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Morphoanatomical and physiological indicators revealed distinct intraspecific phenotypes of *Eugenia patrisii* (Myrtaceae) during germination and post-germination

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Abstract: *Eugenia patrisii* is an Amazonian species belonging to the Myrtaceae family with a high potential for fruit cultivation and use in the recovery of degraded areas due to rusticity and can serve as a source of essential oils for the bioindustry. Based on intraspecific variation, phenotype, this study aimed to provide morphoanatomical and physiological data on germination and seedling vigor to identify marker-differentiating phenotypes. For each phenotype, 60 seeds (n=420) were collected from the city of Marabá (Pará-Brazil). Descriptive, quantitative, and comparative approaches were used to monitor the germination and initial growth rate. The study analyzed 23 characteristics of *E. patrisii*, a species of seedlings, to identify phenotypes. Considering exclusivity, eight notable characteristics were identified, including seed length, width, thickness, germination percentage, seed number, and morphology of the apex and leaf base and border shape of the seedlings. Phenotype Pht7 is the most divergent within the species, exhibiting five distinct characteristics: adherence of the endocarp to the seed, seed length, width and thickness, and germination percentage. In contrast, Pht3, Pht2, and Pht6 are the best for domestication and planting. The morphoanatomical and physiological variables analyzed in the current study helped characterize the intraspecific variations of species *E. patrisii* under cultivation conditions. The variation reveals the potential for the species's cultivation in the Amazonian region, supporting sustainable agriculture and contributing to food security by increasing consumption of the native fruits.

Keywords: Amazonian fruit tree, morphophysiological markers, plant diversity, seeds, seedlings, Myrtaceae, *Eugenia*, phenotype.
Abbreviations: G%_Germination percentage, E. patrisii_Eugenia patrisii, MGT_Mean Germination Time, MVG_ mean velocity of germination, PCA_ Principal component analysis Pht_Phenotype.

Introduction

The Amazon has 15,000 known tree species, of which only 5% are classified in the Myrtaceae (Ter Steege et al., 2023). Amidst this particular tropical diversity is the genus *Eugenia*, with 209 representatives that are recognized for having fleshy-palatable fruits and for its invaluable contribution to humanity in several forms (Silva et al., 2024).

Among their potential uses are such as food security and sovereignty, cosmetics, biocides, pharmaceuticals, and in the recovery of degraded areas, in addition to being the object of studies for the understanding of seed morphophysiology and seedling production (Calvi et al., 2017; Amorim et al., 2020; Costa et al., 2020; Silveira et al., 2021; Kraus et al., 2023; Silva et al., 2024).

One of the main obstacles in the bioprospecting of *Eugenia* is the identification and classification of species. Since its floral composition is similar, this limits the accuracy of traditional taxonomic techniques (Giaretta et al., 2022; Mazine et al., 2023). Morphology and germination data of intraspecific variations also are gaps. The facts that reinforce the importance of this kind of approach are that many important botanical issues are unclear when dealing with intraspecific phenotypes, for instance, what are intraspecific taxa? What is their biological meaning? Are they real entities or merely instrumental units? When should an intraspecific group be ranked and named as an intraspecific taxon? (Reydon and Kunz, 2021).

Due to difficulties in identifying representatives and intraspecific phenotypic variations, some studies have been carried out to characterize representative species morphoanatomically (Puntieri and Gonzales, 2023) using leaf morphology, venation pattern, and histology of the epidermis and mesophyll. Additionally, these studies elaborated dichotomous keys to emphasize the differences and allow for identification (Carvalho et al., 2023; Gonçalves et al., 2024).

Nevertheless, the systematic approaches described were applied to the definition of fruit species, and there are no works that gather accurate data for intraspecific taxonomic groups regarding subgenus and variety and, for fruit plants, phenotypes and genotypes. It should also be considered here that the evolution of the morphology of plant organs tends to be faster due to the environmental conditions of growth and development when compared to the anatomical and physiological ones, which tend to occur later in genetic and adaptive responses (Pires and Dolan, 2012).

Eugenia patrisii Vahl is a shrub that is distributed throughout the Amazon (including Venezuela, Colombia, Bolivia and the Guianas). It is 8 m in height when mature (Silva et al., 2017). Although its fruit is eaten by locals of the region and contributes to food security, no records refer to attempts to establish orchards or crops (Cruz et al., 2022), except for the experiment by Pacheco et al. (2021). The limited information available is reflected in the literature, where there is a notable data deficiency regarding the morphology and physiology of *E. patrisii*. On the other hand, more in-depth studies about the morphophysiological characteristics of this species can be used to characterize it under domestication processes and cultivation conditions. In addition, the cultivation of these phenotypes in orchards could provide enhanced comprehension of intraspecific varieties and phenotypes, as these phenotypes generate fruits with different constitutions and biomass (Pacheco et al., 2021).

Regarding studies on the germination of the seeds of *E. patrisii*, information on the germination parameters and mobilization of primary reserves is fundamental for the formulation of cultivation and management strategies, as well as conservation techniques. For *E. patrisii* and other species in the Amazon biome, we need to apply plant biology knowledge to advance research on species use and conservation (Lima et al., 2008; Carvalho et al., 2022; Carvalho et al., 2023; Gonçalves et al., 2024).

Studies that combine morphoanatomical and physiological information on fruit trees to determine the existence of intraspecific variations prior to domestication are rare, yet they provide valuable insights for various human uses and applications. Furthermore, they are innovative in integrating various morphophysiological traits in plants of the species exposed to identical environmental conditions (Pacheco et al., 2021).

Therefore, we hypothesized that the seven phenotypes of *E. patrisii* have different morphoanatomical and physiological characteristics throughout the germination/post-germination process and the characteristics of the phenotypes can help enhance our understanding of the intraspecific variation within this species. In the present study, we investigated the existence of some characteristics of intraspecific phenotypes in the species under cultivation conditions, using morphoanatomical and physiological data, specifically referring to germination and seedling vigor, to contribute to the prospection and use of Amazonian fruit trees.

Results

Seed morphology and biometry

The fruits of the seven phenotypes of *E. patrisii* show variations in number of seeds, i.e., one (Pht1, Pht2, Pht4, Pht5, Pht6, and Pht7), two or three (Pht3) seeds. The seed adhered to the fibrous endocarp of the fruit, varying in its intensity of adherence, with lower (Pht7) (Fig. 1N) or higher intensity (other phenotypes) of adherence (Figs. 1B, 1D, 1F, 1H, 1J and 1L). Regarding this character, Pht7 stands out from the others.

In terms of the shape and coloration of the seeds, it is possible to categorize: a) Pht1 – spherical to oval, with a pink integument and white cotyledons (Fig. 1C); b) Pht2 – spherical to oval, slightly flattened at the poles, with a purple integument and white cotyledons (Fig. 1E); c) Pht3 – spherical to hemispherical, the latter when there are two or three in the fruit, with a pink integument and white cotyledons (Fig. 1G); Pht4 – spherical, slightly flattened at the poles, with an intense purple integument and brown cotyledons (Fig. 1I); Pht5 – spherical, slightly flattened at the poles, with a yellow to purple integument and white cotyledons (Fig. 1K); Pht6 – spherical, with a red integument and brown cotyledons (Fig. 1M); Pht7 – spherical, slightly flattened at the poles, with a red integument and white cotyledons (Fig. 1O).

Regarding the morphology of the seeds, these present a tegumentary portion that is weakly adhered to the cotyledons and intensely adhered to the endocarp, constituting a tissue of leathery and flexible consistency (Fig. 1P). Internally, they are rigid and colored brown (Pht4 and Pht6), purple (Pht2 and Pht5) and red (Pht1, Pht3 and Pht7) (Fig. 1R). It is not possible to distinguish the testa and tegmen since they are a single, similar cellular assembly (Figs. 1P-1Q).

The second portion of the seed is cotyledonary. It is an exalbuminous seed when mature. The coating of the cotyledons is unistratified, and it is possible to observe the adaxial and abaxial surfaces; the latter in partial contact with the region of the embryonic axis (Fig. 1S). The embryo is pseudo-monocotyledonous, the cotyledon is conferruminated and resembles a unique structure. This joining region is limited by a slight pyramidal prominence on the surface of the cotyledon (Fig. 1S), from which the cotyledon petiole is emitted during germination, and by the hilum region (placentation). Both the

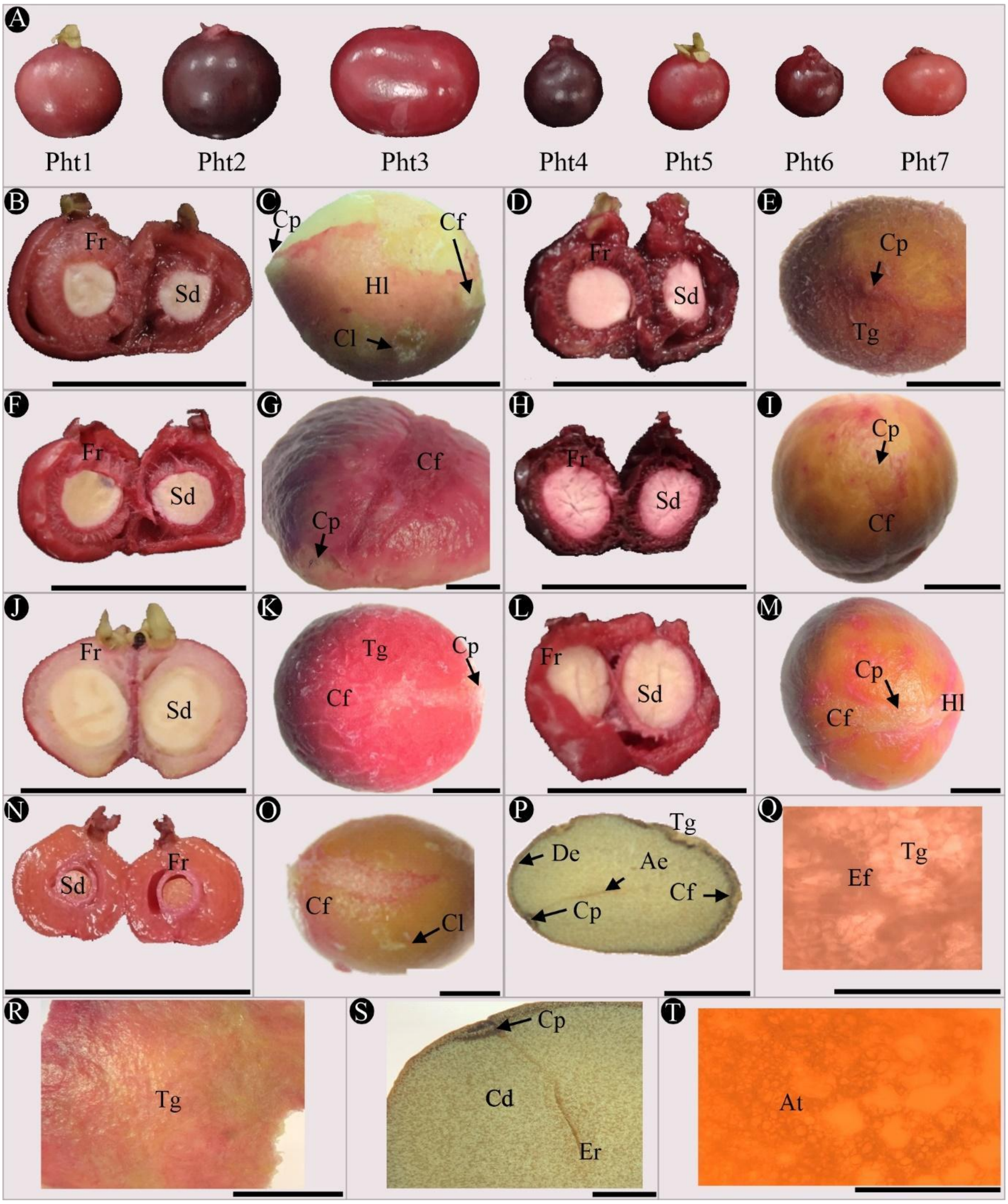

Figure 1. Detail of the fruit and seed morphologies of the seven different phenotypes identified in the species *Eugenia patrisii*. A – phenotypes 1, 2, 3, 4, 5, 6 and 7; B-O – phenotypes 1, 2, 3, 4, 5, 6, and 7; P – Phenotype 2 showing seminal coating and cotyledons; Q – Phenotype 3 showing fibers of the endocarp of the integument cells; R – Phenotype 3 showing the inner face of the integument; S – Phenotype 2 showing the region of the embryonic axis and the abaxial coating of the cotyledons; T – Phenotype 5 showing the amyliferous parenchyma of the cotyledon. Ae – abaxial epidermis; At – amyliferous tissue; Cd – cotyledon; Cf – cotyledonary fusion; Cl – cotyledonary lesion; Cp – cotyledonary petiole; De – adaxial epidermis; Ef – endocarp fibers; Er – embryonic region; Fr – fruit; Hl – hilum; Pht1 – Phenotype 1; Pht2 – Phenotype 2; Pht3 – Phenotype 3; Pht4 – Phenotype 4; Pht5 – Phenotype 5; Pht6 – Phenotype 6; Pht7 – Phenotype 7; Sd – seed; Tg – tegument. Scale: A-P and R-S = 1 cm; Q-T = 20 µm.

prominence, which has the appearance of a micropyle, and the hilum are more evident with the suppression of the integument (Figs. 1C, 1E, 1G and 1I).

Therefore, it is possible to identify the region of the embryonic axis, but not the actual embryonic axis from the cotyledons. The cotyledonary tissue is rich in amyliferous parenchyma and idioblasts and secretes lipophilic substances and phenolic compounds (Fig. 1T). Regarding seed width, Pht4, and Pht3 showed higher values than the others, followed by Pht5 and Pht2 (Fig. 2A). It is noteworthy that Pht5 was statistically similar to Pht4, and Pht3, Pht6 and Pht7 showed the lowest values.

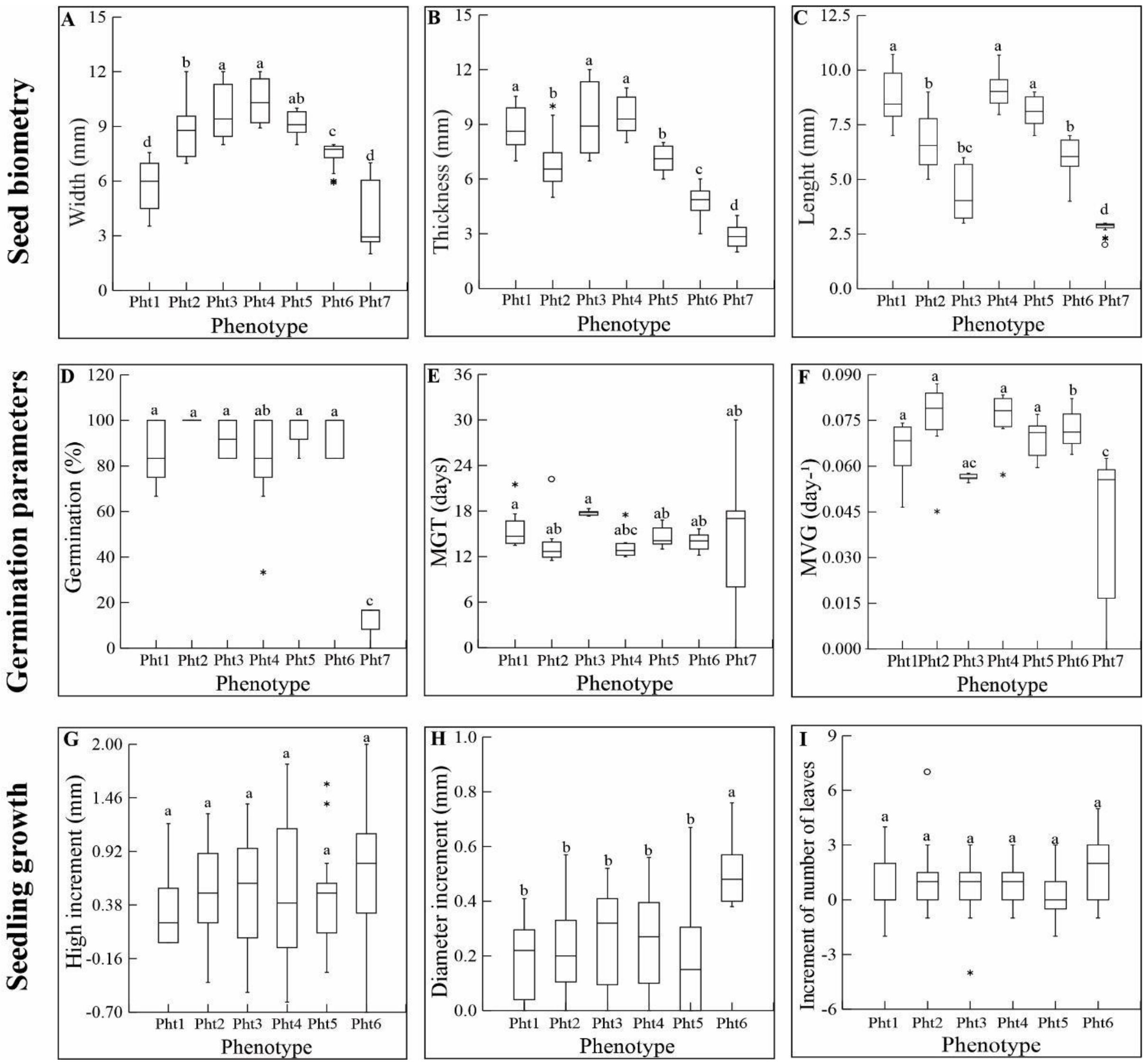

Figure 2. Variations in the three dimensions of the seeds, the germination parameters and initial growth of seedlings of the phenotypes of *Eugenia patrisii*. A – Width; B – Thickness; C – Length; D – germination percentage (%G); E – mean germination time (MGT); F – mean velocity of germination (MVG); G – increment in height; H – increment in the diameter of the collar; and I – increment in the number of leaves.

To seed thickness, Pht3, Pht4, and Pht1 showed higher values among the seven phenotypes (Fig. 2B). Pht2 and Pht5 were statistically similar to each other, while Pht6 and Pht7 showed lower thicknesses. In terms of length, Pht1 and Pht4 showed the two highest values, followed by Pht5, Pht2, Pht6, and Pht3 (Fig. 2C). In general, when considering the three dimensions (width x thickness x length), Pht7 had the lowest values, and Pht4 the highest, distinguishing itself from other phenotypes in terms of seed biometrics.

Germination morphology

In the seven phenotypes of *E. patrisii*, germination was characterized as hypogeal and cryptocotyledonary, and the stages were: 1) radicle protrusion; 2) root elongation; 3) epicotyl emission; 4) elongation of the epicotyl; 5) differentiation of the plumule; 6) opening of the plumule; and 7) extended eophylls. The emission of cotyledonary petiole was on hilum region, which crosses the seminal integument, and, at the base of this structure, the radicle protrusion occurs (Figs. 3A-B). After root protrusion, elongation followed and, at the end, root hair emission started. Concomitantly with the emission of root hairs, on the opposite face of the cotyledonary petiole, a cup-shaped epicotyl is emitted (Fig. 3C), which grows in negative geotropism, and is colored white (Pht3) (Fig. 4E), pink (Pht2 and Pht4) (Fig. 3D) or green (Pht1, Pht5, Pht6 and Pht7) (Fig. 3C).

Subsequently, the emergence of the epicotyl occurred and, finally, the differentiation of the plumule, which began with the pyramidal-shaped dilation of the apex of the epicotyl (Figs. 3F-G). Here, two opposite primordial cataphylls differentiated and remained attached to the epicotyl (Fig. 3H). Then, at the second node, two leaves were elongated (Fig. 3H). These leaf primordia are ovate, about 0.3 cm long and green in color (Fig. 3H).

Finally, there is the extension of the eophyll pair, which already presents itself as a complete leaf with a sheath, petiole and leaf blade, whose shape is either elliptical (Pht2, Pht3 and Pht5) (Fig. 3H) or ovate (Pht1, Pht4, Pht6, and Pht7) (Fig. 3I). Throughout these seven stages of growth and differentiation, the seed remained fixed to the bases of the hypocotyl and

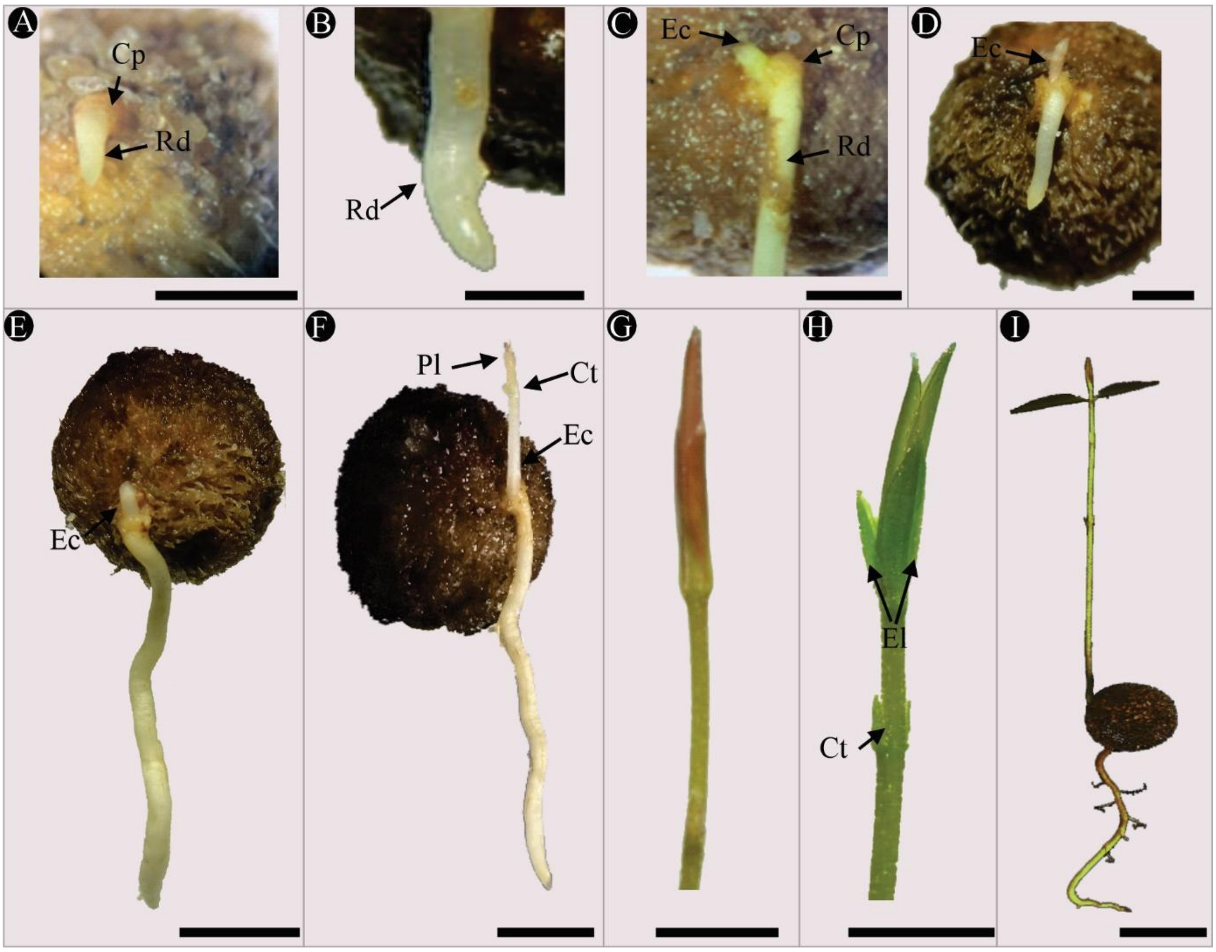

Figure 3. Germination morphology of *Eugenia patrisii* phenotypes. A – Phenotype 1 showing radicle protrusion; B – Phenotype 2 showing root elongation; C – Phenotype 7 showing green epicotyl emission; D – Phenotype 4 showing pink epicotyl emission; E – Phenotype 3 showing white epicotyl emission; F – Phenotype 3 showing elongation of the epicotyl; G – Phenotype 6 showing differentiation of the plumule; H – Phenotype 6 showing opening of the plumule with elliptical-shaped eophylls; I – Phenotype 2 showing fully extended eophylls. Ct – cataphyll; Ec – epicotyl; El – eophyll; Cp – cotyledonary petiole; Pl – plumule; Rdc – radicle. Scale: 1 cm.

epicotyl and did not undergo any dimensional changes (Fig. 3I) and was still attached to the seedling 120 days after the onset of germination.

Germinative parameters

In a comparison among Pht1, Pht2, Pht3, Pht4, Pht5 and Pht6, no significant differences were observed ($p>0.05$). Nonetheless, Pht2 had all seed seeding while Pht7 obtained the lowest mean value for germination percentage when compared with the other phenotypes ($p<0.05$) and was significantly different at 12.5±7.7 (Fig. 2D). MGT were statistically close among all the seven phenotypes ($p>0.05$). However, Pht1 and Pht3 stood out from the others ($p<0.05$). In addition, Pht4 ranked lower than Pht2, Pht5, Pht6 and Pht7 ($p<0.05$) (Fig. 2E). For the mean velocity of germination (MVG), Pht1, Pht2, Pht3, Pht4 and Pht5 had statistically close means ($p>0.05$) and were superior to Pht7, for which germination was slower ($p<0.05$) (Fig. 2F).

Seedings leaves morphoanatomy

The phenotype Pht7 was excluded due to the low germination percentage and vigor of the seedlings, so the morphoanatomical description of the leaves of the seedlings was only performed for Pht1, Pht2, Pht3, Pht4, Pht5 and Pht6. The seedlings of the first six phenotypes of *E. patrisii* had complete leaves, with coloration ranging from light to dark green, the adaxial surface being darker compared to the abaxial one. They are simple leaves with opposite decussate phyllotaxy at different angles. The texture is membranaceous and the surfaces are slightly tomentose (Figs. 4A-F).

The leaf sheath is light to dark green, slightly winged, and weakly adherent to the stem, being easily detachable from the rest of the stem. The petiole follows the color gradient of the sheath, flexible, cylindrical to U-shaped, canalicular in appearance, and slightly shorter when compared to the laminar length (Fig. 4A). The laminar margins are entire and apparently glabrous (Figs. 4A-F). The main venation is prominent for both surfaces and the venation pattern is brochidodromous. The morphology of the leaf blade shows the differences between the phenotypes: a) Pht1 – ovate, with obtuse base and cuspidate apex (Fig. 4A); b) Pht2 – elliptic, with cuneate base and apex (Fig. 4B); c) Pht3 – elliptic, with acute base and cuneate apex (Fig. 4C); d) Pht4 – ovate, with asymmetric base and acute apex (Fig. 4D); e) Pht5 – elliptical, with acute base and acuminate apex (Fig. 4E); f) Pht6 – ovate, with asymmetric base and acuminate apex (Fig. 4F).

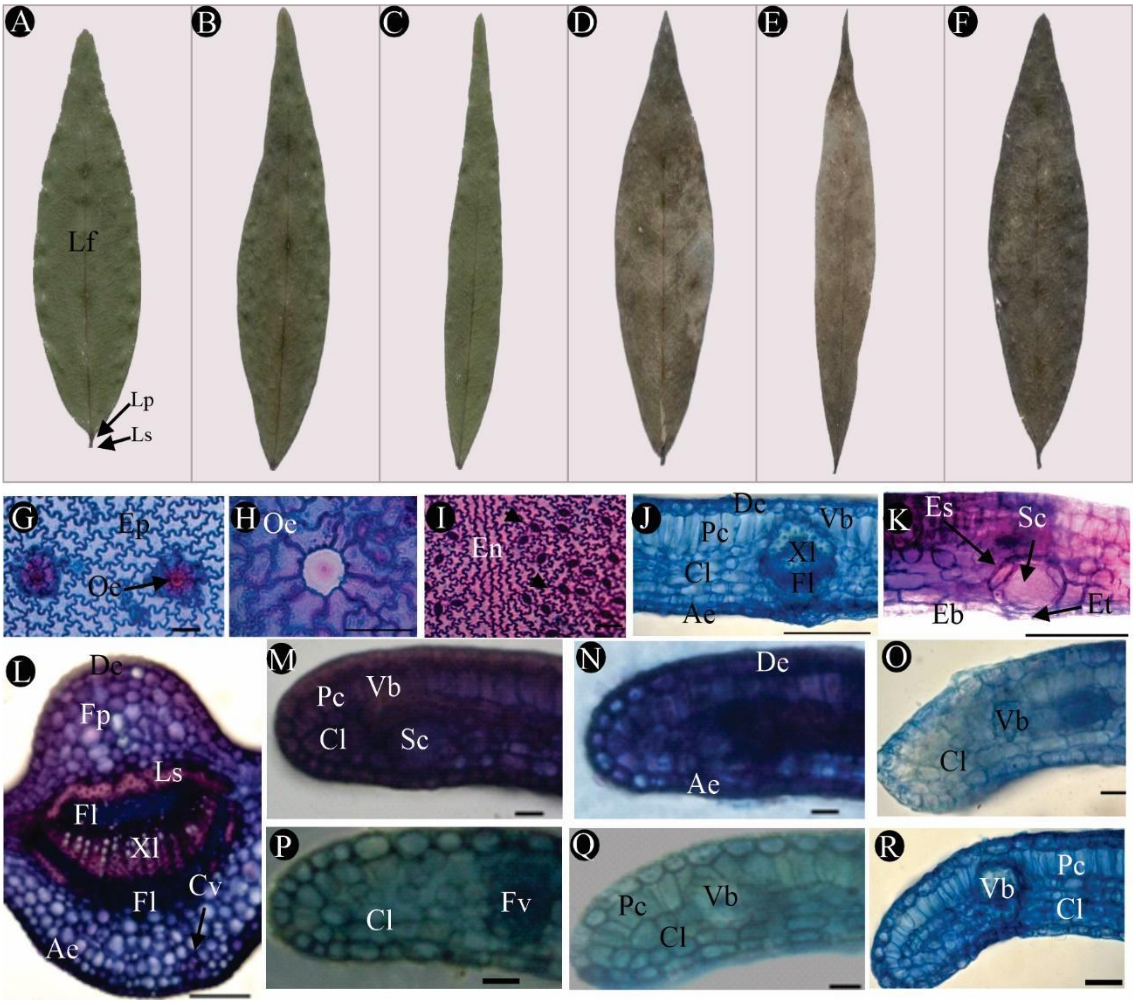

Figure 4. Leaf morphology and leaf blade anatomy of *Eugenia patrisii* phenotypes, in frontal (G-I) and transverse (J-R) views. A-F – morphology of phenotypes 1 to 6, respectively; G – Phenotype 2 details of the adaxial epidermis; H – Phenotype 2 details of the outermost epidermal cells in the secretory cavity; I – Phenotype 3 details of the abaxial epidermis, with stomata complexes (arrow); J – Phenotype 4 details of the mesophyll; K – Phenotype 5 details of the mesophyll with secretory cavity; L – Phenotype 1 details of the central vein; M-R – Margins of phenotypes 1 to 6, respectively. Ep – epidermis; Ls – leaf sheath; Cl – chlorenchyma, Vs – vascular sheath; Fp – fundamental parenchyma; Sc – secretory cavity; Lc – lacunose chlorenchyma; Pc – palisade chlorenchyma; Ae – abaxial epidermis; De – adaxial epidermis; En – epidermal cells in veins; Es – secretory epithelial cells; Ep – epidermis; Oe – outermost epidermal cells; Fl – phloem; Vf – vascular bundle; Lb – leaf blade; Lp – leaf petiole; Xl – xylem. Scale: G-K and M-R = 5 μm and L = 10 μm.

As for the leaf apex, Pht1 had a cuspidate apex and Pht4 had an acute apex. For the leaf base, Pht1 had an obtuse base and Pht2 had a cuneate base. It is important to emphasize that, in the six phenotypes, new leaves tend to have a larger leaf area than the previous one. In addition, lanceolate cataphylls may occur interspersed with leaf nodes, which soon atrophy and acquire dark colors and no longer develop and remain adhered to the stem. Atrophied leaves may occur, but they constitute only one of the opposite pairs.

Regarding leaf anatomy, for all six phenotypes, the frontal views of the adaxial and abaxial epidermis present ordinary cells with sinuous anticlinal walls (Figs. 4G-I). In the secretory cavities, they acquire a polygonal shape, the cytoplasmic content being translucent, and are called outermost epidermal cells in secretory cavities, of which the surrounding ordinary cells present irradiated aspects (Figs. 4G-H). Exceptions also occur in the larger veins, which show a reticular shape and toothed periclinal walls (Fig. 4I). For the stomatal and secretory cavities outermost epidermal cells index, the data were non-parametric. Table 1 shows the mean values for the six phenotypes.

The cross-sectional view of the leaf blade is V-shaped, with the midrib prominent and a lateral arrangement of 10 to 12 vascular bundles of smaller calibers. The epidermis is composed of juxtaposed ordinary cells with outer periclinal walls thicker than the inner ones, of reticular to slightly ellipsoid shapes; the adaxial ones being larger than the abaxial ones (Fig. 4J). This last condition becomes critical in the midrib and margin, where the cells are exclusively ellipsoid and the adaxial cells are much larger (Fig. 4J). Among the ordinary cells, phenolic idioblasts may occur. Surrounding the epidermis is a thin cuticular layer. The stomatal complexes are arranged on the abaxial side and in the plane of the ordinary cells.

The mesophyll is dorsiventral, with one layer of palisade chlorenchyma facing the adaxial surface and five to seven layers of lacunose chlorenchyma (Figs. 4J-K). Interrupting the two chlorenchymatous tissues are conspicuous secretory cavities, which can occur either on the abaxial or adaxial side (Fig. 4J). These structures are composed of a cavity, lined by chlorenchymatous tissue and lipophilic content (Fig. 4K). The distribution is random along the transverse section and affects

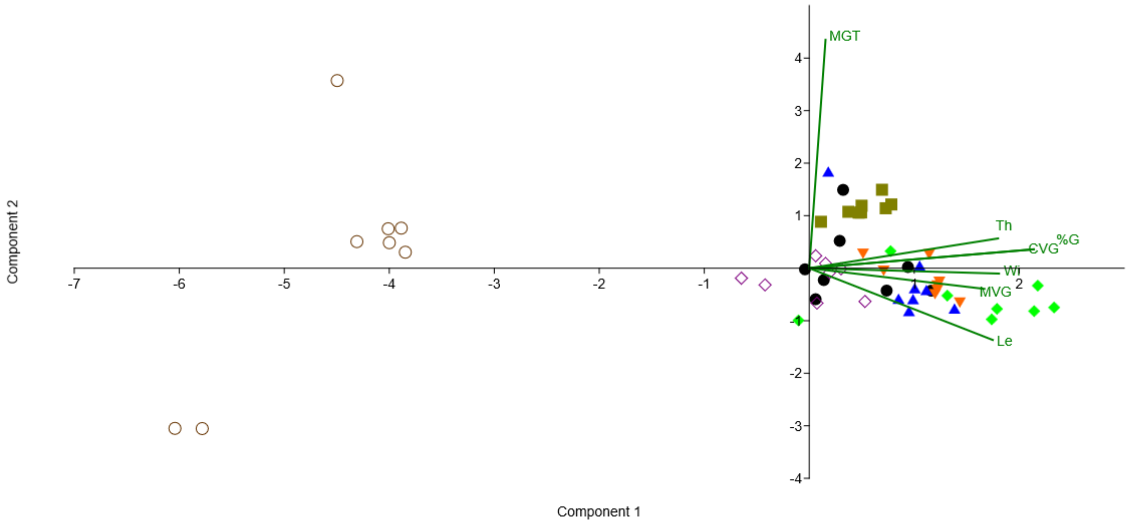

Figure 5. Graph illustrating the principal component analysis of morphoanatomical and physiological characters in the seven phenotypes of *Eugenia patrisii*. Phenotype 1, Phenotype 2, Phenotype 3, Phenotype 4, Phenotype 5, Phenotype 6 and Phenotype 7, respectively.

the margin, apex, base and midrib, always subepidermal. The epidermal cell just above it acquires a rectangular shape (cross-sectional view) and dimensions smaller than the other ordinary cells, in addition to having a translucent cytoplasm (Fig. 4K).

The vascular bundles of the mesophyll are collateral, with different caliber and are surrounded by a fibrous sheath (Fig. 4J). In the midrib, the dorsiventral mesophyll is replaced by a non-chlorophyll fundamental parenchyma and has a single vascular system arranged in an arc and of the bicollateral type, the phloem being covered by a fibrous sheath on the faces facing the two surfaces (Fig. 4L).

The anatomical differences among the phenotypes are in the leaf margin: a) Pht1 – straight margin, with dense chlorenchyma (Fig. 4M); b) Pht2 – straight margin, dense chlorenchyma and epidermal ordinary cells with total thickening in the walls (Fig. 4N); c) Pht3 – involute margin, with slack chlorenchyma (Fig. 4O); d) Pht4 – carinate margin, with slack chlorenchyma (Fig. 4P); e) Pht5 – slightly flexed margin towards abaxial surface, with dense chlorenchyma (Fig. 4Q); f) Pht6 – flexed margin, with dense chlorenchyma (Fig. 4R). However, due to the similarity in the structures described, anatomical profiles applicable to intraspecific segregation are not considered.

Initial seedling growth

The phenotype Pht7 was excluded due to the lower germination percentage and vigor of its seedlings, so only Pht1, Pht2, Pht3, Pht4, Pht5 and Pht6 were used to determine the increments in height, collar diameter and number of leaves. Regarding the height increment, there were no statistical differences between the six phenotypes ($p>0.05$) (Fig. 2G). For the increment in the collar diameter, Pht1, Pht2, Pht3, Pht4, and Pht5 did not show statistical significance when compared to the other means ($p>0.5$). Nevertheless, Pht6 stood out from the others ($p<0.05$) (Fig. 2H). Concerning the number of leaves, the six phenotypes showed mean values that were statistically close to each other ($p>0.5$) (Fig. 2I) and is therefore not a viable character for intraspecific segregation.

Principal component analysis (PCA) and dendrogram UPGMA of morphology and germination

Table 1 summarizes the morphoanatomical and physiological differences between the seven identified phenotypes of *Eugenia patrisii*. A total of 23 characters were observed, among which Pht1 is distinguished from the others by two morphological characters, i.e., apex and leaf base of the seedling; Pht2 only by the morphology of the seedling leaf base; Pht3 by epicotyl color during germination; Pht4 by the leaf apex of the seedling; Pht5 and Pht6 do not present differences regarding the characters analyzed; while Pht7 demonstrated differences in five characters (endocarp adhesion to the seed, seed biometry and germination percentage).

Comparing the physiological components in Tab. 1 (seed biometry and germination parameters), Pht1, Pht2, Pht3, Pht4, Pht5 and Pht6 clustered together, while Pht7 segregated itself from the others. Component one accounted for 53.7% of the results, while component two accounted for 15.3% (Fig. 5). Among the seven variables considered, germination percentage (G%), and mean germination time (MGT) were the ones that most justified the clustering of the phenotypes. The set of information concerning phenotype Pht7 is disparate concerning the others in all quantitative characters and in most of the qualitative ones, which allows us to officially segregate it. The cluster analysis by the UPGMA method considering the mean value of 50% dissimilarity identified three groups (Fig. 6) in which Group 1, Pht7; Group 2, Pht4, Pht5 and Pht6; and Group 3, Pht1, Pht2 and Pht3. As in the PCA, Pht7 was the one that stood out the most from the others, forming a single group.

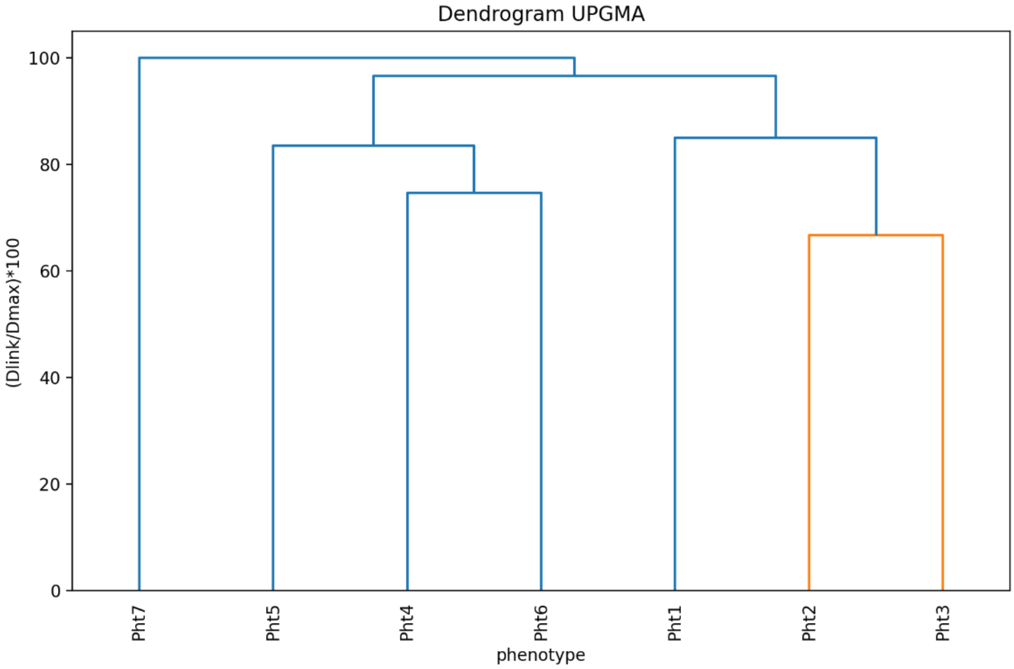

Figure 6. Dendrogram showing the clusters of seven phenotypes of *Eugenia patrisii* elaborated by the UPGMA method from the Euclidean distances calculated based on 23 markers.

Discussion

Regarding the domestication of fruit species in the Amazon, the production of seeds in high quantity, germination and morphological structures related to high temperature are contributing factors to the establishment of orchards (Clement et al., 2021). In *E. patrisii*, only Pht3 stood out for having two to three seeds inside, while the others contained only one seed. For the germination parameters G%, MGT and MGV, and seed dimensions, Pht3 was no different from the others, but it had high values when compared to Pht7. In the morphology and the seedling leaf, Pht3 was the only one with an acute apex and asymmetric base, while the anatomical structure of the leaf border was carinate and the stomatic index was 13.8±8.4. However, when relating the values in the PCA and dendrogram UPGMA, this phenotype did not differ from the others.

Pht6 contained one seed in the fruit, but the G% was 93.8±8.6 and the SI of the leaf epidermis was 24.2±16.8. SI is related to carbon assimilation and photosynthetic activity, and values accompanied by stomatal conductance and plant productivity are suitable for growing plants (Nina-Junior et al., 2024). In terms of domestication, Pht6 has characteristics that can contribute to the establishment of crops. Pht7 was characterized with lower values for the variables and parameters analyzed, distinguishing it from the other phenotypes. Despite this, these differences do not contribute to indicating Pht7 as being suitable for cultivation. In *E. stipitata*, another Amazonian species, there are var. stipitata and var. sororo, and only the latter shows potential for cultivation (McVaugh, 1956)

The phenotypes analyzed have morphofunctional characters that help to distinguish between them and can also be used to select genetic resources that can be domesticated and produce fruit. This genus has some representatives in South America that have gone through different stages of adaptation to backyard cultivation over the last few millennia (Clement et al., 2021). *Eugenia patrisii* lacks information on seeds and germination, which could be clarified in this study. As previously indicated, the seed shape varied among phenotypes, and it was included among the divergences. Justo et al. (2007) state that for *E. pyriformis* Cambess. seed shape is directly related to drying, storage and germination conditions. In the present study, we can affirm that these divergences are not associated with storage or drying conditions since, soon after collection, the morphological characterization of the fruits and seeds was carried out; thus, these are particularities of the phenotypes.

In *E. patrisii*, seeds were cryptocotyledonary, pseudomonocotyledonary, monoembryonic and exalbuminous, and hypogeous germination, with bulky cotyledons rich in carbohydrate reserves. These profiles indicate characters of the species that have not yet been differentiated or particularized in phenotypes. Mendes and Mendonça (2020) observed a similar condition for sexual propagules of *E. stipitata* and added that they are constituent elements of *Eugenia* systematics. Regarding the aspect of germination morphology, the epicotyls presented diverse colorations such as white, pink, or green, and an eophyll of elliptical or ovate forms. Germination diagnoses of *Eugenia* species included the coloration of morphological structures and the shape of the leaf primordium, such as *E. dysenterica* (Mart.) DC. (Duarte et al., 2006), it was possible too differentiates intraspecific phenotypes in *E. patrisii.*

The most significant germination parameter was germination percentage, which segregated Pht7, due to its values below the others. Amador and Barbedo (2015) observed distinctions regarding the percentage of germination between *Eugenia* species (*E. uniflora* and *E. brasiliensis*), indicating this as an important character for distinguishing species and contributing

Table 1. Morphoanatomical and physiological differences between the seven phenotypes of *Eugenia patrisii*.

Characteristics	Pht1	Pht2	Pht3	Pht4	Pht5	Pht6	Pht7
Adhered to the fibrous endocarp	Yes	Yes	Yes	Yes	Yes	Yes	No
Seed shape	spherical to oval	spherical to oval	spherical to hemispherical	spherical	spherical	spherical	spherical
Integument color	pink	pink	Pink	purple	yellow to purple	red	red
Cotyledon color	white	white	White	brown	white	brown	white
Inner integument color	red	purple	Red	brown	purple	brown	red
Seed width (mm)	5.8±1.2	8.7±1.4	9.8±1.6	10.4±1.1	9.1±0.7	7.5±0.6	4.1±1.8
Seed thickness (mm)	8.8±1.0	6.8±1.4	9.2±1.9	9.5±1.0	7.1±0.7	4.8±0.8	2.9±0.6
Seed length (mm)	8.7±1.1	6.7±1.2	4.3±1.1	9.0±0.7	8.2±0.6	6.1±0.7	2.8±0.3
Epicotyl color	green	pink	White	Pink	green	green	green
Eophyll shape	oval	elliptical	elliptical	oval	elliptical	oval	oval
%Germination	85.4±13.9	100±0.0	91.7±8.9	81.3±22.6	95.8±7.7	93.8±8.6	12.5±7.7
MGT (day)	15.6±2.8	13.8±3.5	17.8±0.3	13.4±1.8	14.6±1.4	13.9±1.2	14.5±10.0
MVG (day)	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
Leaf shape of the seedling	oval	elliptical	elliptical	oval	elliptical	oval	-
Leaf apex of the seedling	cuspidate	cuneate	Cuneate	acute	acuminate	acuminate	-
Leaf base of the seedling	obtuse	cuneate	Acute	asymmetric	acute	asymmetric	-
SI (%)	16.6±11.7	14.5±10.3	13.8±8.4	14.3±8.2	13.1±8.2	24.2±16.8	-
CI (%)	0.5±2.8	0.1±1.4	0.2±1.6	0.2±1.6	0.4±2.9	0.4±3.0	-
Leaf margin	straight	straight	Involute	carenade	slightly flexed	flexed	-
Filling of leaf margin	dense chlorenchyma	thickened epidermal cells	slack chlorenchyma	slack chlorenchyma	dense chlorenchyma	dense chlorenchyma	-
Height increment (mm)	10.4±0.4	10.8±0.5	9.4±0.6	6.9±0.8	7.1±0.5	7.4±0.7	-
Diameter increment (mm)	0.2±0.1	0.2±0.2	0.3±0.2	0.3±0.2	0.2±0.2	0.5±0.1	-
Number of leaves	9.5±1.8	9.8±2.0	10.2±1.8	11.7±1.1	11.7±1.5	11.7±1.8	-
Main morphoanatomic markers							

to the knowledge of factors that influence germination inhibition. However, there are no studies that indicate differences between phenotypes, which is unprecedented information.

As shown in the results, the leaf morphology of the seedlings showed divergence among the phenotypes Pht1, Pht2 and Pht4 when considering apex and base. Jorge et al. (2000) and Dias et al. (2012) described the leaf morphoanatomy of *Eugenia punicifolia* and *E. uniflora* from samples that were collected, respectively, in southeastern and northeastern Brazil, and indicated divergences in leaf limb morphometry. The latter authors attributed this difference to the geographic origin of the samples and the life span of the leaves analyzed, which were probably not the same. For the phenotypic samples of the *E. patrisii* seedlings, the geographic point of collection of the seeds and the leaf age are the same, so it can be inferred that the particularities constitute the intraspecific profiles and this is new information for the *Eugenia*.

In our anatomical study of the leaf blade of the seedling, the margins of phenotypes Pht1 to Pht6 are structurally distinct. In *Eugenia florida* DC., Donato and Morretes (2009) used the anatomical particularities of the leaf margin to distinguish two groups within the species, comparing the leaves from sun and shade and considering the marginal span, the presence of vascular bundles, fibrous bundles, and parenchymatic filling. Despite this, Donato and Morretes (2009) considered that the anatomical responses manifested in the leaves of *E. florida* were in response to environmental conditions (sun and shade). Here, all the seedlings were grown under similar environmental conditions and yet manifested distinct differences, providing new insights into the *Eugenia*.

Materials and Methods

Plant materials

The botanical material (fruits and seeds) for the analysis of the species *E. patrisii* was collected from 21 plants used in an agronomic experiment located in the city of Marabá (Pará, Brazil), in November 2022. The original seeds were obtained from specimens that are autochthonous to the municipality (Pacheco et al., 2021). The plants were categorized into seven phenotypes (Pht1, Pht2, Pht3, Pht4, Pht5, Pht6 and Pht7) according to fruit morphology and morphometry (Fig. 1A), with three matrices for each of these.

The taxonomic identification of the species was carried out by a specialist, with specimens of each phenotype incorporated into the collection of the Ático Seabra Herbarium of the Federal University of Maranhão under registration number 11,588.

Samples collection

Using 60 fruits and 60 seeds obtained from each phenotype (n= 420), 12 were randomly selected for morphological and biometric analyses of the seeds. The remaining 48 fruits were used for the germination tests.

The seeds were sown at the Laboratory of Plant Physiology and Biochemistry of the National Institute for Amazonian Research (Manaus, Amazonas, Brazil). The seeds with the endocarp still adhered were washed and soaked in distilled water for 5 minutes, followed by immersion in 1% aqueous sodium hypochlorite for 2 minutes, and finally washed again in distilled water and sown in sterilized sand, in a system of six seeds per basin with eight repetitions (Shukla *et al*. 2008). The sand was kept in 500 mL black bags for 30 days. The seeds were watered every other day with distilled water and kept in a shaded environment with air flow, and the temperature fluctuated between 28º to 33º in daylight. Daily, the seeds were extracted from the substrate to record morphological changes during the 30 days.

Conduction of study

Traits biometry

Seeds were considered germinated when the radicle (radicle protrusion) was between 2-5 mm in length and were evaluated and classified into seven stages of germination, according to Anjos and Ferraz (1999) and Calvin et al. (2017). In addition, germination percentages (G%), mean germination time (MGT) (Labouriau and Valadares, 1976) and mean velocity of germination (MVG) (Maguire, 1962) were calculated.

Soon after seedling formation, after the seventh stage of germination, the following growth variables were evaluated: height (H), collar diameter (CD), and number of leaves (NL). Measurements were taken at 30-day intervals for 120 days, which totaled four measurements (30, 60, 90, and 120 days). For the monitoring of seedling performance, 15 individuals were selected among all the phenotypes (Pht1 to Pht6). However, due to the low germinability of Pht7, it was not possible to select individuals during initial seedling growth. These data were organized and processed in the Jasp 0.17.1 program, and the images were recorded with Corel Drawn Graphic Suit 2022 program.

Traits morphoanatomy

For the morphological descriptions of the seed structures, germination stages and seedlings, specialized literature was consulted and, for the biometry of the transverse and longitudinal sections of Pht1, Pht2, Pht3, Pht4, Pht5, Pht6 and Pht7, a digital caliper, ruler, a binocular stereoscope microscope and digital camera were used. The program Jasp 0.17.1 was used to record and process the performance data.

For the leaf morphological analysis and anatomy of the seedling leaf blade, samples from five individuals (120 days), from Pht1 to Pht6, belonging to the third leaf node were used. After the description, the leaf blades were separated into apex, middle, and base, fixed in 70% FAA (formaldehyde, acetic acid, and ethanol 70%), according to the protocol of Johansen (1940). These portions were then subjected to epidermis dissociation in a solution of acetic acid and hydrogen peroxide (Franklin, 1945) and freehand sections were removed with an aluminum knife.

The sections and the epidermis were depigmented in 1% aqueous sodium hypochlorite, washed in distilled water, and stained with Astra blue (1% aqueous) and Fuchsine (1% aqueous) (Johansen 1940), and then mounted between a glass slide and a coverslip in a 50% glycerin aqueous medium. For the cytochemical and histochemical tests, the protocol of Johansen (1940) was adopted using the Sudan III solution (alcoholic) to indicate lipids, phloroglucinol 1% (aqueous) for lignin, and ferric chloride 1% (aqueous) for phenols in general, and the tested sections were mounted in the aqueous medium between a glass slide and a coverslip. The histological slides were observed under a trinocular microscope and photomicrographs taken with the aid of the Zen 2012 program.

The histological slides of the abaxial epidermis from Pht1 to Pht6 were also used to determine the stomatal index (SI) and secretory cavity outermost epidermal cell index (CI). The former numerically related the epidermal and ordinary cells to the stomatal complexes, while the latter applied the aforementioned situation between ordinary cells and epidermal cells to the secretory cavity, according to Labouriau et al. (1961) with modifications. Fourteen square subfields of 0.28 x 0.28 mm were considered, within eight fields of 1.12 x 1.12 mm, which were determined with the aid of the Zen 2012 program. These data were organized and processed in the Jasp 0.17.1 program, using the direct relationship shown in the following expression for each phenotype subfield.

$SI \ or \ CI \ = \boldsymbol{ns} \ or \ \boldsymbol{no} \ x \ 100$

$(\boldsymbol{ns} \ or \ \boldsymbol{no} \ + \ \boldsymbol{ne})$

ns: number of stomata; no: number of outermost cells; ne: number of epidermal ordinary cells.

$MSI\ or\ MCI\ =\ \boldsymbol{\Sigma SI\ subfield}\ or\ \boldsymbol{\Sigma CI\ subfield}\ \mathbf{112}\quad \mathbf{112}$
MSI: Mean Stomatal Index; MIC: Mean Outermost Cells.

Statical analysis
For these values, the means were compared. To monitor the performance of the seedlings of Pht1 to Pht6, a sample of 15 individuals from each was considered. Height, collar diameter, and number of leaves were recorded. The statistical analysis applied to the physiological parameters of the seeds, germination and seedlings considered the significance (p-value) of 5%, and graphs were made with the aid of the Jasp 0.17.1 program.
The morphoanatomical analyses and physiological parameters were applied in a systematic profile for each of the seven phenotypes of the species in principal component analysis (PCA), also with the aid of the program Jasp 0.17.1. The 23 identify markers were applied to elaborate a dendrogram, which was based on the relative Euclidean distance of standardized data, and grouped by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), using the Google Sheets program online.

Conclusion

The morphoanatomical and physiological variables analyzed contributed to the characterization of the intraspecific variations in the species *E. patrisii* under cultivation conditions. The divergences considered 23 characters that, if considered as a group, are not repeated among the seven morphotypes, phenotypes. However, if we consider only eight are notable among the phenotypes: seed biometry (width, thickness, and length), germination percentage, seed number, and morphology of the apex and leaf base and border shape of the seedling. Pht3 is the best for domestication and cultivation, followed by Pht2 and Pht6, and Pht7 is the most divergent.
Studies revealed that amazonian fruit tree species are in different domestication stages and adapted to new crops through some intraspecific variations, such as *E. patrisii*. Although morphoanatomical and physiological tools were applied to clarify intraspecific differences, complementary studies should also be carried out to observe the occurrence of genetic patterns that can aid the bioprospecting of Amazonian plants.

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Author contributions: PPS - planned and designed the research, performed the experiments, analyzed the data, and writing the text. **JCC -** planned and designed the research, analyzed the data, and editing the text. **EVG -** performed the experiments, analyzed the data. **AVF** – review and editing the text. **AAP -** performed the experiments. **AAR -** performed the experiments. **KDG -** performed the experiments. **ASS -** review and editing the text. **JHFC -** review and editing the text. **JFCG -** planned and designed the research, performed the experiments, analyzed the data, and writing the text.

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