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Morphological and Anatomical Development of True Mangrove Species Generative Organs in Ngurah Rai Forest Park, Bali

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Abstract. Plant phenology is the study of the growth time of plants that occurs repeatedly every year. In the process, growth is accompanied by development at both the cellular and tissue levels of differentiated plants, resulting in the formation of organs with specific functions as a form of environmental adaptation. The lack of mangrove reproduction data motivates this study to investigate the development and differentiation of generative organs in several species of true mangroves within Ngurah Rai Forest Park. Sampling was conducted in the Ngurah Rai Forest Park, while observations were made in the Plant Structure and Development Laboratory at Udayana University. The research method involved observing and comparing changes in the morphological and anatomical structures of the species *Avicennia marina*, *Sonneratia alba*, and *Xylocarpus granatum*. The results found that all species have all the flower's main parts (stamen, pistil, petal, sepal), with some species having nectar around the receptacle to attract pollinators. The fruit development in *X. granatum* formed without any of the flower parts, while in *S. alba*, it clearly involves the calyx and stylus as its main parts of fruit, and *A. marina* only has a small calyx as part of the fruit. Based on the sprout characteristics, *S. alba* and *X. granatum* are classified as normal fruits, while the *A. marina* has cryptoviviparous fruit. As for seeds, the endospermic seed was observed in *S. alba* and *X. granatum*, while the *A. marina* has a non-endospermic seed with fully developed cotyledons.

Keywords: Development; Generative organs; Tissue differentiation; Fertilization; Flowering

I. INTRODUCTION

Mangroves are plant communities that live in areas affected by the tides of seawater, and are naturally distributed across tropical to subtropical regions [1]. Bali is one of the islands in Indonesia with limited mangrove areas. Most of the mangrove ecosystem in Bali is managed by Ngurah Rai Forest Park (Tahura), which spans an area of 1,373.50 ha and stretches from Sanur to Tanjung Benoa [2]. Rehabilitation efforts have been intensively carried out over the past few years by planting mangrove seedlings in the Tahura Ngurah Rai area [3]. The success of mangrove rehabilitation is influenced by various factors, including the availability of a sufficient number of plant seedlings with ready-to-plant sizes, quality, and health at the appropriate time [4].

Mangroves generally bear fruit all year round but can bear massive fruit in certain seasons. Mangrove seeds collected during the fruiting season will be more efficient and have better seed quality [4]. The peak season of mangrove fruiting varies among species and is influenced by climatic and edaphic conditions, which are locally determined by the eco-physiological characteristics of the mangroves. However, phenological data is very necessary, especially in restoration planning, seed collection, and mangrove nursery activities [5].

Plant phenology is a sequence of developmental stages that occur repeatedly each year in response to climate conditions [6]. Mangrove phenology is related to the time of flowering and fruiting until the fruit falls. This process begins with the transformation of the plant's vegetative organ into the generative organ, which involves the pro of flowers, followed by fertilization to form fruits [7]. Phenological studies can be carried out on two components of plants, the vegetative components such as in leaves, and the generative components including flowers and fruits [8]. Duration, intensity of flower formation, and intensity of fruit formation can be used as indicators of mangrove reproduction success, allowing for the maximization of fruit or propagule collection and making mangrove rehabilitation activities more sustainable [9]. Generative phase phenology can indicate the allocation of energy in the formation of fruits and seeds [10].

The phenological phase of plants is closely related to the development of cells and tissues, resulting in the formation of organs that will undergo differentiation [11]. In general, the flowering process of angiosperms consists of the appearance of flower parts, including sepals, petals, stamens, and pistils [12].

The success of the early stage of development will impact the subsequent stages of development. The stages of flowering, as well as the morphological and anatomical characteristics of flower and fruit development, will be studied in this research. Phenological studies that have been carried out are generally not supported by morphological changes and anatomically constituent tissue data; therefore, this research is important to conduct, as anatomical and morphological development are structural responses to environmental changes. Through this research, the differences in development patterns and tissue modifications of each species can be identified and utilized in the preparation of plant breeding explants through ovule culture, allowing for the determination of the optimal time to harvest ovules.

II. METHODS

Study site

This research was carried out from January to May 2024. The locations of several true mangrove generative organs suitable for sampling are in the Ngurah Rai Forest Park area, particularly at Mertasari Beach, Batu Lumbang, and the Mangrove Information Center (MIC). The research continued at the Plant Structure and Development Laboratory, Department of Biology, Udayana University.

Research Tools and Materials

The tools used in this research were a razor blade, a cutter blade, plastic clips, saws, a ruler, black manila paper, and a Canon 1300D camera. The material in this study is generative organs (flowers and fruits) from three true mangrove species (*Avicennia marina, Sonneratia alba,* and *Xylocarpus granatum*). The flowers observed were in the initiation phase, bud phase, and blooming phase, while the fruit was observed in the initiation phase, young fruit phase, and ripe fruit phase.

Research procedure

Each species in each location was tagged to mark the branches of the phenological phases. Observations were made by placing flower and fruit organs on black manila paper aligned with a ruler as a scale and then documented using a camera. Flower and fruit samples were cut longitudinally using a razor blade, cutter blade, or saw, and then compared to the changes in unique morphological and anatomical structures of different species.

In the flowering phase, the flower initiation phase is marked by the emergence of flower protrusion on the axillary leaf, the flower bud phase is marked by the formation of flower primordium bud on the axillary leaf until the flower bud cracked, and the blooming flower phase is marked by the opening of the flower petals accompanied by the fall of flower accessory.

In the fruiting phase, the fruit initiation phase is marked by fertilization of the flower, followed by the fruit initiation. The young fruit phase is characterized by fruit set that changes in shape, and the ripe fruit phase is characterized by fruits that undergo changes in color, shape, and size.

The observed structural changes include the development of existing and reduced fruit seedlings, seeds, and flower accessories [13-15]. The data obtained were analyzed descriptively and presented in the form of images for each development stage.

III. RESULTS AND DISCUSSION

Results

Avicennia marina

Flowering phases

The flower of *A. marina* changed in size, shape, and corolla color from light green during the flower initiation stage to yellow at the flower bud stage as shown in [Figure 1a (A-C)]. At the blooming stage, the flower accessory is open with 4 petals, 5 sepals, and 4 stamens as shown in [Figure 1a (D-E)]. At the initiation stage, the beginning of flower buds is when the perianth, androecium, and gynoecium are formed, as shown in Figure 1b-1c. At the stage of flower buds to bloom, the flower structure consists of a corolla that is composed of petals, stamens (anthers and filaments), pistils (stigmas, styles, and ovary), and a calyx composed of sepals, as in (Figure 1d-f). The change in corolla color to yellow at the flower bud stage, as well as the change in color of the anthers from white to brownish, can be observed in (Figures 1d and 1f).

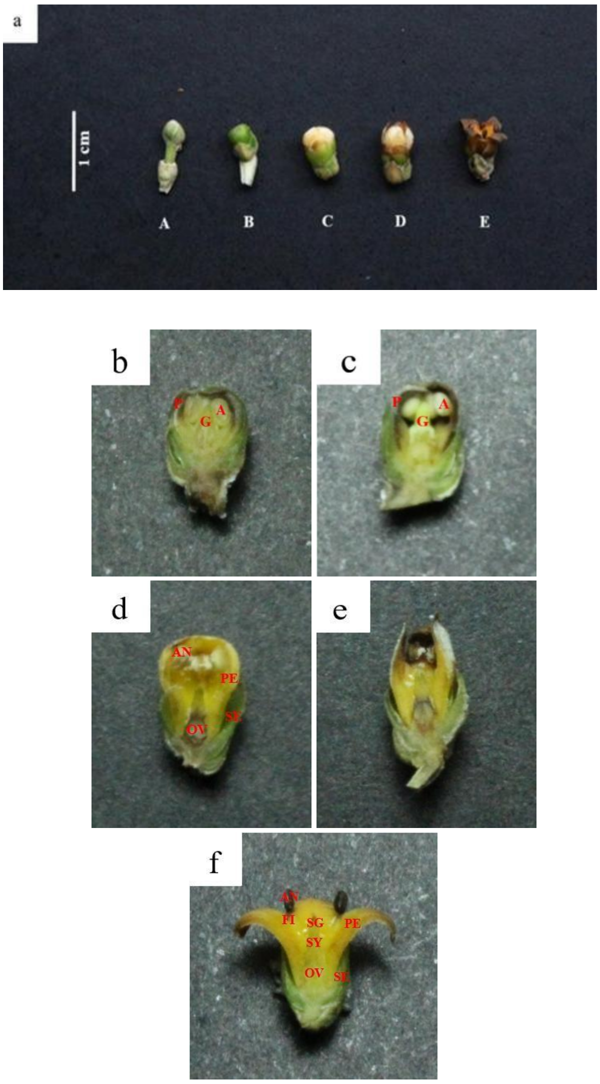


Figure 1. Morphological and anatomical structure of *A. marina* flower (a) morphology of flower (A: initiation, BC: bud, D-E: bloom), (b) anatomical structure of flower at the initiation stage, (c-d) anatomical structure of flower at bud stage, (e-f) anatomical structure of flower at bloom stage (A: Androecium, AN: Anther, FI: Filament, G: Gynoecium, OV: Ovary, P: Perianth, PE: Petal, SE: Sepal, SG: Stigma, SY: Style).

Fruiting phases

The fruit of *A. marina* has a rounded shape with a pointed tip, is green in color, and has a hairy surface. The changes that occur include changes in the shape of fruit from pointed to more rounded, along with the increase in size, as shown in [Figure 2a (A-D)]. The fruit initiation development is marked by the fall of petals and stamens as an indication that pollination has occurred. The structure of the fruit initiation development consists of flower accessories that are still left behind, as in Figure 2b. In the young fruit stage, seeds begin to form, accompanied by an ovary that thickens into a pericarp, as in Figure 2c. At the stage of fruit ripening, the pericarp becomes more depressed and thins along with the growth of the embryo and other seed-making structures. The embryo inside the seed has shown the radicle, cotyledon, caulicle, and plumule as shown in Figure 2d. In addition, it was observed that the fruit was reduced with the pistil still left in (Figure 2e-f). The outer part of the cotyledon can be observed in (Figure 2g), while the appearance of the inside of the 2-layer cotyledon, as well as the presence of plumule, hypocotyl, and radicle, is shown in (Figure 2h).

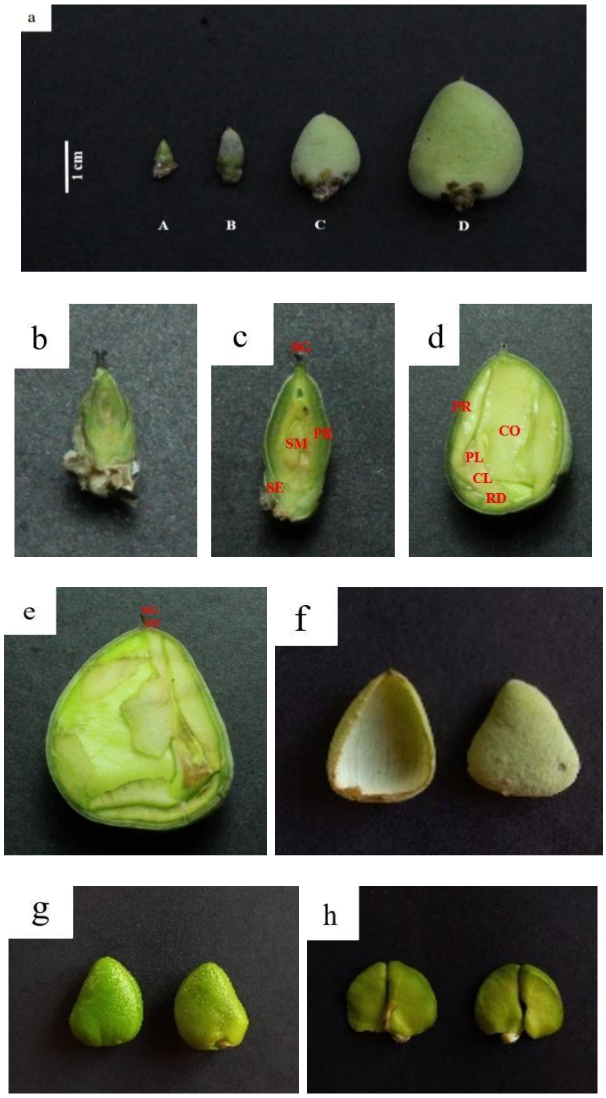


Figure 2. Morphological and anatomical structure of *A. marina* fruit (a) fruit morphology (A: initiation, B-C: young fruit, D: ripe fruit), (b) anatomical structure of fruit at the initiation stage, (c) anatomical structure of fruit at young fruit stage, (d-e) anatomical structure of fruit at ripe fruit and cotyledon stage, (f) reduced fruit, (g) cotyledons with plumule, hypocotyl, and radicle (CL: Caulicle, CO: Cotyledon, PL: Plumule, PR: Pericarp, RD: Radicle, SE: Sepal, SG: Stigma, SY: Style).

Sonneratia alba

Flowering phases

S. alba flowers changed in size during the flower initiation stage development to the early stage of flower buds as shown in [Figure 3a (A-B)]. At the flower bud stage, there is also a change in size as in [Figure 3a (B-C)]. Perianth opens at the blooming stage with 6-8 sepals green on the outside and white on the inside, 6-8 petals in white, long, numerous, and white stamens, and a pistil with a long style as in [Figure 3a (D-E)]. *S. alba* flowers undergo structural changes at the initiation stage and the beginning of flower buds, where the perianth, androecium, and gynoecium are formed, as shown in Figure 3b. At the stage of a flower bud to blooming flower, the flower structure consists of a corolla composed of petals, stamens (anthers and filaments), pistil (stigmas, styles, ovary, and ovule), calyx composed of sepals, and nectar as in (Figure 3d-f).

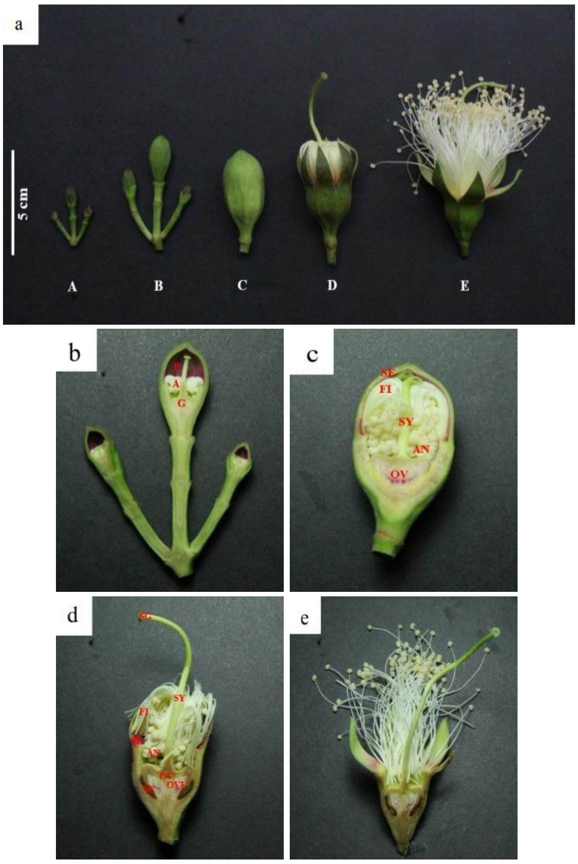


Figure 3. Morphological and anatomical structure of *S. alba* flower (a) flower morphology (A: initiation, B-C: bud, D-E: bloom), (b) anatomical structure of flower at the initiation stage, (c) anatomical structure of flower at bud stage, (d) anatomical structure of flower at bloom stage, (e) stamens released when the flower fully blooms (A: Androecium, AN: Anther, FI: Filament, G: Gynoecium, NE: Nectar, OV: Ovary, OVU: Ovule, P: Perianth, PE: Petal, SE: Sepal. SG: Stigma, SY: Style).

Fruiting phases

The changes that occurred included a change in shape from an oval round to a perfect round, an increase in height and diameter, as well as a change in the color of the perianth that was left behind, namely the calyx from reddish to green and the stigma and style that was light green to dark green as in [Figure 4a (A-D)]. The fruit initiation development is marked by the fall of petals and stamens as an indication that pollination has occurred.

The anatomical structure at the fruit initiation development is composed of pistils (stigma, style, ovary, and ovule), calyx composed of sepals, and nectar, as shown in Figure 4b. In the young fruit stage, seeds begin to form, accompanied by an ovary that undergoes thickening into pericarp, as shown in (Figure 4c-d). At the stage of fruit ripening, the ovary is composed of exocarp, mesocarp, endocarp, and seed, as shown in Figure 4e.

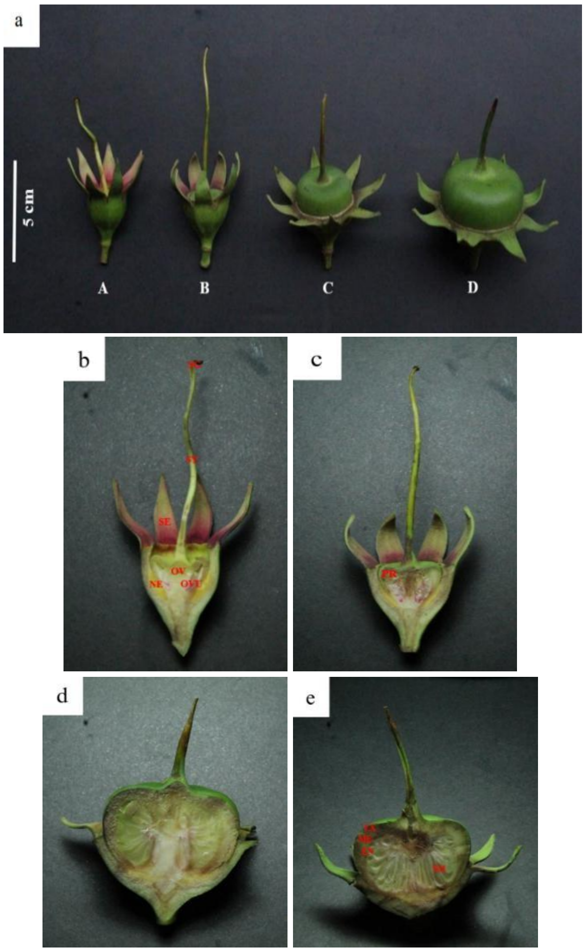


Figure 4. Morphological and anatomical structure of *S. alba* fruit (a) fruit morphology (A: initiation, B-C: young fruit, D: ripe fruit), (b) anatomical structure of fruit at the initiation stage, (c) anatomical structure of fruit at young fruit stage, (d-e) anatomical structure of fruit at ripe fruit stage (EN: Endocarp, EX: Exocarp, ME: Mesocarp, NE: Nectar, OV: Ovary, OVU: Ovule, PR: Pericarp, SE: Sepal, SG: Stigma, SM: Seed, SY: Style).

Xylocarpus granatum

Flowering phases

The flower of *X. granatum* has a change in flower size during the flower initiation stage development to the early stage of flower bud as shown in [Figure 5a (A-B)]. At the flower bud stage, there is a change in the size and color of the calyx from light green to light yellow, as shown in [Figure 5a (C)]. The flower accessory is open at the bloom stage with four white petals, four light yellow sepals, and eight stamens as shown in [Figure 5a (D)]. The flower of *X. granatum* undergoes structural changes during the initiation stage and the early development of the flower bud, when the perianth, androecium, and gynoecium are formed, as shown in Figure 5b-c. At the stage of flower bud to blooming flower, the flower structure consists of a corolla composed of petals, stamens (anthers and filaments), pistils (stigmas, styles, and ovary), calyx composed of sepals, and nectar, as shown in Figure 5d-e.

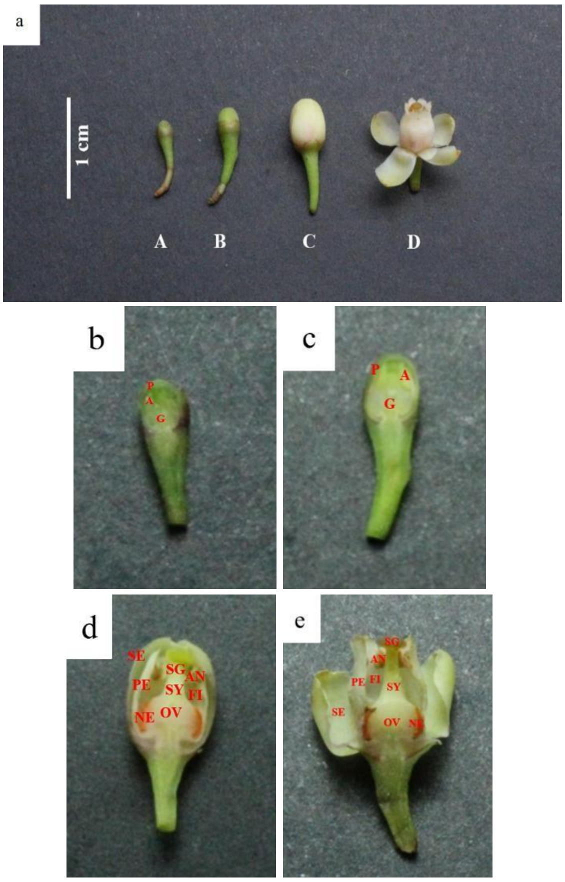
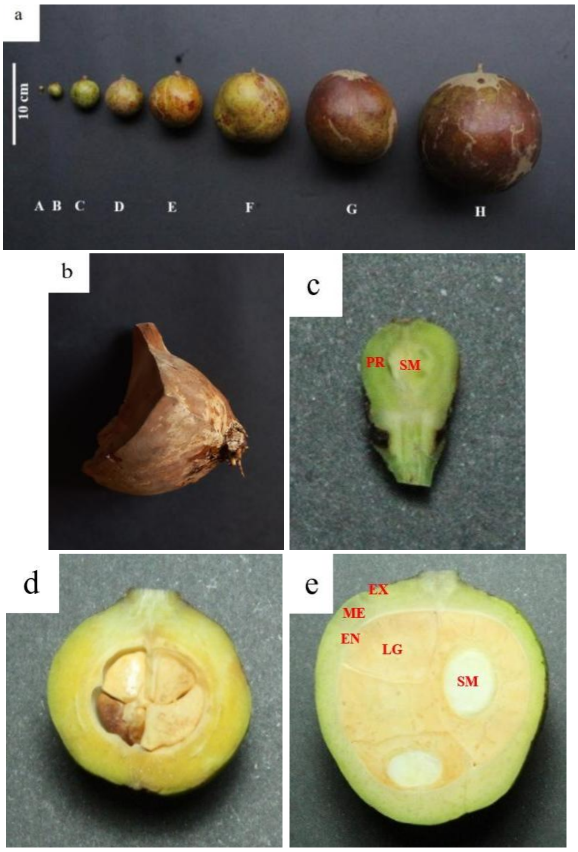


Figure 5. Morphological and anatomical structure of *X. granatum* flower (a) flower morphology (A: initiation, BC: bud, D: bloom), (b) anatomical structure of flower at the initiation stage, (c-d) anatomical structure of flower at bud stage, (e) anatomical structure of flower at bloom stage (A: Androecium, AN: Anther, G: Gynoecium, NE: Nectar, OV: Ovary, P: Perianth, PE: Petal, SE: Sepal, SG: Stigma, SY: Style).

Fruiting phases

The fruit of *X. granatum* is spherical, with morphological changes including changes in size in the form of height and diameter of the fruit, and color changes from light green to yellow to brownish, as in [Figure 6a (AH)]. One fruit consists of 6-16 seeds, each with a thick cork layer, as shown in Figure 6b. The fruit initiation development is marked by the fall of the perianth and stamens as an indication that pollination has occurred. The structure at the initiation of fruit development consists of seeds that begin to form, accompanied by an ovary that undergoes thickening into pericarp, as shown in Figure 6c.

In the young fruit stage, there is a thickening of the pericarp and an increase in seed size, as in Figure 6d. In addition, at the young fruit stage, the pericarp is increasingly depressed and thinning along with seed growth, accompanied by thickening of the cork layer as in (Figure 6e-g). At the stage of fruit ripening, the structure of the fruit and seeds gets harder, accompanied by the appearance of radicles as in (Figure 6h-i). The seeds are coated by a layer of cork and spermoderm. The embryo inside the seed has developed the radicle, cotyledon, and plumule, as shown in Figure 6j.



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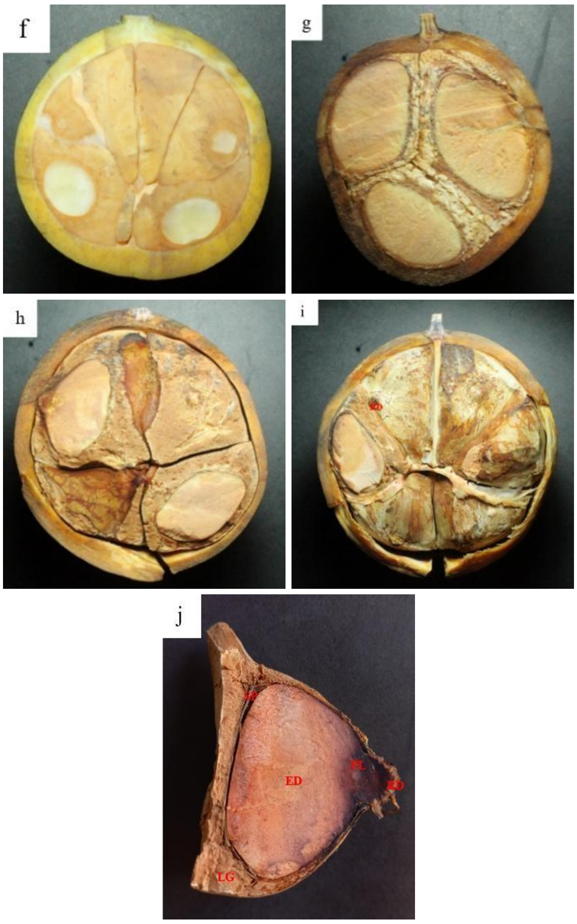

Figure 6. Morphological and anatomical structure of *X. granatum* fruit (a) fruit morphology (A: initiation, B-F: young fruit, G-H: ripe fruit), (b) seed morphology, (c) anatomical structure of split type fruit at the initiation stage, (d-g) anatomical structure of split type fruit at young fruit stage, (h-i) anatomical structure of split type fruit with cork layer covering the seed at ripe fruit stage, (j) anatomical structure of seed with thick cork layer (ED: Endosperm, EN: Endocarp, EX: Exocarp, ME: Mesocarp, PL: Plumule, PR: Pericarp, RD: Radicle, SM: Seed, SP: Spermodermis).

Discussion

Based on the results, the change in flower structure in the three true mangrove species, in general, involves the formation of flower accessories, male reproductive organs (androecium), and female reproductive organs (gynoecium) during the initiation stage of development. As it develops, flowers grow through an increase in size due to cell growth and elongation, differentiation, which involves the emergence of new cell structures with more specific functions, and morphogenesis, which forms special structures in plants [16].

This is in accordance with the development of the flower organ of dicotyledonous plants, where the flower accessory is a modification of the leaf organ, with the sepals formed in the first whorl and petals in the second whorl. In the third whorl, the stamens are formed, while in the fourth whorl the pistils are formed, which are the central part of the flower and end the flower axis [17].

Flowers can grow through the tips of the stalks and or leaf axils, which begin with the appearance of small buds and then enlarge along with growth until fertilization occurs. During the bud stage, swelling occurs caused by the development of the ovary, stamens, and pistils [18]. The increase in flower bud size occurs along with the differentiation process of constituent tissues [19]. The larger the bud size, the larger the flower size [16]. The pollination process in *A. marina* is supported by striking flower colors, while *S. alba* and *X. granatum* are supported by the presence of nectar. It also shows that mangrove species rely on pollinators, especially those with nectar. Nectarine flowers tend to attract insects as the pollinators, so the flowering phenology should align with the pollinator visitation to increase the probability of pollination.

After the fertilization process, there are pistils and sepals that are still left behind and develop into fruit structures. Pistils develop into fruits that serve as food reserves and seed protectors during the development period. The sepals that remain intact provide protection for the fruit from exposure to sunlight so that the fruit does not dry out quickly [20]. There are two types of mangrove fruits obtained, namely the normal fruit type in *S. alba*. and *X. granatum*, as well as the cryptovivipary fruit type in *A. marina.*

Each species has its own unique structure in the fruit and seeds. The fruit wall (pericarp) *of A. marina* thins as the embryo that forms the two cotyledons develops. The fruit of *S. alba* has pom-pom-like sepals as well as reduced nectar chambers during the development phase of the fruit. The sepals of *S. alba* will separate from the rest of the fruit when the fruit falls. The fruit of *S. alba* consists of many locules, so it can produce hundreds of seeds in one fruit. The fruit *of X. granatum* has undergone significant changes in terms of size. X. *granatum* seeds undergo modification of the outer shell of the seed (testa) into a thick cork layer [21].

Angiosperm is characterized by double fertilization, which initiates the development of embryonic and endosperm structures as a result of the zygote and central cells that have been fertilized [22]. Based on the results obtained, endospermic seeds are found *in S. alba* and *X. granatum,* while non-endospermic seeds are found in *A. marina*. Endospermic seeds are characterized by the presence of endosperm as a nutrient provider to the embryo, while non-endospermic seeds are characterized by embryos that get nutrients from the surrounding tissues [23]. In some conditions, the growing cotyledons can take nutrients from the endosperm so that the endosperm is thin and absent when the seeds are ripe [24], but when the embryo sprouts, the cotyledon acts as a reserve of nutrition for the embryo's initial growth.

The characteristics of the constituent tissues are varied and reflect the different functions of each organ [25]. For example, petals are composed of cells that aim to attract pollinators. Morphology at the cellular and tissue level can be plastic and influenced by environmental factors [26]. Under certain conditions, the differentiation of plant cells and tissues is reversible [27]. Plants are sessile organisms, so it has more flexibility due to biotic and abiotic stress conditions [28].

V. CONCLUSION

Flowers are classified as complete flowers of dicot plants with the presence of nectar to attract pollinating insects. The fruit phase is followed by the presence of pistils that are still left behind. Variations in fruit structure include normal and cryptoviviparous fruits with tissue modification in the form of cork layers, while variations in seed structure include endospermic and non-endospermic seeds. The differences in tissue development and modification patterns occur due to tissue specialization, a form of environmental adaptation.

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