Morphoanatomy and biochemistry of seeds of *Dicypellium caryophyllaceum* (Lauraceae) at different stages of fruit maturation

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Key words:
- Amazon native
- Development
- Forest species
- Pau-cravo
- Physiological maturity

Abstract

The "pau-cravo" (*Dicypellium caryophyllaceum*) is a tree species of the family Lauraceae, native to the Amazon rainforest. As a result of overexploitation during the last few centuries, *D. caryophyllaceum* has become extremely rare in Brazil. Taking into account the importance of basic and advanced studies of seeds and the scarce information about this species, the goal of the present study was to define morphological markers of seed developmental stages and to understand the dynamics of reserve deposition in *D. caryophyllaceum* seeds. The species produces a drupe-like fruit due to the presence of a rigid endocarp, as revealed by anatomic examination. The analysis of biochemical properties in mature seeds of *D. caryophyllaceum* showed a lipid content of 19%, being classified as oleaginous. Changes were observed in the contents of proteins, sugars, and starch throughout the maturation stages, with the highest values of Total Soluble Sugars (4.01%) and Reducing Sugars (3.22%) observed in seeds at stage I. The highest values of amino acids (0.13%) and starch (9.5%) were detected in mature fruit (stage II). Biochemical and morphological information from the seeds is relevant for planning conservation, observation, and restoration strategies.

Palavras-chave:
- Nativa da Amazônia
- Desenvolvimento
- Espécies florestais
- Pau-cravo
- Maturidade Fisiológica

Resumo

O pau-cravo (*Dicypellium caryophyllaceum*) é uma espécie arbórea da família Lauraceae, nativa da floresta amazônica. Como resultado da superexploração durante os últimos séculos, *D. caryophyllaceum* tornou-se extremamente raro no Brasil. Levando em consideração a importância de estudos básicos e avançados de sementes e as escassas informações sobre essa espécie, o objetivo do presente estudo foi definir marcadores morfológicos de estágios de desenvolvimento de sementes e entender a dinâmica de deposição de reservas em sementes de *D. caryophyllaceum.* A espécie produziu um fruto semelhante a drupa devido à presença de um endocarpo rígido, conforme revelado pelo exame anatômico. A análise das propriedades bioquímicas em sementes maduras mostrou um teor lipídico de 19%, sendo classificadas como oleaginosas. Foram observadas alterações nos teores de proteínas, açúcares e amido ao longo dos estádios de maturação, sendo os maiores valores de Açúcares Solúveis Totais (4,01%) e Açúcares Redutores (3,22%) observados nas sementes no estádio I. Os maiores valores de aminoácidos (0,13%) e amido (9,5%) foram detectados em frutos maduros (estádio II). As informações bioquímicas e morfológicas das sementes são relevantes para o planejamento de estratégias de conservação, observação e restauração.

Original Papers

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Introduction

The family Lauraceae (Order Laurales, Class Magnoliopsida) belongs to the eudicotyledons clade (APG IV 2016), encompassing approximately 50 genera and more than 2,500 species worldwide (De Souza Assis *et al*. 2005). In Brazil, this family is represented by nearly 27 genera and 467 species (Flora do Brasil 2020, continuously updated), including 239 taxa from four genera regarded as endemic. The laurels stand out among other plant families because of their economic importance, since several species are exploited for furniture (*e.g.*, itauba - *Mezilaurus itauba* (Meisn.) Taub. *ex* Mez), cosmetics (*e.g.*, Brazilian rosewood - *Aniba rosadora* Ducke), food (*e.g.*, avocado - *Persea americana* Mill.), and spices (cinnamon - *Cinnamomum verum J.Presl*, "pau-cravo" - *Dicypellium caryophyllaceum (Mart.)* Nees), among other uses (Marques 2001).

Dicypellium caryophyllaceum is native to the Amazon forests of Brazil, Ecuador, and Peru (Salomão & Rosa 2012), being popularly known as "pau-cravo", "cravo-do-Maranhão", or clove bark as a reference to the clove-like scent produced by all parts of this plant (Salomão & Rosa 2012; Fausto 2013; Donini *et al*. 2016). This tree presents several uses, particularly in the cosmetic and perfume industry, and as food or phytotherapeutics (Fausto 2013). The main products derived from *D. caryophyllaceum* are wood and essential oils. Because of the historical overexploitation of native populations, this tree has become rare in Brazil (Salomão & Rosa 2012) and, therefore, little is known about this species. As a matter of fact, essential information (morphological, biometric, biochemical, germination, and development traits) to infer the diversity and aid in the design of effective policies for the conservation of germplasms and dispersal of this species remains largely overlooked.

Basic studies about native forest plants are a building block of conservation programs and management of biological diversity. In this sense, determining the period and stages of physiological maturation in seeds is useful to understand their reproductive strategies and to define the best moment to harvest high-quality products (Fortado *et al*. 2021). A common indicator that is widely used to identify the maturity of some plant species, particularly those from forest habitats, is the changes in coloration, as reported in several forest trees, such as *Anadenanthera colubrina* (Vell.) Brenan (Fortado *et al*. 2021), *Albizia niopoides* (*Spruce ex Benth.*) Burkart (Ristau *et al*. 2020), *Amburana cearensis (Allemão)* A.C.Sm.. (Lopes *et al*. 2014), and *Jatropha curcas* L. (Rubio *et al*. 2013).

Morphoanatomical and biometric analyses of fruits and seeds also represent an important tool in studies about native species, since these data can be used in botanical identification and the propagation of species (Aquino *et al*. 2009). In turn, the evaluation of biochemical parameters allows us to infer the levels of reserve compounds present in seeds during each development stage (Henning *et al.* 2010). These reserves play a major role in both the maintenance and development of embryos until the formation of seedlings (Oliveira 2015). The main reserve compounds in seeds include lipids, carbohydrates, and proteins. The proportion of these compounds varies among species, even within the same family, as each species differs in reserve mobilization pathways during the formation of physical structures in seeds and seedlings (Corte *et al*. 2006).

Most information about the maturation and biochemical composition of fruits and seeds has been obtained for species of agronomic interest, while few reports are available for forest species. According to Alves *et al.* (2023) studies are needed to elucidate not only the degradation of different reserves, but also the use of metabolites produced in native species. Therefore, understanding the physiological maturation processes and biochemical properties of native forest plants might potentially improve harvesting periods (De Oliveira *et al*. 2020), conservation efforts or the propagation of species, and the production of high-quality seedlings, since vigor and storage abilities are influenced by the content of compounds in seeds (Carvalho & Nakagawa 2012). Given the importance of basic and advanced seed analyses, the aim of the present study was to define morphological markers of seed developmental stages and to understand the dynamics of reserve deposition in *Dicypellium caryophyllaceum* seeds.

Material and Methods

Fruit samples of *Dicypellium caryophyllaceum* were obtained from February to March 2021, from five reproductively mature trees in the municipality of Vitória do Xingu in the state of Pará, northern Brazil (Fig. 1) located at 03º19'12.26"S and 51º56'39.88"W (with permission SISBIO nº 68708-1).

Fruits were collected manually from each tree. After harvesting, the samples were transported to the Laboratory of Biotechnology - BIOTEC at Federal University of Pará, where only fruit samples free of mechanical injuries or plagues and diseases were selected. Furthermore, fertile and sterile branches of *D. caryophyllaceum* were collected for the preparation of exsiccates and deposited in the Herbarium Padre José Maria Albuquerque (HATM) at the Faculty of Biological Sciences, Campus of Altamira (code identification numbers 2325 to 2332).

Morphological, biometric and anatomical analyses

The terminology and methodology used for the morphological study were based on the work of Barroso *et al*. (1999), through the pericarp layers. The classification of fruit coloration was performed according to the catalogue of colors developed by Munsell (1976).

For biometric analyses, the width, length, and weight of fruits and seeds were measured using a digital pachymeter and weighed on an analytical scale. Moisture values were estimated by drying the seeds in an oven at 105 ± 3 °C for 24 h (Brasil 2009). Two developmental stages were identified in samples of *D. caryophyllaceum,* based on morphological and color patterns associated with biometric parameters of the fruits and seeds, seed moisture, and content of reserve compounds. Since it is an endangered species and there are only five matrices in reproduction, 50 fruits and 50 seeds of each stage were placed, with two replicates each.

For the anatomic studies, the seed samples from each stage were fixed in FAA (formaldehyde, glacial acetic acid, and ethanol 50% at a ratio of 1:1:18, v/v) for 48 h and then stored in ethanol 70%, according to the methodology described by (Johansen 1940). The anatomic characterization was based on longitudinal and transversal sections of seeds using slides followed by clarification in sodium hypochlorite diluted in distilled water (1:1). The final cuts were washed in distilled water, stained with toluidine blue 0.05% (pH 4.4) (O'Brien & Feder 1964), and mounted on glass slides and cover slips using glycerin.

Biochemical analyses of seeds

The seeds were dried in a bench oven at 65°C for 48 h. Subsequently, the material was milled for further biochemical analyses. The dried material was stored and sent to the Experimental Forestry Unit at the State University of Feira de Santana (UEFS), in Bahia, northeastern Brazil.

The extraction for the quantification of Total Soluble Amino Acids (AA), Total Soluble Sugars (TSS), and Reducing Sugars (RS) was performed using 0.2 g of dried material homogenized in 10 mL of 0.1 phosphate buffer (pH = 7). The ninhydrin test (Yemm & Cocking 1955) was used to quantify amino acids, using glycine as a standard sample. The reading was performed in a UV spectrophotometer at 570 nm.

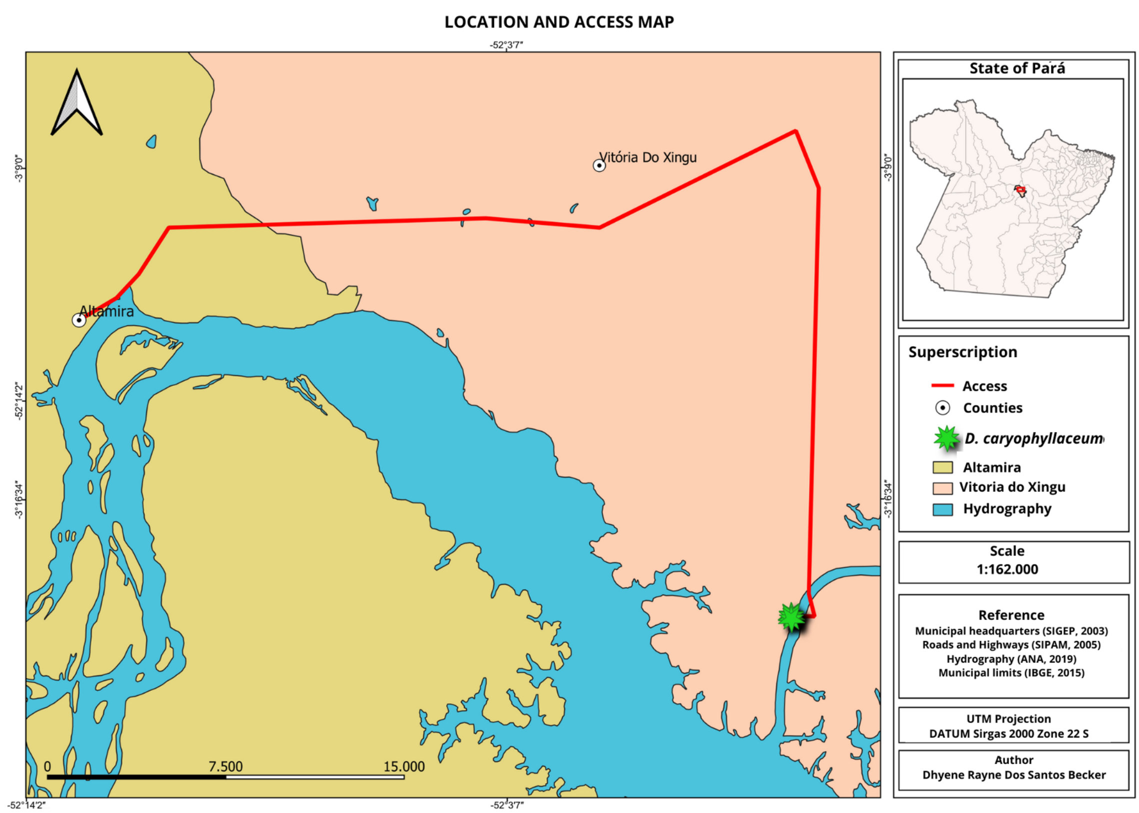

Figure 1 – Collection sites of *Dicypellium caryophyllaceum* in the municipality of Vitoria do Xingu, Para, Northern Brazil.

For the extraction of starch, 0.2 g of dried material was placed in a centrifuge tube, homogenized in 10 mL of 80% ethanol, and incubated at 40 °C for 10 min. The amount of TSS and starch was determined based on the anthrone test, as described by Yemm & Willis (1954), using glucose as a standard carbohydrate and reading parameter at 620 nm in a UV spectrophotometer. The quantification of RS was accomplished based on the procedure reported by Miller (1959), using 3,5-dinitrosalicylic acid (DNS) and glucose as a standard sample. The reading was performed at 540 nm in a UV spectrophotometer.

The protein fractions from seeds were extracted according to their solubility (albumins, globulins, prolamins, and glutelins), following the methodology proposed and adapted by Osborne (1924). The protein fractions were determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard sample, in a UV spectrophotometer at 595 nm.

The extraction and quantification of lipids were carried out in a Soxhlet-type apparatus using the cold extraction method with petroleum ether for 4 h, adapted from the method reported by Silva (1990).

All biochemical assays were performed in triplicate, with samples obtained after harvesting the fruits from the matrices, and mixed to obtain the amount of material necessary for the analyses.

Statistical analyses

For analysis of the morphology of the fruits and seeds, and quantification of lipids, descriptive statistics were performed, showing the mean and standard deviation. The multiple comparisons of means of the biochemical parameters of fruit at both maturation stages was carried out based on the Scott-Knott clustering algorithm, with an error probability considered less than 5%. The analyses were performed in R language (R development core team 2020).

Results

Morphological, biometric and anatomical analyses

Two stages of maturation were defined according to the color pattern of the exocarp (Fig. 2a), as follows: 5 GY 6/4 green (stage I) and 2.5 RP 1/4 dark purple (stage II) (Tab. 1).

The fruit exocarp is thin, smooth, and shiny, varying in coloration. At stage I, fruits were 20.65 mm long and 12.89 mm wide on average, with an approximate weight of 2.23 g. At stage II, the mean length and width of fruit samples were 21.49 mm and 13.42 mm, respectively, with an average weight of 2.40 g. These results are related to the development of fruit, even though no significant differences were observed between the two stages of maturation (Tab. 2). The size of the fruit was variable within each stage, with weight being the most variable trait, presenting a coefficient of variation of 24.66% at stage I.

The humidity values were higher in seeds from stage I (66.1%) than in seeds from stage II (48.7%) when the fruit is completely mature. In general, the seeds of *D. caryophyllaceum* are egg-

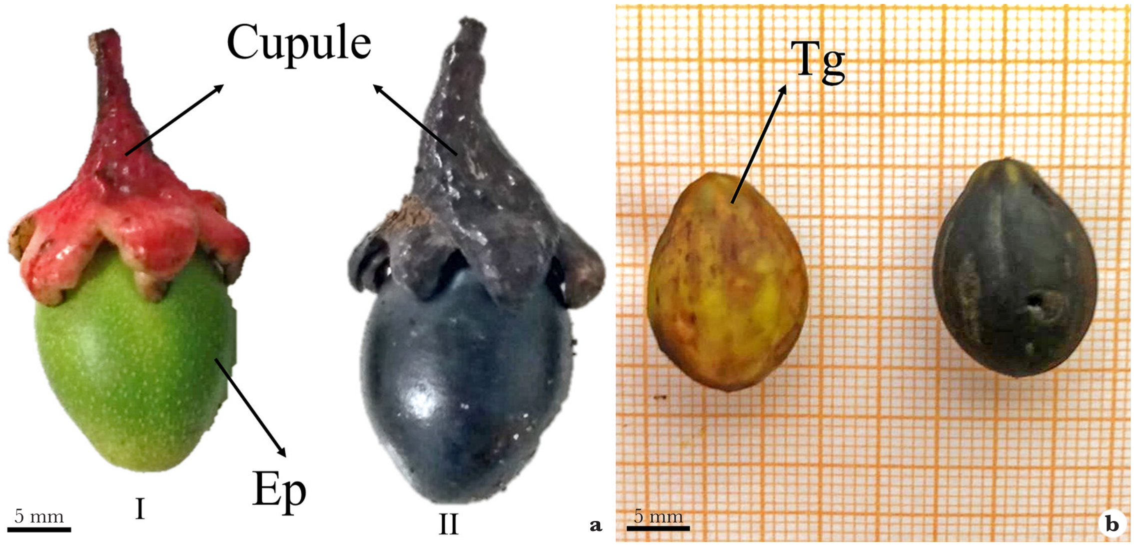

Figure 2 – a-b. Color patterns in fruits of *Dicypellium caryophyllaceum* at the two stages of maturation, according to Munsell 1976; Stage I - 5 GY 6/4 green and Stage II - 2.5 RP 1/4 purple – a. coloration of fruits with the presence of the cupule, Ep = epicarp; b. coloration of seeds of *D. caryophyllaceum* at the two stages of maturation; Tg = Tegument.

Table 1 – Description of epicarp coloration in fruit samples according to Munsell (1976).

Stages	Pigmentation	Coloration
I	Green	5 GY 6/4
II	Dark purple	2.5 RP 1/4

shaped, with a small pore close to their apex, corresponding to the micropyle (Fig. 3a). In addition, the seeds lack endosperm (exalbuminous type), presenting a smooth, dark, and membranous tegument attached to the endocarp. The cotyledons are flat-convex and whitish, being surrounded by the tegument (Fig. 3b). After cutting the seeds, the cotyledons underwent oxidation, turning brown (Fig. 3c). The embryo is axial (Fig. 3d), comprising a large cotyledon (Fig. 3e) and plumule, a cotyledonary node, and a very reduced hypocotyl-radicle axis, as observed in Figure 3f.

The seeds at stage I presented a mean length of 16.29 mm, a mean width of 10.53 mm, and a mean weight of 1.20 g; in seeds at stage II, these values corresponded to 19.72 mm, 11.88 mm, and 1.62 g, respectively. The seeds at stage II were larger and heavier than those from stage I (Tab. 3). Moreover, the seeds from stage I presented wide variation in their weight (CV = 31.59%), this being one of the most variable traits of the analyzed seeds.

The main structures observed in the pericarp of *D. caryophyllaceum* are shown in Figure 4a. The outer mesocarp is wide, with most of the fruit wall being composed of cells of different sizes, while the inner mesocarp is more restricted, being composed of a layer of three small, elongated, and structured cells (Fig. 4b). In this layer, secretory cells were observed along the mesocarp, characterized by notable oleaginous content. The endocarp encompasses prismatic cells arranged in palisades. The cotyledon cells (Fig. 4c-d) presented a round shape and distinct dimensions, and were also characterized by the presence of oil cells.

Biochemical Analyses

The compound content values found in seeds of *D. caryophyllaceum* from each stage of maturation are presented in Figure 5.

The content of total soluble (TSS) and reducing (RS) sugars during the development of seeds of *D. caryophyllaceum* are shown in Figures 5a and 5d. Values of 40.10 mg TSS/g and 19.50 mg AST/g were observed in seeds from stages I and II, respectively. In relation to the reducing sugars, we reported a similar decrease, with values of 32.23 mg RS/g for seeds from stage I and of 12.07 mg RS/g for seeds from stage II, with significant differences between the two stages.

Figure 5b presents the starch content during the development stage in seeds of *D. caryophyllaceum*. The highest value was observed during the last stage of maturation (95.04 mg/g), compared to a value of 76.62 mg/g at stage I, with significant differences between the stages.

The content of amino acids in seeds at distinct stages of maturation are shown in Figure 5c. The lowest value was observed at stage I (0.5 mg of

Table 2 – Descriptive statistics of fruit traits at each stage of maturation (Min. V = minimum value; Max. V = maximum value; S = standard deviation; CV = coefficient of variation).

Stage	Mean	Min. V	Max. V	S	CV (%)
Length (mm)					
I	20.65	15.10	23.03	2.17	10.508475
II	21.49	17.76	23.56	0.88	4.0949279
Width (mm)					
I	12.89	9.36	14.66	1.32	10.240497
II	13.42	10.18	14.9	1.00	7.4515648
Weight (g)					
I	2.23	0.81	2.87	0.55	24.663677
II	2.40	1.98	2.86	0.19	7.9166667

AA/g). The highest accumulation of amino acids (1.3 mg of AA/g) was observed during the final development stage, in mature seeds.

The peak in the amount of Total Soluble Proteins (TSP) was detected during stage I (15.7 mg PST/g), followed by stage II (13.7 mg PST/

Figure 3 – a-f. Overall appearance of fruits of *D. caryophyllaceum* – a. longitudinal; b transversal; c-d. sections of seeds of *D. caryophyllaceum,* the cotyledon (Co), the tegument (Tg), the endocarp (En), the mesocarp (Me), and epicarp (Ep); e. the transversal section of seeds showing the embryonic axis (Ex); f. the hypocotyl-radicle axis (Hr), the cotyledonary node (Nc), the plumule (Pl).

Table 3 – Descriptive statistics of seed traits at each stage of maturation (Min. V = minimum value; Max. V = maximum value; S = standard deviation; CV = coefficient of variation).

Stage	Mean	Min. V	Max. V	S	CV (%)
Length (mm)					
I	16.29	11.88	20.31	2.56	15.71
II	19.72	16.92	21.12	1.16	5.88
Width (mm)					
I	10.53	7.84	13.08	1.63	15.47
II	11.88	10	13.61	1.16	9.76
Weight (g)					
I	1.20	0.58	1.78	0.38	31.59
II	1.62	1.18	2.17	0.22	13.57

g), but without significant differences between the two stages (Fig. 5e). The content of protein fractions (albumins, globulins, prolamins, and glutelins) in seeds of *D. caryophyllaceum* are shown in Figure 6. Glutelins represented the most abundant fraction in both development stages, with values ranging from 11.6 mg/g to 9.9 mg/g, without significant differences between the developmental stages.

Analysis of the biochemical composition in mature seeds of *D. caryophyllaceum* revealed that lipids are one of the main reserves, representing 19% of the seed content, followed by starch (9.5%), TSS (1.95%), TSP (1.31%), RS (1.20%), and total soluble amino acids (0.13%).

Discussion

The coloration of the fruit of *D. caryophyllaceum* underwent changes during the development stages (Fig. 2). According to Anese & Fronza (2015), modifications in fruit color result from the synthesis of pigments by oxidative processes and enzymatic activities in the tegument. Several reports relate fruit coloration to physiological maturity, as described for the forest species *Albizia hassleri* (Ristau *et al*. 2020) and *Albizia niopoides* (Spruce *ex* Benth.) Burkart (Barbeiro *et al.* 2018). These studies observed that the seeds obtained from brown fruits (mature) present increased germination and vigor rates, leading to the production of well-developed seedlings.

The species *D. caryophyllaceum* produces small, fleshy, and monocarpic fruit, typical of Lauraceae, encompassing a double-edged cupule and persistent tepals (Fig. 2a), as similarly reported by Vicentini *et al*. (1999) in fruit of *Dicypellium manausense* W.A.Rodrigues. The coloration of the cupules varied according to the maturation stage of the fruit, being red in green fruit and dark purple when the fruit was mature. The color pattern of seeds obtained at different stages also varied (Fig. 2b), presenting either light or dark coloration in green and mature fruit, respectively.

As pointed out by Silva *et al.* (2012), harvesting seeds at the appropriate time of maturation is one of the key factors to determine their quality, since these seeds should present high physiological potential. Furthermore, color variation can be regarded as a good indicator of the best periods of harvesting, since it allows rapid identification of mature seeds in the field (Lopes *et al.* 2014). This is particularly useful to avoid predation of mature fruit by insects, as observed in ripe samples of *D. caryophyllaceum.*

Fruits and seeds of *D. caryophyllaceum* showed significant differences in biometric parameters. Large variations were observed in both length and diameter, with weight being the most variable trait. The highest values for these parameters were observed in the fruit at stage II, regarded as mature. Similar results were observed in *Inga laurina* (Sw.) Willd. (Schulz *et al.* 2014), since increased diameter and length were found in fruit with yellow colorations, corresponding to stage III of maturation. According to Abud *et al*. (2015), variable values in the biometric data are related to the maturation process, which increases both the weight and size of fruits.

The humidity of the seeds was higher (> 66%) at the initial stage (fruits of green color) and then reduced drastically as the maturity process took place, reaching 48.7%. Similar results were reported by Carvalho (2006), evaluating the humidity content of seeds in green and dark fruit of *Nectandra lanceolata* Nees (Lauraceae), with values of 51.0% and 39.1%, respectively. The water content also decreased in seeds during maturation of fruit in *Anadenanthera colubrina* (Vell.) Brenan (Pires-Neto *et al*. 2016), *Inga laurina* (Schulz *et al.* 2014), and *Jatropha curcas* (Silva *et al*. 2012). The highest humidity is required at the beginning of maturation so the photosynthesized products from leaves are translocated to the formation of seed reserves (Carvalho & Nakagawa 2012; Ducca *et al.* 2015; Silva *et al.* 2020), known as a source-drain relationship between leaves and seeds.

The high moisture content observed in samples from the current study suggest that the seeds of *D. caryophyllaceum* are recalcitrant. Recalcitrant seeds are characterized by the absence of natural desiccation in the mother plant during maturation, thus being dispersed with high water content, which, when drastically reduced, leads to rapid loss of viability and even the death of the seeds (Costa 2009). Similarly, the seeds of tree species from the genera *Nectandra* and *Ocotea* were also classified as recalcitrant by Carvalho *et al.* (2008).

The species of the family Lauraceae are usually characterized by the production of berry-like fruit, comprising a single seed of highly variable shape and size inside the receptacle, which is transformed into a dome (Vicentini *et al*. 1999; Barroso *et al.* 1999). On the other hand, studies in *Ocotea puberula* (Rich.) Nees and *Nectandra megapotamica* (Spreng.) Mez, both representatives of Lauraceae, revealed that the sclerenchymatous tissue that surrounds

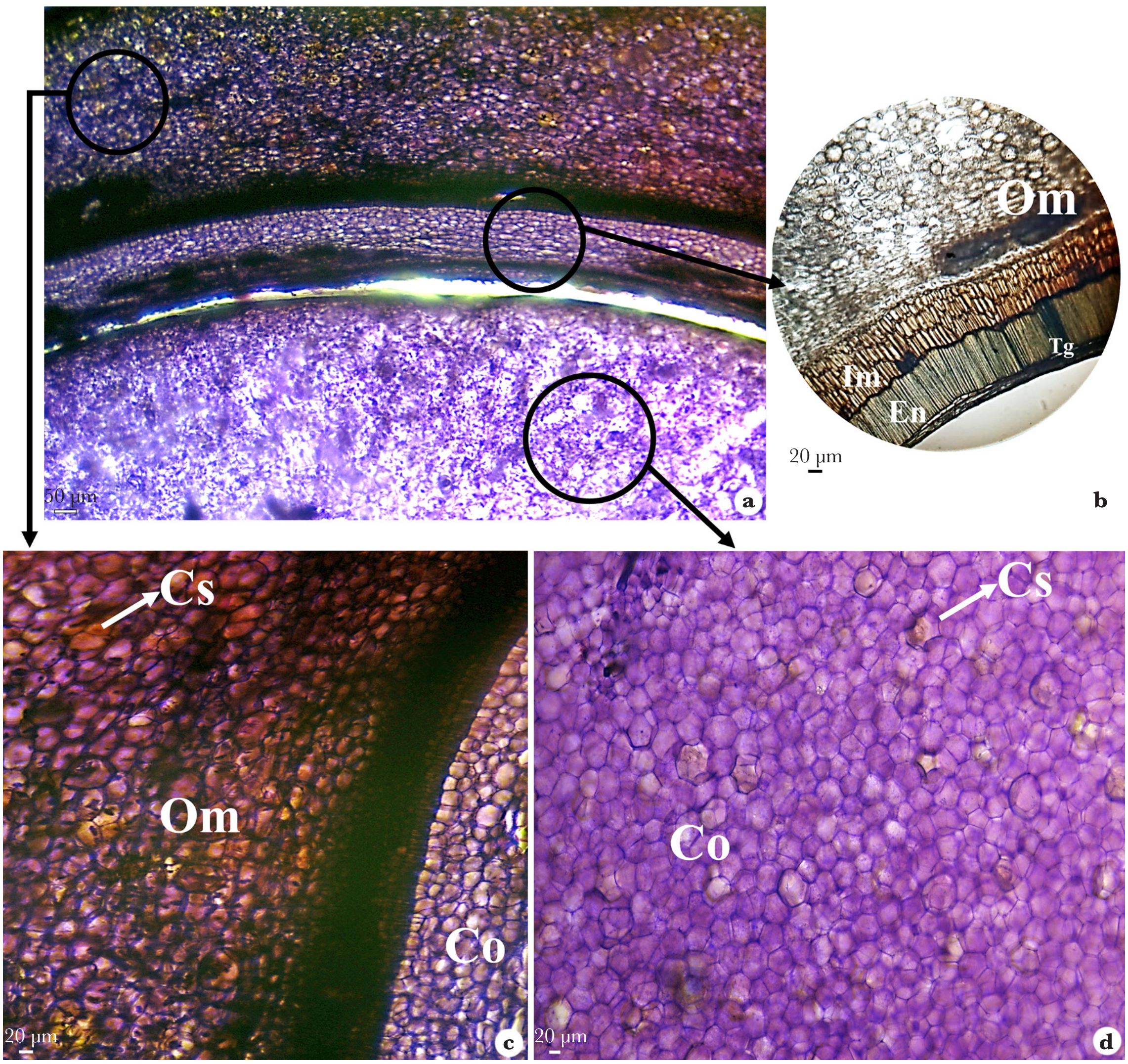

Figure 4 – a. Transversal section of fruit and seed of *Dicypellium caryophyllaceum*. b. Mesocarp of *D. caryophyllaceum*, the Endocarp (En), the Outer mesocarp (Om), the Inner mesocarp (Im), and Tegument (Tg). c. Cotyledon of *D. caryophyllaceum* the Cotyledon (Co), the Outer mesocarp (Om). d. Cotyledon of *D. caryophyllaceum* the Secretory cells (Cs) and the Cotyledon (Co).

the seed is the endocarp, and this unique structure is commonly found in drupes (Souza & Moscheta 2000). While berry-type fruits have a fleshy pericarp and resistant tegument, drupe-type fruits are characterized by a seed protected by a hard and woody endocarp (De Paula *et al*. 2016). The latter pattern was observed in *D. caryophyllaceum*, in which a rigid endocarp remained attached to the tegument when the seed was removed from the mature fruit (Fig. 3e). Therefore, *D. caryophyllaceum* can be considered a species that produces drupe-type fruit. In addition, the anatomical examination revealed a large amount of oil cells in both fruit and seeds, as also reported by Souza and Moscheta (2000), corroborating the high ethereal level of lipids in the seed analysis.

The concentrations of TSS and RS were higher in seeds obtained from immature fruit of *D. caryophyllaceum*. These data are in agreement with those reported by Duarte *et al*. (2020) in seeds of *Lecythis lurida* (Miers) S.A.Mori from early developmental stages. Moreover, these authors pointed out that high levels of sugars during the initial stages of seed maturation also account for the increased concentration of water at the beginning of development, besides assisting the seed growth. During the first embryonic stages, carbohydrates tend to accumulate earlier than other reserves (*e.g.*, proteins and lipids) and then reduce along the development of the embryos (Souza *et al.* 2018). This pattern is related to the importance of available sugars during early maturation stages in promoting embryonic growth, development, and germination (Smeekens *et al.* 2010).

The amounts of TSS and RS were lower at the final development stages of *D. caryophyllaceum*, showing an inverse relationship with starch content, which increased during the maturation of seeds, thus suggesting that a portion of sugars were directed to the synthesis of starch. Similar results were reported during development in other species, like *Pongamia pinnata* (L.) Pierre (Pavithra *et al.* 2014) and *Hevea brasiliensis* (Willd. *ex* A.Juss.) Müll. Arg. (Souza *et al.* 2018), both characterized by increased levels of starch at the final stages of seed maturation. This phenomenon is putatively associated with the preparation of seeds for germination, since they depend on a readily available supply of energy, while the breakdown of lipids takes longer and usually occurs after the depletion of sugars, particularly starch (Souza *et al.* 2018).

Total soluble amino acids were the organic compounds that presented the lowest percentage in seeds of *D. caryophyllaceum* (approximately 0.05% and 0.13% at stages I and II, respectively, as shown in Figure 5). Furthermore, the content of amino acids in the studied species was higher in seeds obtained from stage II, regarded as mature. In general, increased amino acid levels are related

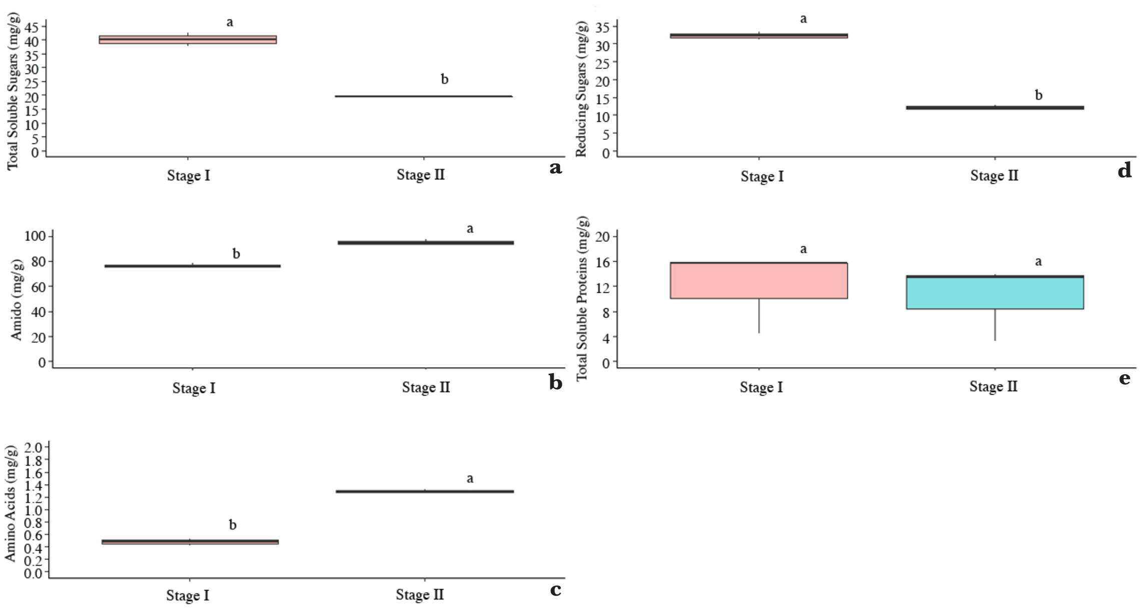

Figure 5 – a-e. Biochemical properties of seeds of *Dicypellium caryophyllaceum* from each maturation stage – a. total soluble sugars; b. starch; c. amino acids; d. reducing sugars; e. total soluble proteins. * Same letters do not differ statistically at 5% probability ($p < 0.05$) Scott-Knott test.

to protein degradation (Azevedo-Neto *et al.* 2009), indicating that the rate of proteolysis was higher than the biosynthesis of proteins at the final maturation stage in seeds of *D. caryophyllaceum* (Fig. 3a). According to Galili *et al*. (2014) the amino acids are used in protein synthesis as the main precursors of multiple secondary metabolites, thus facilitating the production of energy in developing seeds.

During fruit maturation in *D. caryophyllaceum*, the levels of proteins were slightly modified, reaching higher values at the initial stage of seed development (Fig. 5). The reduction in protein content during the seed development is likely to be related to the intense use of proteins in metabolic processes, mainly related to cell division and the formation of new substances (Duarte *et al.* 2020). A similar result was reported in *Jatropha curcas* by Silva *et al.* (2018), who also described reduced amounts of protein during the maturation of seeds.

The protein fractions, divided into glutelins, prolamins, albumins, and globulins play a key role in seed development and germination, since they are used for the formation of new tissues in embryos (Marcos-Filho 2015; Silva 2019). Prolamins are more abundant in grasses, while glutelins are found in cereals, and globulins and albumins are predominant in dicotyledons (Souza *et al.* 2012). Understanding the relationships among these proteins and other biochemical compounds is essential to infer the strategies of energy availability and the potential viability of seeds. Interestingly, glutelins, recognized as the major reserve proteins in monocotyledonous species but uncommon in dicotyledonous plants, were predominant in seeds of *D. caryophyllaceum*, with higher values being observed during stage I. Similarly, studies about the chemical composition of seeds of *Mezilaurus itauba* (Meisn.) Taub. *ex* Mez, another native Amazonian species from the family Lauraceae, also revealed high content of glutelins (86.24 mg.g^{-1}) in seeds, suggesting a species-specific pattern (Souza *et al*. 2012).

According to Pupin *et al.* (2017), the glutelins are high-quality proteins, rich in essential amino acids such as lysine and tryptophan, thus representing a valuable compound in food. In addition, biochemical evaluation during maturation stages allows the detection of the amounts of substances that, from an ecological point of view, would attract or avoid dispersers and predators (Oliveira 2012). In the current study, *D. caryophyllaceum* proved to be particularly vulnerable to predation by insects, suggesting a close relationship between the chemical composition of the fruit and insect behavior.

The seeds of *D. caryophyllaceum* were classified as oleaginous, inasmuch as they presented 19% of lipids. Similar results were reported by Simeone *et al*. (2009) in *Ocotea porosa* (Nees & Mart.) Barroso, another species of Lauraceae native to Brazil, whose seeds were characterized by a lipid content of 19.9%. On the other hand, the amounts of proteins and

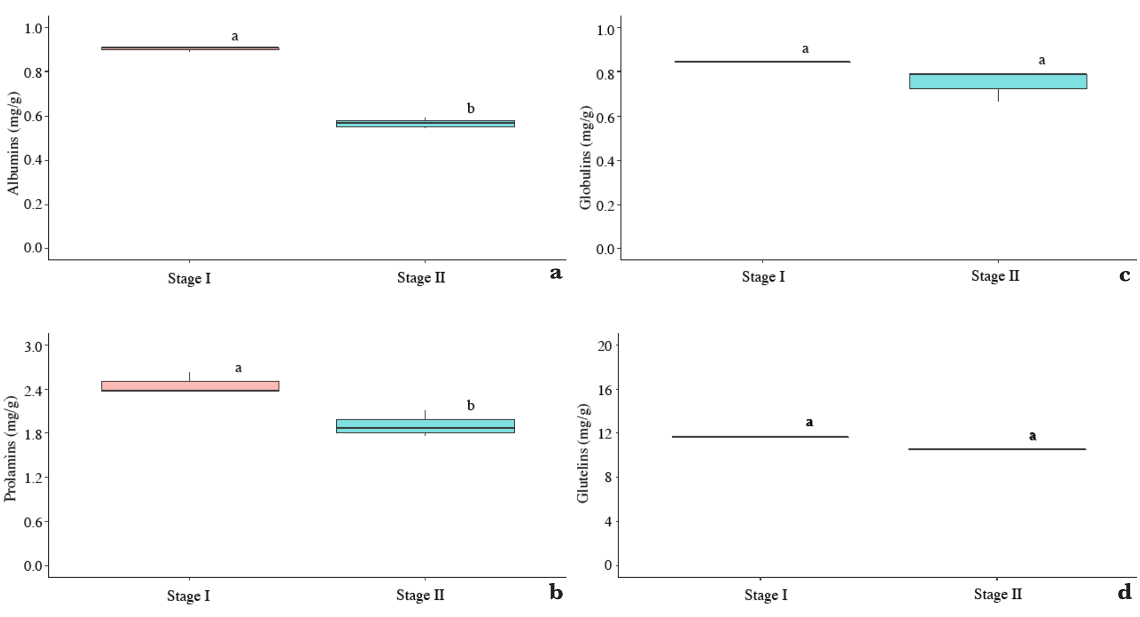

Figure 6 – a-d. Protein fractioning in seeds of *Dicypellium caryophyllaceum* from both stages of maturation – a. albumins; b. globulins; c. prolamins; d. glutelins. * Same letters do not differ statistically at 5% probability ($p < 0.05$) Scott-Knott test.

carbohydrates in mature seeds of *O. porosa* were lower than those reported in the species from the current study, since the former presented values of 3.38% to 2.9% for proteins and 21.20% to 33.4% for carbohydrates (Tonin *et al.* 2006; Simeone *et al.* 2009).

The lack of published data in *D. caryophyllaceum* reveals that little is known about this species. This tree was characterized by the presence of oleaginous seeds because of their high ether content, while starch represented the second most abundant compound (9.5%) in mature seeds. Furthermore, the rates of proteins, sugars, and starch changed during the maturation process, since seeds from immature fruit at stage I presented increased levels of Total Soluble Sugars (4.01%), Reducing Sugars (3.22%), and Total Soluble Proteins (1.57%). Inversely, the highest values of amino acids (0.13%) and starch (9.5%) were observed in seeds from mature fruit (stage II). The visual indicators of maturation proved to be effective under field conditions, being helpful to the early harvesting of fruit with high-quality seeds, and thus avoiding damages by insect predation.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that there is no data sharing of this manuscript.

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