Morphological characterization of fruit, seeds and seedlings of white-seal (Chrysophyllum rufum Mart. -Sapotaceae)

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Abstract: Chrysophyllum is the second largest genus of Sapotaceae, with 81 species distributed in the neotropics. Little data are found in the literature regarding the morphology of seedlings and the early development of this genus. This study aims to morphologically characterize the fruit, seeds and seedlings of Chrysophyllum rufum Mart. Fruits were collected from individuals present in two fragments of the Atlantic Forest, Pernambuco. A sample of 100 seeds and 100 fruits was randomly selected to obtain the morphological data. The seeds were sown in plastic trays in a greenhouse. The fruits are bacoid, obovoid and globose with one or two functional seeds per fruit. The seeds are obovate, with the shape of the hilum ranging from elliptical transverse to oblong transverse. The embryo is cotyledonar, with a spatulated form. The cotyledons are foliaceous and whitish-translucent. The endosperm is abundant and whitish. Germination is epigeal, phanerocotylar and unipolar. The seedling has different characteristics than those of the adult individual, such as the shape and leaf consistency, type of leaf margin, type of venation variation, number of pairs of secondary veins, trichome coloring and abundance of latex.

Keywords: morphological description, germination, propagules, Atlantic Rainforest.

Introduction

Chrysophyllum is the second largest genus, in number of species, of the Sapotaceae family, with 81 species distributed through the neotropics (Pennington 1990). The genus is represented in Brazil by 31 species, including nine subspecies, distributed along the national territory in different phytogeographic domains (Pennington 1990, Carneiro et al. 2015). The taxonomy of the genus appears to be quite undefined and complex, with species characterized by difficult morphological delimitation. Recent studies show that Chrysophyllum does not constitute a monophyletic group either in relation to the generic concept nor in the sections proposed in the last revision of the family (Pennington 1990, Swenson & Andeberg 2005, Swenson et al. 2008). In accordance with this, interest in studying the morphology of the fruit, seed and seedling of Chrysophyllum rufum Mart. has arisen. This species, commonly known as white-seal in the state of Pernambuco, Brazil, has shrubs or trees from 8 to 20 m, with small flowers (2-3 mm), green sepals covered by dense golden-rusty trichomes, and delicate, cream-green petals. According
to the National Environmental Council (Conama 2011), *C. rufum* is an indicator species for different successional stages of the vegetation in the Atlantic Forest and is primarily found in the middle and advanced stages of regeneration. Only in the Pernambuco area does *C. rufum* occur in the initial stages of regeneration (Conama 2011). In small patches of the Atlantic Forest in Pernambuco, the remaining individuals of *C. rufum* are frequently found as part of regrowth, a phenomenon deserving of more attention by scientific research: human pressure in different Brazilian ecosystems, combined with a lack of data about the plant’s conservation status, are currently serious obstacles to maintaining the species. In Brazil, *C. rufum* occurs in the northeastern (Alagoas, Bahia, Ceará, Paráiba, Pernambuco, and Sergipe) and southeastern (Espírito Santo, Minas Gerais, and Rio de Janeiro) regions, at the phytogeographic Caatinga dominium, Cerrado and the Atlantic Forest (Pennington 1990, Carneiro et al. 2015). Despite being a well collected species, little data are available in the literature regarding the morphology of seedlings and the early development of the plants. Thus, this study proposes a morphological characterization of the fruit, seed and seedling of *C. rufum* (white-seal), with consideration that studies following this approach may, besides highlighting characteristics of taxonomic value, serve as support for ecological research related to fauna and flora interactions and the recognition of species seedlings in studies of banked seed and seed rain, among others.

**Material and Methods**

1. **Local collection**

The fruits of *C. rufum* were collected from three individuals present in two fragments of the Atlantic Forest. The first fragment is popularly known as “mata do frio” and is located in the municipality of Paulista, which encompasses Parque Natural Municipal do Frio (Natural Park), the first Conservation Unit of this category in the region. The second fragment is the Ecological Park São José, located at the municipality of Igarassu/Pernambuco (7°40’21.25”– 7°55’50.92”S e 34°54’14.25”–35°05’21.08”W); it is one of the largest reserves of the Atlantic Forest in the Brazilian Northeast and property of the Usina São José (USJ).

2. **Collection and processing of plant material**

Mature fruits were collected directly from trees or on the ground and stored in paper bags. After collection, they were transported to the Laboratory of Floristic Coastal Ecosystems (Laboratório de Florística de Ecosistemas Costeiros - LAFLEC) at the Universidade Federal Rural de Pernambuco (UFRPE), where they were selected properly (discarding immature fruits and/or those with injury/damage by animals), described and photographed. In the laboratory, 100 fruits and 100 seeds were analyzed. The fruits were analyzed in relation to color, brightness, hairiness, dehiscence, shape, form, size (length x width), consistency and texture of pericarp and number of seeds per fruit. The seeds were observed for size (length x width), shape, color, brightness and hairiness of tegument, shape and heel position, and embryo characteristics. The terminology used to describe fruits and seeds follows the guidelines of Barroso et al. (1999). After processing the fruits and obtaining the seeds, seeds were sown in plastic trays (28 x 15 x 8 cm) in a greenhouse. As substrate for germination, we used the soil collected from the forest fragments where the individuals of *C. rufum* were found. From the pool of germinated individuals, the most vigorous individuals were selected to carry out the morphological description of seedlings, these being described using the elements suggested by Roderjan (1983): hypocotyl, cotyledons, epicotyl, ephyll (prophyll) and root. The terminology employed was based on Radford et al. (1974), Duke & Polhill (1981), Oliveira (1993) and Camargo et al. (2008). It is important to mention that the seeds presented a type of dormancy, shown by slow and discontinuous germination and a reduced number of germinated seeds.

**Results and Discussion**

1. **Morphological description of fruits and seeds**

The studied species presents bacoid fruits, light green when immature, which turn brownish-red at later stages and purple when ripe (Figure 1). The fruit originates from a superior ovary, typically six-locular and in rare cases seven-locular, with one ovule per locale. However, not all ovules complete their development. The shape of the fruit varies from obovoid to globose with a round apex and acute-oblute base, and presents a depression in the base due to the seed shape (Figure 1). The size of the fruits vary from 9.35 to 14.25 mm long and from 8.34 to 12.83 mm wide, with one or two well developed seeds. It is important to note that few fruits present two well developed seeds with one apparently viable embryo (Figure 1). However, five to six small and non-functional seeds (Figure 1) can be found inside the fruit. Six to seven persistent and pubescent sepals are observed at the fruit base, with pedicels 5.34 to 6.79 mm long and with pubescent sepals presenting malpighiaceous ferruginous trichomes. The epicarp is smooth (though the voucher specimen has a slightly wrinkled appearance), glossy, thin, and glabrous or with thin sparse trichomes. At the apex of the fruit, one observes the persistent stigma forming a small apical (hairy apicule). The mesocarp is sulcused and soft but slightly fleshy and whitish-purple, without a discernible odor. The endocarp is very thin, membranous, whitish, and not adhered to the seed. The propagule’s characteristics may contain elements that define and separate the different taxa. Almeida Jr. et al. (2010) highlight the importance of obtaining characteristics of fruits, seeds and seedlings to support studies on Sapotaceae, helping with the species identification. In addition, studies that demonstrate the morphology of fruits and seeds of native plant species can be of great use in the production of seedlings for forest nurseries, subsidizing ecological research and forest regeneration after anthropogenic disturbances. The seed has dimensions of 9.8 to 11.95 mm in length and 7.06 to 9.9 mm in width, occupying almost the entire internal cavity of the fruit. Only one seed develops completely inside the fruit (Figure 1). The seed shape is obovate, with a smooth brow, glossy, stiff, and glabrous, with color ranging from cream to light brown (sometimes bi-color). The hilum is evident, wide, depressed, and base-ventral, with its shape ranging from elliptical transverse to oblong transverse. Around the hilum, depressions corresponding to “aborted” seeds that had not finished their development could be observed. Contour lines are observed leading down from the seed apex to the base (around the hilum). The embryo is cotyledonar with a spatulated form, perfectly distinguishing between the hypocotyl-root axis and the cotyledons, which are whitish-translucent, with two oblong, planar and membranous embryonic leaves. The endosperm surrounding the embryo is abundant and whitish in color. The cotyledons are thin and foliaceous (Figure 1). Pennington (1990, 1991) emphasized the importance of seed characteristics to species separation, especially the shape (if laterally compressed or not, the size and shape of hilum, and the presence or absence of endosperm as well as its abundance in the seed). In addition, features such as these, in conjunction with other morphological data, have provided support for the author to subdivide *Chrysophyllum* into five sections. It is also worth noting that the author describes the existence of only one seed per fruit, but the present study verifies that eventually, it is possible to find more than one well developed and viable seed.

2. **Morphological description of seedling and morfofunction**

Germination began 20 – 34 after sowing, with radicle protusion and subsequent formation of the hypocotyl strap (Figure 2). The seedling has epigeal germination, phanerocotylar and unipolar, with axis positioned between the cotyledons. The hypocotyl is slightly elongated (1.5 to 2.5 cm) and starts light green before becoming brown and signified/glabrous.
Morphology of *Chrysophyllum rufum* Mart.

Foliaceous cotyledons are 1.53 to 1.66 cm and photosynthetic and have a petiole that is greenish, opposite, glabrous and 0.26- to 0.31-cm in length. The epycotyl is 0.4 to 0.55 cm long and greenish, becoming light brown with indument pubescent-depressed and with white trichomes becoming ferruginous during its development (Figure 2). Internodes range from 4.64 to 8.91 mm and are greenish in color, with indument and texture similar to that of the epycotyl. The first leaves (16.61-27.10 x 9.14-9.84 mm) are simple, oblanceolate, cartaceous, alternating, and slightly spiraled, while the petiole (2.27 to 3.90 mm) is light green, not canaliculated, and pubescent-depressed, with ferruginous trichomes. Leaf blades are green, smooth on both sides, glossy and glabrescent, with rare ferruginous trichomes near the base and in the mid rib. In the bud and in the beginning of leaf development, trichomes are whitish, becoming ferruginous to golden; leaf bases are cuneate to slightly cuneate, with a cuspidate-acute apex, entire margin (rarely with ciliated margin, trichomes caduceous), and brochidodromous venation. In the beginning of the development of the first leaves (eophyll and metaphyll), only the primary and the secondary venation (arched near the margin) are evident. The following leaves present five to ten pairs of secondary veins, rare intersecondary and reticulate tertiary veins, and a small amount of viscous white latex. A greenish apical bud, always formed by leaf sketches of different sizes, parallels a slightly curved, pubescent indument, with white to ferrugineaous malpighiaceous trichomes.
and largely asymmetric axes. The importance of seedling morphology studies is that many characteristics of young individuals are lost during their development, making the identification of those that germinate in forest areas difficult (especially for those developing research with soil seed bank and seedlings analysis) (Table 1, Figure 3). In the case of the species analyzed here, *Chrysophyllum rufum*, it was possible to observe some characteristics present in the seedling that can distinguish immature from adult individuals. The discussion about the importance of assessing the initial phase of plant organisms is not recent (Rizzini 1965, Ng, 1978, Vogel 1980, Candolle 1985, Miquel 1987). Studies related to the morphology of seedlings are useful in the restoration of anthropic areas or after disturbances and openings of forest clearings, as allowing the correct delimitation of taxa, even young individuals, contributes to support forestry strategies and, consequently, to accelerate the management of conservation actions (Almeida Jr. et al., 2010, Barreto & Ferreira 2010, Lima et al., 2010, Amorim et al., 2006, Guerra et al., 2006). In the case of *C. rufum*, the leaf variation, the amount and color of indumenta and embryo characteristics, and fruit and seed morphology all contribute to the identification of the species in its natural environment and aid its delimitation.
Table 1. Primary differences observed in leaf morphology between seedling and adult individuals of *Chrysophyllum rufum* (Sapotaceae).

<table>
<thead>
<tr>
<th>Characters</th>
<th>Seedling</th>
<th>Adult individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf shape</td>
<td>Oblanceolate</td>
<td>Oblong-elliptic (lanceolate)</td>
</tr>
<tr>
<td>Leaf consistency</td>
<td>Cartaceous</td>
<td>Coriaceous</td>
</tr>
<tr>
<td>Leaf apex</td>
<td>Cuneate to closely cuneate</td>
<td>Acute-acuminate</td>
</tr>
<tr>
<td>Leaf base</td>
<td>Cuneate to closely cuneate</td>
<td>Base obtuse-cuneate</td>
</tr>
<tr>
<td>Leaf margin</td>
<td>Entire or ciliated (trichomes caduceus)</td>
<td>Strongly revolute</td>
</tr>
<tr>
<td>Type of venation</td>
<td>Barely visible initially, then brochidodromous</td>
<td>Eucamptodromous-brochidodromous</td>
</tr>
<tr>
<td>Nº of pairs of secondary veins</td>
<td>5 to 10</td>
<td>8 to 11</td>
</tr>
<tr>
<td>Indument of abaxial face</td>
<td>Glabrescent/ glabrous</td>
<td>Densely tomentose</td>
</tr>
<tr>
<td>Color of trichomes</td>
<td>Golden-whitish</td>
<td>Ferruginous to dark brown</td>
</tr>
<tr>
<td>Latex</td>
<td>Small amounts</td>
<td>More abundant</td>
</tr>
</tbody>
</table>

Figure 3. Comparison of leaf morphology between adult individual and seedling: A, B and C: leaf shape (abaxial and adaxial side, respectively) and bud of adult individual; D, E and F: leaf form (abaxial and adaxial side, respectively) and bud of seedling; G, H, I and J: leaf apex of adult individual and seedling; K: leaf venation of adult individual; L and M: leaf venation during seedling development; N, O, P, Q: leaf base of adult individual and seedling.
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Author Contributions

Liliane Ferreira Lima: collection and species identification, analysis and monitoring of the experiment in the greenhouse and laboratory, orientation methodology, and theoretical contributions; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Renata Gabriela Vila Nova de Lima: support during field collection, practical execution of the experiment in the greenhouse and laboratory, and assistance with personnel and scientific development; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Angelica Cândida Ferreira: support during field collection, practical execution of the experiment in the greenhouse and laboratory, and assistance with personnel and scientific development; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Eduardo Bezerra de Almeida Junior: support for orientation methodology and scientific theoretical contributions; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Carmen Silvia Zickel: support for orientation methodology and scientific theoretical contributions; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Conflicts of interest

The authors declare that there is no conflict of interest related to the publication of the data in this article.

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