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NOTES ON NEGLECTED & UNDERUTILIZED CROPS

Unveiling the structure of *Spondias tuberosa* dispersal units through X-ray imaging

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Abstract Characterizing dispersal structures is crucial for species identification and selecting diverse germplasm. This study aimed to investigate the morphology of *Spondias tuberosa* dispersion units and assess the efficacy of x-ray imaging in characterizing their internal morphology. X-ray imaging successfully revealed the internal structures, enabling the identification of filled, translucent, malformed, and empty seeds. The morphological analysis provided valuable insights into the dispersal units and presented a non-destructive and efficient method for future germplasm research.

Keywords Seed biometry · Aryl · *Spondias tuberosa* · Radiograph

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Introduction

The subfamily Spondioideae of the Anacardiaceae family has a wide distribution in tropical regions. Recent molecular phylogenetic research indicates that Spondioideae is not a monophyletic group but rather consists of several clades, posing challenges in species differentiation based solely on vegetative and floral characteristics. However, the morphology of dispersion units can provide valuable information for distinguishing genera within Anacardiaceae.

Fossils recovered from dispersion units offer insights into the evolution and biogeographic history of the major clades. For instance, a morphological analysis of seeds in the genus *Silene* classified species into three groups (Martín-Gómez et al. 2022).

In Anacardiaceae, all genera are characterized by drupe-like fruits containing sclerified stones, which can vary from uni- to multilocular depending on the genus. Germination within Spondioideae also exhibits variation, with certain species featuring distinctive plug-like opercula, while others possess recessed bilabiate germination valves or open through apical flaps or simple slits (Herrera et al. 2018).

Therefore, research enabling the characterization of dispersal structures is valuable for species and germplasm differentiation and understanding. Differentiating among species within the Anacardiaceae family relies on several essential characteristics, including variations in flowering and fruiting time, fruit color, endocarp shape, sap color, bark texture, chalice pubescence, floral bud shape, number of styles, disc width, and pubescence type (Sork et al. 1999; Souza et al. 2020).

The morphological study of seeds provides valuable information not only about their variability but also their structures, aiding in the understanding of germination, storage, sowing methods, and species identification. In species like *S. tuberosa*, commonly known as umbu, characterizing the internal morphology of endocarps and seeds is crucial. Accessing the internal structure of endocarps typically requires destructive methods and the use of highly resistant saws. The weight of endocarps is correlated with fruit weight, an important factor for the food industry. Morphological characterization results can serve as a basis for selecting plants with desirable traits in breeding programs and contribute to species conservation efforts (Souza et al. 2021).

Image analysis techniques have been utilized to assess internal seed morphology, with x-ray imaging standing out as a quick and efficient method for evaluating seed components in most species (Gomes-Júnior 2010). However, in some cases, the term “seed” may not be appropriate as it is impossible to isolate the botanical seed from the dispersion units. Reports on both seed germination and endocarp anatomy were found for 15 genera in the Spondiadeae tribe, six in the Anacardieae tribe, 30 in the Rhoeae tribe, three in the Semecarpeae tribe, and one in the Dobineeae tribe. The fossil fruit record strongly suggests that the relationships between diaspore dormancy (or non-dormancy), endocarp structure, and taxonomic position within Anacardiaceae extend back to at least the Paleogene (Baskin and Baskin 2022).

The objective of this study was to present fundamental information regarding the physical morphology of *S. tuberosa* dispersal units and evaluate the efficacy of X-ray imaging in characterizing their internal structure.

Materials and methods

- The umbu dispersal units used in this study were obtained from the Canudos Family Agricultural Cooperative (COOPERCUC) in the municipalities of Uauá and Curaçá, Bahia, Brazil. Ripe fruits with a greenish-yellow peel color were selected directly from the trees by COOPERCUC members. The length, width, and thickness of the endocarps were measured individually using a 0.01 mm digital caliper, with 10 replications consisting of 20 endocarps each.
- The dispersal units were analyzed at the Seed Laboratory of the Federal University of Sergipe. A sample of 200 dispersal units, obtained from harvests in various COOPERCUC orchards, was fixed on a styrofoam plate and radiographed at an exposure intensity of 28 kV for 5.2 s, as automatically determined by the equipment. The resulting images were utilized to identify the internal morphology and classify the seeds into categories such as translucent, malformed, filled, and empty (Simak 1991).
- The internal structures of the dispersal units were examined in longitudinal and transverse sections. External characteristics, including shape, color, texture, and consistency, were observed and described. Internal structures, such as cotyledons and hypocotyl-radicle axis, were analyzed. X-ray images were captured using a Faxitron Ultrafocus 60 digital equipment.
- All radiographs were assessed using ImageJ software version 1.49s to facilitate image analysis and gather data on maximum and minimum pixel density. Three-dimensional plots were generated for each identified endocarp, following the methodology described by Abràmoff et al. (2004). Seed filaments were categorized as filled, translucent, or empty. 3D graphs and pixel histograms were examined. It’s important to note that only physical analyses were performed, and no physiological evaluations were conducted to assess the physiological characteristics of the samples.

Results and discussion

Information about the physical morphology (length, width and thickness), weight and variation of *S. tuberosa* dispersion units are presented in Fig. 1.

Regarding the measurements of the dispersal units, there was variation observed in all biometric evaluations. The most frequent distribution of dispersal units was found in the width class of 11.69 mm, length class of 16.66 mm, and thickness class of 9.08 mm (Fig. 1B). A study characterizing umbu dispersal units in Minas Gerais reported average values of 16.60 mm for width and 22.60 mm for

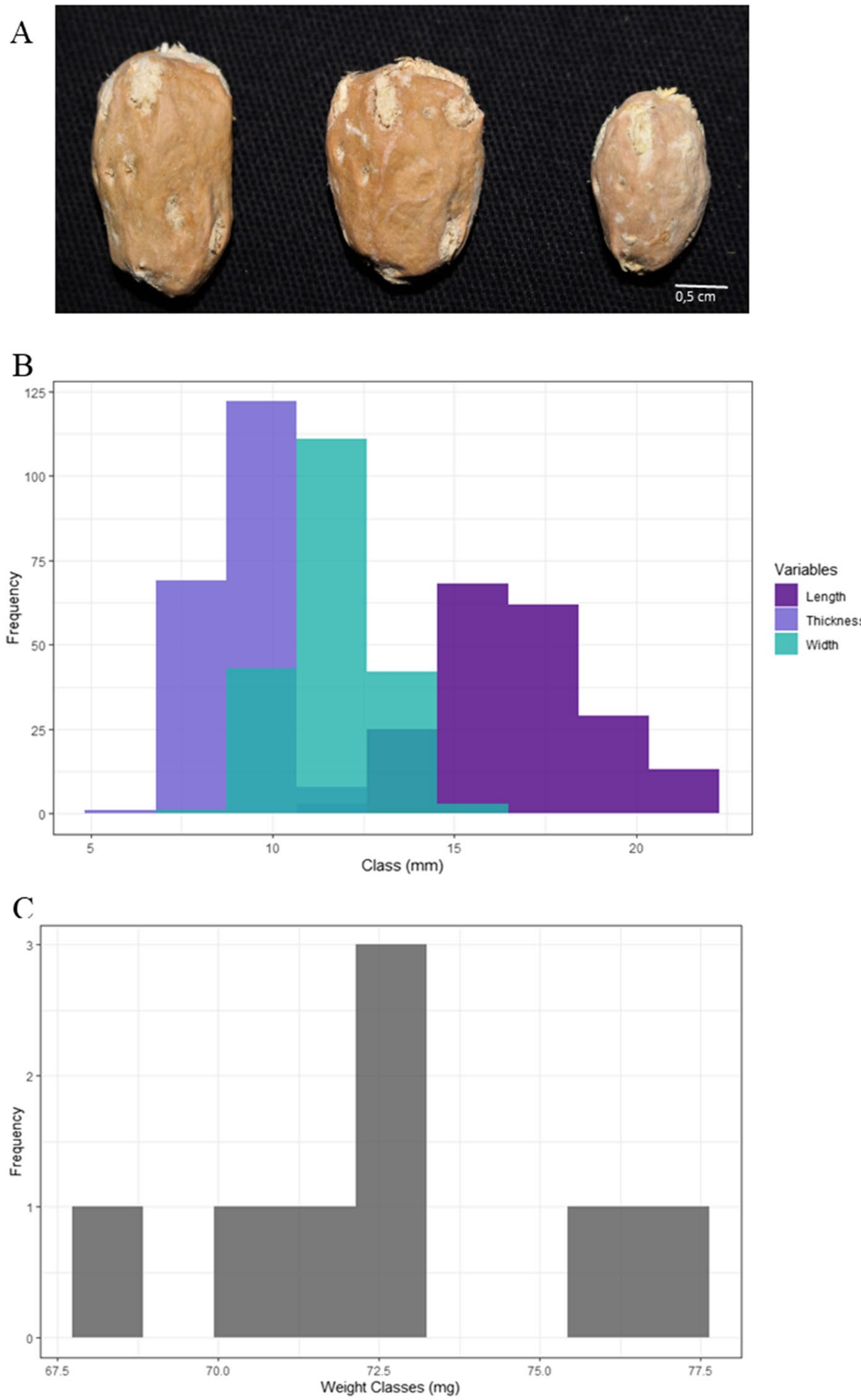

Fig. 1 **A** Variations in the dimensions; **B** Dimensions; and, **C** Weight of *S. tuberosa* Arruda Câmara of dispersion units

length (Nobre et al. 2017). Biometry plays a crucial role in germplasm characterization and enables the identification of phenotypic variations within the same species. These variations may be attributed to the edaphoclimatic conditions during their development and the genetic diversity of the tree sources from which the fruits were collected. This variation provides valuable insights into fruit diversity and could be associated with germplasm variation.

Typically, umbu fruits have a weight ranging from 10 to 20 g, with the pericarp accounting for 22% of the fruit's weight, the pulp (mesocarp) representing 68%, and the endocarp constituting 10% (Lima et al. 2013). Consequently, the dispersal units account for approximately 10% of the total fruit weight. After evaluating the weight of the dispersal units, we observed a minimum value of 68.51 mg and a maximum value of 77.32 mg, with the highest frequency of dispersal units falling within the weight class of 72.68 mg (Fig. 1C).

The dispersal units are fibrous-woody structures that feature distal openings called operculum, which are filled with a fibrous aril (Fig. 2).

However, one of these openings, situated near the hilum region of the seed, is more pronounced, and it serves as the point from which the radicle emerges from the dispersal unit (Fig. 3A). Towards the top of the dispersal unit, there is a small opening that was originally attached to the peduncle. In the proximal region, two additional openings are symmetrically positioned along the longitudinal axis of the seed (Fig. 3B).

The seeds of *S. tuberosa* are elongated and reniform, exhibiting characteristics of being sternospermic, exalbuminous, and bitegmic. They possess a blunted hilum and an inconspicuous micropyle. The funiculus is persistent and has rusty coloration. The basal embryo is invaginated, dicotyledonous, and positioned laterally, with internal, flat, convex, oblong, yellowish cotyledons that display a smooth surface and a brittle texture. The hypocotyl

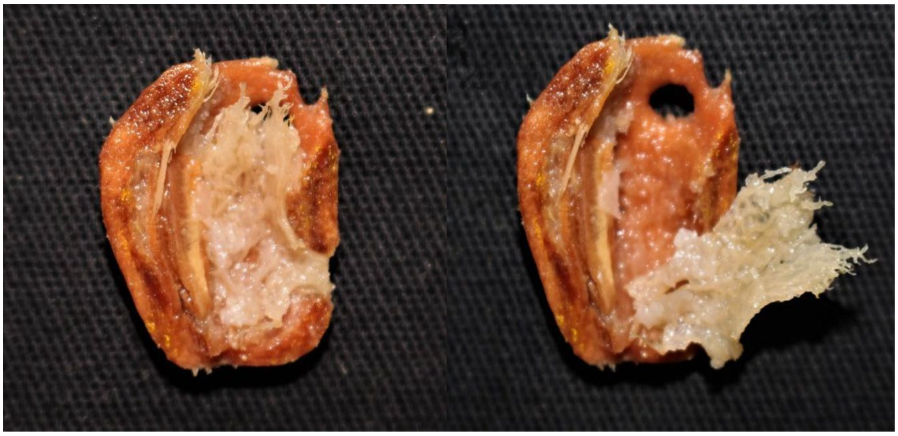

Fig. 2 Dispersion units of *S. tuberosa* Arruda Câmara with details of the internal fibrous aryl

Fig. 3 *Spondias tuberosa* Arr. Câm. dispersion units in apical (distal extremity—**A**) and basal (proximal extremity —**B**) views

axis-radicle is external and short, as depicted in Fig. 4.

In the cross-section of the dispersal units, the presence of five locules can be observed, with four of them being empty and one fertile, containing a single seed in the sample. The cotyledons are enveloped by the tegmen and shielded by the testa, which possesses a smooth, opaque, woody, and glabrous texture, exhibiting a rusty color. The tegmen is thin, rough, opaque, and foliaceous, sharing the same color as the testa. The openings of the dispersal units are filled with a fibrous aril (Fig. 5).

In the study conducted by Azevedo et al. (2004), *Spondias mombin* dispersal units were characterized as euricarps, exhibiting elliptical, obovoid, ovoid, and globous forms. In cross-section, the dispersal units were surrounded by spongy fibers, while the seeds were enclosed by a radial (star-shaped) structure with a woody consistency. Most dispersal units displayed five radially arranged locules, containing two or three viable seeds. The authors described the seeds as elongated, elliptical, and varying in length within the same endocarp. The seeds had a small integument, a straight embryo, a short hypocotyl-radicle axis, and slightly flat-convex cotyledons, conforming to the shape of the seed. The number of locules is associated with the number of seeds per endocarp and can aid in the selection of fruits that yield more seeds for species propagation (Souza et al. 2021).

The extraction of umbu seeds from the dispersal units is a challenging task due to their strong adhesion, which can potentially damage the seeds in the process. Therefore, it is essential to employ techniques that allow for the evaluation of the internal structure of the dispersal units without causing significant harm to the seeds. X-ray imaging is an effective method for studying the internal morphology and visualizing the presence or absence of umbu seeds inside the units. This technique offers a quick and non-destructive approach for examining the dispersal units. The units typically consist of five locules, with four underdeveloped locules and only one well-developed locule containing the seed (Fig. 6).

The dispersion units primarily consist of sclerenchyma tissue, predominantly composed of fibers. The pores within the units are often partially blocked by loosely arranged fibers. In fruits with multiple layers, the dispersion units can exhibit a star-like and spiny

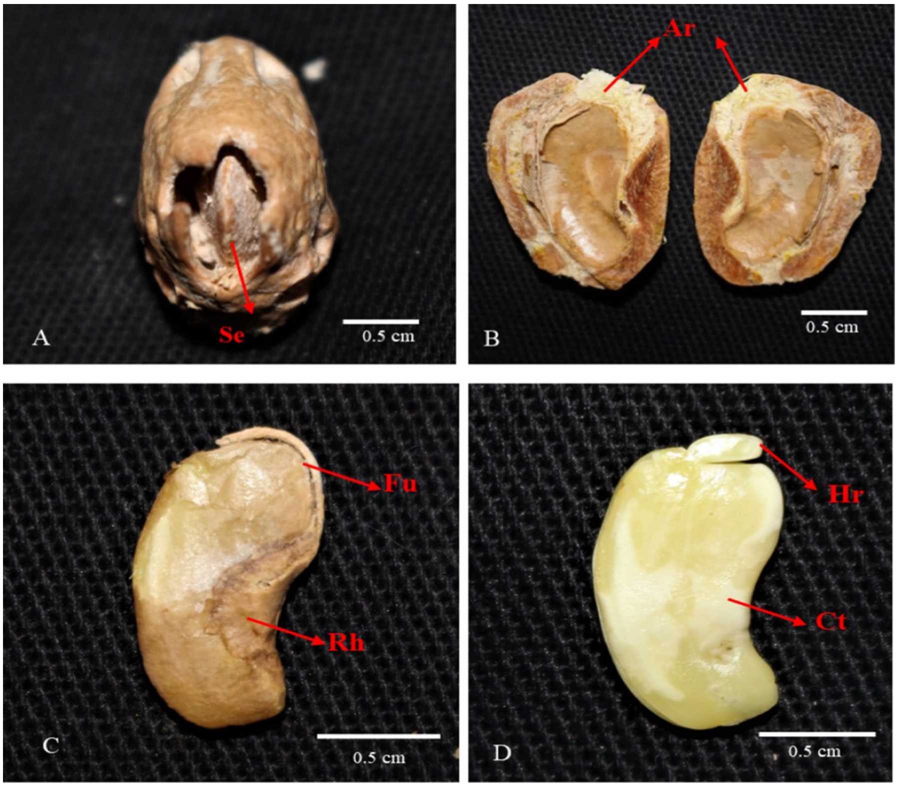

Fig. 4 Morphology of dispersion units of the *S. tuberosa* Arruda Câmara and seed operculum (**A**). Longitudinal section of the endocarp (**B**). Seed (**C**). Embryo (**D**). (Ar: aryl. Ct: cotyledons. Fu: funiculus. Hr: hypocotyl axis-radicle. Rh: hilus. Se: seeds)

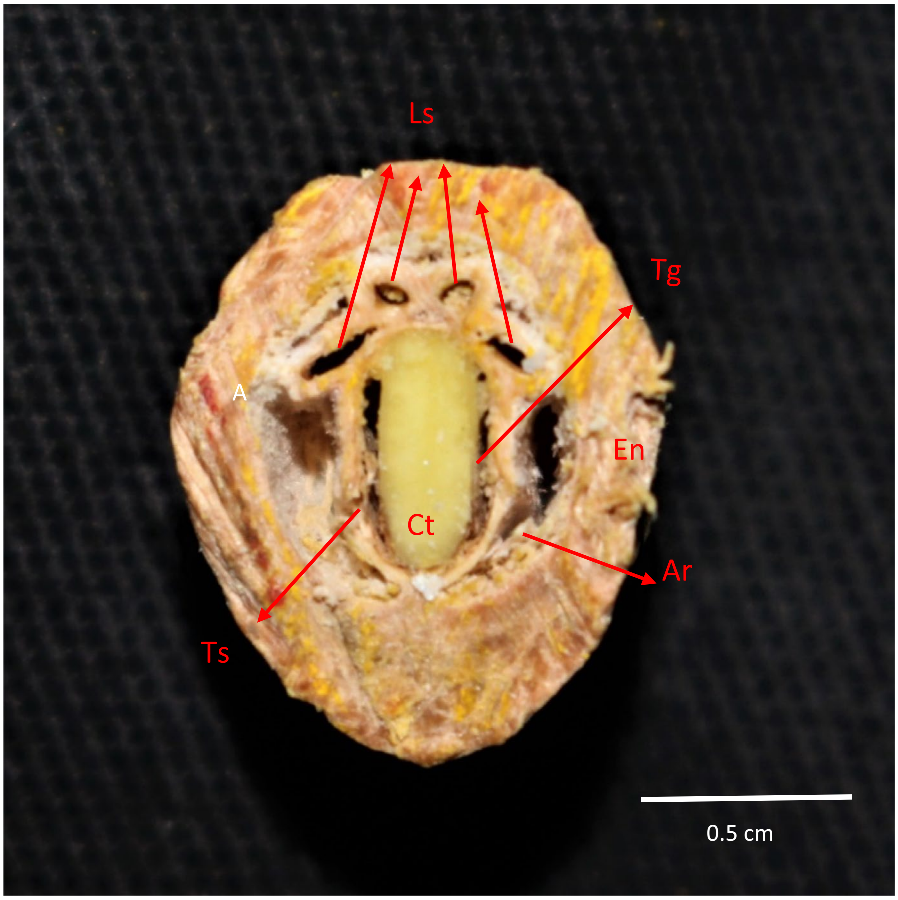

Fig. 5 Cross section of the endocarp of the *S. tuberosa* Arruda Câmara and its internal structures of the endocarp (Ar: aryl; Ct: cotyledons; En: endocarp; ls: seminiferous locules; Tg: tegma; Ts: testa). (Yellow color in endocarps is the ink of the saws)

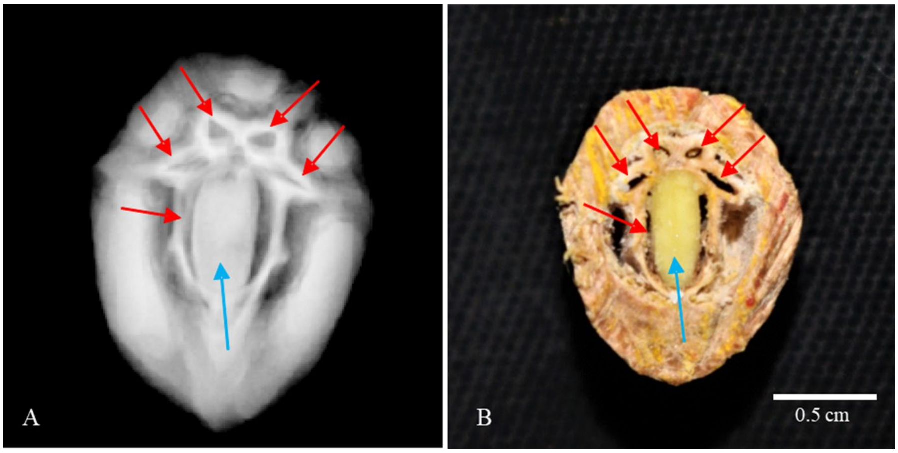

Fig. 6 X-ray images of umbu endocarp (*S. tuberosa* Arruda Câmara) **A** and cross-sectioned endocarp **B** with red arrows indicating five locules and blue arrows for single seed

configuration when viewed in cross-section (Herrera et al. 2018).

Image analysis techniques enable the visualization of the internal morphology of seeds, facilitating the identification of well-formed seeds and those with damage to the storage tissues (Arruda et al. 2016; Noronha et al. 2018). X-ray imaging of umbu dispersion units allowed for the evaluation and categorization of seeds into different groups: filled (well-formed), translucent, malformed, and empty (Fig. 7).

The majority of the seeds (54%) were classified as translucent, followed by malformed (23.5%), filled (21%), and empty (1.5%). The high proportion of translucent seeds may be attributed to low tissue integrity, resulting in lower relative densities (Fig. 2, C to F), which makes them less resistant to X-rays. Consequently, these regions appear more translucent or darkened on the radiograph, indicated by blue and purple colors. This can ultimately lead to the development of abnormal seedlings (Marchi and Gomes Junior 2017). Relative density serves as an indirect measure to assess deterioration, mechanical damage, or non-uniformity of the embryo, as it contributes to increased radioluminescence indices (darker tones) (Medeiros et al. 2019), thus reducing the gray scale (Medeiros et al. 2018). Although there is significant potential for associating x-ray images with the physiological quality of seeds, our primary objective was to analyze the morphological structure of endocarps to understand the presence and formation of seeds (Severiano et al. 2018).

X-ray images were employed to classify *Moquiniastrum polymorphum* (Less.) seeds based on their image color. Dark-colored images indicated unfilled seeds, as they lacked internal mass. Partially light-colored images represented malformed seeds, where only a partial presence of internal mass was observed. In contrast, filled seeds exhibited completely clear or white images due to their higher tissue density (Faria et al. 2019).

The examination of images enabled the identification of the internal components of *Moringa oleifera* Lam. seeds, such as the dense tissue surrounding the embryo, as well as physical and empty damage in the integument (Noronha et al. 2018). However, visualizing critical structures of the seedling, such as the embryonic axis or hypocotyl radicle, can be challenging due to their smaller size relative to the surrounding reserve structures (Vasconcelos et al. 2018).

The examination of histograms using ImageJ software for *S. tuberosa* endocarps revealed distinct pixel density patterns among filled, translucent, malformed, and empty seeds. The pixel density corresponds to the level of radiolucency (dark) or radiopacity (light) in the image, translated into gray values. Figure 8 displays x-ray images of dispersion units with

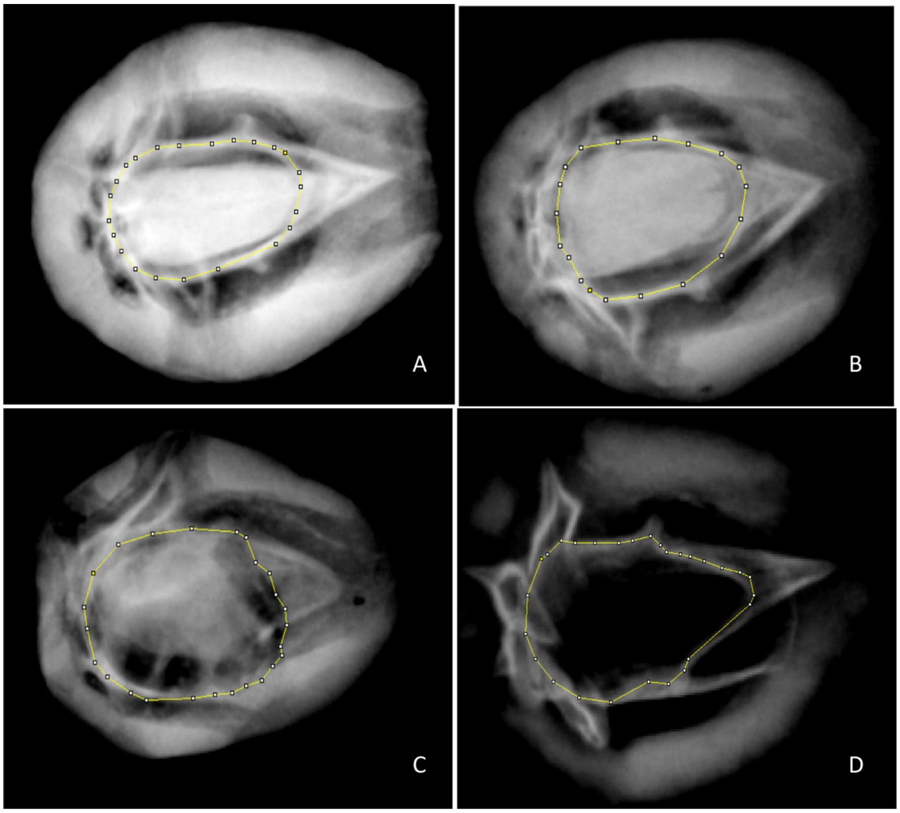

Fig. 7 Endocarps and seeds of *S. tuberosa* Arruda Câmara, categorized as **A** filled; **B** translucent; **C** malformed and **D** empty

seeds from each category, along with their respective three-dimensional (3D) graphs and pixel density histograms. Differences can be observed among the categories, with the maximum pixel density decreasing from filled (255) to translucent (201), malformed (195), and empty seed categories (143). In the 3D graphs, seeds with intact internal structures exhibited the highest density, while the lowest values were observed for malformed or empty seeds. While x-ray imaging has the potential to provide information on seed physiology, our focus solely involved analyzing the morphological structure of dispersion units to understand seed existence and formation (Severiano et al. 2018).

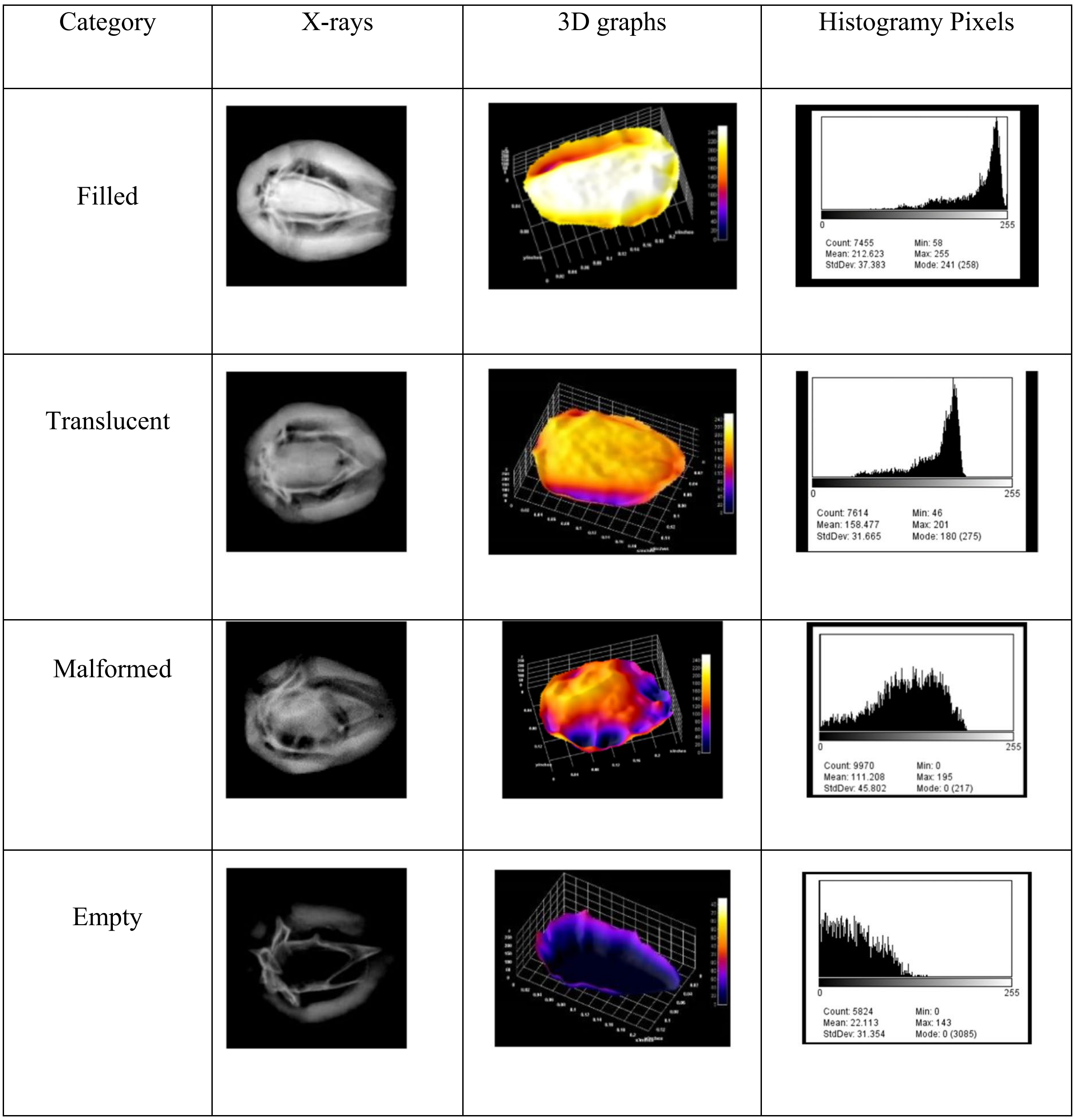

Fig. 8 Dispersion units and seeds of *S. tuberosa* Arruda Câmara classified as filled, translucent, malformed, and empty by X-ray and analyzed using ImageJ software

Grayscale values in x-ray images are indicative of higher tissue density, and previous research suggests that seeds with higher tissue density generally exhibit better physiological quality, as they contain a greater amount of dry seed matter, which reflects the potential germination capacity of the propagule. In seed lots of *Brassica oleraceae* L., a significant proportion of seeds with low relative density were associated with shorter seedling length, indicating lower seed vigor (Abud et al. 2018).

This study represents the first application of x-ray imaging to evaluate *S. tuberosa* dispersion units, making direct comparisons with data and images from other research on the same species or botanical family challenging. Nonetheless, the use of x-ray analysis has proven effective in studying the internal and external morphology of *Leucaena leucocephala* (Lam.) seeds, providing valuable information on physical traits, and generating parameters related to seed physiological quality in a rapid and straightforward manner (Medeiros et al. 2018).

Based on the results of this research, x-ray imaging emerges as a powerful tool for investigating the internal and external morphology of *S. tuberosa* dispersion units. Future research could establish correlations between x-ray images and seed physiological quality through germination and vigor tests. It is worth emphasizing that seeds extracted from the dispersion units did not germinate, highlighting the significance of the entire structure in triggering germination.

Therefore, morphological characterization of umbu represents a vital strategy for the preliminary selection of promising genotypes (Pereira et al. 2021). The analysis of dispersion unit morphology using x-ray images serves as an auxiliary method for evaluating empty and abnormal seeds, as well as for taxonomic research. The density of these structures and internal seed damage can be identified, providing insights into germination potential. For *S. tuberosa*, an allogamous species, assessing fertilization success in seed production through planned crosses could be achieved to predict the effectiveness of double fertilization, thereby informing breeding programs.

Conclusion

The dispersion units of umbu fruits consist of fibrous-woody material and contain distal openings called operculum, which are filled with a fibrous aril. These dispersion units exhibit variations in shape, with an average weight of 0.7289 g. X-ray imaging proves to be an efficient and non-destructive technique for studying the internal morphology of these structures, making it highly suitable for future breeding research, such as germplasm characterization and the identification of stable divergences for genotype selection.

Author contributions AMBR conducted the research and wrote the manuscript. VVN, JLS, MFOT and NAS assisted with photograph registration, translation, and text revision. All authors contributed to the article, approved the final version, and provided valuable input. RSM supervised the study, reviewed the manuscript, and made significant contributions to its writing.

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Data Availability The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no commercial or financial relationships that could be perceived as a potential conflict of interest.

Ethical approval This article does not contain any research with human participants or animals performed by any of the authors.

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