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Fruit and seed morphology and post-seminal development of *Buchenavia tetraphylla* (Combretaceae)

Morfologia de frutos, sementes e desenvolvimento pós-seminal da *Buchenavia tetraphylla* (Combretaceae)

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RESUMO

Buchenavia tetraphylla é uma árvore neotropical amplamente distribuída na América do Sul e Central. Além disso, apesar de sua importância ecológica, potencial madeireiro e paisagístico, estudos têm demonstrado que ela apresenta baixa abundância populacional, bem como que forma agregados sem bancos de sementes e mudas. Portanto, objetivou-se realizar a caracterização morfológica de frutos, pirênios, sementes e desenvolvimento pós-seminal para reunir informações que subsidiem ações voltadas ao manejo sustentável e conservação desta espécie. Em média, 100 amostras de frutos maduros por planta foram coletadas aleatoriamente em subpopulações das espécies aqui investigadas que crescem naturalmente em Brejo Paraibano, Areia-PB, Brasil. Estatística descritiva foi realizada para analisar os dados usando box plots e histogramas, bem como correlação de Pearson entre as variáveis mensuradas. Os resultados evidenciaram que *B. tetraphylla* possui drupas, cuja biometria apresenta baixa variabilidade e apresenta uniformidade no tamanho do fruto e do pirênio. A espécie produz grande número de frutos sem sementes (52%), o que permite inferir que apenas 40% dos frutos apresentam sementes viáveis com germinação desigual, cotilédones epígeos e alta taxa de mortalidade na fase de plântula em condições controladas, com apenas 21% das plântulas normais. A variabilidade biométrica dos frutos e sementes de *B. tetraphylla* que cresce naturalmente no Brejo Paraibano é baixa.

Palavras-chave: *Diásporos; Germinação; Mirindiba; Qualidade fisiológica de sementes.*

ABSTRACT

Buchenavia tetraphylla is a neotropical tree widely distributed in South and Central America. In addition, despite its ecological importance, timber and landscape potential, studies have shown that it has a low population abundance, while also forming aggregates without seeds and seedlings. Therefore, the aim of this study was to determine the morphological features of fruits, pyrenes, seeds and post-seminal development, in order to obtain information to support actions for the sustainable management and conservation of this species. On average, 100 samples of ripe fruits per plant were randomly collected in subpopulations of trees naturally growing in Brejo Paraibano, Areia-PB, Brazil. Descriptive statistics were performed to analyze the data using box plots and histograms, as well as Pearson correlation between the measured variables. Results have shown that *B. tetraphylla* has drupes, whose biometrics exhibited low variability, uniformity in fruit and pyrene size. The species produces a large number of seedless fruits (52%), which lead us to conclude that only 40% of fruits had viable seeds with uneven germination, epigeal cotyledons and high mortality rate at seedling stage under controlled conditions - it only it has 21% of normal seedlings. The biometric variability of fruits and seeds of *B. tetraphylla* that grows naturally in Brejo Paraibano is low.

Keywords: *Diaspores; Germination; Mirindiba; Physiological quality of seeds.*

1. INTRODUCTION

The family Combretaceae is of pantropical distribution and comprises approximately 500 species distributed in 14 genera (Stace, 2010). Among them, one finds *Buchenavia* Eichler, which encompasses approximately 20 species in Tropical America (Maurin et al., 2010). Brazil has 17 species presenting greater diversity in the Northern region (Marquete & Loiola, 2015; Ribeiro et al., 2020), although they also grown in Cerrado, semi-deciduous broadleaved forests (Lorenzi, 2009), Caatinga enclaves moist forests (Loiola et al., 2009), as well as in "carrasco" vegetation, coastal complex, and dry and humid forests (Soares Neto et al., 2014).

Buchenavia tetraphylla (Aubl.) RA Howard naturally grows in neotropical forests (Ribeiro et al., 2017). The species is popularly known as good mouth, imbiridiba, imbiritiba, minguiriba, mirindiba and murunduba. It has pharmacological properties (Pottier et al., 2017; Cavalcanti Filho et al., 2017), as well as cytotoxic and anti-HIV activity (Soares Neto et al., 2014). Although it is used as popular medicine by Caatinga populations (Cavalcanti Filho et al., 2017; Ribeiro et al., 2017), it also has timber potential, which enables using it in civil construction, firewood and charcoal production, as well as in forest reforestation areas (Lorenzi, 2009). It is featured as a 5-to-12-meter-tall emerging tree, whose good-quality timber is used for economic purposes, since it is moderately heavy (specific density 0.61) and resistant (Parresol, 1995).

The species plays an important ecological role, since it helps to maintain resources for the local fauna (Silva et al., 2019). Its fruiting stage lasts approximately three months; it starts at the end of the rainy season and progresses in a non-synchronous way among individuals. Its fruits are consumed by vertebrates and its pyrenes are dispersed by birds and primates. Fruits that were not consumed in the canopy fall naturally and produce the so-called "seed shadows" on the surface of the substrate (Santos et al., 2006).

Studies have shown that *B. tetraphylla* populations are in low abundance and form aggregates without seeds and seedlings (Francis & Lowe, 2000; Silva & Moura, 2021; Cola et al., 2022). They have also pointed out that seed viability *in situ* is restricted to approximately 6-7 months after fruit falling (Santos et al., 2006). In addition, *B. tetraphylla* environments undergo constant deforestation and habitat fragmentation, which increases its risk of extinction in several places, since the species needs a biotic vector for seed dispersal over long periods of time and distances. However, anthropic actions reduce or even extinguish disperser populations at local level, which compromises the final reproductive stage of the species, namely: seedling dispersion and establishment (Santos et al., 2006; López-Vázquez et al., 2024).

In view of the risk of extinction, the morphological and post-seminal characterization of this species is important, as it will guide ex situ conservation actions and those aimed at forest restocking. Additionally, it is possible to infer matters about the species' longevity in natural environments (Santos et al., 2006; Feliciano et al., 2023). It is possible to study adaptive or attractive structures for seed dispersal that are important in areas of natural habitat (Bicalho et al., 2016) or that maintain the moisture content for later germination and complete seed establishment (Mattana et al., 2017) or as dormancy inducers as a strategy for maintaining the species' longevity (Correa et al., 2020).

Despite its ecological, pharmacological, timber and landscape potential, information about the biology of the investigated species remains scarce, since it is restricted to studies about antioxidant compounds, microbial activity, medicinal use and floristic surveys of the subtribe and family (Soares Neto et al., 2014; Ribeiro et al., 2017; Silva et al., 2019). The literature does not provide information about the morphology of its fruits, seeds and seedlings, which is essential to help developing conservation and preservation strategies focused on this species. Therefore, the aim of the current study was to determine the morphological features of *B. tetraphylla* fruits, seeds and seedlings in order to gather information which could lead to studies at the community level and to future actions focused on the sustainable management and conservation of it.

2. MATERIALS AND METHODS

2.1. Study site and selection of individuals

Ten mother plants of *B. tetraphylla* were randomly selected during the fruiting period (October); the sampling took into consideration the distance of 20 m between them. The aforementioned mother trees were grown on Campus II of Federal University of Paraiba, Areia, Paraiba, Brazil. The sampled site has a small remnant of native vegetation species, with prevalence of Caatinga enclaves' moist forest, which is one of the phyto-physiognomies observed in the Atlantic Forest. According to Köppen's classification, the climate in the region is of the AS type - i.e., hot and humid, with minimum average temperature of 18 °C, maximum of 28 °C and annual precipitation between 800 and 1600 mm (Pereira et al., 2015).

2.2. Plant material collection and processing

On average 100 ripe fruit samples per plant were manually collected under the canopy. They were stored in a thermal insulating box to avoid dehydration and transported right away to the Seed Analysis Laboratory of the Agricultural Sciences Center, Federal University of Paraiba (LAS-UFPB), Areia, Brazil. Fruits were sorted in order to discard the underripe ones, as well as fruits presenting lesions or other damage of any kind. Next, they were measured (length and diameter) by using a 0.01 mm precision digital pachymeter. The external (color, texture, epicarp brightness and shape) and internal features of the fruits (mesocarp color and texture, and number of seeds per fruit) were defined, based on the terminology by Barroso et al. (2004).

Subsequently, 100 fruits were selected; each fruit unit was weighed on a precision scale, (0.001 g) pulped, washed in running water to remove pulp residues and left to dry in the shade for three days for endocarp assessment purposes. Endocarp length, width (perpendicular to the length) and thickness were measured with the aid of a digital caliper. Seed presence and viability in fruits were investigated with the aid of mini bench vise; the endocarp of each selected fruit was opened.

The processed seeds were measured in order to have their length, width (perpendicular to length) and thickness determined with a 0.01 mm digital pachymeter. External morphological aspects of the evaluated seeds comprised: color, brightness, texture, shape and scars (hilum and micropyle). On the other hand, internal seed aspects such as embryo structures and incidence of reserve tissues were analyzed.

2.3. Germination test and seedling morphology

The germinative behavior of the seeds was evaluated by a pre-test conducted before the germination test. For the pre-test, paper towel (germitest®) was used as substrate; four repetitions of 25 seeds were sowed at each temperature range (20, 25, 30, 35 and alternating 20-30 °C) in order to determine the best temperature to be used later, with photoperiod of twelve hours.

The pre-test was done in a completely randomized design. Results have shown that the evaluated seeds presented greater germinative potential when they were subjected to temperature of 30 ºC. Based on this outcome, seeds deriving from previously selected fruits were subjected to germination test, which comprised 25 seeds in 4 repetitions. First, seeds were disinfected with 1% sodium hypochlorite solution for five minutes (Muniz et al., 2017) and seed units were washed in distilled water. Each seed unit was identified, placed on paper towels (germitest®) in the form of rolls moistened with distilled water - in amounts equivalent to 2.5 times the dry paper mass (control) - in order to determine germination morphology (Brasil, 2009). The rolls were packed in transparent plastic bags to avoid water loss through evaporation. The test was carried out in the Biological Oxygen Demand chamber at constant temperature of 30 °C, under 12-hour photoperiod (Correa et al., 2020).

Germination was evaluated on a daily basis. Seeds presenting emission of 2-mm primary root were considered germinated (Rehman et al., 1996). The morphology of normal units – i.e., seeds showing full, proportional and healthy development of all essential structures - were analyzed during seedling development, based on (Bekendam & Grob, 1979). Shoot and primary root length were measured with the aid of a graduated ruler (in millimeters), whereas colon diameter was measured with digital caliper (precision of 0.01 mm). Seedlings were classified as intact and normal seedlings with small defects, at the end of the seminal stage, according to Bekendam & Grob (1979).

B. tetraphylla seeds were preconditioned on moistened germination paper, as described in the germination test, and kept in BOD regulated at 25 °C for 16 hours. They were then immersed in a 0.075% tetrazolium solution for 120 minutes at 40 °C in the dark (França-Neto & Krzyzanowski, 2018; Souza et al., 2018). This procedure intended to stain the embryonic axis for easier visualization.

External and internal morphological features of *B. tetraphylla* fruits, endocarps, seeds, germination and seedling stages were recorded in pictures taken with stereomicroscopic loupe (Zeiss Stereo Discovery V20).

2.4. Data analysis

Descriptive statistics was applied to each biometric variable of fruits, endocarps and seeds, as well as to seedling morphology. Quantitative data were represented in boxplot and distributed in class intervals based on frequency histogram. The analyzed variables were correlated to each other based on Pearson's correlation, since data distribution was normal. Analyses were performed in R software v.4.0.0, with the help of the ggplot2 and Performance Analytics packages.

3. RESULTS

3.1. Morphological description of fruits and seeds B. tetraphylla

The fruit is pedunculated and derives from the inferior part of the ovary; it is of the simple drupe type, fleshy, with monospermic pyrene and round shape, as well as slightly obovate. Epicarp at the final ripening stage is thin, smooth, glabrous, greenish, whereas the mesocarp is fleshy and greenish (Figure 1), it has odor and sweet taste, and corresponds to approximately 80% of the fruit fresh mass.

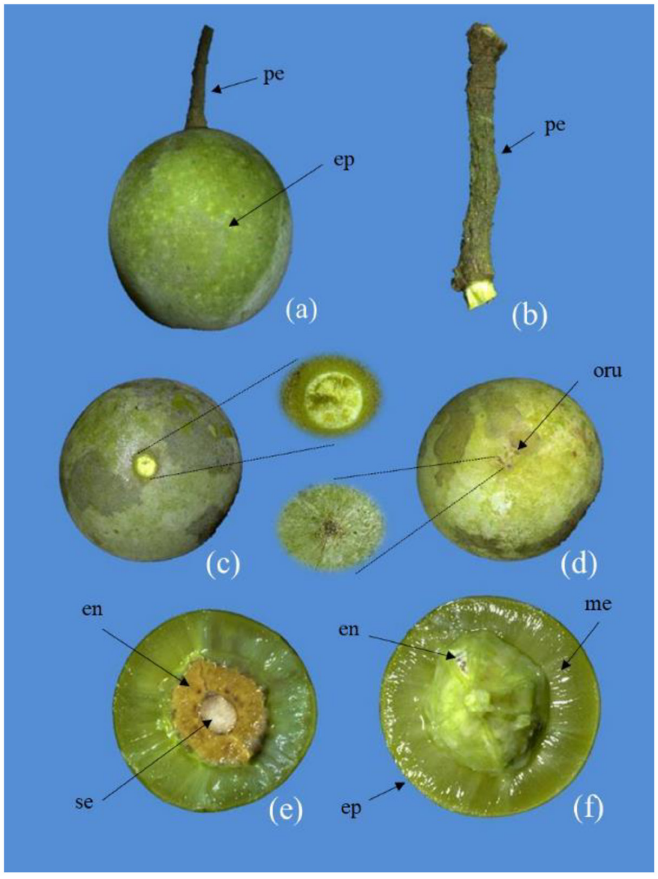

Figure 1. Schematic representation of *Buchenavia tetraphylla* fruits collected in a population grown at the Federal University of Paraíba, Areia, Paraíba, Brazil. a) side view with epicarp (ep); b) peduncle (pe); c) peduncle scar (cp); d) bottom view of the fruit with details of the umbilicated organ (oru); e) cross section highlighting the seed (se); f) cross section with epicarp (ep), mesocarp (me), endocarp (en).

The woody endocarp (pyrene) is oval-oblong, it has longitudinal edges resembling a walnut, irregular surface, dark straw color and a single seed inside of it. The presence of two layers forming the pyrene wall is clearly observed - the outer layer is fibrous and spongy, whereas the inner one is fibrous and dense (Figure 2).

The seed is exalbuminous, oblong, cylindrical, membranous, glabrous and light brown; it has an acute base and obtuse apex, as well as a fine integument (Figure 3). The micropyle is discreet, the chalaza is marked by a brown spot at the tip of the seed, the raphe is brown in color and extends between the hilum and the chalaza (equivalent term to calaza) - the hilum is circular and homochromatic (Figures 3a and 3b). The hilum is imperceptible because the restricted space of the seed locule is connected to the placenta, so it is not possible to perceive the funicle; the ventral raphe shows that the seed derives from an anatropous egg.

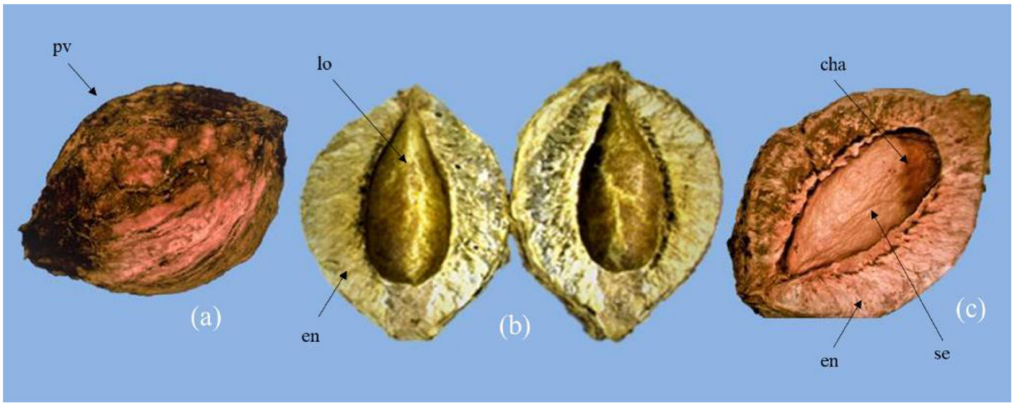

Figure 2. Schematic representation of *Buchenavia tetraphylla* pyrene collected in a population grown at the Federal University of Paraíba, Areia, Paraíba, Brazil. a) Pyrene (pv); b) longitudinal section of pyrene with emphasis on the endocarp (en) and seed locule (lo); c) longitudinal section highlighting the seed in the locule (se), chalaza (cha).

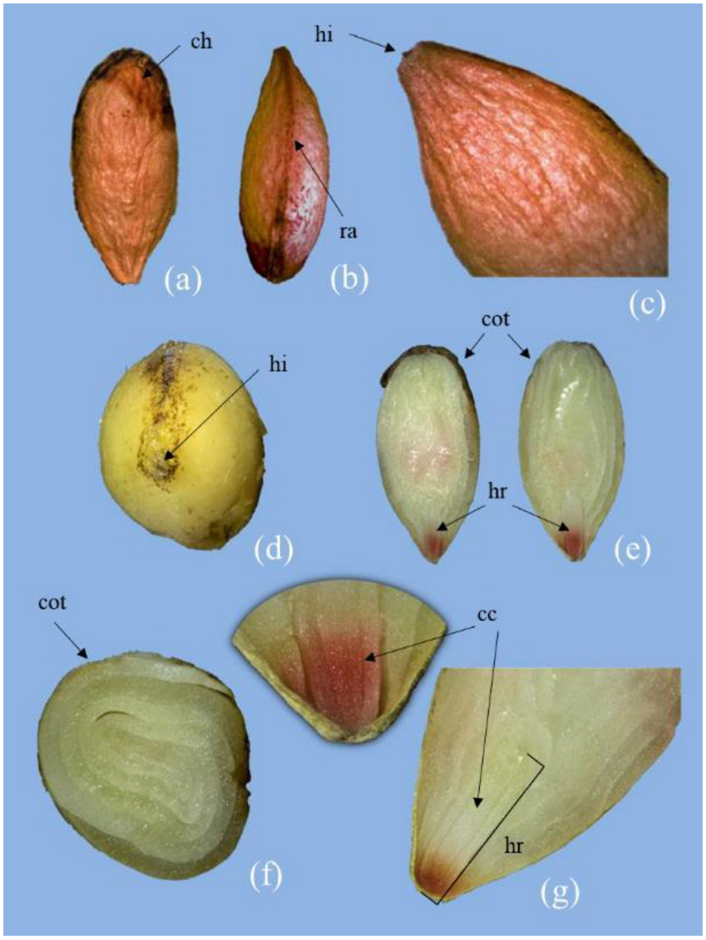

Figure 3. External and internal schematic representation of *Buchenavia tetraphylla* seed collected in a population grown at the Federal University of Paraíba, Areia, Paraíba, Brazil. a) front view of the seed showing the chalaza (cha); b) side view of the seed with the raphe (ra); c) enlarged seed with hilum detail (hi); d) swollen seeds without integument, with emphasis on the hilum (hi); e) longitudinal section of the seeds, with emphasis on cotyledons (cot) and on the axis-hypocotyl-radicle (hr); f) cross section of the seed, with emphasis on convoluted cotyledons (cot); g) longitudinal section showing detail of the central cylinder (cc) and the axis-hypocotyl-radicle (hr) stained with tetrazolium.

The cotyledon embryo is of the complete, starchy, invaginated and straight type; it has a cylindrical axis (<1 mm in diameter), hypocotyl-radicle (HR) axis, which can be linear, straight or slightly curved (approximately 3 mm in length), glabrous, smooth, bright, undifferentiated and partially surrounded by the lobes of the cotyledons that, in their turn, are tortuous and leafy, pearl white, with sagittal base and truncated apex inserted in the hypocotyl end of the embryonic axis (Figures 3c-f). The embryonic axis has a rudimentary plumen and is almost totally surrounded by cotyledons (Figures 3g and 3h).

Biometric analyses have shown wide variations in fruit and endocarp dimensions (diameter and length) and fresh weight - this variation led to the formation of six classes. Fruit length ranged from 21.23 to 30.66 mm and the largest number of fruits (46 fruits) recorded length ranging from 25.96 to 27.52 mm, as shown in the third quartile (Q3) of the box plot (Figure 4a). Similar variation was observed for fruit diameter; however, the incidence of outliers has indicated values outside the maximum and minimum limits set for this feature (Figure 4b). Fruit weight presented a similar data distribution to that of fruit length (Figure 4c). The longest endocarp length (59 fruits) ranged

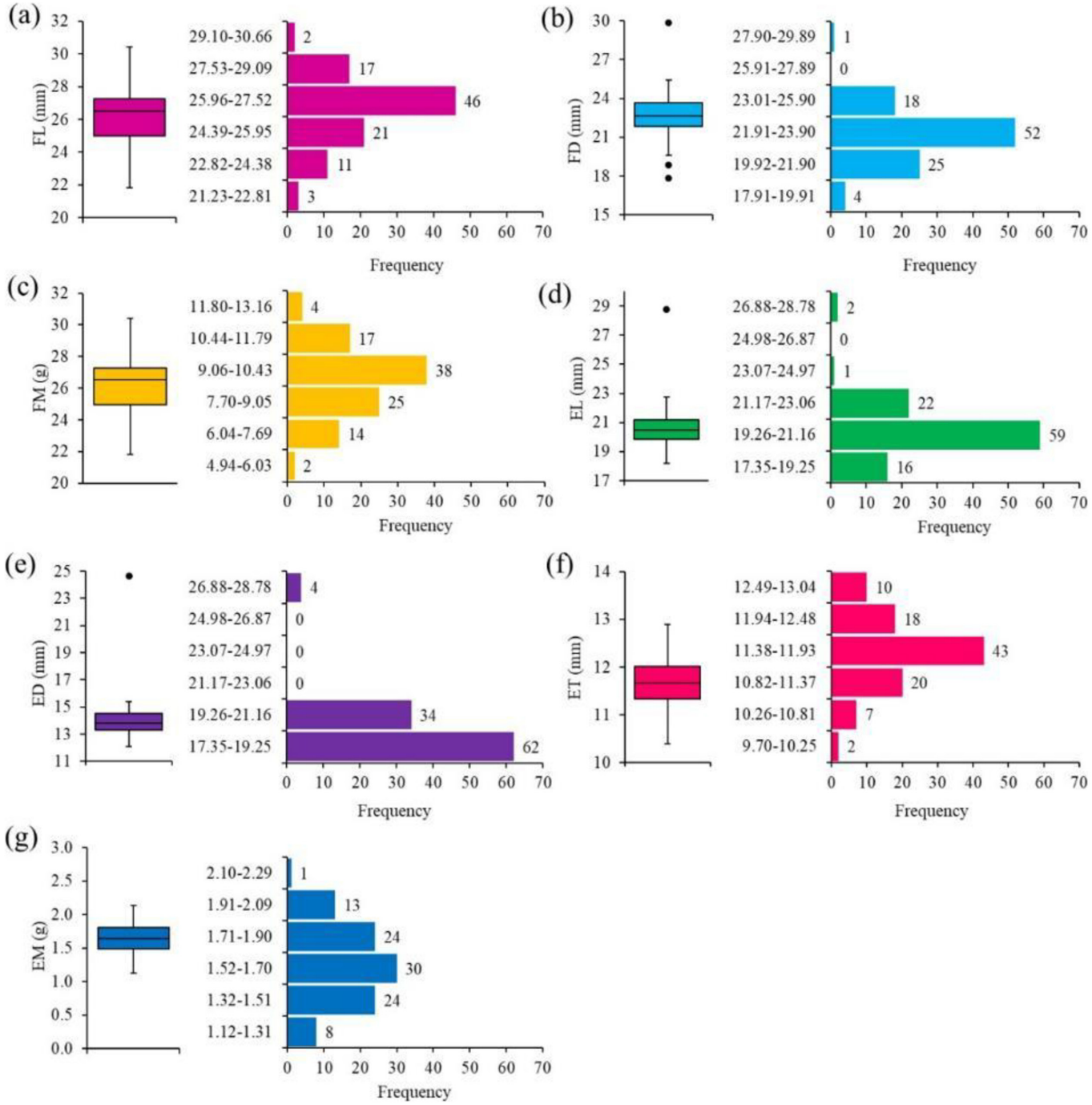

Figure 4. Boxplot and frequency (%) distribution of *Buchenavia tetraphylla* fruit length, diameter and mass (%), as well as of endocarp length, width and thickness, in population grown in Campus II sites of Federal University of Paraíba, Areia County, Paraíba State, Brazil. FL: fruit length (a); FD: fruit diameter (b); FM: fruit mass (c); EL: endocarp length (d); ED: endocarp diameter (e); ET: endocarp thickness (f), EM: endocarp mass (g).

from 19.26 mm to 21.16 mm (Figure 4d). Endocarp diameter was the feature presenting the least variation in the boxplot and histogram (Figure 4e). On the other hand, endocarp thickness and mass have shown wide biometric variation (Figures 4f and 4g).

3.2. Germination description

Germination tests have shown that only 40% of fruits had viable seeds (Figure 5), which generated 21% of normal seedlings (with roots, hypocotyl and cotyledons). *B. tetraphylla* post-seminal development was marked by seed tegument rupture, as well as by protrusion of the primary, cylindrical, glabrous root - which ranged from white to yellowish - and, subsequently, by secondary filiform root formation. In addition, there was a significant cylindrical green hypocotyl growth. The germination is of the epigeal cotyledon type, its primary root grows in a much higher proportion than the hypocotyl. The hypocotyl protrudes above the ground into an upright position, when cotyledons, which were previously curled one under the other in spiral movement, start to expand and take a slightly flabby shape (Figures 6a-g).

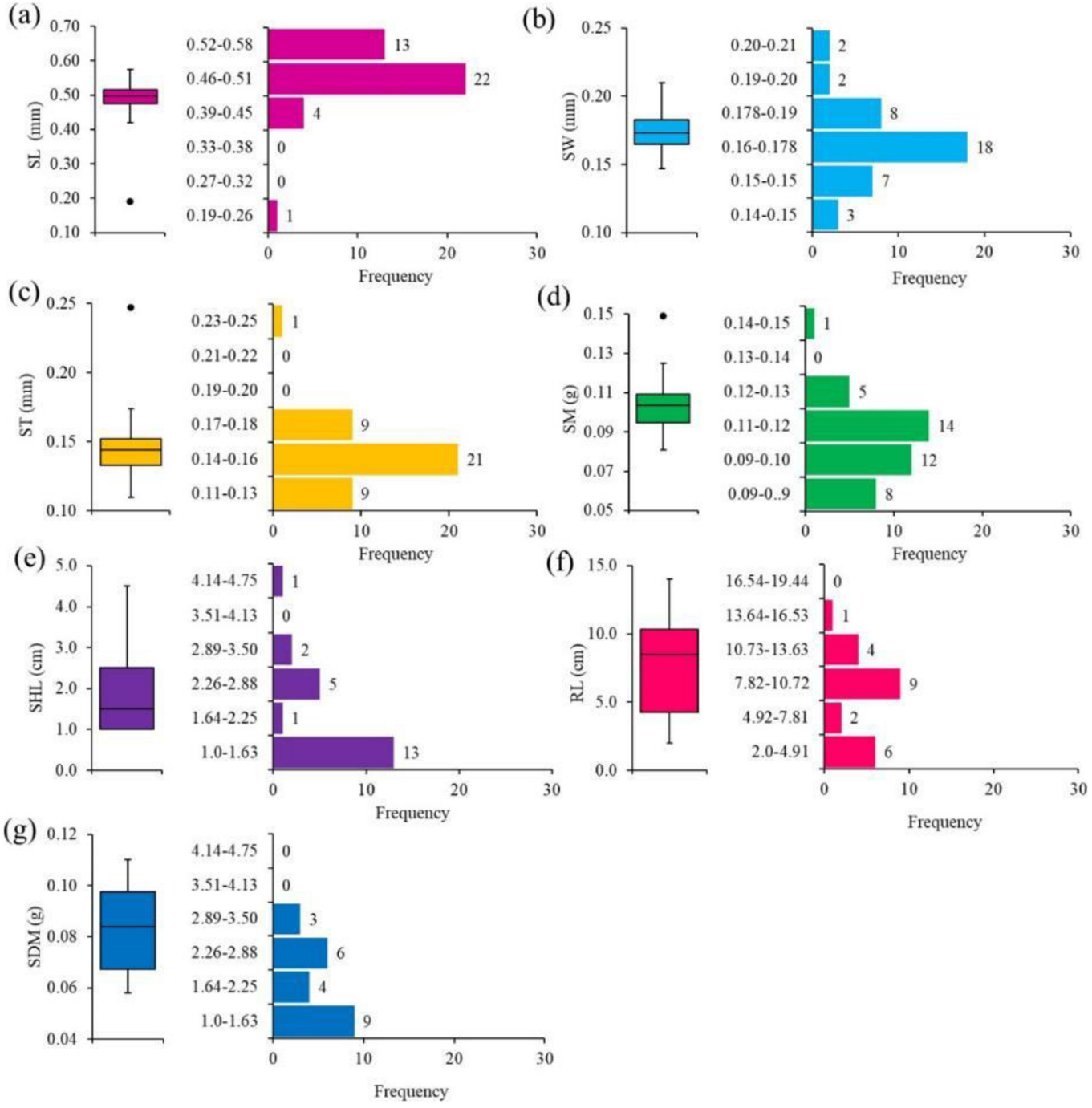

Figure 5. Boxplot and frequency (%) distribution of *Buchenavia tetraphylla* seed length, width, thickness and mass, as well as length and dry mass (in histogram) recorded for sample comprising 21 normal *B. tetraphylla* seedlings collected in a population grown at the Federal University of Paraíba, Areia, Paraíba, Brazil. SL: seed length (a); SW: seed width (b); ST: seed thickness (c); SM: seed mass (d); SHL: shoot length (e); RL: root length (f); SDM: seedling dry mass (g).

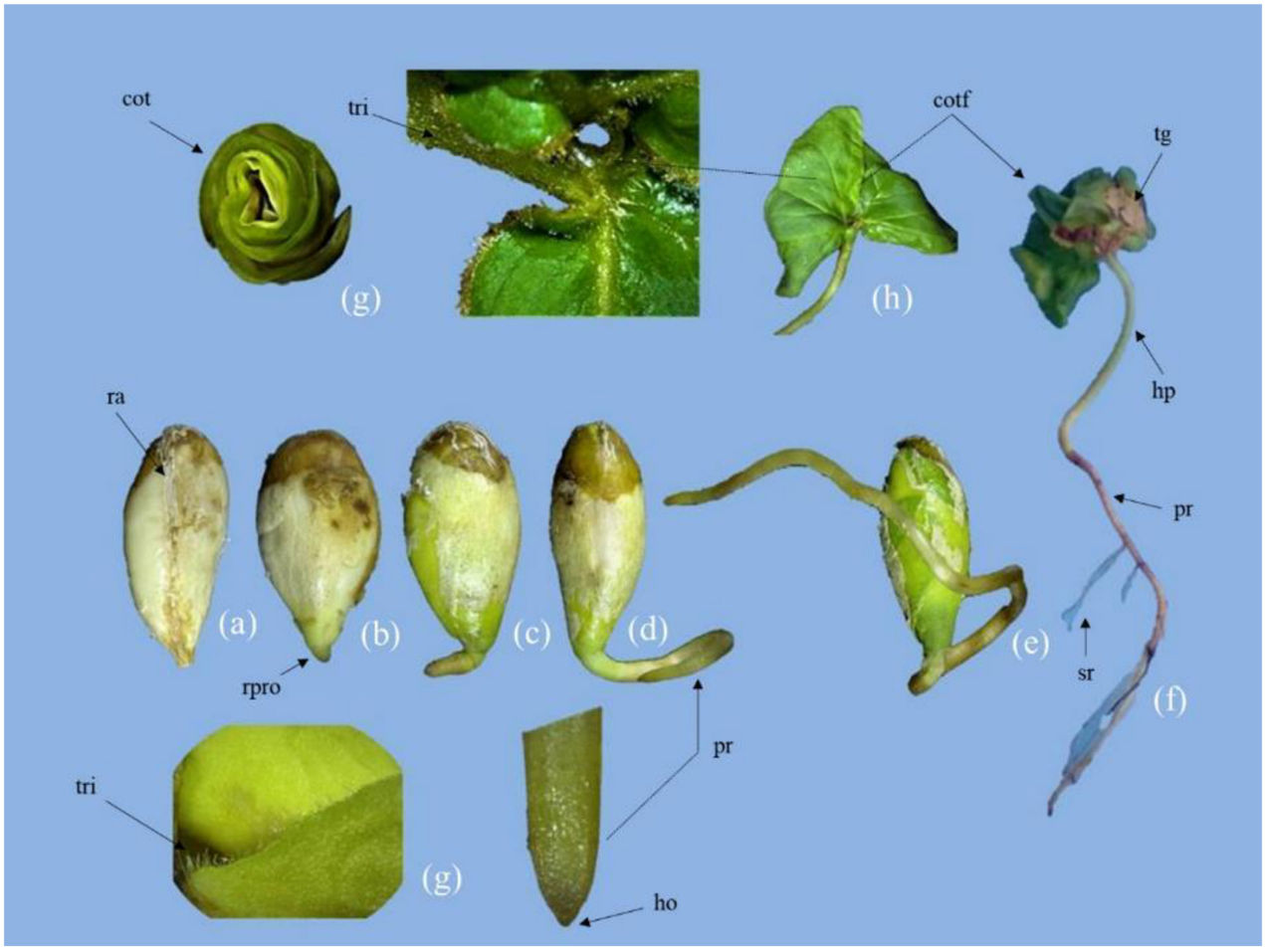

Figure 6. *Buchenavia tetraphylla* seed germination stages in a population grown at the Federal University of Paraíba, Areia, Paraíba, Brazil. a) seed subjected to 48-hour immersion in water; b) primary root protrusion (rpro) 192 hours after sowing; c, d, e) primary root growth 384 hours after sowing; f) seedlings with opened cotyledon leaves; g) cotyledons; h) cotyledon leaf with rusty trichomes; raphe (ra), trichomes (tri), cotyledons (cot), foliated cotyledons (cotf), integument (tg), hypocotyl (hp), primary root (pr), secondary roots (sr), hood (ho).

3.3. Correlation analysis

Correlation analysis applied to biometric features of fruits, seeds and germination has shown that of the 91 analyzed correlation pairs, 21% were significant based on the t-test. Most correlations presented medium magnitude. Correlation higher than 0.7 was only recorded between FL (fruit length) and FM (fruit mass) (r = 0.73), whereas significant negative correlation was only recorded between SW (seed width) and ST (seed thickness) (r = -0.29) (Figure 7).

4. DISCUSSION

The species *B. tetraphylla* has diaspores compatible with animal dispersal due to the presence of a pulp with a pleasant aroma, which is why they play a fundamental role in its dissemination and, consequently, in its natural regeneration (van der Pijl, 1982). The pleasant aroma of the fruits is due to the presence of volatile compounds which are directly associated with the attraction of insects and vertebrates to disperse the seeds (Rodríguez et al., 2013; Pereyra et al., 2022). In the present study, an average value of fresh pulp mass (Figure 4c) was recorded lower than that reported for other species belonging to the same genus, such as *B. tomentosa* Eichler (14.15 g); probably due to genetic characteristics combined with the climatic conditions of the region, as well as a strategy to promote dispersal by smaller animals, such as rodents and marsupials, which is in line with Zuffo Júnior et al. (2016) who also recorded mean diameter and length values of *B. tetraphylla* fruits that were higher (28.37 mm and 34.32 mm, respectively) than those recorded in the present study. This factor can help in the dispersal of the species, especially in anthropic environments with a reduced number of dispersing agents (Camargo et al., 2011).

Fruits of species belonging to family Combretaceae derive from the inferior ovary; therefore, they are involved by the floral receptacle tissue. This extra-carpellar tissue develops after egg fertilization and involves the entire ovary. Something similar happens in apple blossom (family Rosaceae), whose structure is called “amphistego” and accounts for the ontogenesis of different dispersion structures in species belonging to this family (Barroso et al., 2004; Kurokura et al., 2013). These characteristics are compatible with those found in the fruits of *B. tetraphylla* because the species belongs to the same family, therefore its description is important for identification purposes (Figure 1).

The genus *Buchenavia* L. is characterizeded by its round, angular or ridged fruits without amphistego, except for *B. pterocarpa*, whose fruits have two narrow wings (Barroso et al., 2004). According to Barroso et al. (2004), the amphistego comprises three layers: an outer thin layer, an intermediate fibrous one (genus *Thiloa*) or fibrous fleshy layer (genus *Terminalia*) and a spongy one.

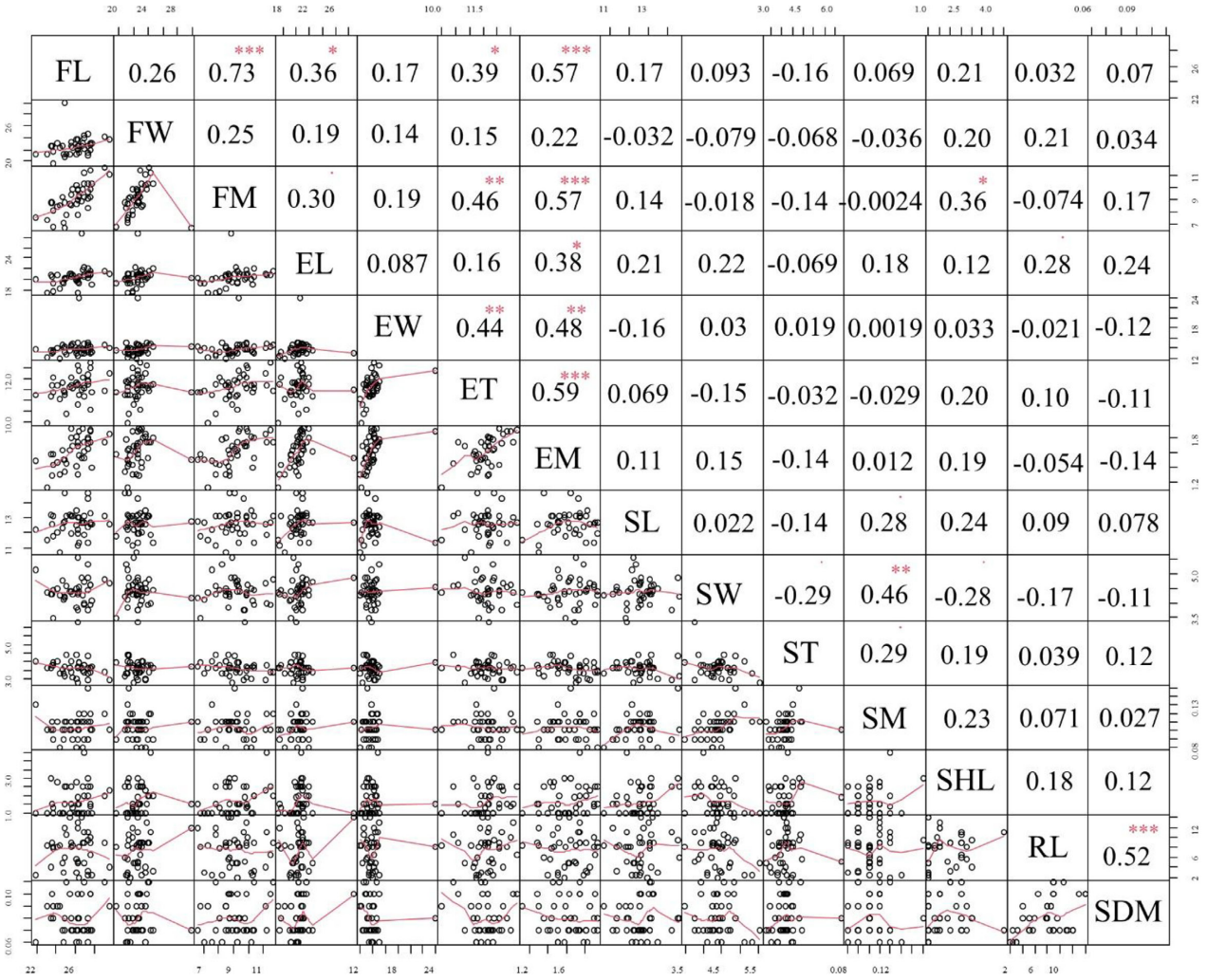

Figure 7. Pearson's correlation coefficients on the upper diagonal and escarteplotes with regression lines on the lower diagonal between biometric elements of *B. tetraphylla* individuals collected in a population grown on the Campus II sites of the Federal University of Paraíba, Areia County, Paraíba State, Brazil. *, ** and *** significant at 5%, 1% and <1%, based on the T test. Fruit length (FL), Fruit width (FW), Fruit mass (FM), Endocarp length (EL), Endocarp width (EW), Endocarp thickness (ET), endocarp mass (EM), Seed length (SL), Seed width (SW), Seed thickness (ST), Seed mass (SM), Shoot length (SHL), Root length (RL), Seedling dry mass (SDM).

The characteristics of the fruits are in accordance with those described for the genus *Buchenavia* L., which are round, angular or striated fruits, without amphistegum, except for *B. pterocarpa*, whose fruits have two narrow wings. According to Barroso et al. (2004), the amphistegus comprises three layers: a thin outer layer, a fibrous (genus *Thiloa*) or fleshy fibrous (genus *Terminalia*) intermediate layer and a spongy layer. The importance of the fruits of B. *tetraphylla* expressing the characteristics of the genus, regardless of origin, is important, especially for species identification purposes.

Thus, it is possible to suggest the existence of amphibian plants belonging to the genus *Buchenavia*, if one takes into consideration that the outer and thin layer evolves to wing formation and makes the anemochoric propagation of most seeds of this species belonging to family Combretaceae easier. On the other hand, the fleshy intermediate layer of the species belonging to genera *Terminalia* and *Buchenavia* promotes zoochoric dispersion and its spongier internal layer (which is the first pyrene layer) makes its buoyancy and dispersion in water easier (Barroso et al., 2004; Taia et al., 2024).

The embryo is of the complete type, that is, it occupies the entire inner surface of the seed, since there is no presence of endosperm, in agreement with the observations of Brasil (2009). The seed coat is very thin, probably for this reason the testa and tegmen are indistinct (Figure 3), a typical characteristic of seeds whose endocarp acts as a mechanical protection tissue, that is, a typical case of drupe fruit, which agrees with descriptions by Barroso et al. (2004). The percentage of seed germination was low (30%) even under ideal conditions, which can be attributed to some type of dormancy, possibly due to mechanical restrictions, as well as the high percentages of seedless endocarp, which led to uneven germination. This characteristic can be understood as a survival strategy adopted by the species to perpetuate itself over time, since its advantage is the gradual establishment and colonization of species in new areas, in order to distribute their germination spatially and temporally or to protect themselves from any damage during the dispersion processes, which is in accordance with the reports of Carvalho & Nakagawa (2012).

The low percentage of germination is a characteristic of this species' seeds, as observed in the study conducted by Santos et al. (2006) in a 380-ha stretch of Atlantic Forest in Northeastern Brazil, 99% of *B. tetraphylla* seeds were found below the crown canopies - seed density ranged from 14.6 m^2 to 29.9 m^2 seeds. Over 18 months of observation, 49.1% of diaspores have germinated until the seventh month in the soil, whereas those that did not germinate were in decomposition. This outcome suggests that seed viability *in situ* was restricted to approximately 6-7 months after fruit drop and that diaspores did not form reserves with persistent seeds in the soil.

The higher values of root length than the aerial part are justified by the need to form a robust root system to provides support for the plant and promotes a more efficient absorption of water and nutrients, so that the seedlings invest most of the of its reserves into the root system. Something important to mention is that biometric values must be correlated with edapho-climatic (Tabarelli et al., 2003), genetic and physiological factors (Moura et al., 2010), and cannot be used as standard values for forest species. However, they can be used to characterize individuals found in the environment, as well as to make inferences about aspects associated with the dispersal dynamics and reproductive success of this plant.

Given the scarcity and incipience of studies about *Buchenavia tetraphylla*, mainly of studies focused on investigating morphological and biometric data of fruits, seeds and seedlings, the current results contribute to improve the investigations about this species, as well as showed wide morphological variability, which was likely associated with genetic factors.

5. CONCLUSIONS

The ripe fruits of *Buchenavia tetraphylla* are pyriform drupes, greenish in color, with a single seed per fruit and surrounded by greenish mucilage. The seeds are exalbuminous, with epigeal germination and the seedlings are phanerocotyledonous.

This species produces a large number of seedless fruits (52%), with irregular germination and a high percentage of mortality at the seedling stage under controlled conditions.

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AUTHOR CONTRIBUTIONS

MFQL: conceptualization, methodology, Investigation, writing – original draft; WSA, ILA and RSS: methodology, investigation; LKSL: data curation, formal analysis; LDAA and EUA: conceptualization, writing – review & editing; RLAB: supervision.