



ORIGINAL ARTICLE

Description of *Platonia insignis*, a fruitful Amazon, by histochemistry test, leaf anatomy and flow cytometry

Descrição de Platonia insignis, uma frutífera da Amazônia, por testes histoquímicos, anatomia foliar e citometria de fluxo

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ABSTRACT: Bacuri, the fruit of the Amazonian bacurizeiro tree (*Platonia insignis*), shows great economic potential for widespread use. It can represent an excellent new addition to the exotic fruit market. Little is known about the anatomy of *P. insignis*, the types of reserves contained in the seeds and the DNA content of species of the family Annonaceae. The aimed was to characterize seeds and seedlings histochemically and anatomically and to determine the DNA content of bacurizeiro leaves. Seedlings were obtained from seeds collected in the Amazon region by Embrapa Roraima and sent to the Federal University of Lavras. The fruits were pulped and their seeds washed and sown in trays of 48 cells containing a sawdust substrate. The seeds remained in a growth chamber at 30 °C for 90 days. The samples were subjected to a histochemical analysis, an anatomical characterization and a determination of the DNA content. The seeds of *Platonia insignis* contain lipid as the primary reserve material, with smaller amounts of starch. The cross sections of the leaf blade show a dorsiventral type of hypostomatic organization, with the stomata paracytic and, to a lesser extent, anomocytic. Several secretory channels were identified in the leaf mesophyll and midrib. The sheets of *P. insignis* contain, on average, 13.30 pg of DNA.

RESUMO: O bacuri evidencia grande potencial econômico pelas amplas possibilidades de uso, podendo transformar-se em nova e excelente alternativa para o mercado de frutas exóticas. Pouco se conhece a respeito da anatomia da planta e do tipo de reserva da semente, como também do conteúdo de DNA de espécies da família anonáceas. O objetivo deste trabalho foi caracterizar histoquimicamente as sementes e anatomicamente a plântula, além de determinar o conteúdo de DNA de bacurizeiro (*Platonia insignis*). As plântulas foram obtidas de sementes coletadas na Região Amazônica pela Embrapa Roraima e enviadas à Universidade Federal de Lavras. Os frutos foram despulpados e suas sementes foram previamente lavadas e semeadas em bandejas de 48 células contendo como substrato pó de serragem, permanecendo em câmara de germinação a 30 °C por 90 dias. Nas plantas obtidas, procedeu-se a análise histoquímica, caracterização anatômica e determinação do conteúdo de DNA. As sementes da *Platonia insignis* apresentam reserva principal lipídica e, em menor quantidade, de amido. As secções transversais da lâmina foliar indicam organização dorsiventral, do tipo hipoestomática, com estômatos paracíticos e, em menor proporção, anomocíticos. Foram identificados diversos canais secretores pelo mesofilo foliar e pela nervura central. As folhas de bacurizeiro apresentam, em média, 13,30 pg de DNA.

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1 Introduction

The bacurizeiro (*Platonia insignis* Mart.) is a fruit-bearing tree from the Amazon. Its wood has good physical and mechanical characteristics (FERREIRA et al., 2009), and its fruits are in high demand in the region. Bacuri fruit shows great economic possibilities for widespread use and can become (in a relatively short period) an excellent new addition to the exotic fruit market (BEZERRA et al., 2005).

P. insignis is one of the few Amazon tree species with vigorous sexual reproduction (via seeds) and asexual reproduction (via shoots growing from roots). In the areas where it occurs naturally, in open vegetation, the density of individuals in the early regeneration phase can reach 40 thousand per hectare because of the growth of the shoots (HOMMA; CARVALHO; MENEZES, 2010). The leaves are coriaceous in texture and sub-leathery, measuring 15 to 20 cm long and 6 to 9 inches wide, forming a simple ellipse in cross section with an opposite arrangement of veins. The venation pattern is of the paxilato type, i.e., with copious secondary veins that are closely spaced and end in a rib that traces the entire periphery of the leaf blade. The petioles are short in length, ranging between 1 and 2 cm (SOUZA et al., 2000). However, despite the social importance and high economic potential of *P. insignis*, much additional research is needed to fully characterize the species. Therefore, it is necessary to intensify research efforts addressing the identification, anatomical characterization, DNA content, and photosynthetic characteristics of *P. insignis*.

To date, studies of *P. insignis* have examined the morphology of fruits, seeds and seedlings (MOURÃO; BELTRATI, 1995a, b); the acceleration of germination (OLIVEIRA et al., 2002); post-harvest characteristics (TEXEIRA et al., 2005); disinfection of root explants in vitro (FERREIRA et al., 2009); the management system for the plant (MENEZES; SCHOFFEL; HOMMA, 2010); the *in vitro* viability of the pollen grains (SINIMBÚ NETO; MARTINS; BARBOSA, 2011); and the antioxidant effect of ethyl acetate (COSTA JÚNIOR et al., 2011). Thus, studies involving plant structures and seeds remain to be conducted in *P. insignis*.

Given that little is known about the anatomy, seed characteristics and DNA content of native Amazonian plant species, this study aimed to histochemically characterize the seeds, to anatomically characterize the seedlings and to determine the DNA content of *Platonia insignis* Mart.

2 Materials and Methods

Fruits were collected from bacurizeiro trees in the Amazon region and sent by Embrapa Roraima to the Federal University of Lavras, where the seeds were extracted, washed and sown in trays of 48 cells containing a sawdust substrate. The seeds remained in a growth chamber at 30 °C for 90 days. After this period, the seedlings were transferred and placed (in pots) inside a greenhouse covered with plastic 70% shade cloth and equipped with an intermittent misting system. The experiment was conducted between September 2011 and July 2012.

Histochemical analysis of seeds - Ten seeds were stored in a refrigerator for 10 days and then fixed in 70% ethanol. The apical and basal tissue regions of these seeds were cut freehand

with a razor blade, and the sections were then processed with various specific dyes: Coomassie Blue for protein detection, iron (III) choride for detection of phenolic compounds, Lugol's solution for starch detection and Sudan IV for lipid detection (KRAUS; ARDUIN, 1997). The sections were mounted on slides, and semipermanent material was observed and photographed under an Olympus CX41 microscope coupled to a Belcam DIV 3000 digital camera.

Anatomical characterization of plants - After 300 days of transplanting, cross-sections were obtained from the young leaves (fully expanded) of several plants. The stems were cut in the region near the node, and the roots were cut at the ends. The samples were fixed in 70% ethanol. Paradermic abaxial and adaxial sections were performed free-hand. The cross sections were made on a table microtome. The sections were placed for 10 minutes in Petri dishes containing 1% sodium hypochlorite. They were then transferred to distilled water for 10 minutes and the paradermic and transversal cuts stained with 1.0% Safranin and Safrablau (1% safranin and 0.1% Astra blue), respectively. Subsequently, semi-permanent slides were mounted using 50% glycerol (KRAUS; ARDUIN, 1997). The material was observed with an Olympus CX41 microscope coupled to a Belcam DIV 3000 digital camera and photographed (15 fields). Measurements were made with ImageTool 3.0 software to evaluate the stomatal density, the relative polar diameter / equatorial diameter of the stomata, the abaxial and adaxial epidermal thickness and the thickness of the palisade and the spongy parenchyma.

Determination of DNA content - Three replicates, each containing 300 mg of leaves (Figure 1) and the same weight of pea (*Pisum sativum*) leaves (reference standard quantity of DNA - 9.09 pg), were ground in a Petri dish containing 1 ml of Marie nuclear extraction buffer (DOLEZEL; BINAROVA; LUCRETTI, 1989). The histograms were obtained with an FACSCalibur flow cytometer (Becton Dickinson) using the Cell Quest program (DICKINSON, 1998). The DNA content of the plants (pg) was obtained with the following equation: Amount of DNA (pg) = (G1 peak position the sample position of the peak/pea G1) × 9.09. The statistical analysis of the data was performed with the WinMDI 2.8 program (Trotter, 2000).

3 Results and Discussion

The dyed histochemical sections showed that the seeds contained lipid (in the embryo and meristem regions). This finding may indicate that lipids are the principal energy reserve of the bacuri seed (Figure 1C). Starch grains (Figure 1A) stained with Lugol solution were found in small amounts throughout the analyzed material. The tests of the material (medullary meristem and embryo) for protein and phenolics were negative (Figure 1B and 1D).

The chemical composition of the reserve material contained in the seeds is of interest because the seeds not only represent a nutritional resource but also may be useful in the manufacture of industrial products (BUCKERIDGE et al., 2010). The histochemical results of this study, although qualitative, are consistent with the findings of Campana, Mourão and Marzinek (2010) in *Clusia lanceolata* Cambess: the lipid content of the seeds of *C. lanceolata* was found to be greater than the content

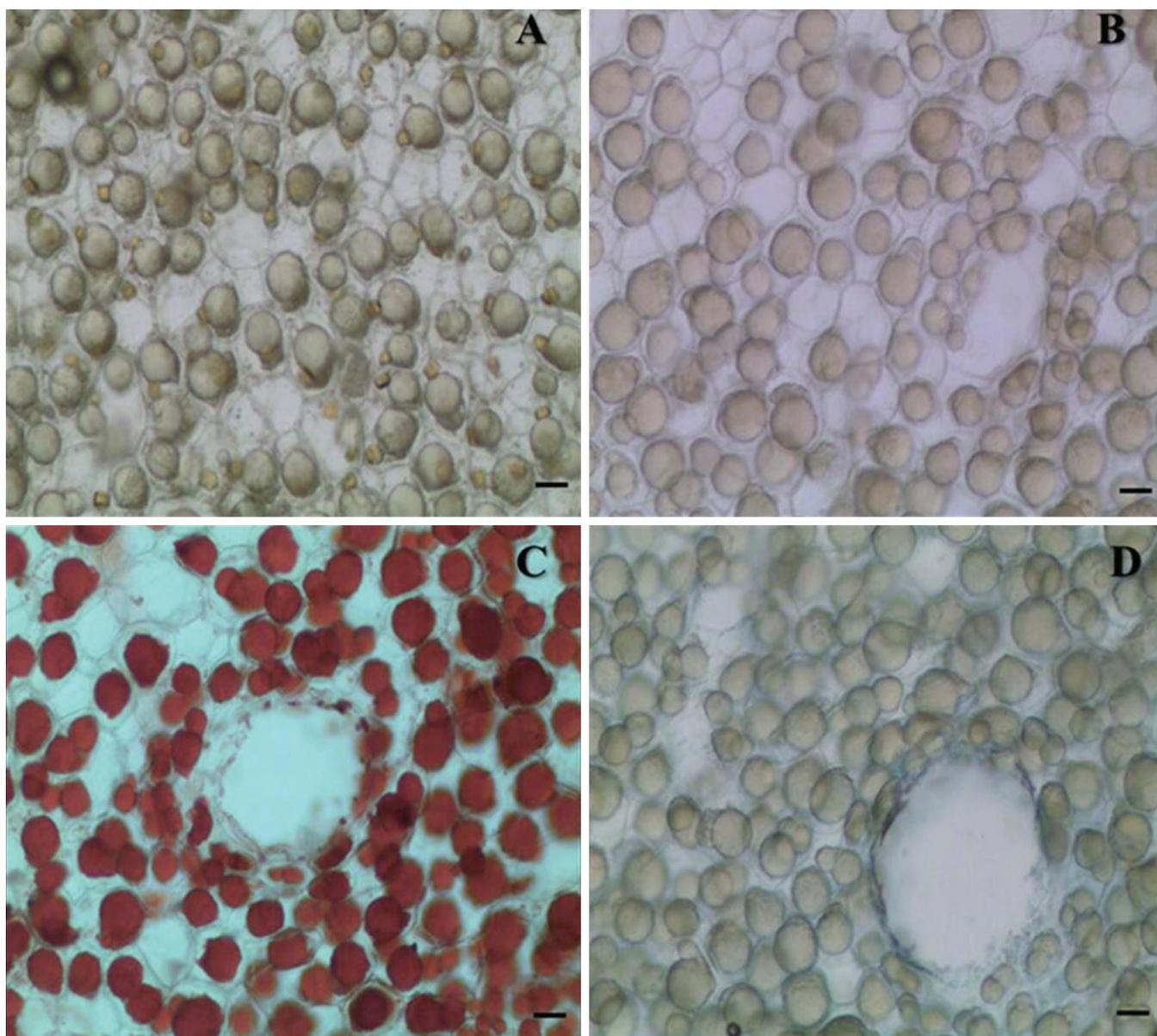


Figure 1. Photomicrographs of cross sections of bacuri seed showing the results of histochemical tests used to identify reserve materials. A) the presence of starch (positive), B) the presence of phenolic compounds (negative), C) the presence of lipids (positive), D) storage proteins (negative). Bar = 20 μ m.

of proteins, carbohydrates or phenolic compounds. According to Villachica et al. (1996), the oil extracted from the seeds has a high percentage of palmitic and oleic acids. According to Mourão and Beltrati (1995a), starch grains may be relatively less common than lipids if the low endosperm is the nuclear type, which is absorbed as the embryo develops and fills the cavity bounded by the seed integuments, whereas the mature seed is exalbuminous.

According to Borges et al. (2005), the total amount of reserves available for seed germination is determined not only by the mass of the seed but also by its chemical composition. The high proportion of lipids in the seeds of *Caesalpinia peltophoroides*, according to these authors, is considered a possible adaptive advantage to the species, allowing seedling establishment in environments with lower light levels due to the

higher content of stored energy. A similar adaptive significance can be inferred for the lipid content of bacuri seeds because the tree is native to dense Amazon forests.

Histochemical tests are very important because they furnish information about the nutraceutical properties and industrial potential of a fruit based on its chemical composition (FANK-DE-CARVALHO; GRACIANO-RIBEIRO, 2005). As the popularity of native plants of the Amazon has intensified, a greater commitment to their study is needed so that the knowledge of the flora of this region is satisfactory and useful for the population. Other types of histochemical tests, such as the detection of the presence of fatty acids, pectin, sucrose, flavonoids and phenolic compounds, are the key to a better understanding of these Amazonian species, which are seldom well studied but can be used for various purposes. Although

the edible portion of the pulp of the bacuri fruit is used as a human food, it is also possible to quantify the histochemical characteristics of the seeds. The chemicals contained in the seeds can be used as defense agents, antioxidants and antibacterials. The byproducts of secondary metabolism can include useful phenolic compounds, including phenols and flavonoids, which can be detected by histochemical tests.

The anatomical characteristics of the *P. insignis* leaf blade were determined from an analysis of cross sections of the blade. The cross sections show a dorsiventral type of organization. The leaves are hypostomatic. Trichomes are absent from all of the faces (Figure 2A and C).

The adaxial surface of the leaf blade is formed by a single layer of cells, rectangular in cross section, followed by a layer of elongated cells which may or may not contain additional material. The hypodermis may be uniseriate. The lower epidermis (6.13 μm) consists of ordinary cells that are relatively smaller than those on the adaxial surface (9.26 μm). The mesophyll (Figure 2C and D) consists of unistratified palisade parenchyma with sections tending to show lobed rectangular to rounded cells in cross section with an average thickness of 31.67 μm . The spongy parenchyma shows seven to eight strata, reduced intercellular spaces and a thickness of 182.56 μm . The fundamental fabric is generally smooth, with few vascular bundles. In a previous study of *P. insignis*,

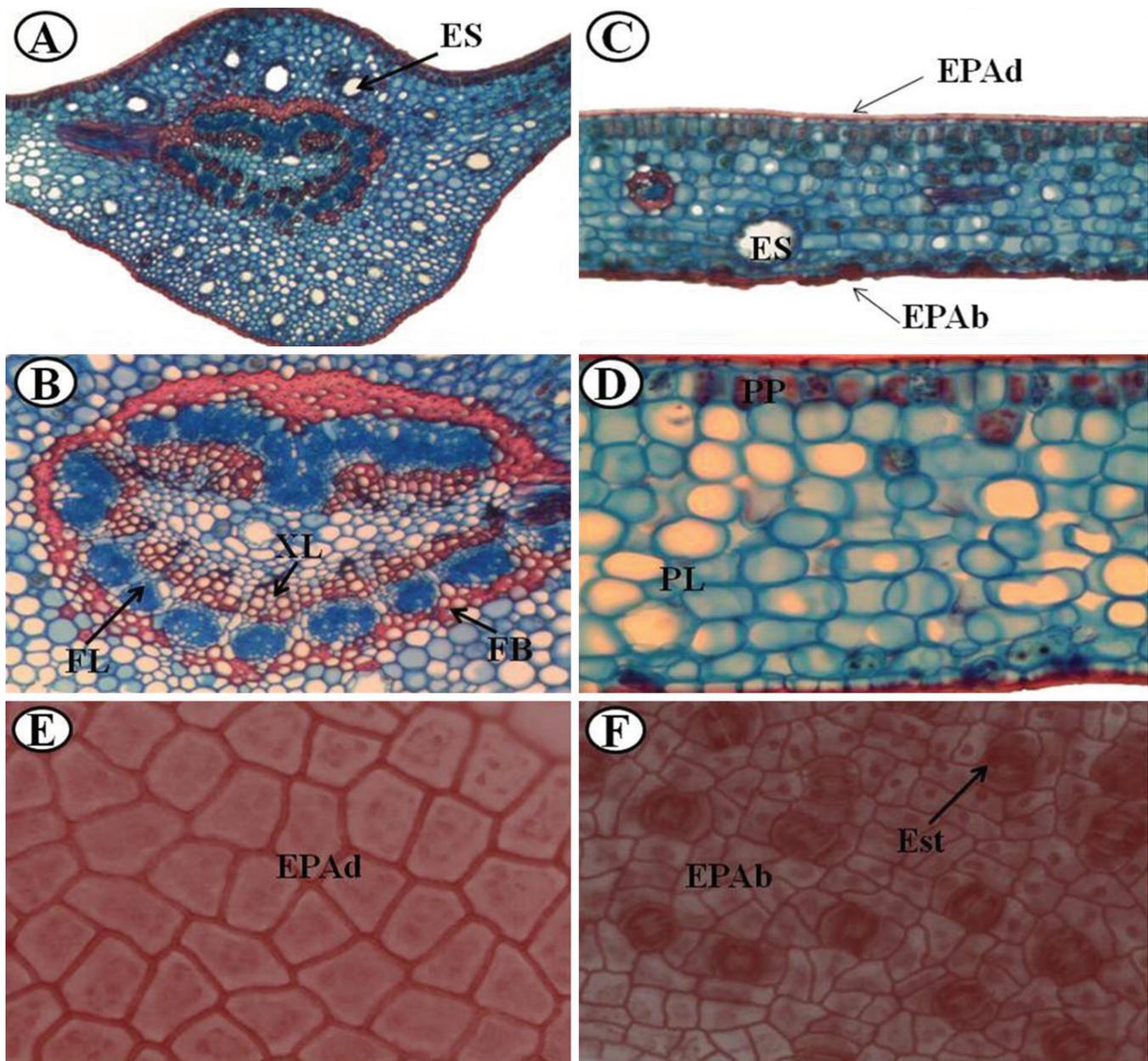


Figure 2. Transverse sections of the leaf blade of *Platonía insignis* Mart. 2A. Overview of midrib showing secretory structure; 2B. Detail of the midrib. Xi = xylem, phloem = FL, FR = fiber; 2C. General view showing mesophyll structure with secretory content. EPAd = adaxial epidermis, ES = secretory structure, EPAB = lower epidermis; 2D. Detail of the mesophyll. PP = palisade, PL = parenchyma. 2E. EPAd = adaxial epidermis, EPAB = lower epidermis, Est = stomata. 2F. EPAB = lower epidermis, Est = stomata.

Alvarez and Potiguara (2013) found that the spongy tissue is more developed near the central region of the leaf; toward the apex, the number of strata tends to decrease, and the central vascular bundle increases in size.

A cross section of the center rib in the cortical region shows two or three layers of angular collenchyma. Xylem arranged in rows is formed in the medullary vascular system. The tracheal elements are radially delimited by fibers and parenchyma cells, and the phloem strip of the vascular system opposite the abaxial face is surrounded by parenchymal cells and other cells common to the tissue. The tissue cells form a substantial central region containing fibers and phloem (Figure 2A and B). The dorsal region of the midrib shows secretory cavities that may or may not contain additional material. According to Cronquist (1981), one of the striking anatomical characteristics of the family Clusiaceae is the presence of schizogenous secretory cavities and channels. These structures are observed in all tissues that produce and secrete secondary metabolites.

The paradermic sections of the leaves of *P. insignis* show abaxial and adaxial surfaces with polyhedral cells and anticlinal walls with a thick contour that is primarily marked on the adaxial surface. The stomata are at the same level or slightly inferior, with small and strongly thickened stomatal cell walls. The epidermal cells show a variable contour as a result of the sinuosity of the cell walls on both sides. Stomata are observed only on the abaxial surface. They are typically paracytic and less frequently anomocytic, and they are randomly distributed (Figure 2E and F). The average stomatal density is 288/mm². The relative polar diameter/equatorial diameter of the stomata is 0.87.

Alvarez and Potiguara (2013) previously analyzed the leaf epidermis of *P. insignis*. They described the epidermal

boundary walls on the adaxial surface of the extremely sinuous blade and the presence of stomata on both sides. The stomata were classified as paracytic, but the stomatal density was not reported. It is possible that the absence of stomata on the adaxial surface of the specimens examined in this study is a form of phenotypic plasticity. The study by these authors and the current study examined material grown in differing environments. These authors examined material from southern Minas Gerais, whereas the material examined in the current study was obtained from the Brazilian Amazon. Certain anatomists have suggested that phenotypic plasticity may represent an adaptive strategy in plants. Nery et al. (2007) observed phenotypic plasticity in the leaves of *Calophyllum brasiliense* Cambess under different levels of shading. Most likely, these variations in shading are responsible for the high plasticity of leaf anatomy in this species.

The amount of nuclear DNA of a given organism, usually termed the C value, is a constant character (BENNET; LEITCH, 2000). It reflects the genome size, i.e., the amount of genetic material present. On average, the leaves of *P. insignis* contain 13.30 pg of DNA, with a coefficient of variation of approximately 0.37 (Figure 3). The amount of nuclear DNA is an adaptive trait and influences the phenotype in two different ways, i.e., via the expression of the DNA content of the nucleus and via the physical effects of the DNA.

The C value is an important biological character, and the typical amount of nuclear DNA of a group of organisms can be useful in various fields, such as molecular and cellular biology, ecology, systematics and phytogeography (BENNET; LEITCH, 2000). As *P. insignis*, despite its great nutritional, industrial and phytochemical potential, is not yet well studied, both anatomical and molecular characterization of the species

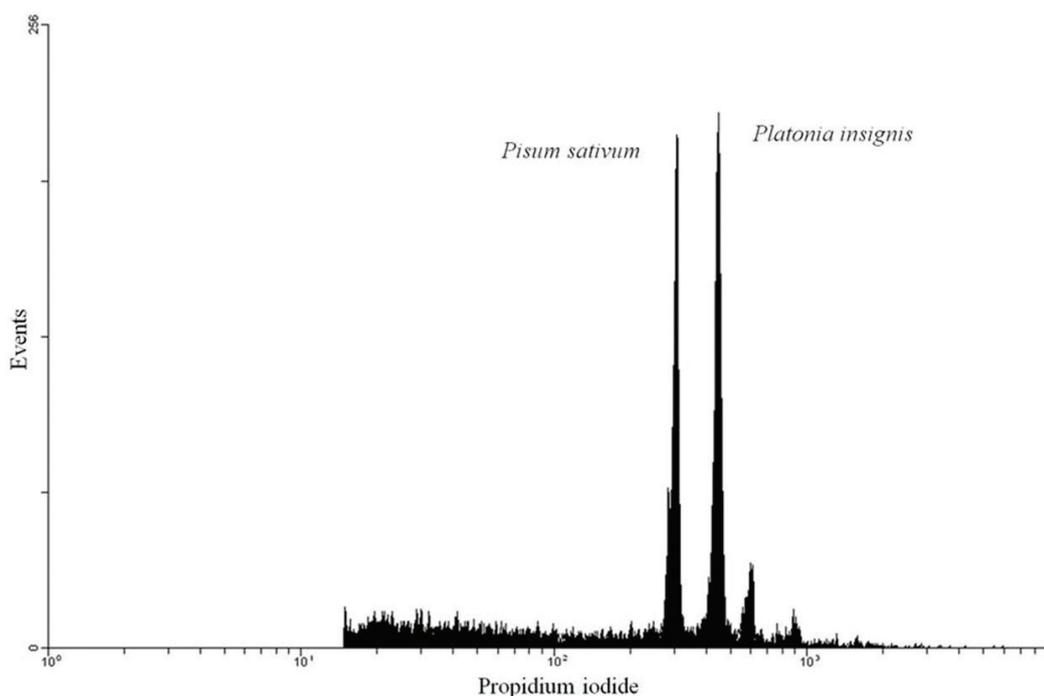


Figure 3. Flow cytometric histogram obtained with Marie nuclear extraction buffer. The first peak is the G1 peak for pea (*Pisum sativum*); the second peak is the G1 peak for bacurizeiro (*Platonia insignis*).

are necessary. Such research facilitates the characterization of the entire germplasm bank of the species as formed in nature. Following the initial process of identification, the determination of the DNA content allows species to be classified into groups.

From a practical standpoint, a determination of the amount of nuclear DNA can replace chromosome counts, especially if very large numbers of individuals are involved, e.g., in Genebank. The knowledge of the number of chromosomes or ploidy level is important for the characterization of germplasm and is essential in plant breeding programs involving planned crosses. Although the determination of the number of chromosomes is relatively simple, it is time consuming. The preferred use of a more efficient technique that gives a similar result, i.e., indicates the ploidy level with an acceptable degree of accuracy, allows several types of work to proceed more efficiently. This is the case both for the maintenance of germplasm banks and for the selection of progeny from crosses between specified individuals having the desired ploidy levels.

4 Conclusions

The seeds of *Platonia insignis* contain lipid as the primary reserve material, with smaller amounts of starch. Transverse sections of the leaf blade show a dorsiventral type of organization. The leaves are hypostomatic. The stomata are primarily paracytic and, to a lesser extent, anomocytic. Several secretory canals occur in the leaf mesophyll and midrib. The leaves of *P. insignis* contain, on average, 13.30 pg of DNA.

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