Rhamnaceae	DE	A	Zoo

Among the selected species, three individuals out of the ten monitored were chosen in a completely randomized way, which were used as seed matrices. Ripe fruits were harvested directly from the coppice of the matrices. They were placed in a polyethylene bag and taken to the Plant Ecophysiology Laboratory of the Caatinga, LEPCaa - UEPB. The fruits were benefited and the seeds extracted, resulting in an amount of 35 seeds per matrix.

The seeds of each matrix were measured for length (C, mm), measured from base to apex, width (L, mm) and thickness (E, mm) measured at seed midline. Measurements were made with the aid of a 0.01 mm digital caliper (Nogueira, Medeiros Filho, & Gallão, 2010). While the measurements were being made some seeds were also photographed (Figure 1). Seed volume (V, mm^3) was calculated from the length x width x thickness ratio.

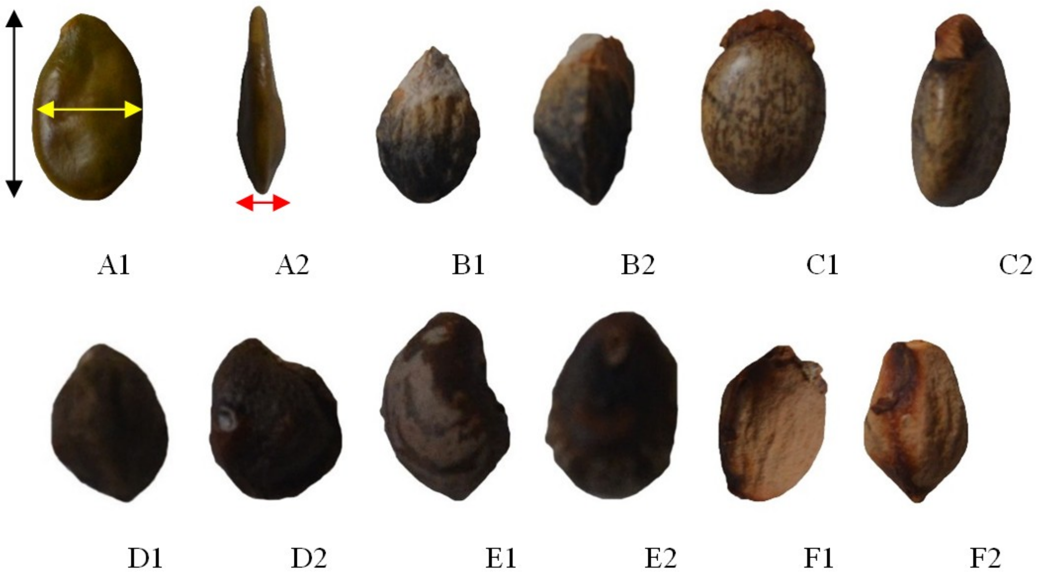

Figure 1. Seeds of the selected species: *Cenostigma pyramidale* (Tul.) L. P. Queiroz (A1-A2), *Commiphora lepthophloeos* (Mart.) J. B. Gillett (B1-B2), *Jatropha molissima* (Pohl.) Baill. (C1-C2), *Monteverdia rigida* (Mart.) Biral. (D1-D2), *Pseudobombax marginatum* (A.ST.-Hil) A. Robyns (E1-E2), *Zizyphus joazeiro* Mart (F1-F2); and biometric variables: length (■), width (🟨), thickness (🟥).

Data were analyzed in two ways: initially, the species data were summed to obtain a simple arithmetic mean for each of the dimensions and volume. From these data, a univariate statistical analysis was performed to obtain position measurements (minimum, maximum, mean and standard error values) and dispersion measures (coefficient of variation). The similarities of the variables between species were observed in BoxPlot graphs. After this analysis, the sum of each variable was performed, which resulted in simple arithmetic averages of each variable per matrix. These averages were normalized, providing data for the realization of a cluster, using Euclidean distance in Ward's method to check if the species were grouped. Following this, a principal component analysis (PCA) was performed to verify which variables provided the species grouping. The analyzes were performed using PASTsoftware (Hammer et al., 2001), version 3.2.

Results and discussion

The mean values of biometric parameters of the species studied are available in Table 2. Data analysis initially shows the accuracy of sampling through the low standard error in all variables, indicating that the sampling size provided reliable data, with little variation in relation to the average found for the individuals of each species.

Table 2. Average values of biometric parameters evaluated, verifying differences within and between species. Biometric Variable (BioV), Length (L), Width (W), Thickness (T) and Volume (V). Number of Seeds (N). Minimum (Min). Maximum (Max). Standard Error (SE). Coefficient of Variation (CV). Length (mm), Width (mm), Thickness (mm) and Volume (mm^3)

Species	BioV	N	Min	Max	Average	SE	CV (%)
Cenostigma pyramidale	L	35	9.73	11.53	10.68	0.06	3.75
	W	35	8.01	10.08	8.75	0.06	4.44
	T	35	1.79	2.91	2.35	0.03	9.51
	V	35	153.01	283.34	219.68	4.67	12.58
Commiphora lepthophloeos	L	35	8.88	9.95	9.56	0.04	2.50
	W	35	6.13	6.71	6.45	0.02	2.24
	T	35	4.25	5.13	4.54	0.03	3.92
	V	35	252.25	317.67	281.41	2.51	5.28
Zizyphus joazeiro	L	35	10.29	12.09	11.22	0.07	3.73
	W	35	7.70	8.82	8.38	0.04	3.45
	T	35	7.41	8.25	7.90	0.03	2.68
	V	35	604.40	858.81	748.16	10.41	8.23
Jatropha molissima	L	35	10.03	12.8	11.25	0.09	5.03
	W	35	7.27	8.46	7.97	0.04	3.5
	T	35	6.05	6.85	6.40	0.035	3.30
	V	35	474.51	689.89	580.18	9.77	9.97
Monteverdia rigida	L	35	4.64	5.47	4.99	0.03	4.01
	W	35	3.28	4.20	3.64	0.03	5.56
	T	35	3.01	4.73	3.39	0.04	8.56
	V	35	50.11	91.93	62.12	1.38	13.19
Pseudobombax marginatum	L	35	5.37	6.36	6.02	0.02	2.77
	W	35	3.43	4.09	3.78	0.02	4.08
	T	35	3.77	4.39	4.03	0.02	3.92
	V	35	74.70	105.50	92.10	1.16	7.51

Volume was the variable with the highest coefficient of variation in *C. pyramidale* and *M. rigida*, differing greatly from *C. lepthophloeos*, which showed a low variation. The thickness also varied substantially when compared to *C. pyramidale* and *Z. joazeiro*.

The variation of the analyzed parameters, length, width, thickness and volume is shown in Figures 2, 3, 4 and 5. The species *Z. joazeiro* and *J. mollissima* showed uniform behavior in all measured parameters, always being among the highest averages and with the largest variations in the data. In contrast to the smallest means and smallest variations, *M. rigida* and *P. marginatum*. *C. pyramidale* and *C. lepthophloeos* were less uniform in their parameters, presenting high lengths, and widths, while thickness and volume observed were between low and intermediate values. Volume was the factor that most influenced the distinction between seeds. The seeds with the largest variation in size (volume) were those of *Z. joazeiro* and *J. mollissima*, which were also the largest in this study. *M. rigida* and *P. marginatum* were the smallest seeds (volume) in this study.

When we compare the biometric characteristics of the seeds from Caatinga with those from other biomes, we found that Caatinga seeds are smaller (Gonçalves, Ferraz, Souza, & Tecchio, 2017; Gonçalves et al., 2013; Zuffo et al., 2017; Marques, Guimarães, Smiderle, & Souza, 2018). This fact in itself characterizes an adaptability to the water stress conditions typical of Caatinga during most of the year. Usually small seeds have a higher percentage of germination than large seeds in environments with water deficit (Costa, Custódio, Machado Neto, & Marubayashi, 2004) due to the higher surface/volume ratio. Beckert, Miguel, and Marcos Filho (2000) reported that the process of soaking smaller seeds is faster and more intense, since the smaller size provides greater total contact of the surface with the moist soil and that under conditions of water restriction, the smaller seeds may be less affected; a small amount of water is sufficient to activate the germination process.

Nogueira et al. (2010) showed that the seeds of *Dalbergia cearensis* (a tree species, widely distributed in Brazil) present advantages for semiarid conditions of the Caatinga because they are small. Small seeds tend to be more easily buried when dispersed, forming seed banks that germinate under favorable humidity conditions (Westoby, 1998; Pérez-Harguindeguy et al., 2013), therefore presenting greater longevity in seed banks (Bakker, Poschlod, Strykstra, Bekker,& Thompson, 1996; Moles et al., 2007) Caatinga soils are shallow, stony soils, which make it difficult for large seeds to be stored in seed banks as well, coupled with the strong solar irradiation due to the large volume, which can more easily desiccate these seeds making them unviable. The ability of seeds to remain viable and stored in the soil provides a great advantage in the establishment process (Wright et al., 2007). Thus, seed longevity is closely linked to water content, since it directly interferes with physiological processes, reducing seed quality, affecting vigor and germination (Marcos Filho, 2005). Thus, under the environmental conditions of the Caatinga, the occurrence of small seeds is expected

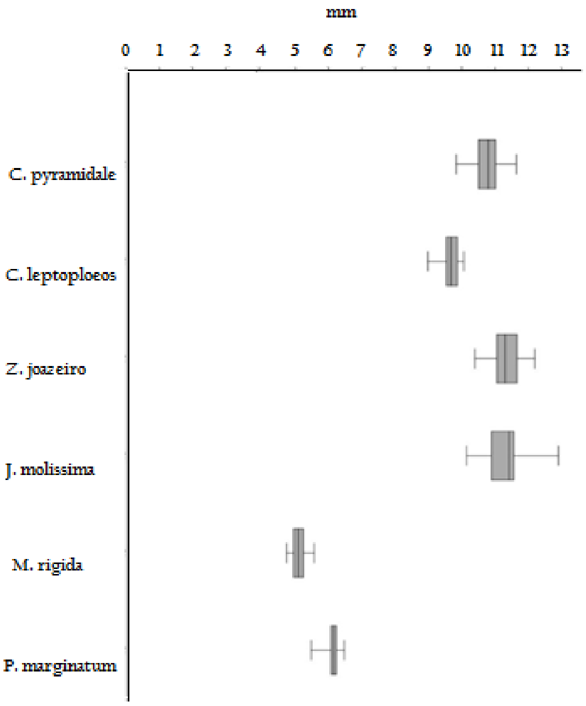

Figure 2. Box plot of Length (mm) values of six Caatinga species.

Euclidean distance showed a separation between the biometric parameters of *M. rigida* and *P. marginatum* seeds and the others. This separation was due to the smallest variation in all parameters in the seeds of these species. It was also possible to verify that there was variation between individuals of each species, and in *C. pyramidale* the individual CAT3 presented greater variation. The cophenetic coefficient was 0.8485, which demonstrates a good fit between the graphical representation and its matrices (Figure 6).

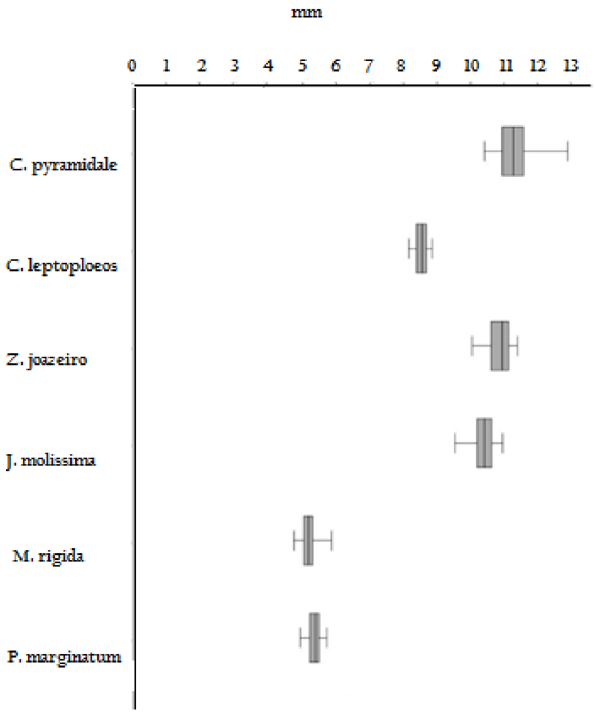

Figure 3. Box plot of Width (W) values of six Caatinga species.

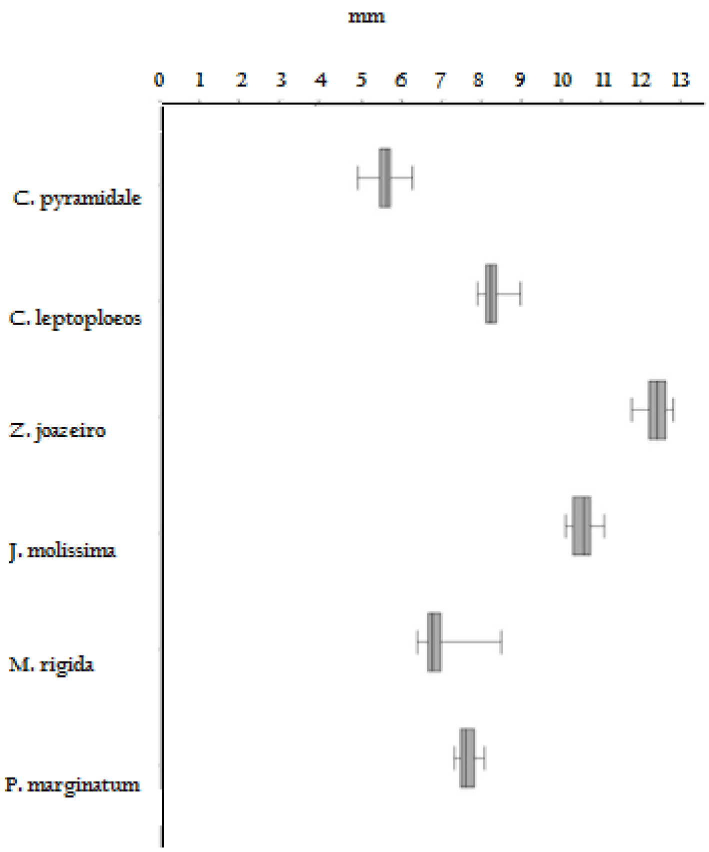

Figure 4. Box plot of thickness (T) values of six Caatinga species.

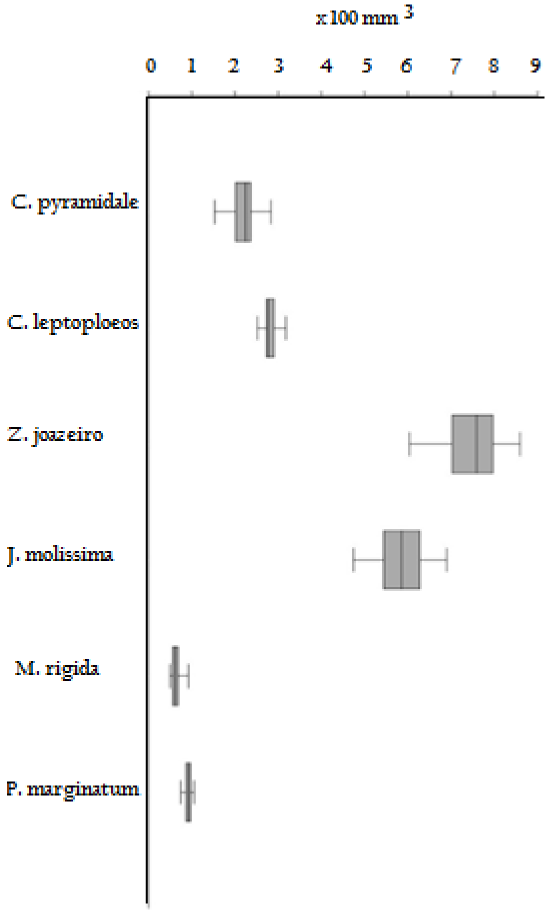

Figure 5. Box plot of Volume (V) values of six Caatinga species.

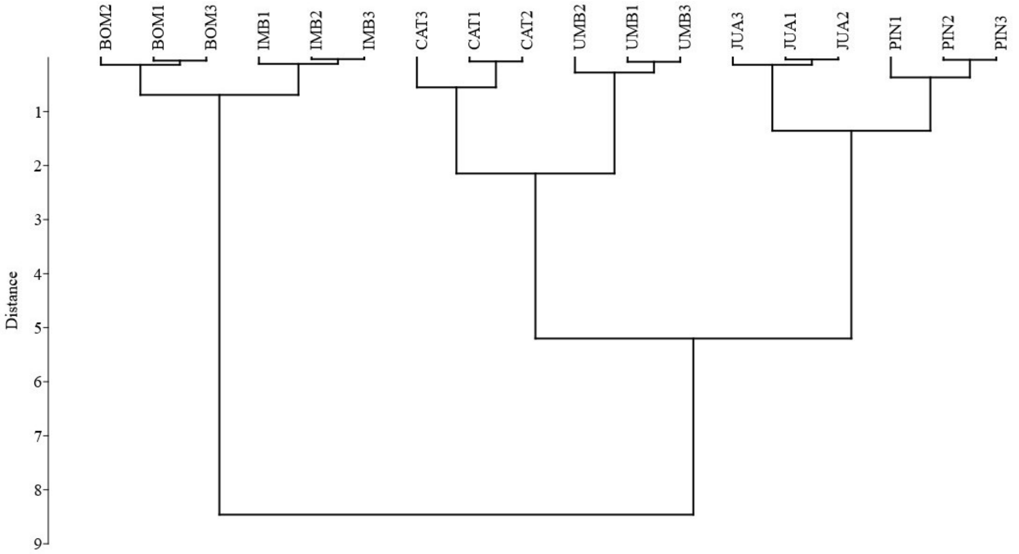

Figure 6. Dendrogram of intra and interspecific similarity in relation to biometric variables (length-width-thickness-volume) of Caatinga species seeds. *C. pyramidale* (CAT), *C. lepthophlocos* (UMB), *Z. joazeiro* (JUA), *J. molissima* (PIN), *M. rigida* (BOM) and *P. marginatum* (IMB). Cofenetic coefficient = 0.8485.

The PCA indicated which variables most influenced the ordering of points on the axes (Figure 7). The analysis of axis 1 with 78.73% of the data variation indicates that the volume variable was the one that ordered the organization of the points. While for axis 2, with 20.27% the variable was the thickness. Therefore, *Z. joazeiro* and *J. mollissima* presented the largest volumes (size) while *M. rigida* and *P. marginatum* presented seeds with smaller volume. The seeds of *C. pyramidale* and *C. lepthophloeos* presented low thickness when compared to the others. The proximity of *C. lepthophloeos* seed data to the point of interception of the PCA axes shows that there is no variation in this data.

We did not identify on the PCA the formation of clusters that strongly characterize a relationship with phenological groups or modes of dispersion. However, when comparing the formed groups (I, II and III) with the works of Carvalho, Souza, and Trovão (2012), Cabral, Sampaio, and Almeida-Cortez (2013) and Silva et al. (2014), in which Caatinga species were categorized according to ecological succession stages, we noticed a tendency to separation by this condition. For example, the species *M. rigida* and *P. marginatum*, which formed group I, are classified as late secondary. Group II formed by *C. pyramidale* and *C. leptophloeos* were characterized as intermediate stage of succession. *Jatropha molissima* and *Z. joazeiro* formed the pioneer group III.

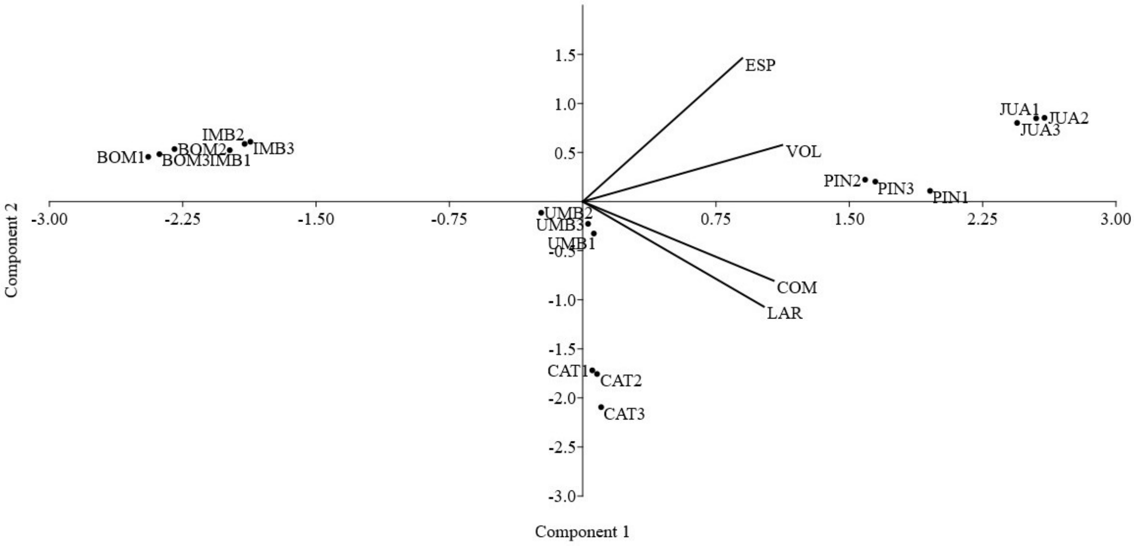

Figure 7. Principal component analysis, showing the distance of Caatinga species matrices as a function of the characteristics: Length (COM), Width (LAR), Thickness (ESP) and Volume (VOL). (*C. pyramidale* (CAT), *C. lepthophlocos* (UMB), *Z. joazeiro* (JUA), *J. molissima* (PIN), *M. rigida* (BOM) and *P. marginatum* (IMB).

The fact that *C. pyramidale* appeared a little further away in the principal component analysis may indicate the versatility of this species in relation to its successional stage. Moreover, it reflects a new perspective on this classification based on this study, where the biometry of its seeds identifies it close to *C. leptoplhoesos*, which in all studies categorizes it as intermediate stage. *C. pyramidale* is a species that does not disappear from the physiognomy as the community establishes, remaining in late successional stages. Their presence is always reported in studies of conserved communities in the Caatinga (Guedes, Zanella, Costa Júnior, Santana, & Silva, 2012; Lima, Coelho, & Oliveira, 2012; Cabral et al., 2013; Sabino, Cunha, & Santana, 2016).

In 1965, Budowski studied ecological succession and adopted seed size as a parameter for the classification of successional stages. However, when it comes to Caatinga, there is no defined standard in the literature for this correlation. Species such as C. pyramidale, which is widely distributed at all successional levels (Cabral et al., 2013), challenges well understood correlation patterns in other vegetation formations.

Another factor is associated with the average height of Caatinga plant species, which is considered low when compared to other plant formations (Olson et al., 2018). Rees and Venable (2007) investigated the relationship between tree size and seed size, arguing that tall trees produce large seeds. According to Rees and Venable (2007), the size of the seeds is related to the evolutionary history of the vegetation, so it would be expected that plants that occur in stressed habitats and with low availability of nutrients, such as the Caatinga, normally grow slowly and bear small fruits and seeds. Thus, the average plant height in the Caatinga, 5 to 15 meters, does not favor the production of large fruits and large seeds. Additionally, the Caatinga species preferentially have wind dispersion (Barbosa et al., 2003) which also makes larger or heavy seeds unfeasible. However, this fact is corroborated by the almost nonexistence of large dispersing mammals (Leal, Tabarelli, & Silva, 2003; Yang et al., 2018) in areas that suffer from anthropic interference, which makes it unnecessary and costly for plants to invest in large fleshy fruits that are usually associated with large seeds.

Conclusion

The seeds of the species analyzed from the Caatinga are generally small. This fact is associated with its adaptations to environmental stresses, especially water stress, which makes seed germination and seedling establishment impossible for species that require large amounts of water. Seeds of common species in early successional stages, such as *J. molissima* and *Z. joazeiro* in secondary succession presented larger sizes, which facilitates their establishment in space due to the absence of competitors and greater nutritional reserve for the embryo, among other factors. As the plant community settles in, other filters emerge. The competition for resources, especially water, common in Caatinga, added to the short period favorable to germination, led the species of this vegetative physiognomy to not invest in large seeds, as this would make the chance of dispersal impossible or reduced. The biometrics of the seeds analyzed was a functional trait that allowed the classification into groups of successional levels of the species and can be an important tool for identification of successional strata in the Caatinga.

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