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ARTICLE

Description and automated seed morphostructural characterization of *Myrciaria dubia* (Kunth) McVaugh: Diagnostic Imaging

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ABSTRACT: (Description and characterization morphostructural automated *Myrciaria dubia* seeds (Kunth) McVaugh: Diagnostic Imaging). The use of diagnostic imaging technology has been presented as an important tool for the rapid and accurate diagnosis of the morphology and uniformity of plant materials such as seeds, fruits and seedlings. Thus, this study aimed to describe and characterize seeds that had been bioprocessed from the pulp extraction of the fruits of *Myrciaria dubia*; these seeds are used in research with technological prospecting and experimental processing in small agro-industries, with the intent of producing quality seedlings for use as raw material for the manufacture of new biotechnology products in the northern Amazon. The study was conducted in waste laboratories and seeds of the EMBRAPA. Representative samples of seeds were selected from December 2013 and were described and characterized by its internal and external parts in the System for Seed Analysis (SAS) by applying the principles of diagnostic imaging techniques. The seeds were 1.72 cm in diameter with an interval of 1.50 cm - 1.99 cm and were 0.49 cm long. The minimum average diameter was 1.18 cm, with intervals ranging from 1.01 to 1.34 cm. For the predominant colors, blue, green and red channels values had values of 31.19, 49.69, and 87.94, respectively, with noticeable visual observations that demonstrated the maturation phase. The diagnostic imaging technique allows for the efficient and effective characterization and classification of morphostructural *M. dubia* seeds compared to the conventional method.
Keywords: Northern Amazon, camu-camu, quality, remaining bioprocessed, SAS.

RESUMO: (Descrição e caracterização morfo-estrutural automatizada de sementes de *Myrciaria dubia* (Kunth) McVaugh: Diagnóstico por imagem). O uso da tecnologia de diagnóstico por imagem vem se apresentando como importante ferramenta para uma análise rápida e precisa referente a morfologia e uniformidade de materiais vegetais tais como sementes, frutos e mudas. Assim, neste trabalho objetivou-se descrever e caracterizar as sementes, remanescente bioprocessado, proveniente da extração da polpa dos frutos de *Myrciaria dubia*, utilizados em pesquisas com prospecção tecnológica e processamento experimental em agroindústrias de pequeno porte, com vistas a produção de mudas de qualidade e uso como matéria-prima para fabricação de novos produtos biotecnológicos na Amazônia setentrional. O estudo foi realizado nos laboratórios de resíduos e sementes da EMBRAPA. Amostras representativas de sementes foram selecionadas a partir de dezembro de 2013, sendo descritas e caracterizadas no Sistema para Análise de Sementes (SAS) aplicando-se os principios da técnica de diagnose por imagem. As sementes apresentaram 1,72 centímetros de diâmetro, com um intervalo de 1,50 cm - 1,99 centímetros e 0,49 cm de comprimento. O diâmetro mínimo médio foi de 1,18 cm, com intervalos de 1,01-1,34 cm. Para as cores predominantes, os canais de valores azuis, verdes e vermelhos tiveram os valores de 31,19, 49,69, 87,94, respectivamente, perceptíveis com observações visuais, demonstrando a fase de maturação do fruto. A técnica de diagnose por imagens permite a caracterização e classificação morfoestrutural da semente de *M. dubia*, de forma eficiente e eficaz, quando comparada ao método convencional.
Palavras-chave: Amazônia setentrional, camu-camu, qualidade, remanescente bioprocessado, SAS.

INTRODUCTION

The use of diagnostic imaging technology has been presented as an important tool for fast and accurate diagnosis in several areas. It is based on the utilization of high resolution images and techniques of pattern recognition. It captures, treats and interprets images with software that collects information such as color, shape, texture, morphology and uniformity of several plant materials including seeds, fruits, seedlings and plants. In the area of seeds, imaging analysis can be extremely useful, since the development of simple, fast and non-destructive methods for evaluation of the quality enables, according to Carvalho (2010), an assessment of the potential for storage or for development in the field. Additionally, analysis has great importance in the establishment of dynamic and efficient quality control programs.

Digital images have been utilized in the ordinary analyses of quality patterns because of the possibility

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of verifying parameters such as size, color and texture and eliminating the subjectivity of the analysis and the dependence on the human visual system, which in turn is influenced by environmental conditions that can lead to inconsistencies (Antonelli *et al*. 2004, Du & Sun 2004, Milanez 2013). According to Carvalho (2010), digital imaging processing techniques provide a greater analysis sensitivity that is inherent to the computational processing, allowing the acquisition of characteristics that would be impossible to observe by human eye alone.

The imaging analysis has proved to be a promising technique, mainly because of its technological evolution stage, which reflects on advances in the capacity of image capture, treatment and interpretation. The speed in digital image detection and informatized data processing, in addition to decreased costs, has made image analysis systems more appropriate in the automatic evaluation of the quality and classification of products (Carvalho 2010). As an example, the Seed Analysis System (SAS) was developed recently in Brazil and has the capacity to extract a number of pieces of information based upon image diagnosis technology, such as color, shape, texture, morphology and uniformity.

Brazil is one of the greatest agricultural producers in the world and has managed to significantly improve its production capacity recently. Nevertheless, in response to this advance in the agro-industrial sector, the generation of residues or by-products also increased, and their inadequate elimination may present environmental impacts, causing losses of raw materials and energy.

Raw material and waste have the same function for different organisms, providing the minimization of losses and the best environmental efficiency (Gameiro & Silva 2007). Therefore, what is produced in parallel to a main product, whether because of the process or because of the processing, can possibly present a potential for the addition of economic and environmental value. Specifically, according to ANFALPET (2011), this value could be diversification and better use of raw materials.

The fruit of the species *Myrciaria dubia* that is utilized for pulp extraction, the main product, presents a high ascorbic acid content, with a range up to 8,000 mg 100 g^{-1} higher than acerola (Chagas *et al*. 2013) and generates skins and seeds that are called remaining bioprocessed materials or coproducts. These, by definition, are considered raw materials that are generated from the processing of products (primary) that were generally originated from food, siderurgical and metallurgical industries, among others, according to ANFALPET (2011).

The seeds of *M. dubia* are normally utilized for species propagation (Chagas *et al*. 2012); nevertheless, there is a scarcity of more detailed information, particularly with Good Laboratory Practices, that provides identification, preservation, transportation and storage conditions of the sample before analysis. In addition, there is minimal information of these seeds when they are characterized as co-products, e.g., when raw material is generated from the processing of the fruit of *M. dubia*.

The fruit that gives rise to the seeds is popularly known as camu-camu. It is named caçari in the state of Roraima and in other states of the Amazon region; according to Yuyama (2002) and Bacelar-Lima (2009), it is known as araçá, araçarana, araçazinho, sarão, socoró, azedinha, etc.

Thus, considering that currently the choice of a product is essentially motivated by its value and by the beneficial properties related to the value (Milanez 2013), obtaining methodologies capable of evaluating the quality and authenticity of products in a simple, fast and low cost way becomes necessary.

This work aimed to describe and characterize the seeds that had been bioprocessed from the pulp extraction of the fruits of *Myrciaria dubia*; these seeds are used in research with technological prospecting and experimental processing in small agro-industries, with the intent of producing quality seedlings for use as raw material for the manufacture of new biotechnology products in the northern Amazon.

MATERIALS AND METHODS

The study was conducted in the residues and seed laboratory that is located in the seat of the Brazilian Corporation of Agricultural Research, situated at 02°42'30"N and 47°38'00"W, 90 m of altitude; representative samples of seeds were selected in December 2013 that had their internal and external parts described and characterized in the system for Seed Analysis (SAS) by applying the principles of diagnostic imaging.

Randomly, 10% of the total samples received in the residue laboratory corresponding to 1,000 g of seeds were selected. From that amount, subsamples were selected for use and application of the diagnostic imaging technology. Anatomic and pictorial morphological descriptions of the *M. dubia* seeds were obtained, and iconographic methods were used for verification of the color, presence or absence of endosperm, and the type, shape and color of cotyledons.

First, from the sample, some seeds were selected at random (Fig. 1A-B) for longitudinal sectioning and consequent visual observation and characteristic recording.

In the Seed Analysis System (SAS), the analysis of four parameters was performed, namely: color, geometry, image and texture moments. In this analysis, the combination of image measuring modules and an item of software that utilizes artificial intelligence to process the information collected, was utilized (TBIT 2015).

In the analysis via SAS, five replications were utilized; each of them were made up of thirty seeds that were consecutively distributed onto a tray made with transparent film, type A4 – 210 x 297 mm with the dimensions of 19 cm x 26.5 cm x 1 cm (Fig. 2).

Afterwards, the process for the pre-analysis screen was started by pressing the button "New Analysis in the software". This screen enabled real-time presentation of the samples that were already in the measuring modules, allowing for the analysis of seed positioning and the choice of the analysis setting, in addition to the

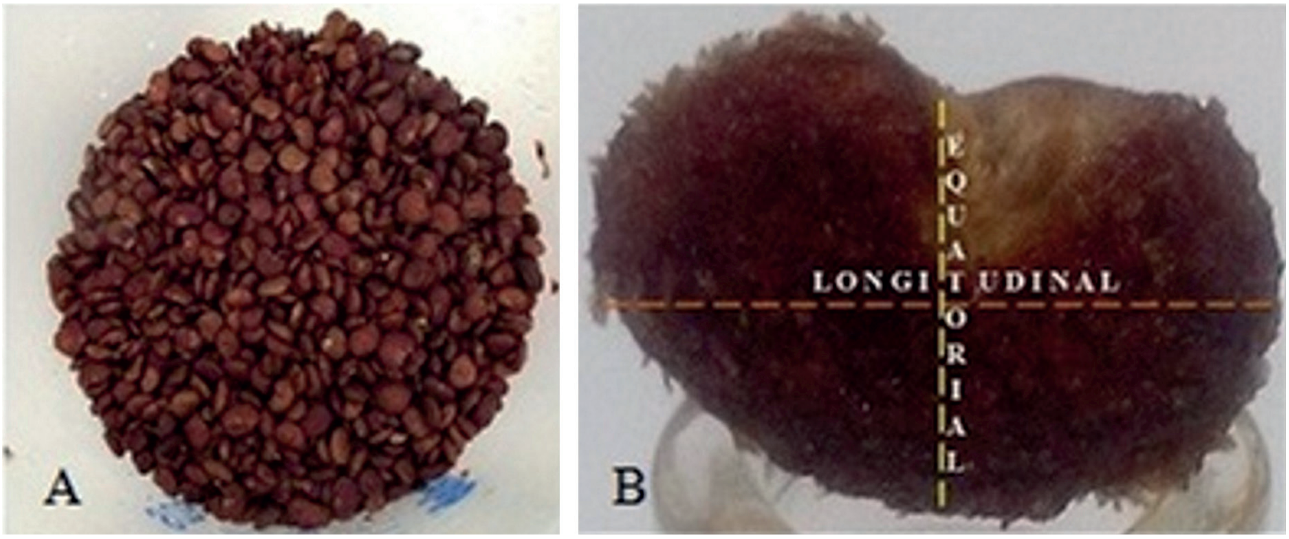

Figure 1. Seeds of *M. dubia* (A) samples selected (Sousa 2013) for morphoanatomical characterization and (B) enlarged seed, showing dimensions to be measured (Silva 2014). Source: Prepared by Sousa & Silva (2015).

exportation of information generated in the system to spreadsheets (Fig. 3).

The SAS detail screen allows access to the tools of the system for exportation of the data including shapes, variables, plots**,** statistics and colors. After obtaining the images (Fig. 3), they were decomposed into histograms containing distributions of the levels of colors allowed to a pixel. Three modules for the color of a pixel were utilized: red-green-blue (RGB), matrix-saturation-intensity (HSI) and shades of gray. Linear discriminant analysis (LDA) was utilized for the development of classification models on the basis of a reduced variable subset.

For variable selection purposes, two techniques were utilized: successive projection algorithm (SPA) and stepwise (SW). Models based upon partial least square analysis (PLS-DA) that were applied to the complete histograms (without selection of variables) were also utilized for the purpose of comparison.

To present the results, a set of descriptive statistics based on the characteristics drawn from the objects (samples) was generated in the SAS. The representations of the geometric information such as perimeter, convex perimeter, maximum diameter, minimum diameter and centroid, are demonstrated in images obtained from the SAS. In these demonstrations, it is also possible to observe a representation of the geometry, LBP histogram

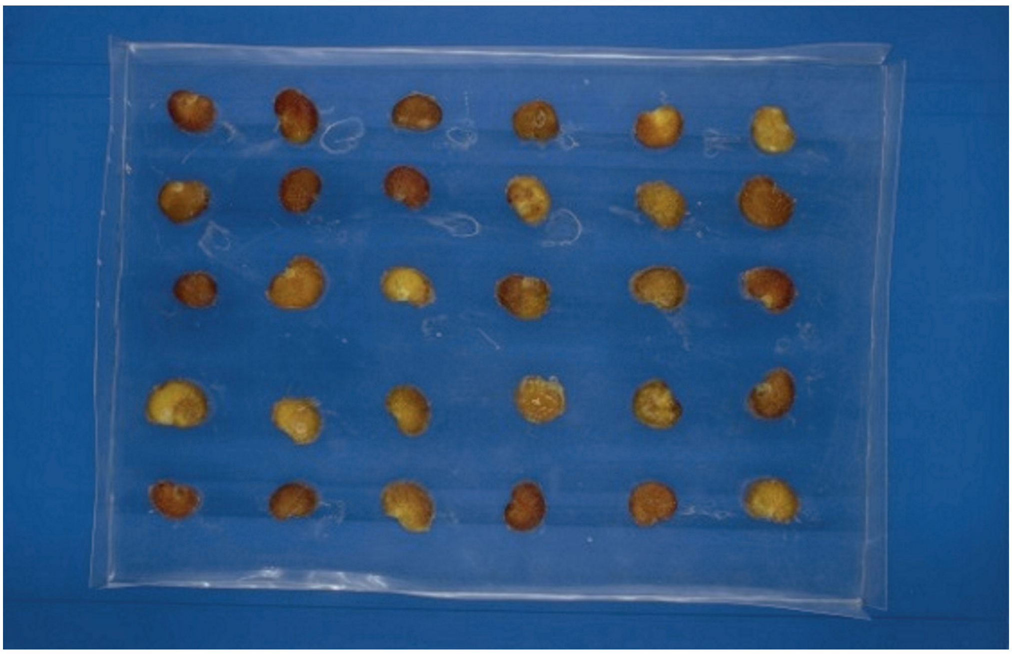

Figure 2. Tray made on transparent film (19 cm x 26.5 cm x 1 cm) containing samples of seeds of *M. dubia* in the measuring modules for analysis in SAS. Source: Prepared by Sousa & Silva (2013).

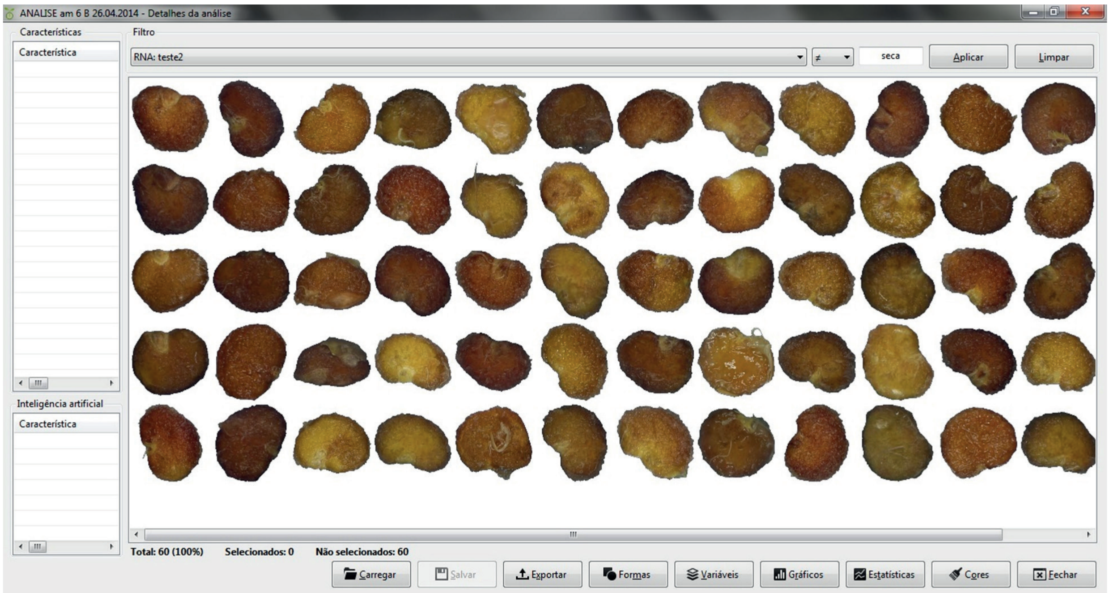

Figure 3. Samples of *M. dubia* seeds distributed on the detail screen of the SAS. Source: prepared by the authors in SAS (2013).

and LBP topology of each object (seed) in the study.

On the detail screen for obtaining analysis results (Fig. 3), one can select other characteristics to be studied as the variable "color" of the structure of the "hilum" and the variable "texture"; it is possible to obtain numerical results either singly or grouped in the form of a histogram.

RESULTS

The simplified morphoanatomical longitudinal section of the internal and external surface of a *M. dubia* seed that was obtained via an iconographic technique (Fig. 4) showed that the seed has two cotyledons that were apparently fused. Externally, the seed has a light brown color, is kidney-shaped and is conspicuously flattened with a thin, permeable and absorbent integument; the rough surface was covered by a network of fibrils. The hilum is medium-sized, opaque and lateral-ventral, emitting fiber extensions that are dispersed throughout the pulp.

For interpreting the data obtained via SAS relative to the comprehension of the geometric information (Fig. 5) of the *M. dubia* seed, the perimeter of the object under study (seed) is defined by the sum of all the sides of the

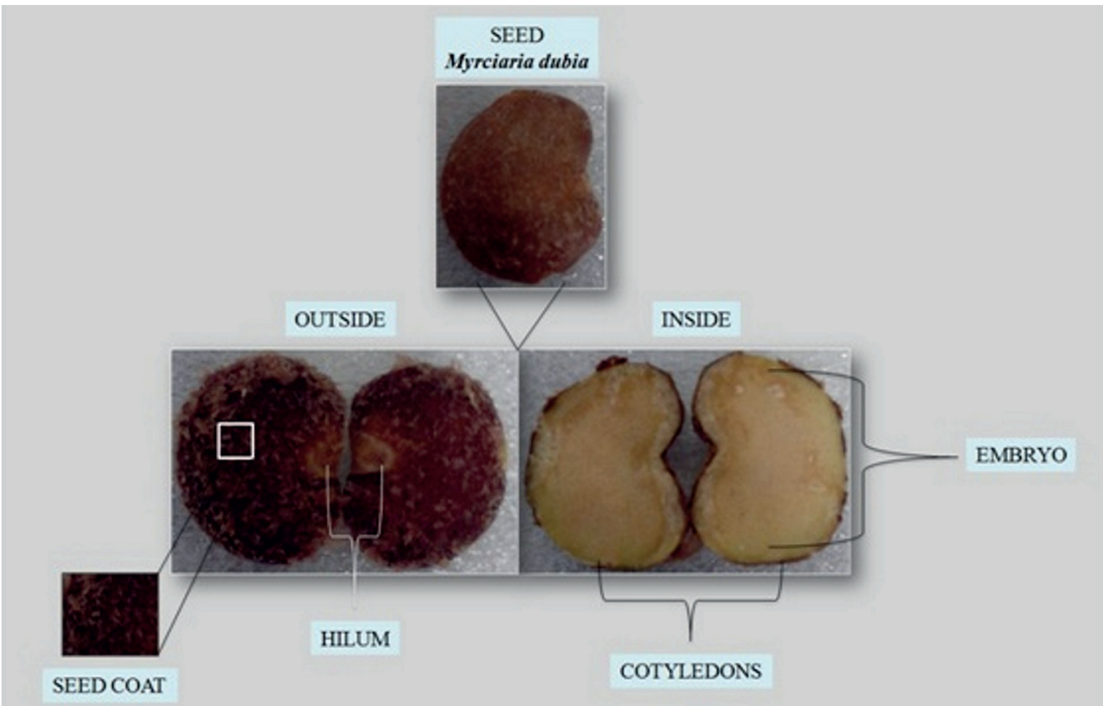

Figure 4. View of external and internal surfaces of *M. dubia* seeds in a longitudinal section. Source: Prepared by Sousa & Bacelar-Lima (2015).

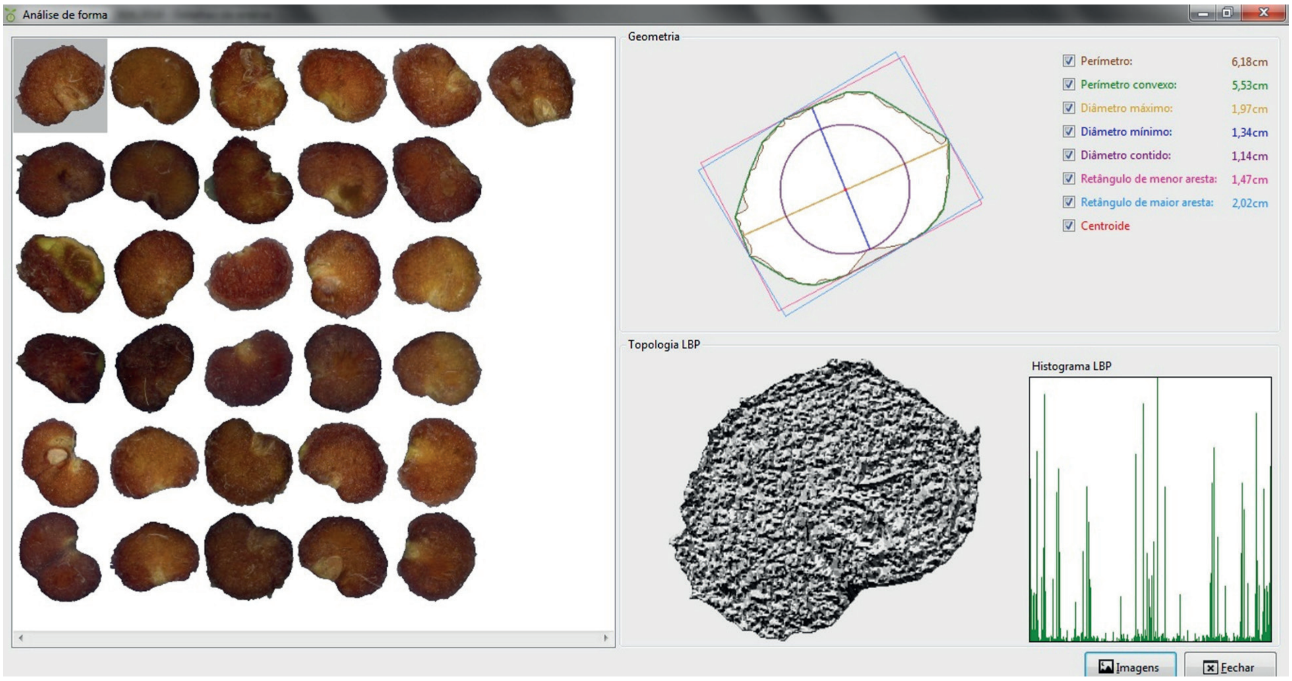

Figure 5. Analysis of geometry, topology LBP and LBP histogram seeds of *M. dubia in natura* obtained by image selection shown on the left in the SAS screen. Source: prepared by the authors in SAS (2013).

object; the diameter is any straight line passing through the center of a figure and the centroid is the point on the inside of a geometric shape that defines its center. However, the LBP topology of each object of the study (seed) is a code that represents the relationship between the pixels. These codes are plotted as a histogram to represent the surface texture (TBIT 2015).

Topology is one of the newest branches of mathematics and therefore assumes ever-increasing importance in the investigation of the secrets of nature (Borges 2005). It aims to study the properties of space independent of any idea of measurement. It is popularly called rubber sheet geometry or geometry without a measurement. Under certain conditions, there is no distinction between a drawing and its copy, even if it is badly done; an example is shown in figure 5 with images of *M. dubia* seeds that were analyzed in the SAS. It is a case of describing the space itself and its properties (Valente 2000), for example, a circle is topologically equivalent to an ellipse.

The information obtained for *M. dubia* seed samples (*in natura*) concerning the size (geometry) in centimeters (cm), the predominant color, texture and color variation given in numerical codes (Table 1) were extracted or filtered from characteristics listed in the SAS. The "geometry" displays the maximal and minimal diameters and perimeter (cm); the "predominant color" shows channels with blue, green and red, and the color variation and the "SFM texture" display values for the measures of frequency space and modified frequency, respectively. In relation to color and texture, the values (codes) are based on the color chart of plant tissue (Munsell 1977).

Figure 5 shows the geometric representation of *M. dubia*

Table 1. Results of variables selected for *M. dubia* seeds in SAS regarding dimensions (geometry) in centimeters (cm), predominant color, texture, and color variation in numerical codes.

Character		Mean	StanDev	Med	Mod	Var	Max	Min	Leng	Total
Geometry	MD	1.72	0.13	1.72	1.68	0.02	1.99	1.50	0.49	27.47
	MI	1.18	0.11	1.20	1.15	0.01	1.34	1.01	0.33	18.93
	PER	5.41	0.44	5.45	5.14	0.19	6.12	4.64	1.49	86.55
Color	BC	31.19	5.33	30.00	30.00	28.43	43.00	22.00	21.00	499.00
Pred	GC	49.69	14.92	45.00	45.00	222.63	82.00	30.00	52.00	795.00
	RC	87.94	27.96	81.50	51.00	781.53	138.00	47.00	91.00	1407.00
Texture SFM	MFS	4.54	0.85	4.30	3.73	0.72	6.23	3.68	2.55	72.70
	MMF	2.58	0.67	2.30	2.09	0.46	4.19	1.94	2.25	41.22
Color variation		0.06	0.05	0.04	0.11	0.00	0.17	0.00	0.17	0.92

Abbreviations: DMA, maximum diameter; DMI, minimum diameter; PER, perimeter; BC, blue channel; GC, green channel; RC, red channel; SFM, Spatial Measurement Frequency; MFS, Mean frequency space; MMF, Mean modified frequency; Color Pred, Color predominant; Stan Dev, Standart deviation; Med, Median; Mod, Mode; Var, Variance; Max, Maximum; Min, Minimum; Leng, Length.

seeds: perimeter convex perimeter, maximum diameter, minimum diameter, and centroid. It also presents a histogram of the LBP and LBP topology of each object (seed *in natura*) in the study that was obtained by image selection as indicated in the SAS fabric. However, camu-camu seeds presented an average 1.72 cm diameter, with a range of 1.50 cm - 1.99 cm and were 0.49 cm long. The minimum average diameter was 1.18 cm, with the range varying from 1.01 to 1.34 cm.

The color of an object/sample obtained by a mathematical formula relates to its greater occurrence that allows formation of color groupings or channels that can also be extracted from pixels. SFM from the English *Spatial Measurement Frequency* refers to the overall level of activity of an image. This calculation is done by covering the picture from left to right and from top to bottom for the frequencies in rows (R) and columns (C) given in conventional or modified mathematical formulas as specified in the "Summary" window of the SAS by browsing in "Calculated Characteristics: Textures".

In the analysis, it was observed that for the predominant colors, the blue, green and red channels presented values of 31.19, 49.69, and 87.94, respectively (Table 1). These colors are perceptible with visual observations and show the maturation stage of the fruit. The variables "frequency space" and "modified frequency" concerning "texture", presented 4.54 and 2.58 as the mean values, respectively (Tab. 1).

In figures 6 and 7, it is possible to verify the numerical results obtained either singly or grouped in the form of a histogram, relative to the variable "color" of the structure of the "hilum" and the variable "texture" of the samples of seeds of *M. dubia* analyzed via SAS.

DISCUSSION

Few works, such as Villachica *et al*. (1996), Maciel *et al*. (2009) and Smith *et al*. (2014), have covered morphological, eco-physiological and histochemical studies on the primary method of spreading *M. dubia* seeds.

M. dubia seeds, and all dicots, have no developed endosperm. The embryo fills its entire volume with an axial position that is invaginated, i.e., straight and is composed of a green cotyledon mass that has a massive, thick covering hiding the hypocotyl-root axis lying around the reserve material that is stored in a bulky closed cotyledon that is fused, making it difficult to separate without damage (Maciel *et al*. 2009).

By analyzing the SAS, it is possible obtain important informational characteristics on the *M. dubia* seed, such as "color" and "texture" of the heel, in addition to conventional morphometric analysis. The seeds of camu-camu showed variation in size and feature that were also observed by Maciel *et al*. (2009), but with lower values reported as a length of 13.72 ± 1.53 mm and a width of 10.71 ± 1.48 mm above the fresh weight of 1.51 ± 0.96 g. According to Pê *et al*. (2003), the physical characteristics such as seed size and shape are of great interest for the control of equipment automation that aims to improve the product quality, add economic value and, consequently,

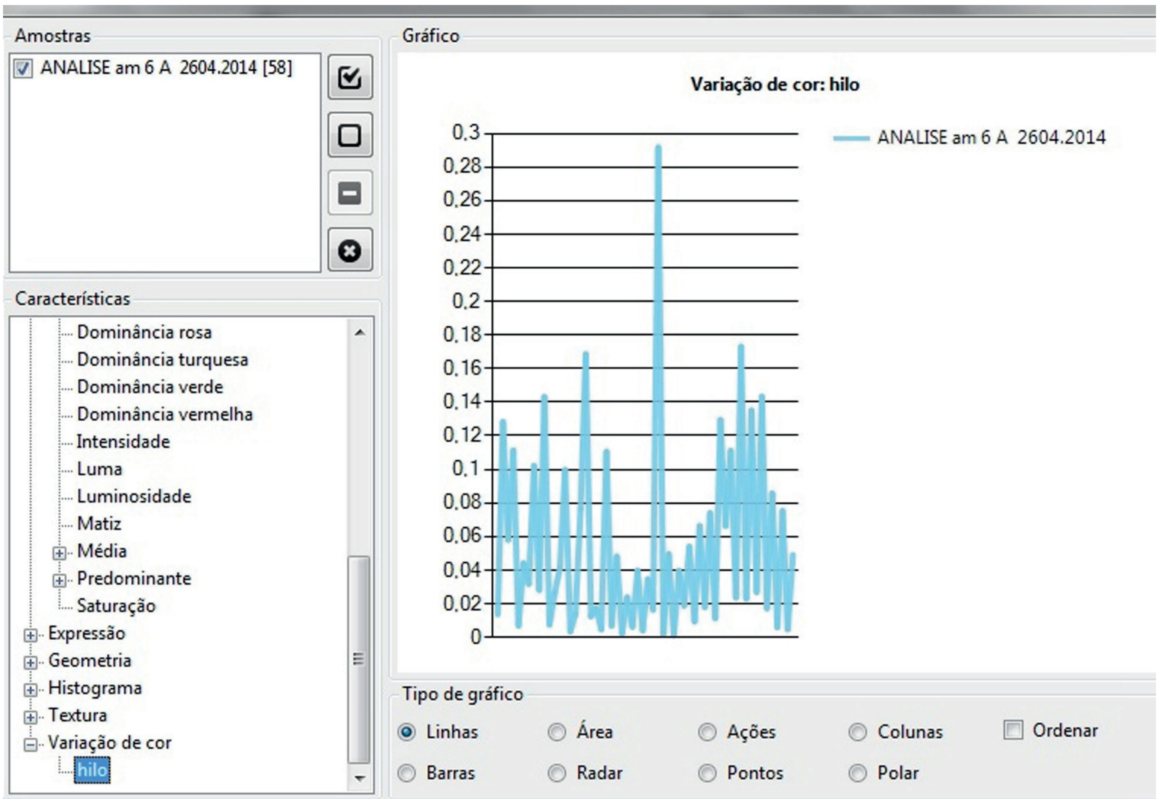

Figure 6. Analysis hilum color obtained in *M. dubia* seed-samples (*in nature*) in the SAS screen. Source: prepared by the authors in SAS (2013).

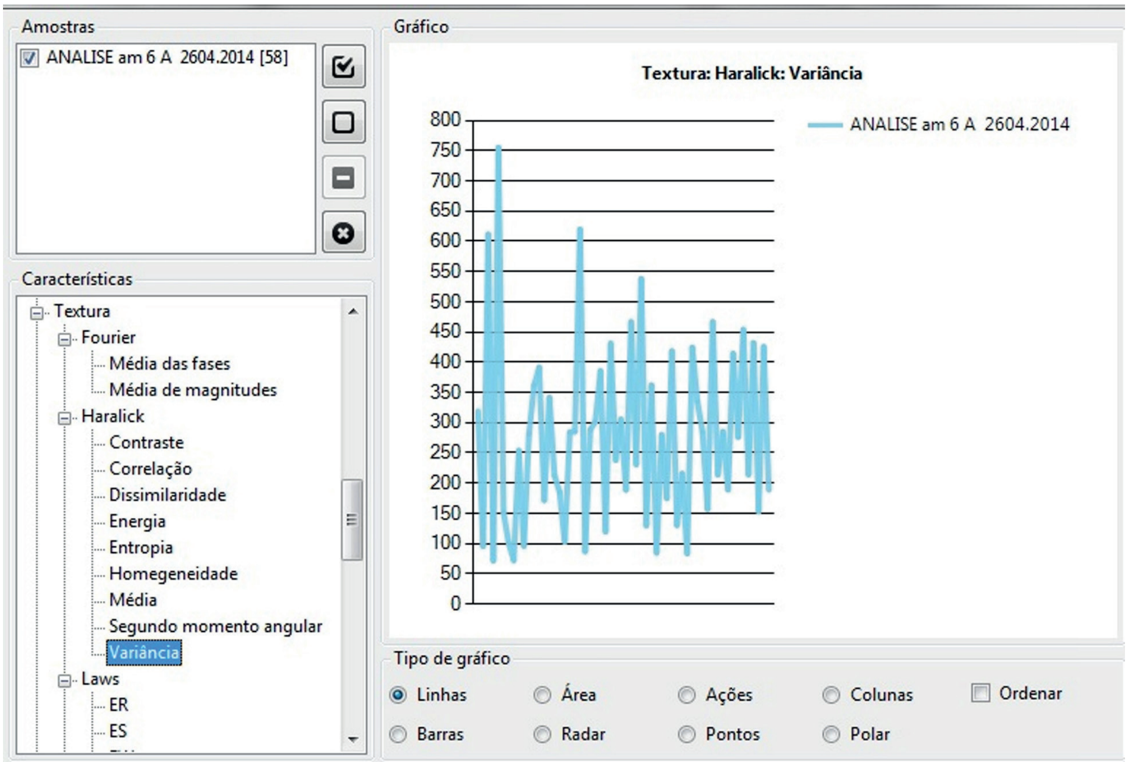

Figure 7. Texture analysis of variance obtained from seeds of wet *M. dubia* (*in nature*) in the SAS screen. Source: prepared by the authors in SAS (2013).

reduce the cost of labor and processing and post-harvest operating times.

These aspects are relevant in the use of techniques that are utilized in the storage and building of silos and other storage devices of those products, helping to solve problems related to heat and mass transfer during drying and aeration steps (Goneli *et al*. 2003).

It was found that the SAS allows numerous reliable morphological and biometric characterizations of seeds and their structures in a short time, especially for work with populations that have several samples to be compared.

Several studies have been conducted to obtain information to assist with the domestication and breeding of this species, but according to Soares *et al*. (2013), Sousa *et al*. (2013), Rodriguez *et al*. (2014) and Neves *et al*. (2015), there is still a need for basic studies on the natural occurrence and distribution areas as well as reviews of agronomic studies of natural populations of these fruits and systematization of information on this species in the state of Roraima. Therefore, it was found that the diagnosis technology provides the selection matrices for future work to improve and complement the active species Genebank (BAG).

CONCLUSION

The diagnostic imaging technique allows efficient and effective characterization and classification of morphostructural *M. dubia* seeds compared to the conventional method. Additionally, the diagnostic imaging technique enables obtaining information about color, texture, shape, complete geometry, among others that is impossible by visual and rapid assessment in existing methods. They are new parameters that are important to be added for product quality characterization.

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