# COMPARATIVE ANATOMICAL STUDIES ON SOME SPECIES OF THE GENUS *Artocarpus*

Dian Palupi[1)], Riska Desi Aryani[1)], Sri Lestari[1)]
[1)]Faculty of Biology, Jenderal Soedirman University
email: dian.palupi@unsoed.ac.id

***Abstract***

*The genus Artocarpus is a member of the Moreceae family that is spread in tropical and subtropical regions, which are spread across Southeast Asia and Asia Pacific, including Indonesia. This research was conducted in ten districts in the Banyumas Regency area. Sampling of plants was done randomly selected (purposive random sampling), then the leaves were made preparations for preserved anatomy to determine the anatomical character. The data obtained from the anatomical observations were then analyzed descriptively to obtain anatomical data. There are five members of the genus Artocarpus found in the Banyumas district, namely Jackfruit (Artocarpus heterophyllus Lam.), Cempedak (Artocarpus integer (Thunb) Merr.), Breadfruit (Artocarpus altilis [Parkinson] Fosberg), Kluwih (Artocarpus camansi Blanco) and Benda (Artocarpus elasticus Reinw.exe. Ex Blume. shows anatomical variations in cuticle thickness, epidermal thickness, palisade ratio, and mesophyll thickness.*

***Keywords:*** *anatomy, Artocarpus, comparative*

## 1. INTRODUCTION

*Artocarpus* is a genus of the Moraceae which has members of about 50 species, many of which produce edible fruit. *Artocarpus* members that are well-known as consumption fruits include *A. heterophyllus* (jackfruit), *A. integer* (cempedak), *A. altilis* (breadfruit), A. lakoocha (lakoocha), *A. kemando* (pudau) and *A. odoratissimus* (terap). Several species of *Artocarpus* produce wood which can be used as a building material, or for making furniture. *Artocarpus* originates from South Asia, Southeast Asia, Papua and the South Pacific Islands. (Somashekhar et al., 2013).

*Artocarpus* research that has been carried out in Indonesia is more about variations in morphological characters, namely in *Artocarpus heterophyllus* (jackfruit) conducted in West Kalimantan (Safitri et al., 2017), *A. integer* (cempedak) (Muchils et al., 2017) and *A. atilis* (breadfruit) in Yogyakarta (Estalansa et al., 2018). Research on *Artocarpus* in Indonesia is not only based on morphological characters, but also research using molecular markers, namely *A. heterophyllus* (Palupi, 2019) and *A. champeden* (Hakim, 2005).

The anatomical approach can show a correlation between anatomical characters and other characters; therefore, this data can be used to strengthen taxon boundaries, especially for taxonomic evidence such as dubious morphological characters. Generally, anatomical characters provide a reliable basis for differentiating species, but usually these anatomical characters have great use in infrageneric taxon. These characteristics are fairly constant and can be diagnostic. Anatomical characters are used both for identification practice and to determine phylogenetic relationships (Judd, 2003).

Comparative studies of plant structure, morphology and anatomy have become the backbone of plant systematic efforts to explain diversity, phylogeny and evolution. The leaf is an organ that can be used as a growth parameter because it is easy to adapt to environmental changes (Dickison, 2000). Research on the anatomical characters of *Artocarpus altilis* and *A. communis* can be used to identify and describe the two species, anatomical characters can be used to obtain special characters that can be used to limit 2 taxa. The specific anatomical characters to differentiate between *Artocarpuas altilis* and *A. communis* are the vascular tissue in the roots, the tissue in the leaf spines, the density

of the trichomata, and the abaxial and existing epidermal cells (Akinloye et al., 2015). Trichomata on *Artocarpus heterophyllus* are found in the upper and lower leaf epidermis layers. There are two types of trichomata in *A. heterophyllus*, the first type has a bicellular head with a unicellular stalk, while the second type of trichomata has a unicellular head with a uniseriate stalk (Reddy et al, 2015).

Information about the *Artocarpus* genus in Banyumas Regency is expected to add information about the wealth of germplasm and become the basis for management efforts, development of its potential and benefits, especially for science and for society in general. The purpose of this study was to compare the anatomical characters between members of the genus *Artocarpus* in Banyumas Regency.

## 2. RESEARCH METHODS

### a. Place and Time of the study

This research was conducted in the Banyumas Regency area. Preparation and observation of anatomical characters were carried out in Laboratory of Plant Structure and Development, Faculty of Biology, Jenderal Soedirman University from August 2019 to February 2020

### b. Sampling of Plants

The method used was a survey method with a purposive random sampling technique. *Artocarpus* plant samples were taken in several areas of Banyumas Regency, namely Cilongok, Ajibarang, Sumbang, Kembaran, Kemranjen, Somagede, Patikraja, Banyumas, Kedungbanteng, Karanglewas districts. *Artocarpus* plant samples were then taken from the 3rd leaf from the shoot in the middle of the tree branching and anatomical preservation preparations were made to determine its anatomical character.

### c. Making Leaf Anatomical Preparations

Observation of the leaf anatomy character was carried out by making preparations using the paraffin method, staining with 1% safranin in 70% alcohol. *Artocarpus* leaves are cut 1 cm long with a razor blade, then fixed with FAA fixate solution for 24 hours. Dehydrate with alcohol levels ranging from 70% alcohol to absolute alcohol for 30 minutes each. Dealkoholization with alcohol-xylol solution in a ratio of 3: 1, 1: 1, and 1: 3 for 30 minutes each. Infiltration with a xylol-paraffin mixture with a ratio of 1: 9 for 24 hours, then replaced with pure paraffin which is carried out in an oven at a temperature of 57 $^0$C. Making paraffin blocks, making small boxes of cardboard, then pouring liquid paraffin into small boxes that have been smeared with glycerin. The pieces of the *Artocarpus* leaves are put in a box and their position is arranged in such a way. The chilled paraffin block is removed from the mold and then cut and attached to the holder on the rotary microtome. An incision was made with a thickness of 10 µm. The paraffin strips containing the preparation are placed on a glass object which has previously been dripped with glycerin and albumin and dried on a heating box until the paraffin bands stretch. Staining or staining by dipping a glass object containing the preparation into pure xylol is repeated 2 times, then xylol-alcohol with a ratio of 3: 1, 1: 1, and 1: 3, then treated with alcohol decreasing from 100% alcohol to alcohol 70%. Then put in 1% safranin dye in 70% alcohol for 1-2 hours. Washing with 70% alcohol and dehydrating with 70% alcohol to 100% alcohol, alcohol-xylol with a ratio of 3: 1, 1: 1, and 1: 3, then the pure xylol is repeated 2 times for 5 minutes each. Labeling includes the name of the preparation and the direction of the slices of the preparation.

### d. Data Analysis

The data obtained from the anatomical observations were then analyzed descriptively to obtain anatomical variations from members of the genus *Artocarpus*.

## 3. RESULT AND DISCUSSION

The anatomical characters that have been observed are the size and density of the stomata and the density of the trichomata per 1 mm$^2$ of area. Parameters were cuticle thickness, epidermal thickness, palisade ratio, and mesophyll thickness.

**A. Stomata**

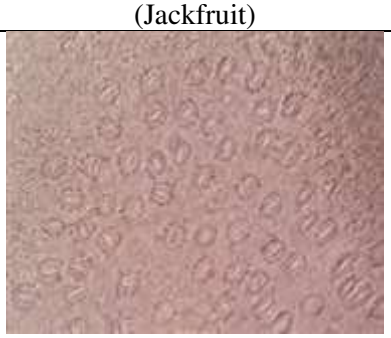
Based on the observation of the paradermal incision, the leaf stomata of the five members of the genus *Artocarpus* are only found on the abaxial (hypostomatous) side of the leaf, arranged scattered, tightly, and bounded by leaf bones. All members of the genus *Artocarpus* were observed to have anomocytic stomata type. Anomocytic stomata type is characterized by guard cells surrounded by cells that are indistinguishable from epidermal cells. Stomata with anomocytic type are also found in nagka and *Artocarpus altilis* (breadfruit) in Malaysia (Sikarwar et al. 2015). The form of guard cells of the stomata of the genus *Artocarpus* is a kidney with elliptical stomata and a few rounded stomata.

Tabel 1. Average character length, width and density of stomata on *Artocarpus* leaves

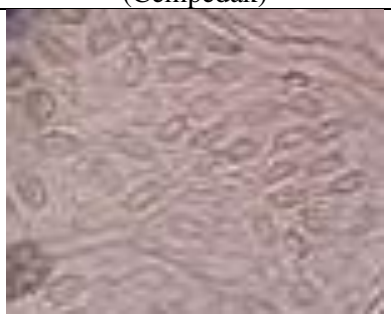
| No | Plant Name | Stomat length (µm) | Stomat a width (µm) | Stomata Density ($mm^2$) |
|---|---|---|---|---|
| 1 | Jackfruit | 16.80 | 7.3 | 59.8 |
| 2 | Cempedak | 15.65 | 6.65 | 55.4 |
| 3 | Breadfruit | 18.90 | 7.4 | 39.8 |
| 4 | Kluwih | 14.55 | 6.7 | 39.2 |
| 5 | Benda | 16.70 | 6.65 | 30,8 |

The length of the stomata differs in the five members of the genus *Artocarpus*. The results of the measurement of stomata length in the five members of the genus *Artocarpus* which have the longest average length of the stomata on the leaves is 18.90 µm in breadfruit plants. The kluwih plant has the shortest stomata length, which is 14.55 µm. Cempedak has an average stomata length of 15.65 µm. Jackfruit has an average stomata length of 16.80 µm. Objects have an average stomatal length of 16.70 µm. Stomata in the same family members as *Artocarpus*, namely awar-awar as an outgroup, have an average stomata length of 17.90 µm. The results of the measurement of the stomata width of the five members of the genus *Artocarpus* which had the widest average length of the stomata on the leaf, was 7.4 µm in breadfruit plants. Cempedak, object, and proof plants have the shortest stomata width, namely 6.65 µm. Kluwih has an average stomata width of 6.7µm. Jackfruit has an average stomata width of 7.3 µm.

*Artocarpus heterophyllus* Lam. (Jackfruit)

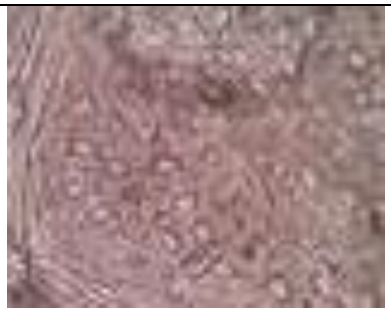


*Artocarpus integer* (Thunb) Merr. (Cempedak)



*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg (Breadfruit)

*Artocarpus camansi* Blanco (Kluwih)



*Artocarpus elasticus* Reinw. ex Blume (Benda)

Figure 1. Paradermal incision of the abaxial side of the *Artocarpus* leaf shows scattered stomata. (400x magnification)

**Stomata Density**

The highest stomatal density was found in jackfruit, with an average of 59.8 / $mm^2$ and the lowest stomata density in object plants, which was 30.8 / $mm^2$. Character stomata density can change according to environmental conditions such as light and air $CO_2$ levels). *Artocarpus elasticus* has a smaller stomata density under shade than in full sun (Kardiman and Raebild 2018). *Artocarpus* stomata have the same structure and are found only on the underside of the leaves, these stomata are not found on the top of the leaves.

**B. Trichomata**

Based on the paradermal incision, there are two types of trichomata found in *Artocarpus* leaves, namely non-glandular, unicellular trichomata, tapered tip, rounded base, and radially surrounded by several epidermal cells and glandular trichomata, multicellular head cells consisting of 4-8 cells, and surrounded by several epidermal cells radially. The base of the trichomata is characterized by a hexagon shape with an oval in it. The base of the trichomata in the paradermal incision is thought to be part of the stem cells of the glandular trichome when viewed transversely.

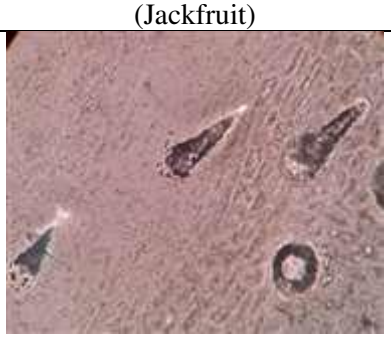
Tabel 2. Non-glandular trichomata densities on *Artocarpus* leaves

| No | Plant Name | Average Non-Glandular Trichoma density (Upper) | Average Non Glandular Trichomata Density (Lower) |
|---|---|---|---|
| 1 | Jackfruit | 15.82 | 19,67 |
| 2 | Cempedak | 16.64 | 19.84 |
| 3 | Breadfruit | 18.83 | 20.52 |
| 4 | Kluwih | 18.54 | 20.25 |
| 5 | Benda | 17.55 | 19.73 |

Tabel 3. Glandular trichomata densities on *Artocarpus* leaves

| No | Plant Name | Average Glandular Trichoma density (Upper) | Average Glandular Trichomata Density (Lower) |
|---|---|---|---|
| 1 | Jackfruit | 7.85 | 10.58 |
| 2 | Cempedak | 8.59 | 12.85 |
| 3 | Breadfruit | 12.24 | 14.62 |
| 4 | Kluwih | 12.14 | 15.53 |
| 5 | Benda | 10.52 | 12.65 |

*Artocarpus heterophyllus* Lam. (Jackfruit)



*Artocarpus integer* (Thunb) Merr. (Cempedak)

*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg (Breadfruit)

*Artocarpus camansi* Blanco (Kluwih)

*Artocarpus elasticus* Reinw. ex Blume (Benda)

Figure 2. Cross Section of Trichomata on *Artocarpus* Leaves (400x magnification)

**Trichomata Density**

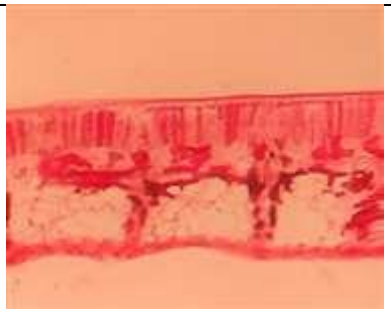
The density of non-glandular trichomes was higher than glandular trichomes on both the adaxial and abaxial sides of the five *Artocarpus* plants, while the density of glandular trichomes was higher than that of non-glandular trichomes on the abaxial side. The results of measurements of the upper and lower glandular trichomata density of the five *Artocarpus* leaves showed that the highest and lower non-glandular trichomata densities were 18.83 and 20.52, while the smallest non-upper and lower glandular trichomata density was found in jackfruit, namely 15.82 and 19.67. The results of measurements of the upper and lower trichomata density of the five *Artocarpus* leaves showed that the highest and lower gland trichomata densities were 12.24 and 14.62, while the smallest upper and lower gland trichomata densities were found in jackfruit, namely 7.85 and 10.58.

**Observation of Leaf Transverse Incision Microscopic Preparations**

The results of transverse incisions of the five leaves of the genus *Artocarpus* show a similar structure. The leaves of *Artocarpus* have the structure of the epidermis, mesophyll and vessels. In accordance with the function of leaves in plants, which play a role in the photosynthesis process, which is to convert solar energy into carbohydrates as an ingredient in growth and development. The cuticle on the leaves is formed by changes in environmental factors that are less favorable for the plant, the thickness of the cuticle on the plant is formed as a form of adaptation in reducing the transipiration rate in the leaves. The cuticle on the *Artocarpus* leaf is found on the top of the leaf, while the cuticle layer is not visible on the underside of the leaf.

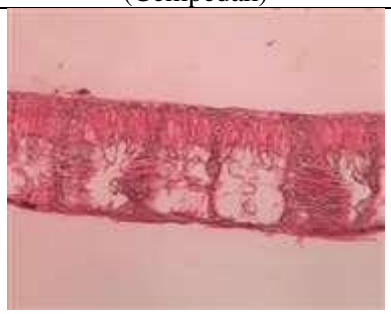
Table 4. Average of cuticle and epidermal characters on *Artocarpus* leaves

| No | Plant Name | Thickness Cuticles (µm) | Thickness of Upper Epidermis (µm) | Thickness of the lower epidermis (µm) |
|---|---|---|---|---|
| 1 | Jackfruit | 6.3 | 10.9 | 7.9 |
| 2 | Cempedak | 5.7 | 10.5 | 7.5 |
| 3 | Breadfruit | 6.5 | 14.5 | 8.5 |
| 4 | Kluwih | 6.7 | 15.0 | 8.8 |
| 5 | Benda | 6.9 | 15.3 | 8.9 |



*Artocarpus heterophyllus* Lam. (Jackfruit)

*Artocarpus integer* (Thunb) Merr. (Cempedak)



*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg (Breadfruit)

*Artocarpus camansi* Blanco (Kluwih)

*Artocarpus elasticus* Reinw. ex Blume (Benda)

Figure 3. Leaf Anatomy of *Artocarpus* (magnification 100x)

**C. Cuticles**

The results of measuring the cuticle thickness of the five members of the genus *Artocarpus* showed that the object plants had the largest mean cuticle thickness of 6.9 µm, while the smallest cuticle thickness was found in cempedak, which was 5.7 µm. The cuticle has a major role in reducing the evaporation of water on the leaf surface due to direct sun exposure and an increase in air temperature in the environment.

**D. Epidermis**

The results of measuring the thickness of the upper and lower epidermis of the five members of the genus *Artocarpus* showed that plant objects had the largest average upper and lower epidermal thickness, namely 15.30 µm and 8.90 µm, while the smallest upper and lower epidermal thicknesses were found in cempedak plants, namely 10.5 µm and 7.5 µm. The epidermis on the leaves of *Artocarpus* is found on the upper and lower surfaces of the leaves, which has an important role in protecting the organs and protecting the underlying tissues from both biotic and abiotic disturbances from the environment.

**E. Palisade ratio**

**Tabel 5**. Average of palisade ratio and mesophile thickness on *Artocarpus* leaves

| No | Plant Name | palisade ratio | mesophile thickness (µm) |
|---|---|---|---|
| 1 | Jackfruit | 9.85 | 274.5 |
| 2 | Cempedak | 8.64 | 260.7 |
| 3 | Breadfruit | 10.45 | 288.9 |
| 4 | Kluwih | 12.14 | 298.8 |
| 5 | Benda | 12.03 | 293.7 |

The results of measuring the palisade ratio of the leaves of the five members of the genus *Artocarpus* showed that the kluwih plant had the largest average palisade ratio of 12.14, while the smallest palisade ratio was found in the cempedak plant, which was 8.64. Palisade on *Artocarpus* leaves is one of the mesophyll derivatives that functions in photosynthesis. Palisade cells are located under the upper epidermis to get full sunlight and contain lots of plastids (chloroplasts) which function to capture light and the site of photosynthesis.

**F. Mesophiles**

The measurement results of the leaf mesophyll thickness of the five members of the genus *Artocarpus* showed that the kluwih plant had the largest average mesophyll thickness of 298.8 µm, while the smallest mesophyll thickness was found in the cempedak plant, which was 260.7 µm. The mesophyll tissue of *Artocarpus* leaves differentiates into palisades and sponges with the dorsiventral type. Dorsiventral mesophyll type has also been observed in jackfruit leaves in India (Reddy et al. 2015). Palisade tissue on *Artocarpus* leaves is rectangular, compactly arranged, and extends vertically, while spongy tissue is irregular in shape and has a lot of space between cells.

## 4. CONCLUSION

The five species of the genus *Artocarpus* found in the Banyumas district are Jackfruit (*Artocarpus heterophyllus* Lam.), Cempedak (*Artocarpus integer* (Thunb) Merr.), Breadfruit (*Artocarpus altilis* [Parkinson] Fosberg), Kluwih (*Artocarpus camansi* Blanco) and Benda (*Artocarpus elasticus* Reinw.ex Blume shows anatomical variations in cuticle thickness, epidermal thickness, palisade ratio, and mesophyll thickness.

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