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SEED, SEEDLING AND FRUIT MORPHOLOGY AND SEED GERMINATION OF *Psidium sobralianum* PLANTS OF THE SÃO FRANCISCO VALLEY, BRAZIL[1]

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ABSTRACT - The Northeast region of Brazil has the second highest number of species of the Myrtaceae family. It is mostly covered by the Caatinga biome, which is very degraded, making it difficult to preserve species of this family. Thus, the objective of this work was to describe the seed, seedling, and fruit morphology, and seed germination of *Psidium sobralianum* Landrum & Proença plants of the São Francisco Valley, Brazil. The fruits were collected in an area of the Brazilian Agricultural Research Corporation (Embrapa Semiarid), in Petrolina PE, Brazil. The evaluations consisted of determinations of fruit shape, consistency, and number of seeds; seed form, cotyledons, hilum, hypocotyl-radicle axis, and embryo type; epicarp, mesocarp, endocarp, and seed staining; longitudinal, transversal, and ventral diameters of fruits and seeds; fruit, pulp, and seed fresh and dry masses; germination test; first count of germinated seeds; germination speed index; shoot and root lengths; shoot and root fresh and dry masses; and imbibition test. *Psidium sobralianum* has polyspermic, berry fruits, subclassified as solanidium, with persistent sepals and globular shape, consisting of epicarp, mesocarp, endocarp and seeds. The fruits have green with orange epicarp, pale-green mesocarp, and white endocarp. The seeds have a pilose and bony aspect, a pimentoid type of embryo, foliaceous cotyledons, presence of operculum and hilum, and pale-yellow tegument. The germination is epigeal phanerocotiledonar, with root protrusion from 26 days after sowing (DAS); it presents a short, glabrous, thick radicle, and a rounded, pale-green apex. The germination is slow, probably due to the mechanical barrier of the tegument, and stabilizes at 90 DAS.

Keywords: Myrtaceae. Araçá. Vigor test. Imbibition test.

MORFOLOGIA DE FRUTOS, SEMENTES, PLÂNTULAS E GERMINAÇÃO DAS SEMENTES DE *Psidium sobralianum* DO VALE DO SÃO FRANCISCO, BRASIL

RESUMO – O Nordeste do Brasil é a segunda região em número de espécies registradas da família Myrtaceae, porém a Caatinga está bastante degradada, o que dificulta a preservação das espécies deste grupo. Objetivou-se caracterizar a morfologia de frutos, sementes, plântulas e a germinação de araçá (*Psidium sobralianum*) do vale do São Francisco. Os frutos foram coletados na Embrapa Semiárido, Petrolina, PE e submetidos as análises: formato, consistência e número de sementes por fruto; forma, cotilédones, hilo, eixo hipocótilo-radícula e tipo de embrião das sementes; coloração do epicarpo, mesocarpo, endocarpo e semente; diâmetros longitudinal, transversal e ventral de frutos e sementes; massas frescas e secas de frutos, polpa e sementes; teste de germinação; primeira contagem; índice de velocidade de germinação; comprimento das partes aérea e radicular; massas frescas e secas aérea e radicular; teste de embebição. *P. sobralianum* possui fruto solanídio, pétalas persistentes, formato globular, polispérmico, composto de epicarpo, mesocarpo, endocarpo e sementes. O epicarpo tem coloração verde com laranja, mesocarpo verde-claro e endocarpo branco. Sementes com aspecto piloso e ósseo, embrião pimentoide, cotilédones foliáceos, presença de opérculo, hilo e tegumento amarelo-claro. Germinação epígea fanerocotiledonar, com protrusão radicular a partir dos 26 dias após a semeadura (DAS), radícula curta, glabra, espessa, ápice arredondado e verde-claro. A germinação é lenta, provavelmente devido a barreira mecânica do tegumento, estabilizando-se apenas aos 90 DAS.

Palavras-chave: Myrtaceae. Araçá. Teste de vigor. Teste de embebição.

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INTRODUCTION

Species of the family Myrtaceae are widespread in Brazil, including the genus *Psidium*. The Northeast of Brazil has the second highest number of species of this family, with significant presence in all states of this region (SOBRAL et al., 2015).

Fruits of species of the genus *Psidium* are widely consumed by the population and avifauna of the Caatinga biome in the Northeast of Brazil (LUCENA; MAJOR; BONILLA, 2015; MENDES; SOUZA, 2016). Several studies report the characteristics of essential oils of these species because they are very promising for antioxidant, antimicrobial, cytotoxic (SIMONETTI et al., 2016), and antifungal applications (MORAIS-BRAGA et al., 2016).

The species *Psidium sobralianum*, is known as araçá in the São Francisco Valley, and as little guava in Crato, and Barbalha, in the state of Ceará (CAMPOS et al., 2016), and is used as a medicinal plant to treat sore throats and flu in the Chapada do Araripe (LANDRUM; PROENÇA, 2015) in the Northeast of Brazil. It was discovered and described by Landrum and Proença (2015); according to these authors, it is found in regions of Dry Highland Forests, Carrasco vegetation, and Wet Forests, in Northeast Brazil.

Although species of this genus has ecological, commercial, and medicinal value, the Caatinga biome has been degraded due to the removal of native species for agriculture and livestock activities, and deforestation and burning practices, which result in the environmental imbalance of the region (LUCENA; MAJOR; BONILLA, 2015; MENDES; SOUZA, 2016).

In this context, the objective of this work was to describe the seed, seedling, and fruit morphology, and seed germination of *P. sobralianum* plants in the São Francisco Valley, Brazil.

MATERIAL AND METHODS

Fruits and seeds of *P. sobralianum* were acquired in an area of the Brazilian Agricultural Research Corporation (Embrapa Semiarid), in Petrolina PE, Brazil (9º09'S, 40º22'W, and altitude of 365 m). They were analyzed morphologically at the Laboratory of Plant Ecophysiology of the State University of Ceará (UECE). The fruit epicarp and mesocarp color were evaluated using the Munsell primer (1994), which expresses the color in three parameters: value (V), chroma (C) and hue angle (H). The fruit shape, pericarp consistency, and number of seeds were also determined (CHITARRA; CHITARRA, 2005). The seed tegument color (MUNSELL, 1994), form, cotyledons, presence of hilum, hypocotyl-radicle axis, and embryo type were characterized (BARROSO et al., 2012). The fruit longitudinal (LD) and transversal (TD) diameters, and seed LD, TD, and ventral (VD) diameters were measured with a digital caliper ruler.

The fruit, pulp (epicarp + mesocarp + endocarp), and seed fresh and dry masses were measured in an analytical balance (0.001 g). The plant materials were placed in a drying oven at 60 °C until constant mass to evaluate their dry masses (LUCENA et al., 2007).

Some fruit and seed samples were taken to the Laboratory of Shrimp Culture of the UECE for photographic records using a DFC295 camera coupled to a M50-Leica microscope.

Germination tests were carried out at the Laboratory of Ecology of the UECE, using four replicates. Twenty-five seeds were disinfested using a 5% commercial solution of sodium hypochlorite ($NaClO$) (2.5% a.i.) for five minutes and seeded on two filter papers in previously autoclaved Petri dishes; they were moistened and monitored according to the Rules for Seed Analysis (BRASIL, 2009).

A 12-hour photoperiod was used in the biochemical oxygen demand (BOD) germinator, which was set with alternating temperature of 20 to 30 ºC, with the highest temperature in the light period simulating daylight by fluorescent lamps (4 x 20 W). The first count of germinated seeds in the germination test (GT) was carried out and the germination rate index was determined at 60 days after sowing (DAS) (MAGUIRE, 1962). Germinated seeds were those with root protrusion of 2 mm. Photographic records of the seedling development were taken during the GT and the shoot length (SL) and root length (RL), shoot fresh mass (SFM), root fresh mass (RFM), shoot dry mass (SDM) and root dry mass (RDM) was evaluated at the end of the GT (CARVALHO; NAKAGAWA, 2012).

A similar test to the GT with 10 seeds and two replicates was conducted in a complete randomized experimental design to evaluate the seed imbibition in water, using 41 imbibition times (0, 6, 12, 18, 24, 36, 48 hours, and every 24 hours up to 864 hours). Petri dishes were wrapped in polyvinyl chloride film to avoid water loss by evaporation and the water content was monitored daily and replenished uniformly when necessary in all plates, using an amount equivalent to two times the substrate mass. The moisture content of the seeds was determined gravimetrically at all imbibition times and expressed as percentages of the fresh mass. The seeds were weighed in analytical balance (0.0001 g) and dried in an oven at 105 ± 3 ºC for 24 hours (BRASIL, 2009).

The data of imbibition as a function of time were subjected to analysis of variance, by the test F. Treatments with significant means were subjected to polynomial regressions. The ESTAT Software

(System for Statistical Analysis) was used for the calculations.

RESULTS AND DISCUSSION

Morphological characterization

P. sobralianum has a bacoid (berry) fruit, with persistent sepals and globose shape (Figure 1A-C). However, Landrum and Proença (2015) characterize the fruit format as subglobose. According to Barroso et al. (2012), South American species of the Myrtaceae family have bacoid, fleshy, indehiscent fruits, which may be globose, obovoid, oblong, piriform, ellipsoid, or lageniform; and the bacoid fruits of *Psidium* can be classified in the subtype solanidium, which have fleshy and developed placentas, reduced loci, and numerous ovules involved by pulp.

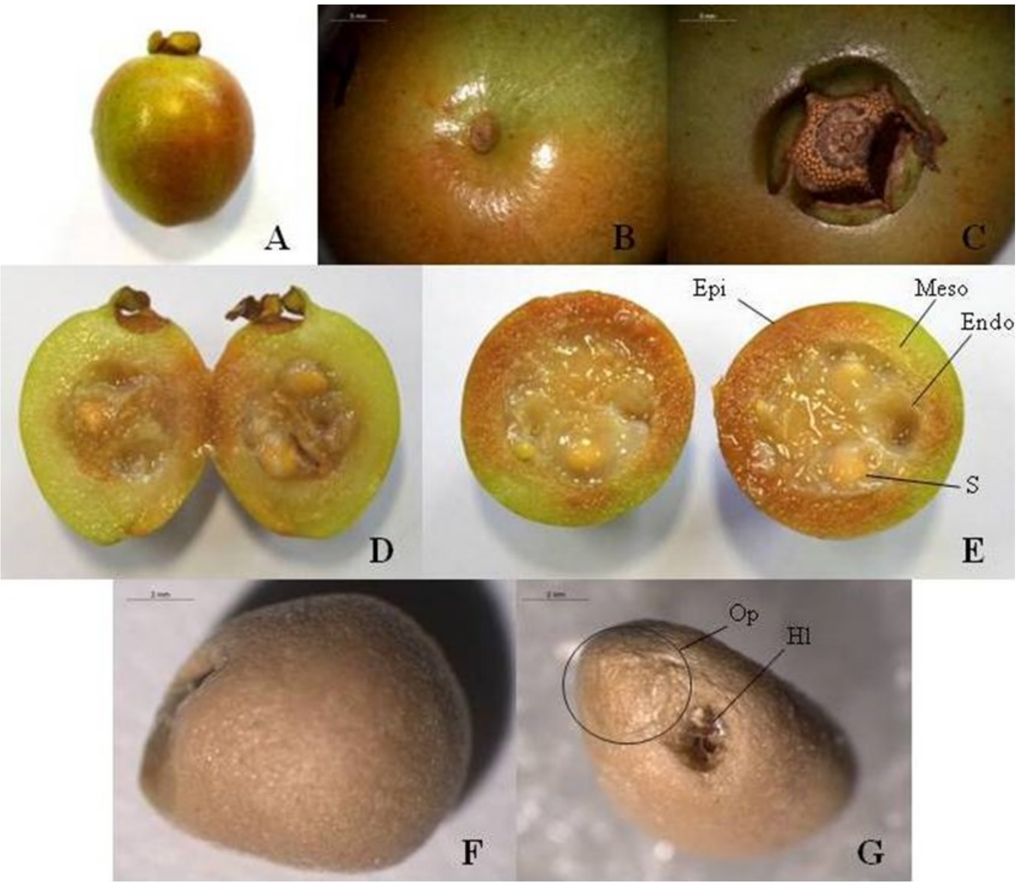


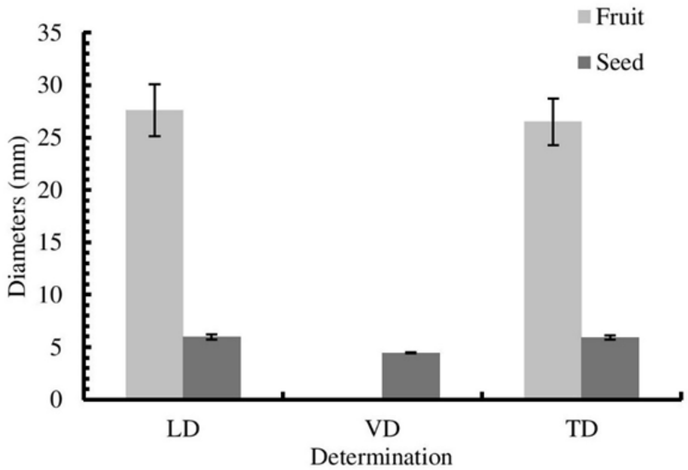
Figure 1. Fruits and seeds of *Psidium sobralianum* Landrum & Proença: A. external appearance of the fruit; B. Region of insertion of the peduncle in the fruit; C. Opposite region to the insertion of the peduncle in the fruit, with persistent floral sepals; D. Longitudinal cut of the fruit; E. Cross section of the fruit showing the epicarp (Epi), mesocarp (Meso), endocarp (Endo) and seed (S); F. Longitudinal aspect of the seed; G. Transversal aspect of the seed showing the operculum (Op) and hilum (H1).

The globular fruit of *P. sobralianum* is polyspermic, containing 3 to 6 seeds, but for Landrum and Proença (2015) these fruits may have 1 to 10 seeds. According to Figure 1, the mesocarp, endocarp, and seeds of ripe fruit of *P. sobralianum* shows green epicarp (V = 7, C = 6, and H = 5YG), orange equatorial region (V - 5, C – 6, and H - 5GY), pale-green mesocarp (V = 8, C = 4, and H = 5GY), and white endocarp.

The seeds have a pilose and bony aspect, with pimentoid type of embryo that can be found in the genus *Psidium*, *Calycorectes*, *Acca*, *Campomanesia,* and *Pimenta* (BARROSO et al., 2012). Landrum and Proença (2015) describe the seeds as smooth, with rounded and flat surfaces. These seeds presented foliaceous cotyledons, confirming the results of Gomes et al. (2015), who describes the *P. cattleianum* Sabine species as having foliaceous cotyledons. The tegument color is cream (V = 8, C = 4, and H = 5YR) (Figure 1F-G).

P. sobralianum fruits presented longitudinal diameter (LD) of 27.593 ± 2.4685 mm, and transversal diameter (TD) of 26.493 ± 2.2212 mm (Figure 2), as in the classification of Landrum and Proença (2015). Diniz et al. (2017) found similar diameters (LD of 32.8 mm ± 16.0, and TD of 33.6 mm ± 17.0) for *Eugenia cribrata* McVaugh. However, they were smaller than those found for

Eugenia uniflora L. (width of 10-12 mm and length of 12-15 mm) (SMIDERLE; SOUZA; SOUZA, 2016), and *Eugenia dysenterica* DC. (LD of 27.36, and TD of 32.52 mm) (CAMILO et al., 2014). These results denote interspecific and intraspecific differences between fruit sizes and masses (SMIDERLE; SOUZA; SOUZA, 2016; BIANCHINI et al., 2016).



The vertical line on each bar indicates the standard deviation (±) of the mean of four replicates of 25 fruits or seeds.

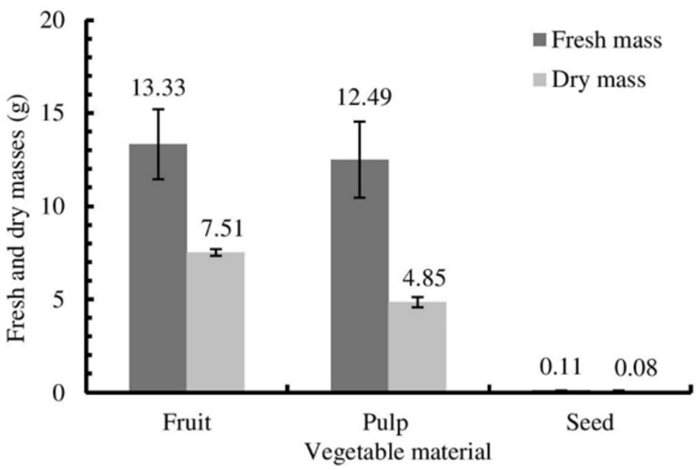
Figure 2. Longitudinal (LD), ventral (VD) and transversal (TD) diameters of fruits and seeds of *Psidium sobralianum* Landrum & Proença.

The seed diameters found (LD of 5.954 ± 0.2512, VD of 4.453 ± 0.0644, and TD of 5.901 ± 0.1766 mm) confirm the seed lengths (5 to 7 mm) described by Landrum and Proença (2015).

The fresh fruit, pulp (epicarp + mesocarp + endocarp), and seed masses were: 13.32 ± 1.88 g, 12.49 ± 2.03 g, and 0.10 ± 0.004 g, respectively. The dry fruit, pulp (epicarp + mesocarp + endocarp), and seed masses were: 2.43 ± 0.27 g, 2.38 ± 0.28 g, and 0.07 ± 0.003 g, respectively (Figure 3).

The fresh fruit mass of *P. sobralianum* was lower than that found for *Eugenia cribrate* (24.28 ± 2.85 g) (DINIZ et al., 2017), and *Campomanesia phaea* (Berg) Landr. (23.73 to 82.33 g) (BIANCHINI et al., 2016). These masses were similar to that found for *Eugenia dysenterica* (18.08 g) (CAMILO et al., 2014), and well above that found for *Campomanesia cambessedeana* O.Berg (4.00 g) (MORZELLE et al., 2015).

The larger the fruit size, the higher the pulp mass (CAMILO et al., 2014), thus, the pulp masses were also smaller than those found for *Eugenia cribrata* (17.51 ± 2.56 g) (DINIZ et al., 2017). The seeds had lower masses than those found for *Eugenia uniflora* (0.5 to 1.29 g) (SMIDERLE; SOUZA; SOUZA, 2016).



The vertical line on each bar indicates the standard deviation (±) of the mean of four replicates of 25 fruits or seeds.

Figure 3. Fresh and dry fruit, pulp (epicarp + mesocarp + endocarp), and seed masses of *Psidium sobralianum* Landrum & Proença.

Germination

The *P. sobralianum* seeds had epigeal phanerocotiledonar germination. It seems to be very common in the Myrtaceae family; it is found in other species of this genus, such as *Psidium cattleianum*, and in species of different genus, such as *Acca sellowiana* (O.Berg) Burret. (GOMES et al., 2015), and *Myrcia cuprea* (O.Berg) Kiaersk. (FERREIRA et al., 2013).

The operculum opening occurred at about two days before the root protrusion (Figure 4A). *P. sobralianum* began to show root protrusion from 26 DAS (Figure 4B), whereas *P. cattleianum* starts germination at 10 DAS (GOMES et al., 2015). However, the radicle appearance (4C-D) of *P. cattleianum*—short, glabrous, thick, rounded apex (GOMES et al., 2015)—is similar to that of *P. sobralianum*, differing only in color, which is white in *P. cattleianum*, and pale-green in *P. sobralianum*.

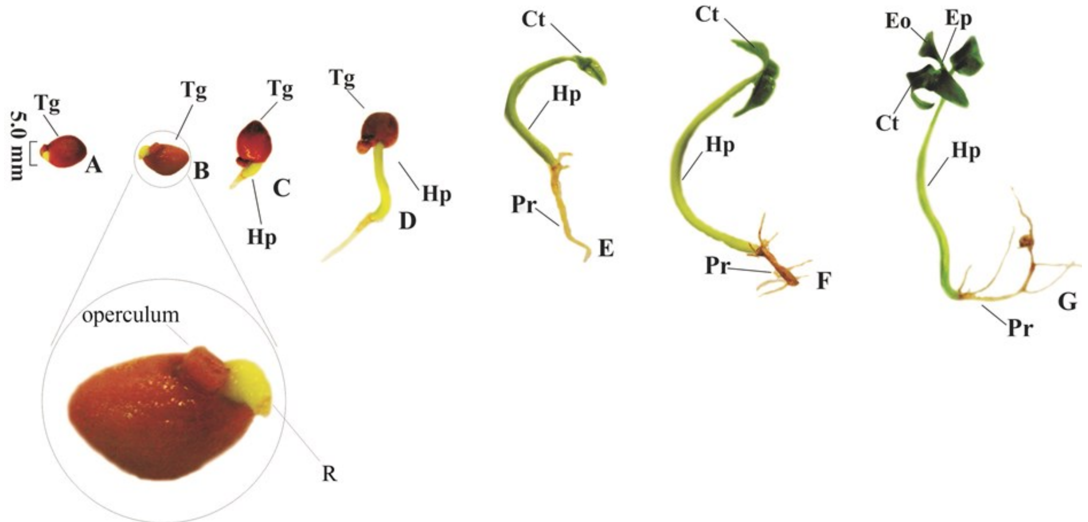


Figure 4. Seedling development of *Psidium sobralianum* Landrum & Proença. A. Beginning of the development with operculum opening; B. Root protrusion; C. Root and hypocotyl differentiation; D. Hypocotyl and radicle development; E. Seedlings with paracotyledons free from tegument; F. Cotyledon color differentiation; G: Seedling with the first pair of Eophiles. Tg = Tegument; R = radicle; Hp = Hypocotyl; Pr = Primary root; Ct = cotyledons; Eo = Eophiles; Ep = Epicotyl.

Approximately two days after root protrusion (Figure 4C), the primary root begins to differentiate from the hypocotyl, becoming pale yellow and thin at the end where the hood is, which also has a pale-yellow color, while the hypocotyl has a pale-green color and glabrous aspect.

After four more days (Figure 4D), the root hairs are well visible at the end of the hypocotyl and as the radicle lengthens, the hypocotyl develops. After 10 days of radicular protrusion (Figure 4E), the foliaceous cotyledons begin to come out completely from the tegument, they are still small and of the same color of hypocotyl, which is a little thinner in the region near the cotyledons.

As the cotyledons and primary root grow larger, about four days after the tegument's release (Figure 4F), their color becomes darker. Eophiles, epicotyl, and thinning of the hypocotyl from the cotyledon to its center were observed at the end of the germination test (Figure 4G).

The differentiation of foliaceous cotyledons, which change their color from pale green to dark green during their development (Figure 4E-G), denotes the high photosynthetic capacity of the seedlings during their development (GOMES et al., 2015).

The germination test (GT) showed 83% germination at 90 days after sowing (DAS) (Figure 5), which was higher than that found by Gomes et al. (2016), using the same temperature, for *Eugenia involucrata* DC. (56% to 72% at 49 DAS), *Acca sellowiana* (69% to 76% at 143 DAS), *Eugenia pyriformis* Cambess. (56% to 72% at 126 DAS) and *Campomanesia xanthocarpa* (Mart.) O.Berg (44% to 78% at 58 DAS).

The first count of germinated seeds occurred at 60 DAS, showing 58% germination (Figure 5). The results of the first count were higher than those found by Gomes et al. (2016) at 15 DAS for *Acca sellowiana* (20% to 24%), *Campomanesia xanthocarpa* (16% to 28%), *Eugenia involucrata* (16% to 24%), and *Eugenia pyriformis* (16% to 24%). The Instructions for Analysis of Seeds of Forest Species (BRASIL, 2013) of the genus *Psidium* present recommendations for first and final counts for *Psidium acutangulum* Mart. ex DC. (not determined and 100 DAS), *Psidium cattleianum* (32 and 90 DAS), and *Psidium myrtoides* O. Berg. (21 and 64 DAS). Different number of days for first count was choose for this study, since the first count at 32 DAS and 45 DAS would show only 1% and 23% germination, respectively.

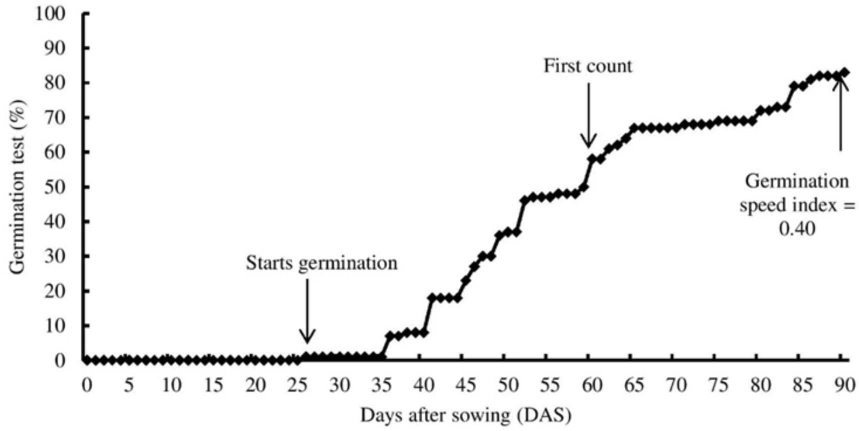


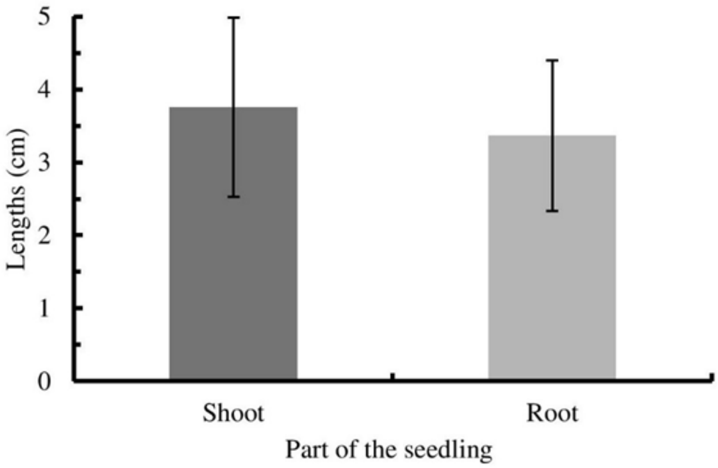
Figure 5. Germination test, first count of germinated seeds, and germination speed index of *Psidium sobralianum* Landrum & Proença seeds.

The germination rate index for *P. sobralianum* was 0.40 (Figure 5), which is consistent with the range found for *Psidium guineense* Swartz. (0.07 to 0.68) (SANTOS et al., 2015), and lower than those found for *Psidium guajava* L. in different substrates (0.75 to 6.50) (ALVES; SILVA; CANDIDO, 2015).

The mean shoot (SL) and root (RL) lengths were 3.757 ± 1.233 cm, and 3.370 ± 1.033 cm, respectively (Figure 6). These results differed from those found for *Acca sellowiana*, which grows rapidly and had 0.5 cm of roots before 30 days of testing (GOMES et al., 2015), since the germination test for *P. sobralianum* in the present study had 90 days; however, the germination process started only at 26 DAS (Figure 5).

According to Ferreira et al. (2013), the radicle and hypocotyl lengths of *Myrcia cuprea* vary depending on the substrate used; the highest lengths were found in vermiculite substrate, presenting radicle length of 10.34 ± 1.43 cm and hypocotyl length of 2.51 ± 0.52 cm. These lengths are higher than those found for *P. sobralianum* probably due to the different germination times, because *Myrcia cuprea* presented the plumule without the tegument adhered to it, at 14 days after sowing.

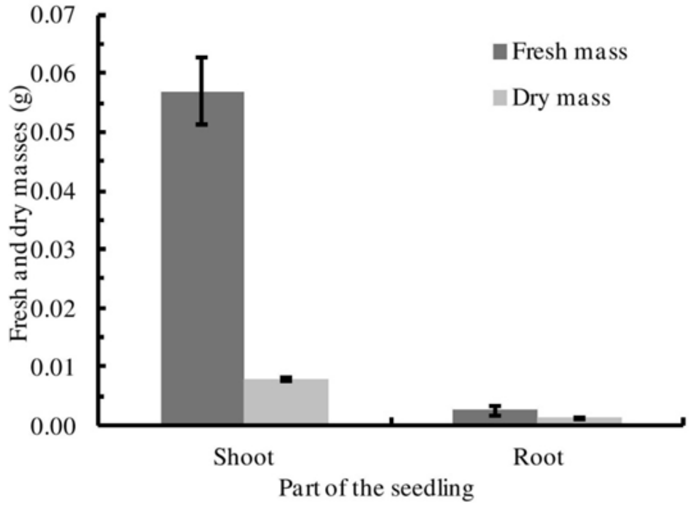
According to Periotto and Gualtieri (2017), *Campomanesia aromatica* (Aubl.) Griseb. seedlings present well developed shoot and radicular organs, and shoot and root lengths, and dry masses varying according to the substrate used for their germination after 120 DAS. They reported higher stem and root lengths in seedlings grown in coconut fiber/ vermiculite and sand substrates, with 3.55 cm for stems in both substrates, and roots with approximately 31 cm in the coconut fiber/ vermiculite, and 34 cm in the sand substrate. These lengths were higher than those found for *P. sobralianum.*



The vertical line on each bar indicates the standard deviation (±) of the mean of four replicates of 25 seedlings.

Figure 6. Shoot (hypocotyl + epicotyl + eophile) and root (primary root) lengths of *Psidium sobralianum* Landrum & Proença seedlings.

The fresh shoot mass found was 0.056 ± 0.0058 g, and the fresh root mass was 0.002 ± 0.0008 g. The shoot dry mass found was 0.007 ± 0.0004 g, and the root dry mass was 0.001 ± 0.0002 g (Figure 7). The summed fresh shoot and root masses (0.058 g) were lower than that found for *Eugenia pyriformis Cambess*. (approximately 4 g), which were also measured after 90 DAS (SCALON et al., 2012), indicating that they were less vigorous. Therefore, according to Carvalho and Nakagawa (2012), normal seedlings that present greater average fresh and dry masses are more vigorous; and vigorous seeds provide greater transfer of materials from their reserve tissues to the embryonic axis in the germination phase, generating seedlings with higher masses.



The vertical line on each bar indicates the standard deviation (±) of the mean of four replicates of 25 seedlings.

Figure 7. Fresh shoot (hypocotyl + epicotyl + eophile) and root (primary root) masses, and dry shoot and root masses of *Psidium sobralianum* Landrum & Proença seedlings.

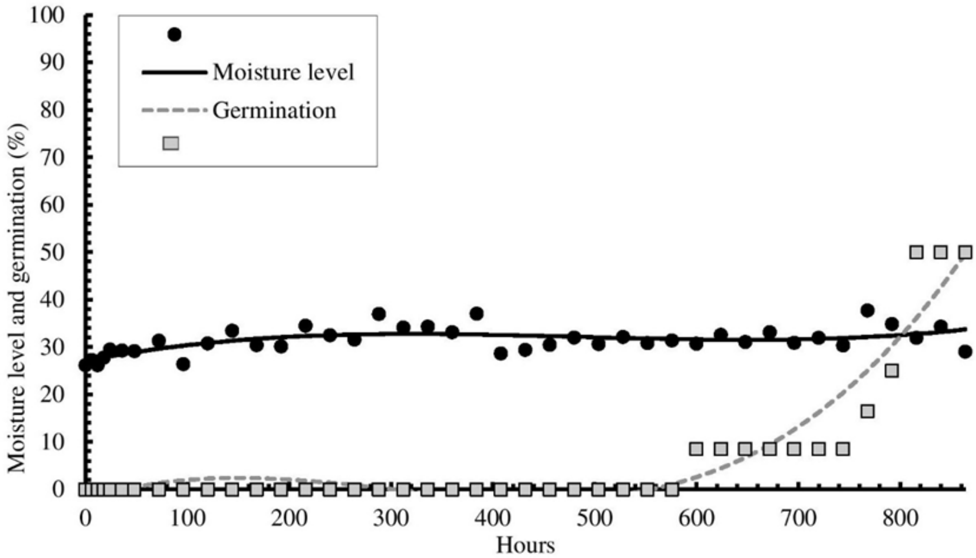
Periotto and Gualtieri (2017) evaluate stem and root dry masses of *Campomanesia aromatica* in different substrates and found higher stem mass in seedlings grown in vermiculite, with masses of approximately 0.023 g, and higher root mass in seedlings grown in sand, approximately 0.12 g. The *P. sobralianum* growth was slow when compared to that species, since despite their evaluation been carried out at 120 DAS, the length and dry mass found in *C. aromatica* were higher than that found in the present study for *P. sobralianum* (90 DAS).

Seed imbibition in water

The initial moisture content of the *P. sobralianum* seeds was 26.18% (Figure 8), which is below than those found for *Campomanesia pubescens* (Mart. Ex DC.) O.Berg (42%) (DOUSSEAU et al., 2011), and *Eugenia pyriformis* Cambess. (45%) (SCALON et al., 2012).

Justo et al. (2007) found increase of 7% (240 h) in the initial seed moisture content of *Eugenia pyriformis* Cambess seeds, considering the moisture as a function of the fresh mass; this increase was sufficient to start the germination. Similarly, the increase of 5.23% (516 h) in the moisture level of the *P. sobralianum* seeds was low, but also satisfactory to start the germination.

The minimum moisture level for germination of seeds whose reserve tissue is part of the embryo (cotyledons) is generally high (CARVALHO; NAKAGAWA, 2012), as shown in this test, in which the seeds needed to reach approximately 30% moisture to start the germination. The moisture and germination presented cubic behavior as a function of the time, with increases up to 200 hours, then stabilization, and increases again after 600 hours. This imbibition follows a three-phase water absorption pattern in the seed germination (BEWLEY; BLACK, 1994): phase I (0 to 200 hours) with only water absorption, a physical process that occurs because of matric forces; phase II (201 to 600 hours) with practically no water absorption; and phase III (601 to 864 hours) with a resumption of water absorption, culminating with the elongation and emergence of the radicle.



$Y_{Moisture\ level} = 27.076 + 0.04223892\ x - 0.00009837168\ x^2 + 0.00000006755\ x^3,\ R^2 = 0.3994^*$.
$Y_{Germination} = -2.243155 + 0.06978368\ x - 0.0003117164\ x^2 + 0.0000003478356\ x^3,\ R^2 = 0.9056^{**}$.

*, **Significant, respectively, at 5 and 1% of probability by the F test.

Figure 8. Imbibition test (moisture level) of *Psidium sobralianum* Landrum & Proença seeds, and germination during the test.

CONCLUSIONS

Psidium sobralianum has polyspermic, berry fruits, subclassified as solanidium, with persistent sepals, and globular shape, consisting of epicarp, mesocarp, endocarp, and seeds. These fruits have green with orange epicarp, pale-green mesocarp, and white endocarp.

The seeds have a pilose and bony aspect, a pimentoid type of embryo, foliaceous cotyledons, presence of operculum and hilum, and pale-yellow tegument.

The germination is epigeal phanerocotiledonar, with root protrusion from 26 days after sowing, presenting presents a short, glabrous, thick radicle, and a rounded, pale-green apex.

The germination is slow, probably due to the mechanical barrier of the tegument, and stabilizes at 90 days after sowing.

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