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#### rutidosperma ANTHER DEVELOPMENT ANATOMY OF Cleome DC. (CLEOMACEAE) IN LOMBOK

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#### ARTICLE INFO **ABSTRACT** Article history Cleome rutidosperma DC is a plant native to tropical Africa, India, and Indochina. This research aimed to determine the Submission 2024-07-29 anatomical development of the C. rutidosperma anther. This Revision 2025-02-25 observation was conducted in April-June 2024 at the Accepted 2024-04-09 Advanced Biology Laboratory, Mataram University, Keywords: Lombok. The samples used are flower buds of C. Cleome rutidosperma rutidosperma, divided into seven development phases. The Cleomaceae results of the observation of the anatomical structure of the Anther development anther of C. rutidosperma plants were analyzed Lombok descriptively. It's include various development of flower anther development, anther transverse slice preparations of each development phase, and anther locus of each development of C. rutidosperma plants presented in images and descriptions. Each phase, there was a change in the structure of the anther tissue, occurring alongside the development of pollen. Phase 2 indicated that the pollen was still in the division stage and was still fusing. The division stage was completed in phase 3, with the pollen having separated. Mature pollen occurred from phase 6, and the stomium tissue structure had formed. The theca formed in phase 7, which co-occurred with the horizontal opening of

of pollen.



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## **INTRODUCTION**

the stomium, a structure that supported the outward release

The Cleomaceae family consists of 2 genera that live worldwide in tropical and subtropical regions, e.g., the Cleome and Cleomella genera (Plants of the World Online, 2024a). Cleomaceae plants are tropical African plants that were later brought in and are now naturalized in many places in Asia, Australia, America, and the West Indies. Cleomaceae has about 300 species distributed in tropical to warm climates (Gosh et al.,

Cleomaceae has about 300 species distributed in tropical to warm climates (Gosh et al., 2019). It grows worldwide but often in warmer places such as arid deserts, grasslands, and moist forests (Bayat et al., 2018).

One of the genera of the Cleomaceae family is Cleome. The Cleome genus consists of more than 200 species that grow in various regions of the earth. It is the largest genus in the Cleomaceae family. Cleome species are annual or perennial bushy plants or trees (Singh et al., 2018). *C. rutidosperma* is a Cleome species native to Cameroon, Central African Republic, India, Liberia, Myanmar, Nigeria, Sri Lanka, and Uganda. It was later introduced to Bangladesh, Brunei, Brazil North, Cambodia, South Central China, Jamaica, Eastern Himalayas, Florida, Hawaii, Laos, Malaysia, and Mexico. In Indonesia, it was invasive as a weed plant to Andaman Island (Aceh), Sabang, Java, Kalimantan, Sumatra, and Lesser Sunda Islands (Plants of the World Online, 2024b).

Maman Lanang, or spider flower (*C. rutidosperma*), has a herbaceous habitus with a height of up to 1 m. Leaves are arranged alternately with rhomboid to elliptic or lanceolate shapes. The tip of the leaf is pointed or tapered with a cuneate base. Flowers are located in the leaf axils with flower stalks up to 2.5 cm long. The petals are lanceolate and usually have fine hairs. The four-crowned crown is white, pink, lilac, violet, or blue. Seeds have a diameter of 2 mm with a smooth (glabrous) surface. Seeds are reddish brown, dark brown, or black (Umar et al., 2014). The habitat is generally in areas with low altitude, humid, hot conditions, and can grow at altitudes up to 400 meters above sea level. This plant can be found on roadsides, rice fields, and fields. It is also found living as an epiphyte on rocks and wood.

C. rutidosperma is a medicinal plant found in subtropical and tropical areas. This medicinal weed is called because C. rutidosperma acts as a medicinal plant with the ability of biological activity. This is due to the phytochemical and nutritional compounds it contains. The potential possessed by C. rutidosperma is what makes this plant often used in ethnomedicine (Ghosh et al., 2019). The compounds in question are alkaloids, flavonoids, tannins, steroids, polyphenols, proteins, and phenolics (Leboe et al., 2018; Prabha et al., 2017). These can be anti-microbial, anti-oxidant, anti-inflammatory, anti-cancer, anti-diabetic, and anti-plasmodial agents (Aftab, 2019). This plant can be an anti-plasmodial, which inhibits the growth of malaria parasites or plasmodium (Maximus et al., 2021). Some believe C. rutidosperma can cure earache, skin irritation, and

convulsions (Nguyen, 2023). *C. rutidosperma* has other contents, namely vitamins and minerals (Akinsola & Oluwafemi, 2022). It is also a food ingredient, often processed into cooked vegetables or added to soups (Aftab, 2019). This plant has an application role in health-related diseases and in increasing agricultural productivity by controlling pests (Ngatimin, 2020).

The formation of the anther primordium undergoes separation into layers that will develop into the main parts of the anther. The cells inside the anther primordium undergo a series of mitotic divisions that will form structures such as pollen sacs or lobes in a mature anther. The cells inside the pollen sacs begin to differentiate into various cell types that play a role in pollen formation (Walbot & Egger, 2016). Sporogenic cells will become pollen, tapetum cells that supply nutrients and support for the pollen cells, and vascular cells that provide structural support and resources for another development. The sporogenic cells undergo meiosis to produce haploid cells, which then develop into pollen. The anther is generally oval, consisting of four lobes, which usually open at the top to release pollen when ripe. Each anther lobe has two chambers (pollen sacs) that contain pollen. These chambers are usually located at the bottom of the lobe (Marchant & Walbot, 2022). This research aims to determine the anatomical development of the C. rutidosperma anther. The developmental anatomy of this anther will provide information about the process of sexual reproduction, including cleistogamy or chasmogamy flowers, and help us understand more about the adaptation of this plant to its environmental conditions.

### MATERIALS AND METHODS

This research was conducted in April-June 2024 at the Advanced Biology Laboratory, Faculty of Mathematics and Natural Sciences, Mataram University, Lombok. *C. rutidosperma* flower buds were sampled at Karang Pule Village, Mataram, Lombok (Figure 1).



**Figure 1.** Map of Sampling of *C. rutidosperma* flower buds at Karang Pule Village, Mataram. Notes: samples are taken along the ref L-shaped line.

The tools used include stationery, petri dishes, XS max mobile camera, glass objects, cover glass, brushes, a Zeiss Primo Star microscope, tweezers, and razor blades. The materials were used samples of flower buds of *C. rutidosperma* flowers in Phase 1, phase 2, phase 3, phase 4, phase 5, phase 6, and Phase 7, equates, alcohol 70%, Toluidine Blue 0.025% (acl 70%), and tissue.

This research is used in a qualitative descriptive study. The initial activity carried out was the process of collecting specimens. The sample used was seven different sizes of *C. rutidosperma* flower buds. The next stage of the procedure is fixation; the samples were fixed by soaking them in 70% alcohol for 24 hours. After that, the anther part of the fixed flower bud was taken. The slicing used the freehand section method. The anther was then a thinly transversal section, and the section was placed in the water in the petri dish to find the thinnest section. The preparation was made using the fresh slide method.

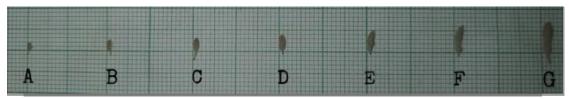
The thin slice was placed on a glass object and covered with a cover glass, then observed under a microscope. The next step was staining with Toluidine Blue O, 0.025%. This staining was dropped at the edge of the cover glass and left for 20 minutes. After that, the rinsing step was done by dropping 70% alcohol at the edge of the cover glass and absorbing it with tissue paper. This process was repeated until the stain became light. The next stage was preparation observation with a binocular microscope, ZEISS Primo Star, with a magnification of 40x, 100x, and 400x. Photos were taken with an iPhone XR mobile camera. The results of these images could be observed in detail as material for describing the anatomy of the anther of development of *C. rutidosperma* 

plants. Each photo was accompanied by a scale bar using ImageJ Version 1.8.0\_172 (NIH, 1995 in Qurrahma et al., 2022).

Data from observing the anatomical development of the anther of *C. rutidosperma* plants were analyzed descriptively. The data taken in this observation are as follows: epidermis, endothecium, middle layer, tapetum, pollen, locus or theca, stomium, and connectivism. In this observation, the *Embriologi Angiospermae* book by Utami (2023) was used.

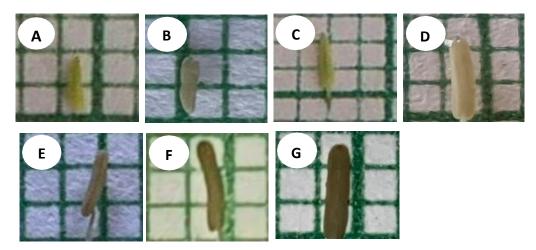
#### RESULTS AND DISCUSSION

Results include various flower bud development, anther development, anther transverse slice preparations of each developmental phase, and anther locus of each development of *C. rutidosperma* plants, presented in pictures and descriptions.



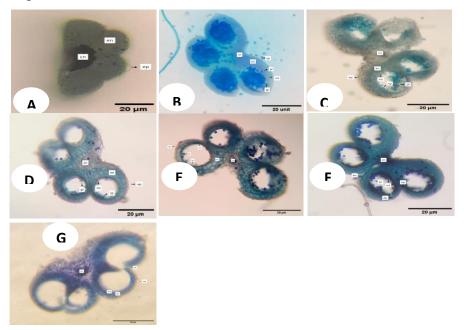
**Figure 2.** Flower bud development of *Cleome rutidosperma*. Notes: A. Phase 1; B. Phase 2; C. Phase 3; D. Phase 4; E. Phase 5; F. Phase 6; G. Phase 7

In this study, *C. rutidosperma* was divided into seven developmental phases based on the size of the flower buds. Flower buds from phase 1 to 7 (Figure 2-3). Flower bud length× width: phase 1: 2 mm × 1 mm; phase 2: 2.5 mm × 1.5 mm; phase 3: 3.3 mm × 1.9 mm; phase 4: 4 mm × 2 mm; phase 5: 5 mm × 2 mm; phase 6: 6 mm × 2 mm; phase 7: 7 mm × 2.5 mm. Each flower bud was taken another in each developmental phase 1 to 7 (Figure 3). Length × width anther size of phase 1: 1.5 mm × 0.5 mm; phase 2: 1.8 mm × 0.7 mm; phase 3: 2.2 mm × 0.8 mm; phase 4: 2.3 mm × 0.9 mm; phase 5: 2.4 mm × 1 mm; phase 6: 2.4 mm × 1 mm; phase 7: 2.6 mm × 1 mm. It can be seen that the size of flower buds from phase 1 to phase 7 was getting bigger, indicating that the flowers of this plant experience several phases of development, including the anther.



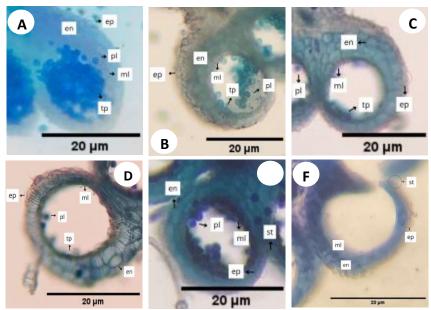
**Figure 3.** Various Anther Development of *Cleome rutidosperma*. Notes: A. Phase 1 anther development; B. Phase 2 anther development; C. Phase 3 anther; D. Phase 4 anther development; E. Phase 5 anther development; F. Phase 6 anther development; G. Phase 7 anther development

Another structure of *C. rutidosperma* has four loci, where the two loci above appear larger than the two loci below. The size of the two-locus above is due to the tissue that makes up the anthers larger than the two below. The connective separates the right and left locus, so the connection appears between the four loci. Several tissues cover the locus; the tissues from outer to inner include the exothecium or epidermis, endothecium, middle layer, and tapetum.



**Figure 4.** Cross-section of *Cleome rutidosperma* anther development. Notes: A. Phase 1 anther; B. Phase 2 anther; C. Phase 3 anther; D. Phase 4 anther; E. Phase 5 anther; F. Phase 6 anther; G. Phase 7 anther; ep. Epidermis; en. Endothecium; ml. Middle Layer; tp. Tapetum; pl. Pollen; st. Stomium; cn; Connectivum.

The epidermal cells appear round at the beginning of the phase (Figure 4 A), but as the phase progresses, the cells appear flatter in shape so that in the final phases, such as in phase 6 (Figure 4 F), they already appear flat. The endothecium tissues appear as significant and tetragonal-shaped cells; this can be seen in phase 4 (Figure 4 D). In phase 5 (Figure 4 E), the endothecium cells look different, where the endothecium cells form a vertical striped pattern on their cells. As the phase progresses, these patterns increase so that the endothecium cells appear to have an unclear shape in the final phase (Figure 5 F). The middle layer is under the endothecium tissue; this layer will look increasingly flatter as the phase increases.



**Figure 5.** Locus of *Cleome rutidosperma* anther development, Notes: A. Phase 2 anther; B. Phase 3 anther; C. Phase 4 anther; D. Phase 5 anther; E. Phase 6 anther; F. Phase 7 anther; ep. Epidermis; en. Endothecium; ml. Middle Layer; tp. Tapetum; pl. Pollen; st. Stomium.

The tapetum appeared to be attached by pollen in several phases, but the tapetum was depleted by phase 6 (Figure 4 F). The pollen seen in phase 2 (Figure 4 B) was still united, in contrast to phase 3 (Figure 4 C), where the pollen has separated. The pollen experienced a maturation process in the next phase, marked by the thickening of the exine wall, and the colour became dark blue, visible in phase 6 (Figure 4 F). As the pollen matures, changes also occur in the structure of the tissue that makes up the anther, such as the opening of the septa of two loci to become the theca and the presence of a stomium structure, which was visible in phase 7 (Figure 4 G).

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The other *C. rutidosperma* had several stages of development, which, in this study, can be seen from the slide of phase 1 to phase 7. The overall anatomical structure of the anther included connectivum, epidermis, endothecium, middle layer, tapetum, pollen, and stomium. The connective separated the locus on the left and right sides; the locus was the compartment where the pollen was stored (Thpanorama, 2024). The connectivum is where the filament is attached to the anther (Mulyani, 2016). The connective was composed of a vascular bundle consisting of xylem and phloem. These tissues channel water and nutrients necessary for other development (Nehemy et al., 2022).

Phase 1 showed that the locus was protected by several tissues that make up the anther, namely the epidermis and endothecium. Epidermal cells in this phase looked round. The tissue inside the endothecium seems like a mass of cells due to the incision made at the age of the anther, not right in the middle. The endothecium cells were larger than other protective tissues of the locus (Utami et al 2023), so only the endothecium cells were seen. Inside the endothecium cells was a collection of homogeneous cell masses (Costryco & chwil, 2021).

Phase 2 showed that the surrounding epidermal cells were still rounded to relatively flattened. This was because the higher the phase, the flatter the epidermis became. The endothecium cells appeared to be tetragonal in shape, and there was a middle layer just below the cell layer. The pollen in this phase still looks fused, so the pollen is still in the division stage (Wardhana, 2015). In phase 3, the pollen separated. According to Haque and Ghosh (2017), the pollen division process is completed in the two telophase stages of meiosis, forming four separate pollen grains. The formed pollen was still immature and was still attached to the tapetum around the locus. The tapetum functioned as a provider of nutrients and supported the pollen maturation process. The tapetum visible around the locus did not dissolve, indicating a secretory type of tapetum (Yao et al., 2022).

Maturation of previously formed pollen occurred in the next phase. Phase 5 began to show that the endothecium cells form a vertical striped pattern. Not all plants had this pattern on their other endothecium. This pattern appeared increasingly denser as the phase increased, so the endothecium cells' shape appeared irregular. In phases 4 and 5, it could be seen that there was still a tapetum around the locus. This was the effect of using Toluidine Blue dye, which will give a dark blue colour to the lignin content (Reitz, 2021),

so that the pollen in phase 5 has begun to thicken the exine walls of the pollen, giving rise to a dark blue colour.

In phase 6, the anther structure began to show signs of pollen maturity. Among them was a change in the shape of epidermal cells, which became flat. By Utami et al. (2023), the epidermis would flatten when the structure of the anthers had matured. The tapetum in this phase had begun to degenerate. The stomium appeared to have formed in this phase, with its flat cells aligned anticlinally. The stomium is the tissue that opens to support pollen release from the anther (Mulyani, 2016). However, structurally, in this phase, the locus had not yet united into theca, thus indicating that the pollen was not yet fully mature and not ready to be released.

In phase 7, the dividing tissue between the locus disappeared, and the previously formed stomium opened sideways or horizontally. As a result, the septa of two loci opened to become theca. The theca was a storage space for mature pollen (Muniraja et al., 2018). So, in phase 7, with a flower bud size of 7 mm x 2.5 mm and an anther size of 2.6 mm x 1 mm, the pollen was wholly mature and ready to be released when the stomium opened vertically. Compared with the anthers of the Brassicaceae tribe *Arabidopsis thaliana* phase 7 in Sanders et al.'s research (Sanders et al., 1998), there were differences in development. The visible difference was that the tapetum in phase 7 *of A. thaliana* anther was still there and had not experienced the degeneration stage. Meanwhile, in phase 7, *C. rutidosperma* anthers, the tapetum, was gone or degenerating. This was caused by determining the number of phases used; in Sanders et al.'s (1999) research, there were more phases used than in this study.

Formation of the theca and opening of the stomium meant pollen was spread out of the theca and ready to pollinate the stigma while the flower was still in bud. C. rutidisperma might have cleistogamy (CL) or pseudo-cleistogamy flowers. The environment influences the flower shape, which could change chasmogamy (Bernetski, 2015). Environmental conditions could inhibit the development of CH flowers before the flowers open and fail to open the flowers mechanically so that fertilization occurs in flower buds (CL flowers) (Love & Ferris, 2024). In contrast to dimorphic cleistogamy, there were no morphological differences between CL and other CH flowers compared to the lack of flower expansion and anthesis in CL flowers. The shift from CH to CL flower production occurred more rapidly during unfavourable conditions such as drought and

low temperatures, which often encouraged CL flower production and relatively high humidity (Zhang et al., 2018), as well as reduced light and temperature conditions, could fail flower buds to open (Penso et al., 2020).

#### **CONCLUSION**

A slide of *Cleome rutidosperma* in phase 2 shows that the pollen was still in the cleavage stage, as indicated by the pollen still being fused. The cleavage stage was completed in phase 3, with the pollen having separated. Ripe pollen occurs from phase 6, and the stomium tissue structure has been formed. The theca was formed in phase 7, which appears simultaneously as the stomium opens horizontally.

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