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Article

Anatomy of *Cleome rutidosperma* DC. (Cleomaceae) Ovary Development in Mataram City

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Abstract. *Cleome rutidosperma* is one of the species that belong to famili Cleomaceae and ordo Brassicales. It was first discovered in West Africa. More recently, it has been found in the Caribbean, Thailand and Malaysia. In Indonesia, this plant was first discovered in Java, precisely in Tanjung Priok in 1946, then it spread rapidly in Indonesia. In Lombok, West Nusa Tenggara, *C. rutidosperma* is known to be an invasive plant found growing in open fields and roadsides. The purpose of this research is to observe the ovary development of *C. rutidosperma* and analyse the differences in each growth phase. *C. rutidosperma* is belong to Brassicales order. Ordo Brassicales is well known for having cleistogamy type of flowers. This research is going to prove *C. rutidosperma* belongs to cleistogamy or chasmogamy pollination type. However, the best solution is to research until a new study is obtained that discusses the development of *C. rutidosperma* ovaries and *C. rutidosperma* flower type. The results of this study can also open new insights and open further research. The results shows in first phase the ovaries were already in the early developmental stage such as protrusions. This development continues to complete and expand its structure. Some of these include the growth of the funiculus which continues to change until it reaches a phase where the funiculus already appears to fully support the ovule. In the last two phases, it can also be seen that structures such as the inner integument and integument begin to appear. The development has greatly increased from the previous phase.

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1. Introduction

Cleome is the largest genus in the family of Cleomaceae, consists of 207 species. One of the species is *Cleome rutidosperma*. It was first discovered in West Africa (Guenia, Congo and Angola). More recently, it has been found in the Caribbean, Thailand and Malaysia. In Indonesia, this plant was first discovered in Java, precisely in Tanjung Priok in 1946, then it spread rapidly in Indonesia. This plant can also be found in Madura, precisely in Telang, Bangkalan Regency. This plant is known as Spider flowers plant and often found growing in open fields and roadsides at an altitude of 4 masl [1-2]. *C. rutidosperma* is known as an invasive plant that grows in abundance. It prefers disturbed and rough environments, especially in humid and hot climates, in areas with annual rainfall of 1.700-3.000 mm [3].

This plant is also belong to ordo Brassicales. Brassicales order is well known for having Cleistogamy type of flowers. Flowers based on their pollination type are divided into cleistogamy and chasmogamy. In cleistogamy, the flower usually produces seeds without requiring interaction with pollinating insects or wind. Chasmogamy is the process that occurs when both the pistil and pollen ripen after the flower has bloomed. It can occur between pollen and pistil from the same flower, or from different flowers once they have bloomed. Chasmogamous flower can opens up and allows pollination by insects or wind. Chasmogamy is also called open pollination. Meanwhile, cleistogamy is pollination that occurs when the flower is still in the bud phase [4]. This type of pollination is also called closed pollination. The term was first used by Kuhn in 1867 to describe a bud-like flower that never opens but develops into a fruit [5].

Cleistogamy has several types, namely preanthesis, pseudocleistogamy, complete and true cleistogamy. Preanthesis cleistogamy is a type where pollination occurs during the bud stage, but the flower eventually blooms. Pseudocleistogamy is type where one flower plant can have both closed pollination (CL) and open pollination (CH) flowers. Pseudocleistogamy can occur due to environmental factors. Complete cleistogamy is the type where a plant will produce flowers with closed pollination (CL). The last type, true cleistogamy, is where the CL and CH flowers are morphologically different. Unlike the CH flowers which are open and relatively large, the CL flowers are closed and small [6].

This spider plant is a perennial plant that can reach heights of up to 2 metres. It grows upright, creeping, or crawling with capsule-shaped stems. The leaves characterised by trifoliate leaves, where each petiole has three small leaves, commonly found in the *Cleome* genus [7]. It is grow alternately along the stem on long stalks. Usually, these leaves are compound and consist of small lanceolate or elliptical leaves. The stem of this plant has many branches from the base and has a subglabrous, pubescent or villous surface. The flowers are small, pink, purple or white in colour, and arranged in a long terminal form, with claw-like pointed corolla leaves [8]. This flowers are single flowers, and grow near the leaf axils (axillary). The species have complete flowers and zygomorph symmetry. It is commonly found at low altitudes up to 1,500 metres and has tolerance to harsh environmental conditions [9]. The fruit is a cylindrical capsule containing globular-reniform seeds that are orange-brown to dull black in colour. The seeds are asymmetrical in shape and dull black in colour, which is one of the main features in the identification of this species [10-11].

C. rutidosperma commonly cultivated due to its natural occurrence in the wild. Some people use it as herbal medicine to treat swelling, pain, or irritation in the eyes. In some countries such as Ghana and India, the plant is used as a traditional medicine to treat ear pain and deafness [4]. The parts of this plant, including the leaves and roots, are used to treat muscle spasms, skin diseases, and ear symptoms, including deafness and earaches. The leaf extract is also effective for seizures [10]. This plant has long been recognized for its potential to alleviate various medical conditions, with previous studies highlighting its anti-inflammatory and analgesic properties. Moreover, it contains active compounds such as alkaloids, flavonoids, and tannins that are beneficial to health [8].

Alkaloids and flavonoids are both potentially negative regulators of oncogenes (a group of cell cycle regulatory genes) and positive regulators of tumour suppressor genes, hence their potential as anti-cancer agents. *C. rutidosperma* also has antioxidant properties and antimicrobial activities that make it a candidate for drug development. This combination of benefits makes *C. rutidosperma* a valuable plant in traditional medicine and modern drug development [7] [12-13].

The ovary in *Cleome* flowers is located on the underside of the pistil and is where the ovule develops. In some *Cleome* species, the ovary is often supported by a structure called a gynophore, which is a stalk that supports the ovary, providing distance between the ovary and the rest of the flower [3]. The anatomical development of ovulum consists of a nucellus surrounded by one or two integuments and attached to the placenta by a stalk called the funiculus. At the free end of the ovule there is a small gap called a microfil. The area where the integument is attached to the funiculus is called the khalaza. The nuselus cells are usually found under the outermost layer at the end of the micropyle, and are called megaspore mother cells. Therefore, the nuselus is considered a megasporangium [14]. The ovulum develops from the placenta of the ovary. The ovule primordia originate from pericline cleavage of cells under the surface layer of the placenta The inner integument begins to develop and pericline cleavage begins to occur in the protoderm. At first, the integument looks like a ring at the edge then grows towards the tip and covers the nucellus except for the micropyle. The beginning of the outer integument occurs due to the cleavage of the pericline layer below the surface. The development of both integuments is the same [15] [16].

It is important to observe *C. rutidosperma* especially in analysing the development phase of the ovary because because it can cause morphological and anatomical variations of the ovules of these plants [17-18]. Research on the variation of ovule development in each phase will also answer the question of whether the flower type of this plant is chasmogamy or cleistogamy. Besides the uniqueness of this plant that belongs to invasive plants with its seeds that can be spread to various regions is quite interesting to study. Because of its uniqueness, research can be carried out on the developmental stages of the ovaries, which are the reproductive organs and the beginnings of the growth of the seed [11] [19-21]. Also, the urgency in this research is because until now there is still no research that specifically discusses the development of *C. rutidosperma* ovaries. However, the best solution is to research until a new study is obtained that discusses the development of *C. rutidosperma* ovaries. The results of this study can also open new insights and open further research.

2. Method

The research of the ovarian anatomy of *C. rutidosperma* was done at the Advance Biology Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University. The sampling place is located on Karang Pule, Mataram City, Lombok (-8.603759,116.089285). This research began in April to June 2024.

2.1 Materials and Tools

The materials used in this study are the samples used are flower buds of various sizes of *C. rutidosperma* phase 1, phase 2, phase 3, phase 4, phase 5, phase 6, and phase 7, alcohol 70%, Toluidine Blue O 0,025% (aq) and tissue. The tools were stationeries, Petri dishes, iPhone XR mobile phone, camera, glass objects, cover glass, brushes, Zeiss Primo Star microscope, tweezers, and new razor blades.

Figure 1. The map of *Cleome rutidosperma* collection sites

2.2 Research Method

The research method used in this study are divided into slicing methods and preparation methods. The preparation method that been use is fresh preparations. For the incision method was use hands free section method. Hands free section method provide a quick assessment of the anatomical organization of the specimen. This method is done by slicing the specimen as thin as possible using a tool in the form of a new razor blade [22].

2.3 Data Analysis

Data analysis was carried out using descriptive data methods, identifying the type of *C. rutidosperma* using several literatures such as journals (Angiosperm ovules: Diversity, Development, Evolution) [23], (Embryological Studies in Cleome monophylla Linn.) [24] and guidebooks (Plant Anatomy) [25]. Data taken in the form of anatomical structures of ovary development by recording characters from the presence and type of trichomes, epidermis, carpium, type of ovule, funiculus, and the presence of integument. The processed data is presented in the form of photos and descriptions according to the results obtained.

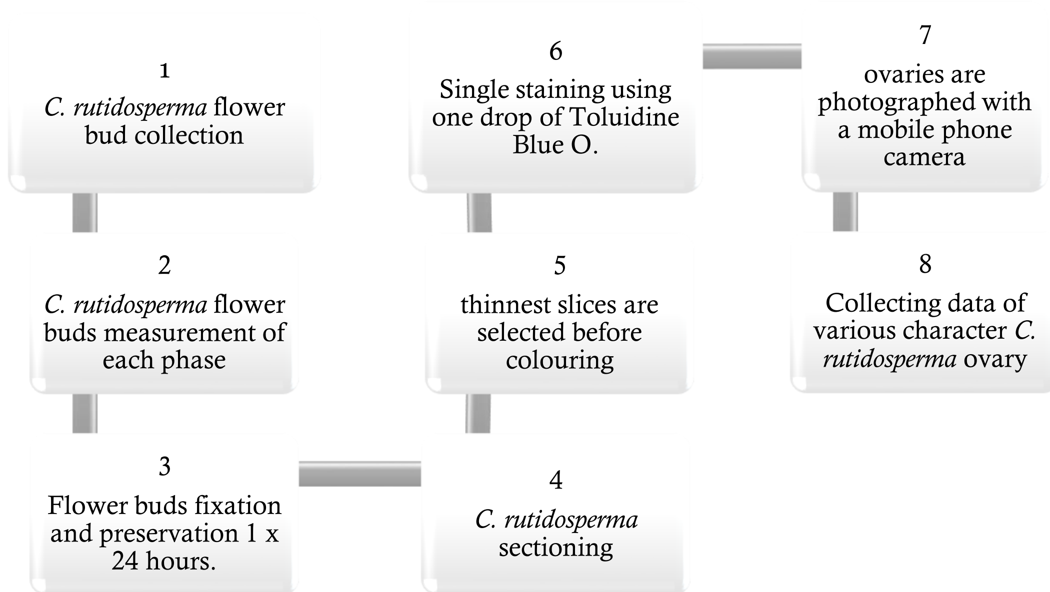

Figure 2. Flowchart of research

3. Results and Discussion

According to the research that has been conducted, the results of the study of flower bud development of *C. rutidosperma* (Figure 3), ovary development of *C. rutidosperma* (Figure 4) and anatomical development of *C. rutidosperma* ovary (Figure 4) from the first phase to the seventh phase with various sizes were observed. In the first phase of *C. rutidosperma*, the ovary is at an early stage of development. During first phase, the flower buds measure 1.3 mm × 1.2 mm (Fig. 3A) with ovaries measuring 1.2 mm × 0.2 mm (Fig. 4A). The ovary is at an early stage of development. The development of the ovary is only visible in the form of a mound on the edge of the carpel (marginal). At this stage, the ovary shows early signs of differentiation and the formation of important structures that will develop further at later stages.

In this phase the epicarpium, mesocarpium and endocarpium are still forming and differentiating. The epicarpium is seen as a protective outer layer, the mesocarpium as a middle layer that stores nutrients and water, and the endocarpium as an inner layer that protects the ovule (Fig. 5A). The outermost part of the ovary is the epidermis, in the form of a layer of small and tight cells that serve as the main protection from physical damage and pathogenic infections [26]. Trichomes of this plant can also be seen with a multiseriate stalk and the head. Because on their appearance they are grouped as multicellular glandular head type (Fig. 6A). In the early phase, the ovule is formed at or near the edge of the carpium (marginal), and first appears as a small mound, where the ovule begins its development from the inner morphological surface of the carpel (Fig. 6A) [23]. According to the results of several studies, the ovule will be small round or mound-shaped in the early stages [18]. In this phase there are still many parts that have not been seen because this phase is the initial phase and is still in the formation stage.

In second phase, the flower bud measures 3.5 mm × 1.4 mm (Fig. 3B) with an ovary that measures 2.0 mm × 0.4 mm (Fig. 4B). The ovary shows that the ovule which was originally mound-shaped will begin to elongate. Ovule attached to the edge of the carpel or marginal carpium. In phase two, the ovule has begun to appear funiculus which is still short. The funiculus length will develop in line with the development of the ovary phase [23]. Funiculus plays an important role in attaching the ovule to the ovary wall and supporting the ovule (Fig. 6B) [25]. Funiculus also has a role to transport nutrients as it is connected the ovule with the ovary [27]. The trichome structure is clearly visible which has a multicellular glandular headed type. In addition, the epidermis and pericarpium can be seen which form three layers, namely epicarpium (outer part), mesocarpium (middle part) and endocarpium (inner part). The preparation clearly shows the lines that divide the three layers of the pericarpium (Fig. 5B) [26].

In the third phase of the ovary, the flower buds are 5.0 mm × 1.5 mm in size (Fig. 3C) with an ovary size of 2.3 mm × 0.7 mm (Fig. 4C). There is a development in the ovule and funiculus, the ovule is more developed with a larger size. The increase in ovule size is a form of development that will soon lead to the addition of various other structures [28]. In addition, there is a striking development in the length of the funiculus, which is longer than phase two where in phase two the funiculus is still very short. In this phase there is an elongation of the funiculus, from initially very short to longer in this phase (Fig. 6C). The funiculus functions as an ovule connector and is a nutrient pathway from the parent plant to the ovule [23] [27].

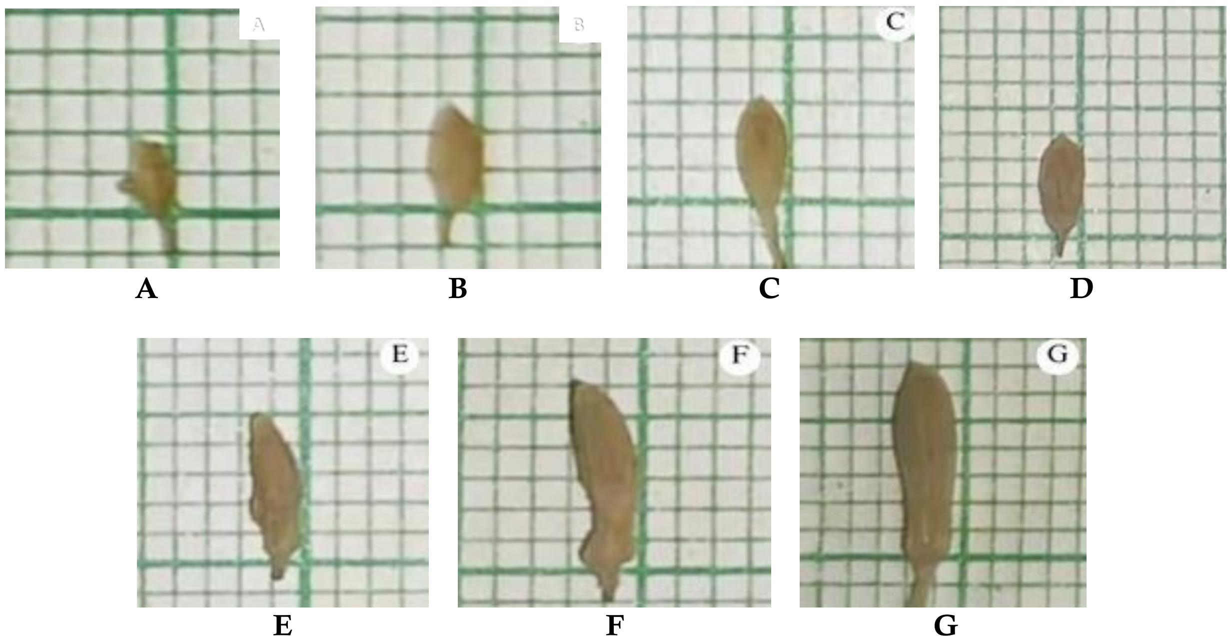

Figure 3. Flower bud development of *C. rutidosperma* with various size. (A)First phase, (B) Second phase, (C)Third phase, (D)Fourth phase, (E) Fifth phase, (F) Sixth phase, (G) Seventh phase.

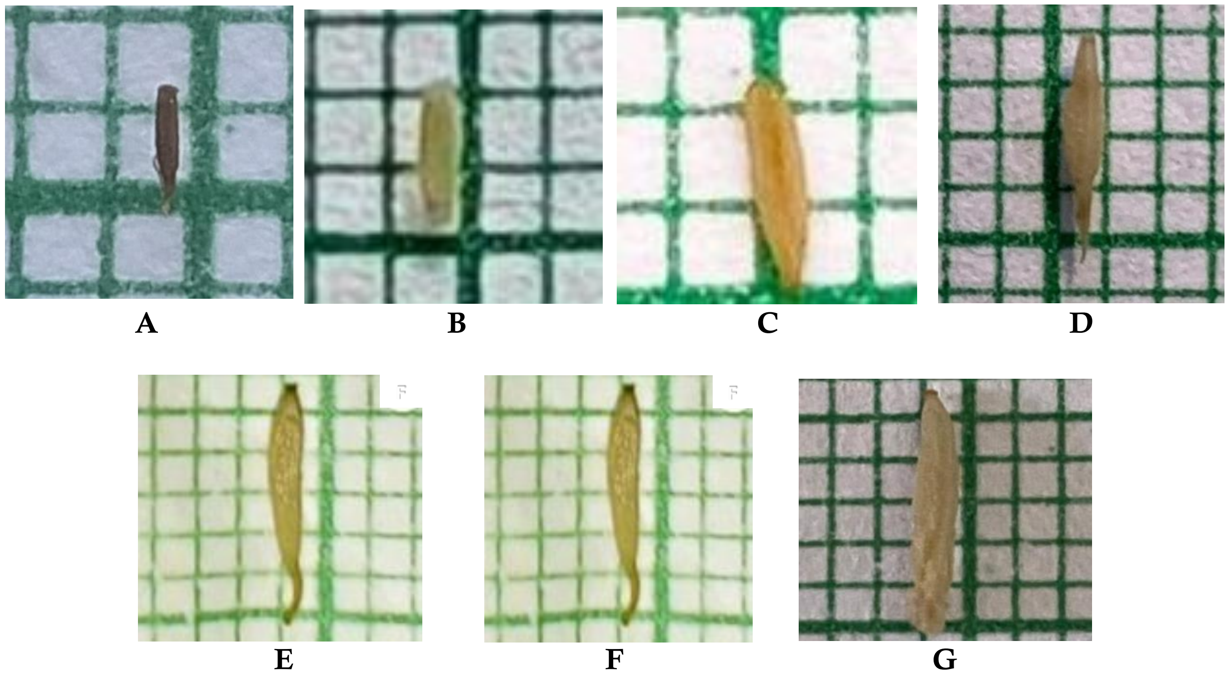

Figure 4. Ovary development of *C. rutidosperma* with various size. (A) First phase, (B) Second phase, (C) Third phase, (D) Fourth phase, (E) Fifth phase, (F) Sixth phase, (G) Seventh phase.

In fourth phase ovaries, the flower bud measured 5.5 mm × 1.6 mm (Fig. 3D) with an ovary size of 4.0 mm × 0.9 mm (Fig. 4D). In fourth phase ovaries, the ovules were larger than phase three. The trichomes are also longer than phase three (Fig. 5D). The ovules are facing sideways so they are not neatly arranged as in other phases, the ovules are hemianathropes [14]. The development of ovules when compared to plants from the family of Bromeliaceae, the ovary wall begins thickening of the collenchyma in the epidermal cell wall, ovules are anatropic and bitegmic, many ovules are found in each locule, and the presence of aerenchyma in the ovary wall (Fig. 6D) [16] [25] [29].

At the fifth phase, the flower bud measured 6.0 mm × 1.8 mm (Fig. 3E) and the ovary measured 4.2 mm × 1.0 mm (Fig. 4E). The ovule appears clearly visible and the funiculus can also be seen clearly. The outer integumen and inner integumen in this phase are not clearly visible, but the epicarpium, mesocarpium and endocarpium which can be seen clearly with the dividing line (Fig. 5E) [26]. When compared to the previous phase, the ovule in this phase appears more clearly. This ovulum is located attached to the carpel placenta (Fig. 6E) [25]. In this phase, trichomes are clearly visible, these trichomes have a multicellular glandular type. When comparing the trichome found in with several species belonging to the Cleomaceae family, there are similarities, such as the type of trichome is multicellular glandular type [30]. Comparisons were also made in some other species such as *C. spinosa*, it can be seen that the trichomes found in *C.spinosa* are more complex than the trichomes found in *C.rutidosperma* [31].

In the sixth phase, the flower bud measured is 7.0 mm × 2.0 mm (Fig. 3F) and the ovary size is 5.5 mm × 1.3 mm (Fig. 4F). The ovary in sixth phase underwent significant development. The external structure of the ovary such as multicellular glandular head type and the carpium structure in the form of epicarpium, mesocarpium and endocarpium appear more clearly in this phase (Fig. 5F). When compared to the development of the ovary in the previous phase, there is a prominent difference with the beginning of the appearance of the outer integument and the inner integument. The integument consists of two layers of thick cells [23]. The integument is the outermost layer of the ovule that will later develop into the seed coat [31]. The outer integument and inner integument can be seen clearly marked by different colour gradations, which are located at the tip of the ovule (Fig. 6F). The presence of the outer integument plays an important role for the curvature of the ovule. The presence of integument shows significant development of the ovule. Integument is divided into three types, such as bitegemic which has two layers and is commonly found in angiosperms, unitegemic which has only one layer and ategemic which has no integument [27] [30].

Cleomaceae especially the *C. monophylla* species, the inner integument develops first, followed by the outer integument [32]. The ovule is marginally attached to the carpium which is arranged in parallel. Ovule contained in this phase is slightly curved which is a type of hemianathrop ovule [14] [25]. Ovule development that occurs in this sixth phase aims to help efficiency in the process of pollination and fertilisation because it optimises the position of the ovulum in the ovary. The development that occurs in this phase occurs because the ovary has begun to approach the mature phase in the next phase before finally developing into a flower (Fig. 6F) [23].

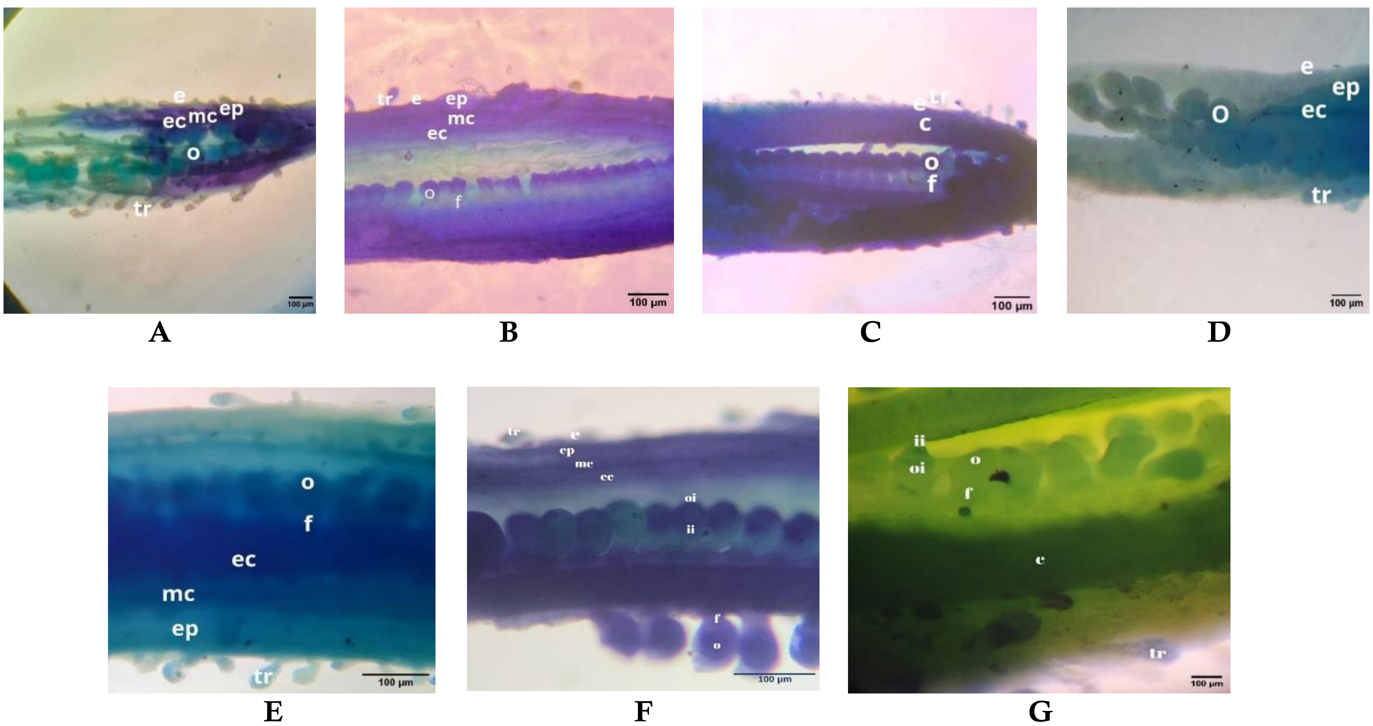

Figure 5. Anatomical development of *Cleome rutidosperma* ovary with various size (A) First phase, (B) Second phase, (C) Third phase, (D) Fourth phase, (E) Fifth phase, (F) Sixth phase, (G) Seventh phase.

tr. Trichome; e. epidermis; ep. Epicarpium; ec. Endocarpium; f. funiculus; mc. Mesocarpium; o.ovule; oi. Outer integument; ii. Inner integument

During this seventh phase, the size of the flower bud is 9.0 mm × 2.5 mm (Fig. 3G), while the size of the ovary is 6.0 mm × 1.5 mm (Fig. 4G). Several structures are also visible, including trichomes, carpium, ovule, funiculus, inner integument, and outer integument. Carpium or seed coat of this plant appears to be coated. There are also several ovules that are quite numerous here. The ovule has a much more advanced development compared to the previous phase from the size and the presence of inner integument and outer integument at the end of the ovule (Fig. 5G) [27]. The last phase of ovary development was compared with *C. viscosa* and *C. gynandra*. The trichomes of *C. rutidosperma* have a multicellular glandular head type while in *C. gynandra* and *C. viscosa* plants [33] both have pubescent glandular trichomes (Fig. 5G). For ovule structure, as known ovule is a structure that develops from ovarian placenta. In this *C. rutidosperma* plant, the ovule grows and attaches the marginal placenta. For the ovule itself there are types that distinguish it, in *C. rutidosperma* the type is hemianathrop (Fig. 6G1). The same type of ovules is also found in *C. monophylla* [24]. However ovules in some Angiospermae plants are anatrop [23]. In the (FIG. 6G2) there is one anomaly ovule located at the end of the ovary which is considered belongs to the anathrop type [14] [25]. This preparation also shows the funiculus or stalk that supports the ovule with a much larger size than the previous phase and is more clearly visible.

The next structures to appear seventh phase are the inner integument and outer integument. There are variations of the inner and outer integument in some species of Cleomaceae, for example in *C. monophylla* there are two integuments and in *C. horrida* only has one inner integument [24]. In *C. rutidosperma*, although there are inner and outer integuments, there is still no visible microfil development, same goes with most plants, that outer integument does not reach the microfil (Fig. 6G1, Fig. 6G2) [14] [20]. When compared with growth first phase to seventh phase, it can be seen that this phase is much more developed and more complete than the previous phase. Seventh phase is quite similar to sixth phase, the difference is that the integument is much more developed and also in terms of the size of the ovary which is larger in diameter.

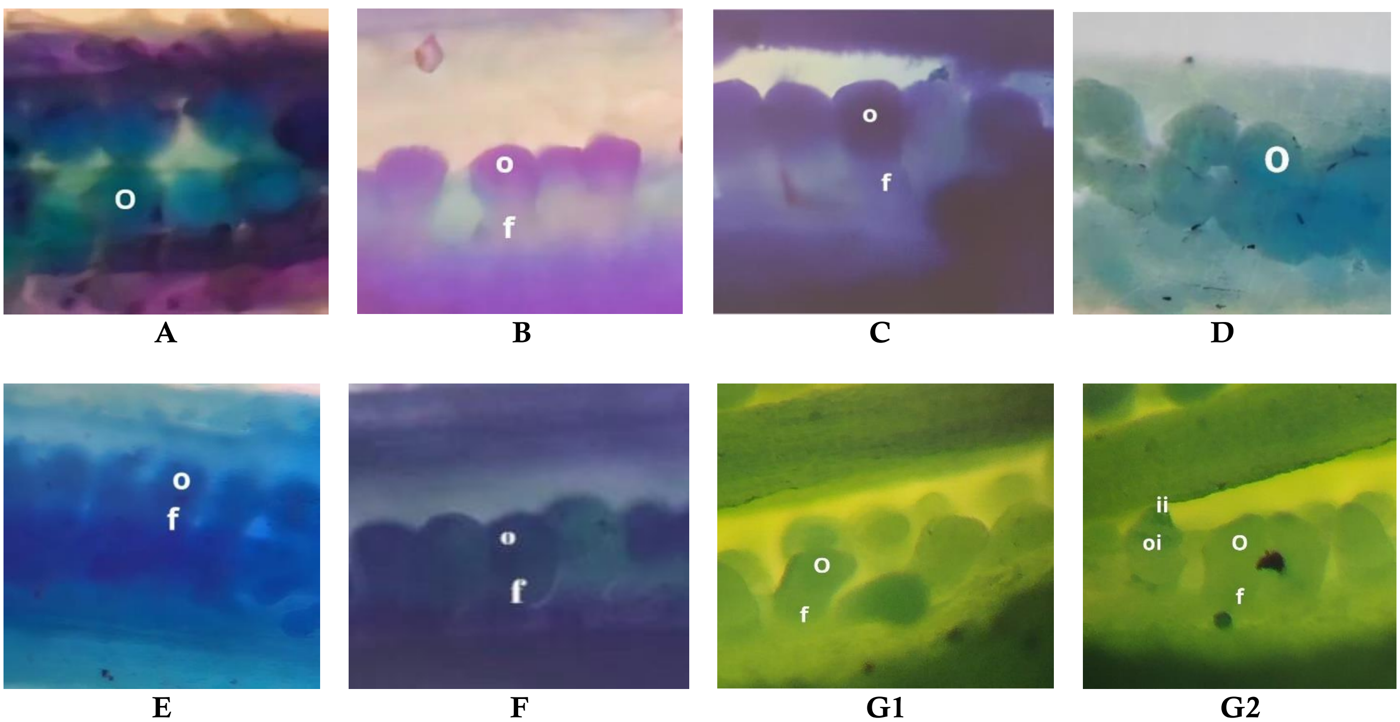

Figure 6. Anatomical development of *C. rutidosperma* ovule with various size. (A) First phase, (B) Second phase, (C) Third phase, (D) Fourth phase, (E) Fifth phase, (F) Sixth phase, (G1) Seventh phase, (G2) Seventh phase.

From this study that has been done, the results show that in the last phase of ovary development of *C. rutidosperma*, the ovule is immature. This can be seen from the integument structure that has not covered the nuselus [24]. This condition can also be used as proof that the ovule has not yet reached the mature stage and not ready to be pollinated. From these results it can be said *that C. rutidosperma* belongs to the chasmogamy flower type. Chasmogamy is a type of flower that pollinates when the flower has bloomed [5]. To refine this statement, further research is required more.

4. Conclusion

The results showed that in all phase of *C. rutidosperma* ovary development there is significant growth in each phase. In the early stages, the ovary appears as a small protrusion, gradually growing larger with each phase. The funiculus begins to develop in the second phase and continues to grow throughout the later stages. By the sixth and seventh phases, both the inner and outer integuments become clearly visible, marking the final stages of ovary development. The ovule is primarily hemianatropous, with one anomalous anatropous ovule observed. *C. rutidosperma* is classified as a chasmogamy pollination type.

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