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Morphology of starch grains of cassava cultivars (Manihot esculenta Crantz, Euphorbiaceae) – Pará, Brazil

Morfologia dos grãos de amido de cultivares de mandioca (Manihot esculenta Crantz, Euphorbiaceae) – Pará, Brasil

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ABSTRACT
Cassava (*Manihot esculenta* Crantz, Euphorbiaceae) is a species widely grown in Brazil, mainly by traditional communities of the Amazon, the cassava plots being home to a large number of cultivars yet to be investigated. This study aimed to characterize the morphology of starch grains found in the roots of five cultivars of cassava, coming from the Caxiuanã National Forest, Pará, Brazil, to verify the similarity among them. The starch grains were measured, described and illustrated in light microscopy and scanning electron microscopy. They were found to be smooth surfaced, non-compound, presenting miter, cup and rounded forms. Although the morphologies of the starch grains were quite similar among the cultivars studied, the statistical treatment applied showed significant differences in diameter, allowing the five cultivars to be narrowed down to only four.
Keywords: Euphorbiaceae; Caxiuanã National Forest; morphometry of amiloplasts.

RESUMO
A mandioca (*Manihot esculenta* Crantz, Euphorbiaceae) é uma espécie amplamente cultivada no Brasil, principalmente pelas comunidades tradicionais da Amazônia, que abrigam em seus roçados grande número de cultivares ainda pouco explorados. Objetivou-se caracterizar a morfologia dos grãos de amido encontrados nas raízes de cinco cultivares de mandioca, procedentes da Floresta Nacional (Flona) de Caxiuanã, Pará, Brasil, a fim de verificar a similaridade entre eles. Os grãos de amido foram medidos, descritos e ilustrados em microscopia de luz e eletrônica de varredura. Constatou-se que são de superfície lisa, não compostos e apresentam as formas mitra, cúpula e arredondada. Embora a morfologia dos grãos de amido tenha se mostrado bastante semelhante entre os cultivares estudados, o tratamento estatístico aplicado evidenciou diferenças quanto ao diâmetro, sendo possível delimitar os cinco cultivares em apenas quatro.
Palavras-chave: Euphorbiaceae; Flona de Caxiuanã; morfometria de amiloplastos.

INTRODUCTION

Cassava (also called manioc or yuca), *Manihot esculenta* Crantz (Euphorbiaceae), is a species widely grown in tropical America and Africa. It originated on the South American continent, probably in Central Brazil, and presents significant genetic diversity, particularly in Latin America and the Caribbean (FUKUDA; SILVA, 2002).

In the Caxiuanã National Forest, located in the municipality of Melgaço (Pará, Brazil), the cultivation of cassava is a major source of livelihood for communities. This type of family farming is typical of the Amazon environment, inherited from past generations and important in economic, ethnic and cultural terms. Brenha (1986) indicated that early Amazonian communities consider cassava as an important source of carbohydrates and vitamins.

In these traditional Amazonian communities, a wide variety of cultivars are found and receive different names according to the region. Leitão Filho (1970) discussed this aspect, coupled with the fact that environmental conditions can also cause morphological changes in a given cultivar, driving to different popular names. The existence of cultivars, besides hindering improvement programs directed towards cassava cultivation, represents one of the biggest obstacles to the taxonomy of the species (ALBUQUERQUE, 1969).

In recent years, the search for new species rich in starch reserves has fueled the development of advanced technologies derived from starch grains. However, data available from the literature shows that most plants domesticated by indigenous peoples of the Amazon, despite their elevated biological richness, have not yet been sufficiently evaluated in terms of composition, nutrition or technological potential.

Various plant species manifest the starch grain as the main reserve sugar. This compound, when observed under light microscopy or scanning electron microscopy, displays a wide diversity of forms and structural types. Esau (1972) and Galliard (1987) emphasized that the structure of starch grains is extremely variable among different species, mainly with respect to morphological pattern and arrangement, which interfere with the shape and diameter of these grains. These characteristics are used to clarify doubts about the identification of species, since the plastids of starch grains can serve as diagnostic tools, facilitating taxonomy (ESAU, 1972).

The morphology of starch grains was studied for comparison between current species and archaeological materials, checking for possible changes over time (FREITAS, 2002). Other works contributed to the area of starch grain morphology as well, mainly those of Rosenthal *et al.* (1974) and Cereda and Franco (2002), which investigated the morphology of starch grains of different botanical families, including the Euphorbiaceae, with an emphasis on *Manihot esculenta* Crantz.

Assessing the importance of the species for the Amazon region, this research was designed to characterize and describe the morphology of starch grains of five cultivars of cassava *Manihot esculenta* (Crantz), in order to ascertain the similarity among them and to contribute to a better delimitation of the cultivars.

MATERIAL AND METHODS

Roots were collected from five cultivars of *Manihot esculenta* from the Caxiuanã National Forest, located in the region of Melgaço, coordinates 1°42'30'' S, 51°31'45'' W (figure 1). The cultivars investigated were from the same plot, four years old, named by the farmers: Tartaruga, Jabuti, Açaí Açu, Jaçanã and Domingo Tauá.

Figure 1 – Location map of the collection area (dashed) – Caxiuanã National Forest – Melgaço, Pará, Brazil.

To study the morphology of the starch grains, cassava roots were scraped and the scraps deposited on slides with aqueous glycerin in the proportion 1:1 (v/v) (JOHANSEN, 1940). Five slides were prepared for each cultivar.

The largest diameters of 100 starch grains of each cultivar were measured, totaling 500 grains.

Photomicrographs were obtained using a ZEISS Axiolab microscope, fitted with a digital camera. For the scanning electron microscopy analyses, macerated samples of the cultivar roots were deposited on slides mounted on stubs, coated with a 2.5 nm layer of gold, and then examined in a LEO 1450VP scanning electron microscope.

The descriptions of the forms of the starch grains follow the terminology used by Carvalho and Jochimek (1975).

In order to assess the degree of similarity among the cultivars, an analysis of variance and comparison of means was performed, based on the starch grain measurements. The NTIA program version 4.2.1 was used, developed by Embrapa – Campinas, applying Tukey's test at 5% probability.

RESULTS AND DISCUSSION

The starch grains of *Manihot esculenta* are mostly rounded, presenting also miter and cup forms, smooth surfaced, clustered, non-compound and navel apparent (figures 2 and 3).

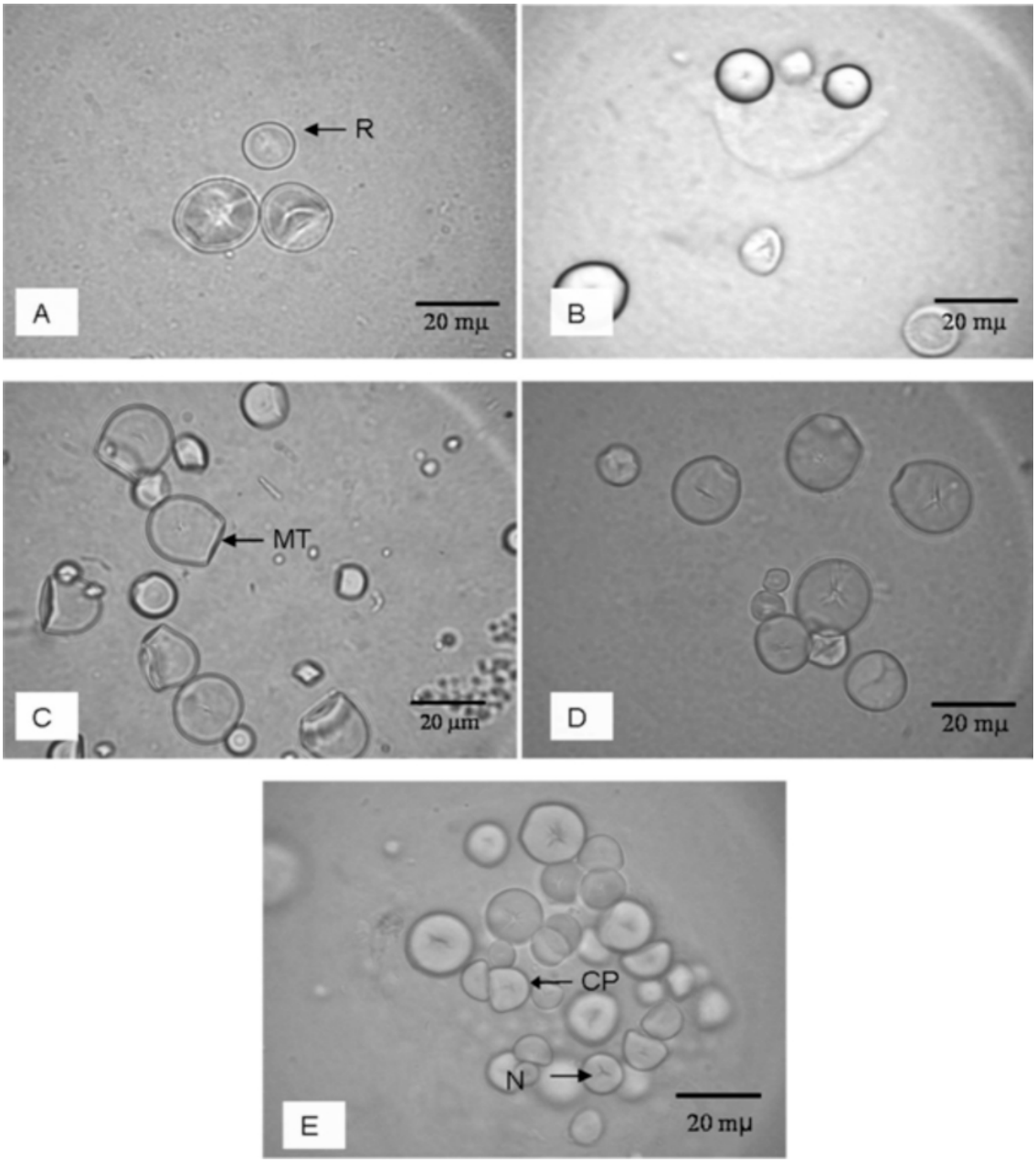

Figure 2 – *Manihot esculenta* Crantz. Photomicrographs of starch grains. Details of the morphology of the grains: A) Tartaruga cultivar; B) Jabuti cultivar; C) Açaí Açu cultivar; D) Jaçanã cultivar; E) Domingo Tauá cultivar and the navel apparent; CP = cupuliform grain; MT = mitriform grain; N = navel; R = rounded rain.

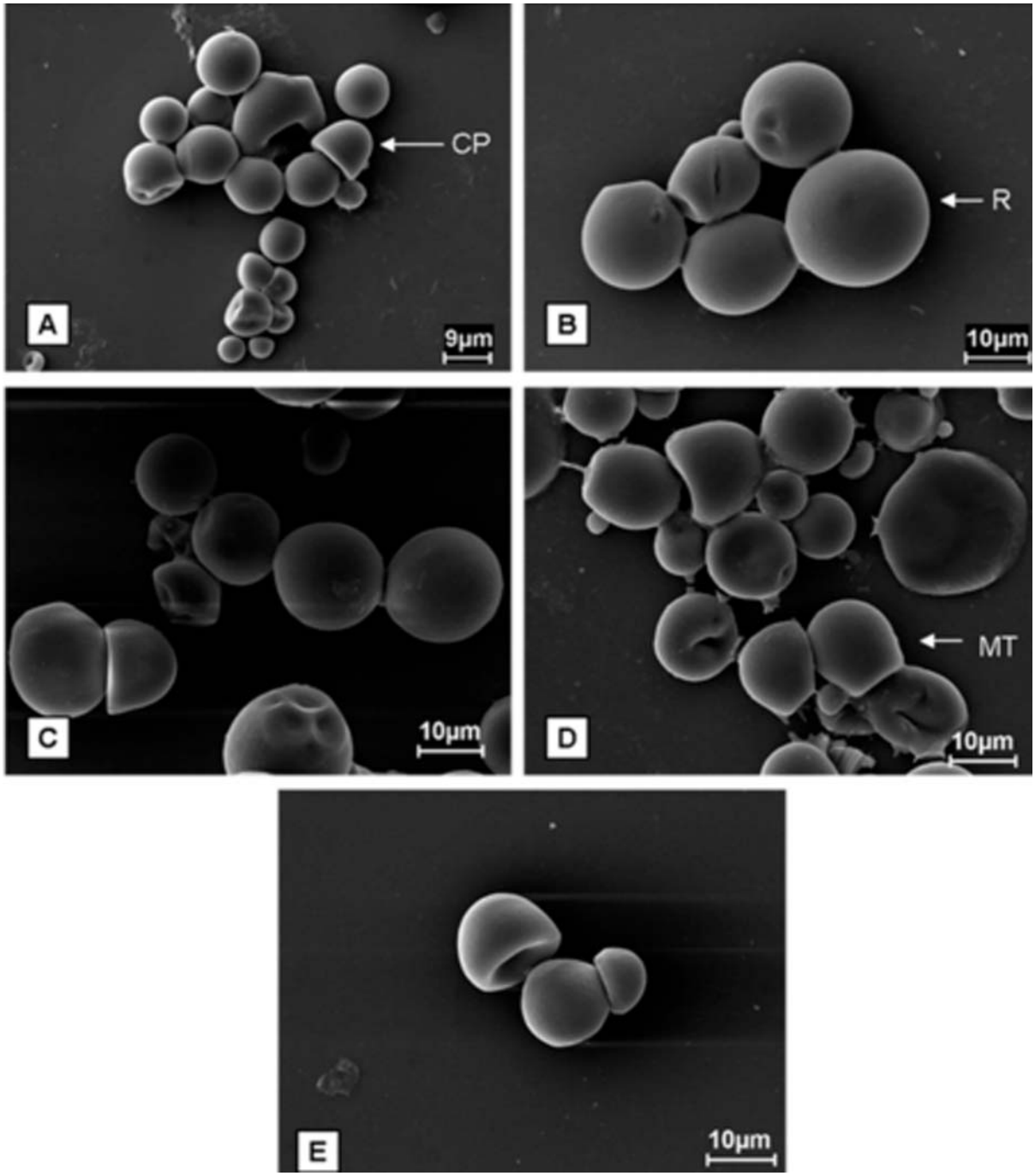

Figure 3 – *Manihot esculenta* Crantz. Electron micrographs of starch grains. Details of the morphology and surface of the grains: A) Tartaruga cultivar; B) Jabuti cultivar; C) Açaí Açu cultivar; D) Jaçanã cultivar; E) Domingo Tauá cultivar; CP = cupuliform grain; MT = mitriform grain; R = rounded grain.

The rounded shape is the most common among the five studied cultivars, with specific representation of the miter and cup forms, according to the type of cultivar. In Jabuti and Jaçanã the mitriform is the second most frequent, in Domingo Tauá and Açaí Açu cupuliform is the second and in Tartaruga, the three forms are well characterized.

Navel vestiges were seen in grains of all the cultivars, with greater emphasis in Domingo Tauá and Jaçanã.

Rosenthal *et al.* (1974), investigating starch grain cultivars of *Manihot esculenta*, verified that they are manifested in round, cupuliform, mitriform, sacciform and rounded pentagonal forms. The data obtained in this study corroborate, partly, the data of the authors mentioned above, although the sacciform and rounded pentagonal forms were not observed here.

Regarding the diameter of the starch grains, the average diameter values presented by each cultivar were: Domingo Tauá 12.1 µm, Açaí Açu 15.9 µm, Jabuti 16.4 µm, Jaçanã 19.3 µm and Tartaruga 15.6 µm (figure 4), with the cultivar Domingo Tauá having the lowest average diameter value.

The variation in the diameter of the grains from the studied cultivars was from 2.5 to 25 µm, with Açaí Açu showing the smallest diameter, while the lowest average was in the Domingo Tauá cultivar (table 1). Also regarding diameter, it was observed that the cultivar Jaçanã exhibited the largest diameter of all the cultivars.

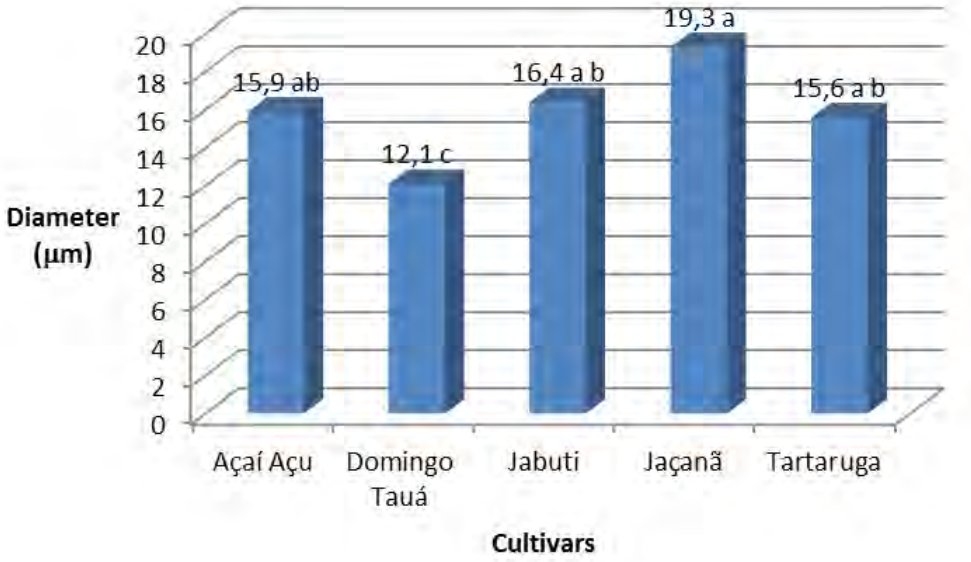

Figure 4 – Average diameter in µm of starch grains of cassava. "a", "b" and "c" are graphical representation of the data obtained by Tukey's test (α = 0,05) demonstrating the degree of similarity among cultivars.

Table 1 – Variation in diameter measurements of starch grains of each cultivar. Values of diameters are: minimum – average – maximum.

Cultivars	Diameters (µm)		
Acaí Açu	2,5	15,9	22,5
Jaçanã	10,0	19,3	25,0
Jabuti	10,0	16,4	22,5
Tartaruga	7,0	15,6	22,5
Domingo Tauá	6,0	12,1	22,0

The diameter of starch grains of cassava cultivars was also investigated by Sriroth *et al*. (1999), that found average diameters of 15 µm, with a distribution of diameters between 8 and 22 µm for four cassava cultivars grown in Thailand, values similar to those obtained in this work. Other studies related to the diameter of the grains were found, performed with farina starch from *Manihot esculenta*: Garcia *et al.* (1997) who showed that the diameter of farina starch grains of cassava varies from 3 to 30 µm with an average of 15 µm; Defloor and Delcour (1998) who obtained average grain diameter ranging between 9.5 and 13.6 µm; Sriroth *et al.* (1999) who determined the average diameter of starch grains at 12 µm with normal distribution of diameters between 7 and 28 µm. These variations probably occur due to the techniques and mode of selection of the grains and to the influence of edapho-climatic conditions of the location where the cassava plants were grown.

This high degree of similarity in the morphological data leads to question and evaluate the crop handling conditions commonly used in the cassava plots in Caxiuanã. In these places, the spread of plant material is predominantly vegetative, with cultivars close to each other in the planting areas, and with the possibility of the same cultivar being introduced into other cassava plots, in an exchange of

goods between growers. Such factors are capable of generating significant homogeneity among the cassava cultivars. Thus, aspects of external morphology of the plant, which largely appear so distinct from one cultivar to another, such as size and coloration of roots, number of leaf lobes and stem color, do not correspond to marked differences in internal morphology.

However, variations in the values of the grain diameters allowed the separation of the cultivars. The related arithmetic averages among Açaí Açu, Jabuti, Jaçanã and Tartaruga showed that they are significantly similar and this was not observed in relation to Domingo Tauá, which is statistically isolated from the other cultivars, as a separate group. Açaí Açu and Jabuti are the closest, demonstrating a significant degree of affinity. Jaçanã and Tartaruga cultivars share their average with Açaí Açu and Jabuti, despite showing values that individualized them as separate cultivars. The verification of this segregation must, in the future, be investigated in order to verify if related to geographical cultivars distributions.

CONCLUSION

The study of starch grains forms of different cassava cultivars, aiming to observe differences between the cultivars in light microscopy and in scanning electron microscopy, showed morphological patterns quite similar to one another between the cultivars. However, in relation to the diameter of the starch grains, it was possible to verify similarities and differences among the cultivars. According to the diameter of the starch grains, Açaí Açu and Jabuti had sufficient similarities to form a single cultivar.

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