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Morphology and anatomy of the fruit and seed of *Cananga odorata* (Lam.) Hook.f. & Thomson

FERAWATI OKTIA NURHAYANI[1,♥], ARUM SEKAR WULANDARI[2,♥♥], TATIEK KARTIKA SUHARSI[3,♥♥♥]

[1]Program of Tropical Silviculture, School of Graduates, Institut Pertanian Bogor. Jl. Lingkar Kampus, Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8621947, ♥email: oktiaferawati@gmail.com

[2]Department of Silviculture, Faculty of Forestry, Institut Pertanian Bogor. Jl. Ulin, Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8621947, Fax.: +62-251-8621947, ♥♥email: rr_arum@yahoo.com

[3]Departement of Agronomy and Horticulture, Faculty of Agronomy, Institut Pertanian Bogor. Jl. Meranti, Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8629354, Fax.: +62-251-8629352, ♥♥♥email: tatiek.kartika55@gmail.com

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Abstract. *Nurhayani FO, Wulandari AS, Suharsi TK. 2019. Morphology and anatomy of the fruit and seed of* Cananga odorata *(Lam.) Hook.f. & Thomson. Biodiversitas 20: 3199-3206. Cananga odorata* (Lam.) Hook.f. & Thomson is one of the Annonaceae family which has high benefits for humans such as constructions, furniture, cosmetics, perfumes, and traditional medicines. The planting efforts were needed to support the existence of these plants. These plants have low regeneration and germination. The morphology and anatomy of fruit and seed could enhance the understanding of the reproductive biology in the species. There is little information about the morphology and anatomy of fruit and seed of *C. odorata* var. *odorata* forma *genuina.* The aim of this study is to understand the morphology and anatomy of fruit and seed of *C. odorata*. The morphologic description was made from the analysis of the fresh fruits and seeds. The anatomical description was made from the analysis of permanent slides with longitudinal sections of the fruits and seeds. The samples were fixed, dehydrated, alcoholized, embedding in paraffin, and stained with safranin-fast green. Data analysis consists of the morphological observation (shape, color, size and the number of seeds per fruits) and the anatomical observation (the pericarp layers of fruit, the seed coat layers, and the structure in the seed). The fruit of *C. odorata* var. *odorata* forma *genuina* was fleshy, ovoid or obovoid, 0.98-2.75 cm long, 0.42-1.63 cm in diameter, and contain 1-12 seeds. The seeds were pale brown, flattened, ovoid, and hard. The fruit anatomy showed that the pericarp layers derived from parenchymatous cells and differentiated into three zones, i.e. exocarp, mesocarp, and endocarp. The seed has ruminated endosperm with irregular folds. The seed coat was divided into three integuments, i.e. outer, middle, and inner integument. The information about the fruit and seed anatomy of *C. odorata* var. *odorata* forma *genuina* might enhance the understanding of future studies regarding natural reproduction and conservation programs with generative propagation.

Keywords: Anatomy, *Cananga odorata*, fruit, morphology, seed

INTRODUCTION

Cananga odorata (Lam.) Hook.f. & Thomson is one of the Annonaceae family. It has two varieties, i.e. *C. odorata* var. *fruticosa* and *C. odorata* var. *odorata*. *C. odorata* var. *fruticosa* is a widely grown garden shrub which is a dwarf form and seems never to set seed. *C. odorata* var. *odorata* consists of forma *macrophylla* from Java and forma *genuina* from the Philippines which is known as "ylang-ylang". Both forma were trees and set seeds. *C. odorata* var. *odorata* forma *macrophylla* produced cananga oil while *C. odorata* var. *odorata* forma *genuina* produced ylang-ylang oil. Cananga oil is inferior to ylang-ylang oil in perfumery (Turner and Veldkamp 2009). *C. odorata* which used in this study was *C. odorata* var. *odorata* forma *genuina*.

Cananga odorata var. *odorata* forma *genuina* was fast-growing species, a medium-sized evergreen tree, typically 10−20 m in height but occasionally up to 40 m in natural forests in its native Indo-Pacific range. It produced a single main trunk and an uneven spreading crown of drooping branches and twigs bearing leaves in two rows. It was easily recognized by its odd-shaped, very fragrant yellow or greenish-yellow flowers and distinctive aggregate fruit consisting of 8−15 clustered green or blackberries (Parrotta 2009). It grew well in more humid lowland tropics or moist valleys, sometimes with other evergreen and teak trees. It preferred well light places, fertile sandy loam and volcanic soils with altitude 1-1800 m asl, mean annual temperature is 20-27 ºC, mean annual rainfall is 650-4000 mm, and soil with pH 4.5-8. In Java it grew gregariously in moist evergreen forest and in teak forest. The native distribution of *C. odorata* is Cambodia, Indonesia, Laos, Malaysia, Myanmar, Papua New Guinea, Philippines, Solomon Islands, Thailand, Vietnam. Then this plant was distributed to other countries such as Cameroon, China, Comoros, Ivory Coast, India, Jamaica, Madagascar, Reunion, Seychelles, Sri Lanka (Orwa et al. 2009).

Cananga odorata have high benefits for humans. In Malaysia, it used as street tree. In the Pacific Island of Pohnpei, it is used as an agroforestry system that combined with yam (*Dioscorea* spp.). The flowers are used to make garlands and headdresses in Samoa and other Pacific Islands (Parrotta 2009). In Tonga and Samoa the bark is used to treat stomach ailments and as a laxative (Manner and Elevitch 2006).

The woods of *C. odorata* var. *odorata* forma *genuina* are used for construction, furniture, tool handles, wooden shoes, and boxes (Nurfadilah et al. 2017). The flowers are commonly used as an ornamental plant because they are beautiful and fragrant (Handayani 2018). The flowers also used for religious ceremonies such as “Oke Sou” ceremony which is a tradition of welcoming maturity girl in Lako Akediri Village, West Halmahera, Indonesia (Wakhidah et al. 2017); “Balimau Tradition” ceremony by communities in Pariaman, West Sumatera, Indonesia (Hulyati 2014); and “Sura Month Welcoming” ceremony by communities in Nganjuk, East Java, Indonesia (Ayuningtyas and Hakim 2014). The flowers (also leaves and fruits) produce an important essential oil (contain 1-2% volatile oil) widely used in the manufacture of numerous beauty products (Brokl et al. 2013). The essential oil of *C. odorata* can be used as aromatherapy and materials in various industries (Anggia et al. 2018); possessed potential for development as novel natural insecticide/fumigant for stored products (Cheng et al. 2014); can be used to inhibit the growth of bacteria such as *Staphylococcus aureus* (Maulidya et al. 2016); as lotion and perfume manufacture that are more effective in repelling insects because the oil that produced by *C. odorata* var. *odorata* forma *genuina* contain benzyl benzoate, caryophyllene, linalool and eugenol (Budi et al. 2018); as an insect repellent (Sari and Supartono 2014); natural antibacterial because it contains flavonoids and saponins (Dutsuria et al. 2016); antimicrobial, antibiofilm, antifertility, anti-inflammatory, antimelanogenesis activities, effective in treating depression, high blood pressure, anxiety (Tan et al. 2015); antidepressants for humans (Zhang et al. 2016); and as mild natural antioxidant (Pujiarti 2015).

Even though several studies have been conducted in *C. odorata* var. *odorata* forma *genuina*, information about this plant is scarce and mainly limited to essential oil content. The morphology and anatomy of fruit and seed of *C. odorata* var. *odorata* forma *genuina* has never been undertaken. The planting activities to increase their availability in the field depend on generative propagation, so the availability of seeds plays a very important role. A complete morphological and anatomical study of the species could enhance the understanding of the reproductive biology in the species, which is under human threat and has a very low regeneration and germination rates. The aim of this study is to understand morphology and anatomy of fruit and seed of *C. odorata*.

MATERIALS AND METHODS

Materials

The mature and immature fruits of *C. odorata* var. *odorata* forma *genuina* were collected from individual trees which is about 20 years old in February 2019 at IPB University (6º33’23.0”S 106º43’55.0”E). The other materials used in this study were HNO_3 (nitric acid), FAA (formaldehyde acetic acid alcohol), alcohol concentration of 70%, 96%, and absolute alcohol, xylol, paraffin, Haupt adhesive, safranin (2%), fast green (1%), and entellan (rapid mounting medium for microscopy).

Procedures

Morphology

The morphology characters of the fruits and seeds were described and illustrated from 100 fruit, collected from an individual tree. A digital caliper was used for fruit and seed measures (length and diameter). The number of seeds per fruit was also determined.

Anatomy

The anatomical description was made from the analysis of permanent slides made with longitudinal sections of the fruits and seeds. The method used to make the permanent slides followed Sass (1951) with modifications. The fruits were cut longitudinally and soaked in water. Samples were fixed in the HNO3 (3: 1) for 24 hours and covered with aluminum foil. Then, samples were put in the vacuum, fixed in FAA for 24 hours, and dehydrated through alcohol series (alcohol concentration of 70%, 96%, and absolute alcohol) for 24 hours each. Samples were de-alcoholized by xylol-alcohol combinations (1: 3, 1: 1, 3: 1, 1: 0, and 1: 0) for 24 hours each. Then, samples were infiltrated consisting of two stages. They were solid infiltration and liquid infiltration. Samples that have been soaked in xylol solution were given paraffin powder slowly until the solution was saturated. The samples were put in an incubator (± 60 oC) for liquid infiltration. The liquid infiltration was replacing the previous solution with liquid paraffin slowly. This stage starts from ¼, ½, ¾, and 1 full part of the solution. The next process is embedding by putting the sample into liquid paraffin and leaving it until solid. Then, the samples were cut longitudinally with microtome (thick = 20 μm), placed on object-glass which has given Haupt adhesive, dropped with aquadest, and stored on hot plate (temperature = 40 oC) for ± 2 days. Sections were stained with safranine and fast green. The staining starts from : (1) xylol, (2) xylol, (3) xylol: alcohol (3: 1), (4) xylol: alcohol (1: 1), (5) xylol: alcohol (1: 3) , (6) absolute alcohol, (7) 96% alcohol, (8) 70% alcohol, (9) safranin, (10) 70% alcohol, (11) 96% alcohol, (12) absolute alcohol, (13) fast green, (14) absolute alcohol, (15) alcohol: xylol (3: 1), (16) alcohol: xylol (1: 1), (17) alcohol: xylol (1: 3), (18) xylol, and (19) xylol. The sections that have been stained were dropped with entellan (rapid mounting medium for microscopy) and covered with cover glass. Finally, the permanent slides were observed with light microscope to define the pericarp layers and seed description.

Data analysis

The observation of morphologic characters consists of shape, color, size (length and diameter) and the number of seeds per fruits. The anatomical observation was description of the pericarp layers of fruit, the seed coat layers, and the structure in the seed.

RESULTS AND DISCUSSION

Fruit and seed morphology

The fruits of *C. odorata* var. *odorata* forma *genuina* were clustered, fleshy, and ovoid or obovoid shape. The immature fruits were green, and the fruits become blackish when ripe. The fruit has 0.98-2.75 cm long and 0.42-1.63 cm in diameter. There are 1-12 seeds in one fruit of *C. odorata* var. *odorata* forma *genuina* (Figure 1.A-B). The seeds which have been extracted were pale brown, flattened, and ovoid in shape. The seed coat structure was hard (Figure 1.C).

Fruit anatomy

The fruit of *C. odorata* var. *odorata* forma *genuina* developed anatomically, but the changes that occurred were not significantly. The immature fruit was composed of pericarp layers that were small and still compact (Figure 2.A). Then the pericarp layer continued to develop into a layer with larger cells. The fruit develops with increasing in size because of cell expansion (Figure 2.B-D). The pericarp of fruits of *C. odorata* var. *odorata* forma *genuina* was differentiated into three zones, i.e. exocarp, mesocarp and endocarp (Figure 3.A).

The exocarp was composed of unicellular layers. They were irregular and plano-convex cells. The plano-convex cells consisted of 17-20 layers of parenchymatous cells. The exocarp was 0.72-0.83 mm thick, the mesocarp was 0.5-1 mm thick and the endocarp was ≤ 0.1 mm (Figure 3.B). The mesocarp was composed of many layers of parenchymatous cells which had a plano-convex shape, smaller and thinner than the exocarp layers. The mesocarp was fleshy and contained small granules (Figure 3.C). The endocarp was a thin tissue that consisted of 6-8 layers and surrounding the seeds (Figure 3.D).

Seed anatomy

The seeds in the immature fruits continued to develop until the fruits were mature. The seed coat of the immature fruit had a thinner layer ≤ 0.03 mm because it did not become thickening. The seed from immature fruit did not have a peculiar feature on the surface of the endosperm. Then, the seed develops with the endosperm liked irregular folds and continued to develop occupying most of the seed. the seed of *C. odorata* var. *odorata* forma *genuina* had flattened shape and pitted surface (Figure 4.A). The endosperm was in the middle of the seed and the surface was irregular folds.

The seed coat was derived from integuments. They were differentiated into three zones, i.e. the outer, the middle, and the inner integument. The thick of the seed coat of *C. odorata* var. *odorata* forma *genuina* was 0.1-0.5 mm. The outer integument was formed by the cube-shaped cell. The middle integument derived from thin tissues. The inner integument derived from thinner tissues than the outer integument (Figure 4.B).

Discussion

There are similarities between the observation and the literature on the variable fruit color. The fruit color of *C. odorata* var. *odorata* forma *genuina* is the same as the literature that is dark green to black (Table 1). Several fruits developed from each inflorescence. These compound fruits have consisted of 6−12 (occasionally up to 20) in axillary clusters. The fruit of *C. odorata* var. *odorata* forma *genuina* was pendulous consisting of 7-16 separate about 25 mm by 15 mm on stalk 10-20 mm long. The mature seed was flattened ovoid, pale brown, surface pitted, with a rudimentary aril, had ± 6 mm diameter, arranged in one or two rows, and embedded in an oily nearly tasteless yellow pulp (Orwa et al. 2009; Parrotta 2009).

Figure 1. The morphology of fruit and seed of *Cananga odorata* var. *odorata* forma *genuina*. A. The fresh fruit when immature, B. The fruit which has mature blackish and fleshy, C. The seeds which have been extracted. Bar = 3 cm

The result from observation on the variable length and the number of seeds in fruit was different (Table 1). It can be occurred because of several reasons, such as fruit growth and maturation was influenced by the pollination process (Wietzke et al. 2018). The measures of the fruits from observation were longer or shorter than literature can be caused by different climatic conditions and growing sites. Rodrigues et al. (2018) argued that the climatic conditions of an area influenced the development of pollen. The longer duration of the rainy season can support pollination. The physical characteristics of the fruit produced in the rainy season were also greater than in other seasons. There was smaller size of fruit, which found in this observation so there was fruit with smaller number of seeds than literature.

The fruit of *C. odorata* var. *odorata* forma *genuina* developed and increased in size until it was ripe. Handa et al. (2012) argued that fruit development can be divided into three phases. The first phase was the development of the ovary and the initiation of the cell division, together called a fruit set. In the second phase, cell division was the predominant feature. During the third phase, fruit increased in size by cell expansion. Once the fruit cells were fully developed and the ripening process ensued.

The pericarp of the fruit may be divided into layers, referred to as exocarp (outer layer), mesocarp (middle layer), and endocarp (inner layer) (Silva and Souza 2009). The pericarp as a whole or a major was composed by parenchymatous. The exocarp derived from the ovarian epidermis that may be smooth, often covered by an epicuticular wax and a cuticle, or only by cuticle (Krishnamurthy 2015). The exocarp was a dorsal ovarian epidermis, which consisted of subepidermal layers of the mesophyll ovary. These cells had very thick walls with simple holes visible. The mesocarp derived from ovarian mesophyll that had fibrous because of the differentiation of fibers around the vascular bundles (Marzinek and Mourao 2003). The mesocarp was derived from parenchymatous cells, which were large, isodiametric, and thin walls (Retamales et al. 2014). The result showed that the mesocarp contained small granules that consistent with the reported by Murwani (2012) for *Annona muricata, A. reticulata*, and *A. squamosa* who indicated that the small granules in the mesocarp were starch because when they were reacted with KI_3 (potassium iodide) solution they turn blue (Murwani 2012). The endocarp was composed of the ventral epidermis of the ovary wall, whose cells undergo a gradual elongation and lignification (Marzinek and Mourao 2003).

In all species of Annonaceae, the raphe/antiraphe formed a hoop that encircled the seed (Johnson and Murray 2018). The seed of *C. odorata* var. *odorata* forma *genuina* had irregular folds form the rumination. It was consistent with the statement of Rao (1983) that some seeds from the family of Annonaceae had ruminate endosperm. *Annona squamosa* had endosperm that was cellular, ruminate, occupied most of the seed, and was the main reserve tissue. The endosperm presented voluminous cells with different sizes, but generally isoradiometric, meristematic, with dense cytoplasm that were differentiated in an anticlinal sense. The slightly thickened walls presented cells with unidentified reserve structures, presumably of a lipid and pectin content. In the micropylar region, cells with smaller size and density were located, comprising no more than eight endospermatic cell layers, separating the embryo from the micropylar plug (Martinez et al. 2013).

Svoma (1997) argued that rumination served to supply water and nutrients to the embryo and endosperm. However, there are doubts because the supply of nutrients and water during seed development and in the initial stages of germination was only through perichalaza. Meanwhile, Bayer and Appel (1996) argued that rumination, often consisted of oil and phenolic substances that make seeds less attractive to seed predators. The increasing contact between the integument and the endosperm facilitated the supply of water, oxygen, and nutrients to the development of the endosperm or embryo. In addition, the rumination was more permeable to gas than endosperm, facilitating gas exchange (Svoma 1997).

The rumination was separated from each other by inclusions or transverse folds of the outer and inner integuments. The folds were observed in the inner and outer integument consist of transverse fibers that penetrate the endosperm to produce ruminations that are distributed along the length of the seed (Martinez et al. 2013). The cause of rumination was the rest of the seed coat or the endosperm. The rumination also due to localized meristematic activity during post-fertilization stages resulting in ingrowths or infoldings of the seed coat and ridges furrows in the endosperm. These formations often involve (1) differential radial elongation of the component cell of any one layer (or the only layer) of the seed coat, or (2) the participation of the entire seed coat (Krishnamurthy 2015). The ruminate developed because of a small plate that runs along the side of the seed. The immature seeds form ruminate folds through intensive anticlinal cell division in one of the deep integument layers. Furthermore, the middle part of the inner integument layer forms the folds of ruminate (Svoma 1998). In Annonaceae, the rumination formed by the ingrowths is formed either by the elongation of the constituent cells (Krishnamurthy 2015). The seed of Annonaceae had a small embryo that was considered underdeveloped and immature, because of this immaturity, it was likely that more time was needed for its embryo growth and germination after dispersal than in other species (Martinez et al. 2013).

Table 1. The comparison of result from observation and literature of *Cananga odorata* var. *odorata* forma *genuina*

Variables	Observation	Orwa et al. (2009)	Parrotta (2009)
The color of fruit	Green to blackish	Dark green to blackish	Green to black
The length size of fruit	7.40-27.5 mm	10-20 mm	15-23 mm
The number of seed in fruit	1-12 seeds	2-12 seeds	2-12 seeds

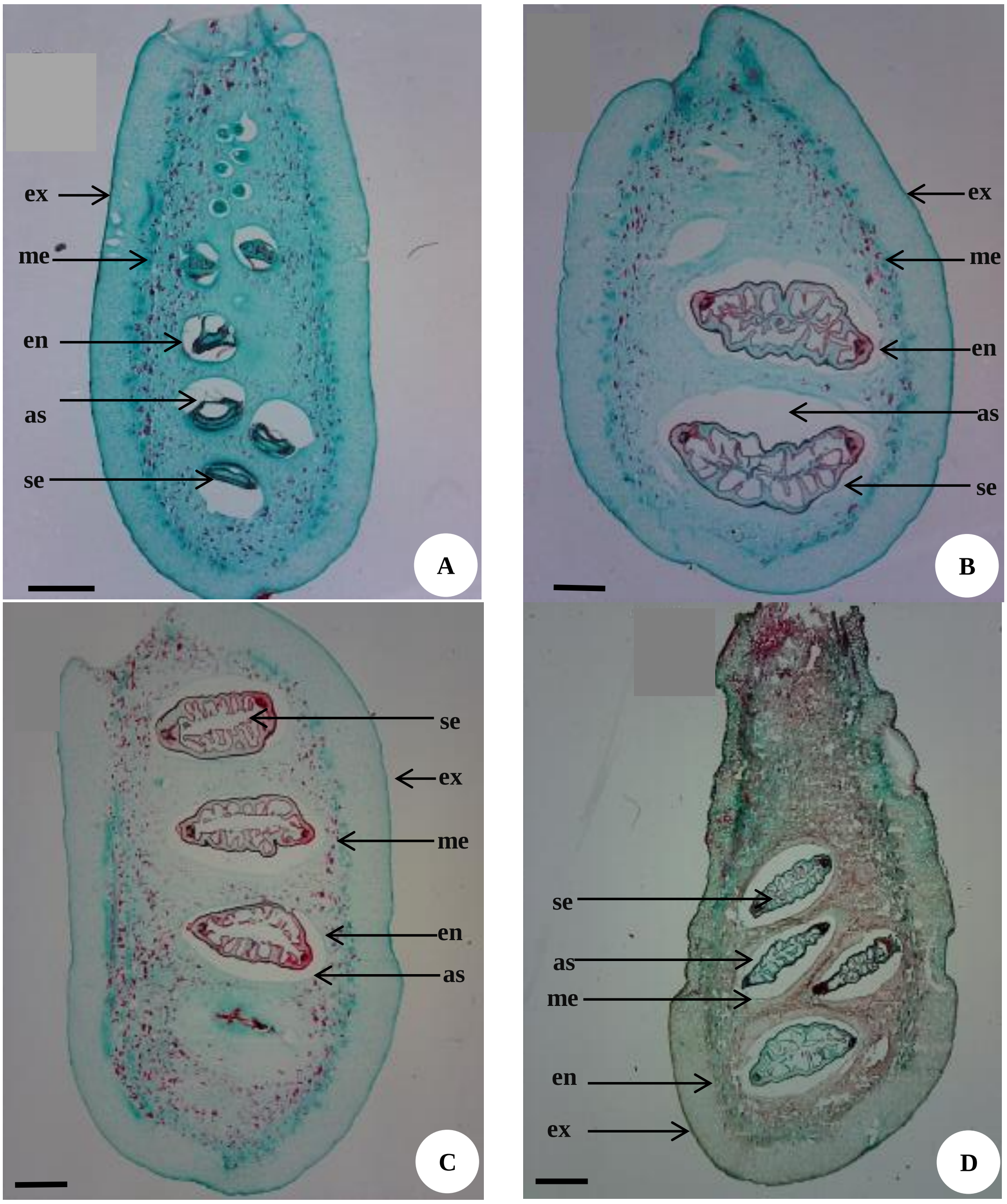

Figure 2. Longitudinal sections of fruit of *Cananga odorata* var. *odorata* forma *genuina* in different stages. A. Immature fruit with 1 cm long, B. Immature fruit with 1.2 cm long, C. Immature fruit with 1.5 cm long, D. Mature fruit with 1.5 cm long. as: air spaces, en: endocarp, ex: exocarp, me: mesocarp, se: seed. Scale bars = 1 mm

The seed of *C. odorata* var. *odorata* forma *genuina* had hard, thick, and fibrous characteristics as the main protector of the seed coat (Rao 1983). The seed coat was derived from the integuments. The term testa was often applied to the seed coat originated from the only integument in unitegmic ovules or to the seed coat originated from the outer integument in bitegmic ovules. The inner integument formed the tegmen that again formed a part of the seed coat. Many people denote the entire seed coat (derived from both integuments) as testa. externally the seed coat varies in color and features of the seed surface that may be smooth, wrinkled, striate, ribbed, furrowed, reticulate, tuberculate, hairy, spinescent, gummy, etc. The seeds with fleshy or very hard testa of the component cells were complicated and diverse. The outermost layer of hard seed was usually made up of thick-walled cells, often covered by a thick cuticle. The thick-walled cells were sclereids (Krishnamurthy 2015).

The seeds of Annonaceae show high tissue differentiation resulting in a complex seed structure. The outer integument and adjacent raphe were differentiated into three regions called exotesta, mesotesta, and endotesta. The outer epidermis was referred to as the exotesta, the inner epidermis as the endotesta, and all the intervening layers are placed in the mesotesta (Svoma 1998).

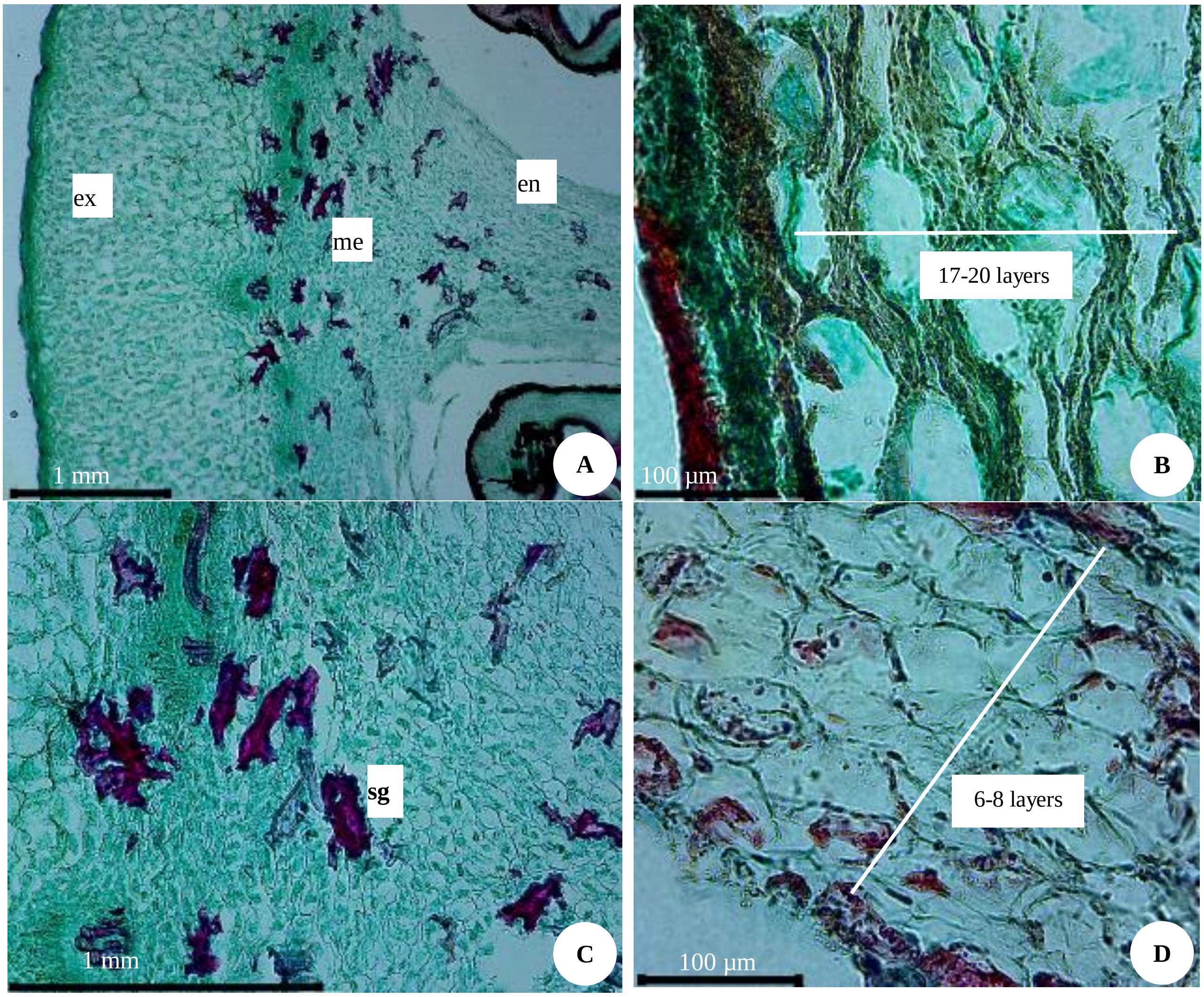

Figure 3. The pericarp of mature fruit of *Cananga odorata* var. *odorata* forma *genuina*. A. Longitudinal section of pericarp, B. Detail the parenchymatous cells of exocarp, C. Longitudinal section of mesocarp, D. Longitudinal section of endocarp. en: endocarp, ex: exocarp, me: mesocarp, sg: small granules

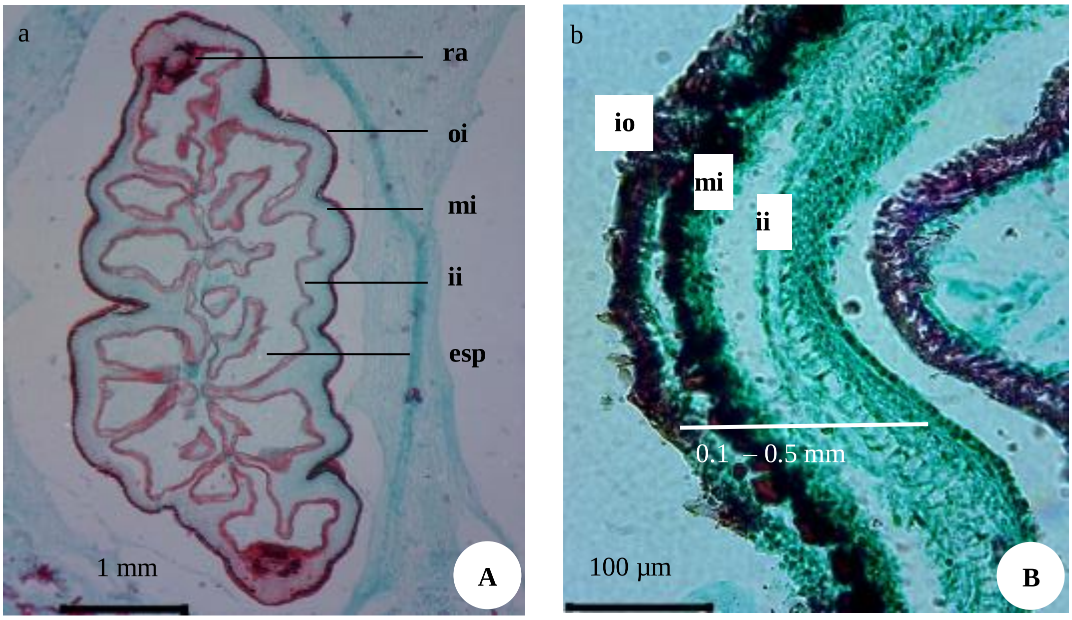

Figure 4. The seed of *Cananga odorata* var. *odorata* forma *genuina.* A. Longitudinal section of the seed, B. Detail of the integument. esp: endosperm, ii: inner integument, mi: middle integument, oi: outer integument, ra: raphe

The observation of *C. odorata* var. *odorata* forma *genuina* was consistent with the reported for Martinez et al. (2013) for one of species of Annonaceae family (*Annona squamosa*) who indicated that, the outer integument called exotesta is uniseriate, consisted of isoradiometrics, tangential elongated, and cuboid cells with thin walls and thickening of the periclinal in the outer wall and subsequently anti clinical elongated cells as palisade tissue. The hypodermis consisted of one or two layers of thin walls, compressed and crystalline in appearance. The mesotesta had two layers with lignified fibers and is interrupted by elongated, longitudinal and transversal, oblique, very dense cells that might correspond to macrosclereids subsequent to brachysclereids covered with thin cuticles and one to three layers of hypodermic cells. Endotesta consisted of transversal, isoradiometric fibers with thickened multilayer walls, but it is less than mesotesta. In the longitudinal medium of the endotesta, perichalaza fragments were observed submerged in the outer integument, where it is possible to identify more than two rows of vascular tissue and thin-walled parenchyma cells with small intercellular spaces. The endotesta rumination consists of proliferation and extends to the micropyle region, where the microcapillary plug has many appearances.

The percentage of seed germination of *C. odorata* var. *odorata* forma *genuina* was still low at 58.05 % (Handayani 2008). This study showed that the seed coat of *C. odorata* var. *odorata* forma *genuina* was thick, so the low germination can be caused by dormancy from the thick seed coat. The thickening and the level of lignin content in the seed coat was one of the limiting factors in seed germination which can cause seed germination to be low (Yulianti et al. 2015). From this study we can decide that the most appropriate physical or chemical methods were needed to breach seed dormancy of *C. odorata* var. *odorata* forma *genuina*, so we can get the fast and high seed germination. The morphology of *C. odorata* var. *odorata* forma *genuina* can be used as information to find out that the availability of seeds is enough. The regeneration of this species depends on generative propagation, so the information about seed is very important for planting activities and conservation programs. Incorporating these anatomical findings in future studies, can provide a better understanding of the evolution of this species. The comprehensive use of anatomical characters in the broad phylogeny of *C. odorata* var. *odorata* forma *genuina* is recommended.

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