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FLORIBUNDA
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IS THE FLOWER OF PULASAN (NEPHELIUM RAMBOUTAN-AKE) A PROTOGYNY OR PROTANDRY?

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Nina Ratna Djuita, Alex Hartana, Tatik Chikmawati & Dorly. 2021. Apakah Bunga Kapulasan (*Nephelium ramboutan-ake*) Bertipe Protogini atau Protandri?. *Floribunda* 6(7): 239–247. — Kapulasan merupakan tumbuhan androdioecious yang mempunyai bunga jantan dan bunga hermafrodit pada individu yang berbeda. Penelitian ini bertujuan untuk mendapatkan data tentang anatomi bunga kapulasan dan untuk membuktikan apakah bunga kapulasan mempunyai pematangan putik dan benang sari yang bersamaan atau tidak. Pengamatan anatomi menggunakan bunga majemuk yang berasal dari tiga pohon jantan dan tiga pohon hermafrodit. Tiap pohon diambil dua bunga majemuk yang masih kuncup, hampir mekar, dan yang sudah mekar. Bunga diamati dalam potongan melintang dan membujur. Hasil penelitian menunjukkan bahwa kelopak bunga kapulasan terdiri atas satu lapis epidermis dan banyak lapis sel parenkima berbentuk polihedral. Perkembangan benang sari dimulai dari kepala sari diikuti dengan pembentukan tangkai sari. Serbuk sari dari bunga hermafrodit matang terlebih dahulu daripada putik. Perkembangan putik dimulai dari pemanjangan sel-sel meristem di bagian pusat bunga dan berakhir dengan pelengkungan kepala putik.
Kata kunci: Anatomi bunga, androdioecious, bunga hermafrodit.

Nina Ratna Djuita, Alex Hartana, Tatik Chikmawati & Dorly. 2021. Is the Flower of Pulasan (*Nephelium ramboutan-ake*) a Protogyny or Protandry?. *Floribunda* 6(7): 239–247. — Pulasan is an androdioecious plant that has both male and hermaphrodite flowers on separate plants. The objectives of this study were to obtain data about the anatomical structure of pulasan flower and to investigate whether the pistil and the stamens of pulasan flower reach maturity at different times. The anatomical observation was done on compound flowers taken from three male trees and three hermaphrodite trees. Two compound flowers that still in buds, about to bloom and fully bloom were picked from each tree. Flowers were observed in a cross and longitudinal section. The results showed that the sepals of pulasan flower comprised of the uniseriate epidermis and multilayered polyhedral parenchymal cells. Stamen development started from the anther followed by the formation of the filament. The pollen of hermaphrodite pulasan flowers reached maturation earlier than the pistil. The pistil development started from the expansion of meristem cells in the center of the flower and ends with the warp of the stigma.
Keywords: Androdioecious, floral anatomy, hermaphrodite flower.

The terms andromonoecious, androdioecious, gynomonoecious, and gynodioecious are known among plants related terminology (Dellaporta & Calderon-Urrea 1993). Rambutan, a member of Sapindaceae (*Nephelium lappaceum*) and pulasan (*Nephelium ramboutan-ake*) are androdioecious plants, which are plants with male and hermaphrodite flowers in different individuals. Male and hermaphrodite pulasan flowers do not have petals, thus it is an incomplete flower.

Researches on pulasan are generally about genetic and morphological diversity only. The morphological structure of pulasan has been studied by Djuita *et al.* (2016). It was reported that pulasan showed a varied amount of flower parts such as the petals, stamens, and pistils. In addition to morphological data, it is also necessary to conduct research on the flower anatomy to obtain

complete information about pulasan flower.

Anatomical studies on the plant relatives of pulasan have been carried out on *Cardiospermum, Urvillea*, and *Dodonae*a from Sapindaceae. Some unique features that can only be seen by observing the inner structure of the flower were discovered. *C. grandiflorum* and *U. chacoensis* had tetrahedral microspores (Soltis *et al.* 2010). The tissues of *C. grandiflorum* are not differentiated, and the nectar has stomata. *U. chacoensis* had apparent nectariferous tissue, and the abaxial side was equipped with nectarostomata, while the other side was not exist (Soltis & Ferrucci 2009). Flowers of *D. viscosa* had glandular trichomes with multicellular stalks on the ovary (Karkare-Khushalani & Mulay 1964).

Although the flower anatomical information of close relatives is available, the anatomy of the pulasan flower has never been studied. Therefore, the flower anatomical unique features are not recognized. It is unknown whether the pistils and stamens of hermaphrodite pulasan flower reach the maturity simultaneously. This information can be useful to find out the tendency of pollination of pulasan, then it can be used in plant breeding to get superior outward cultivars. The objectives of this study were to obtain data about the anatomical structure of pulasan flower and to investigate whether the pistil and the stamens of pulasan flower reach maturity at different times.

MATERIALS AND METHODS

The anatomical structure of the pulasan flower was observed on three male trees and three hermaphrodite trees. Two compound flowers that still in buds, about to bloom and fully bloom were picked from each tree in Mekarsari Fruit Park and Cipaku Experimental Garden, Bogor. Pulasan flowers were stored in 70 % alcohol in bottles for 1 day, then the flowers were fixed into a solution of formalin, acetic acid, and alcohol (FAA) and were let to sit for one week.

The pulasan flower buds which had been fixed in FAA were dehydrated based on the Johansen procedure (1940). After that, the sample was infiltrated into paraffin blocks then soaked in Gifford solution for 4–6 weeks. Blocks containing flower samples were then sliced into the cross and longitudinal sections as thick as 10 µm using Yamato RV 240 rotary microtome. The next step was the preparation of flower staining using safranin 2 % for 2 nights and in fast green 0.5 % for 8–10 minutes. After the staining, the slides were given entellan then covered with a cover glass and left to dry and photographed.

RESULT AND DISCUSSION

Pulasan has racemes and panicles inflorescences. There are different sized flower buds in an inflorescence, large buds interspersed with smaller buds. Each flower has one bracteole (Figure 1). Bracteole consisted of 2–3 layers multiseriate epidermis and several layers of homogeneous parenchymal cells. The epidermal and parenchymal cells are rectangular, with a smaller size of epidermis. There are 4–8 sepals inside the bracteole.

Several flower buds in different developmental stages were observed in the longitudinal section (Figure 1). Some flower buds were forming sepals and some others are also forming stamen. These stages are almost identical, except in the formation of buds for both male flowers and hermaphrodite flowers. The pulasan flower consisted of sepals, stamens, and pistils on hermaphrodite flowers, while the male flowers consisted of sepals, stamens, and pistillode. Pistillode is a simple pistil and reduced in size and function. The previous literature (Leenhouts 1986; Seibert 1992; Lim 2013) only described parts of flowers without distinguishing male flowers from hermaphrodite flowers and mentioning of pistillode.

In the early development of the sepals, meristem cells divided periclinal to the surface. These cells then expanded and formed a dome (Figure 2) with identical cell form. This dome will eventually develop and produce cells differing in shape between the intercalary meristem and lateral meristem. The cells in the middle are shaped polyhedral and large, while the cells at the side are rectangular and smaller. The dome-shaped structure will form the sepals from the sides.

Pulasan flower sepals are composed of several types of plant tissues. They are comprised of the uniseriate epidermis in the outer part and multilayered polyhedral parenchymal cells in the inside. There are uneven sized vascular bundles among the uneven parenchymal tissues, one large vascular bundle is in the middle of the sepals, while the small bundles are from the middle to the tip of the sepals.

In time with the development of flower sepals, stamens are formed gradually (Figure 3) with varying amounts of stamens. The maximum amount of pulasan stamens formed is 9. In the early stages, meristems are in the form of small

dome which will get bigger eventually. The next stage is the flattening/leveling of the dome and the growth of the cells in the middle that develop to the upper part. These cells grow further and produce a higher dome. At the edge of the meristem, there is a twist that will produce two stamen primordia. The flattening of meristem is repeated to produce four stamen primordia. This stage keeps repeating until the maximum number of stamens is produced in the flower.

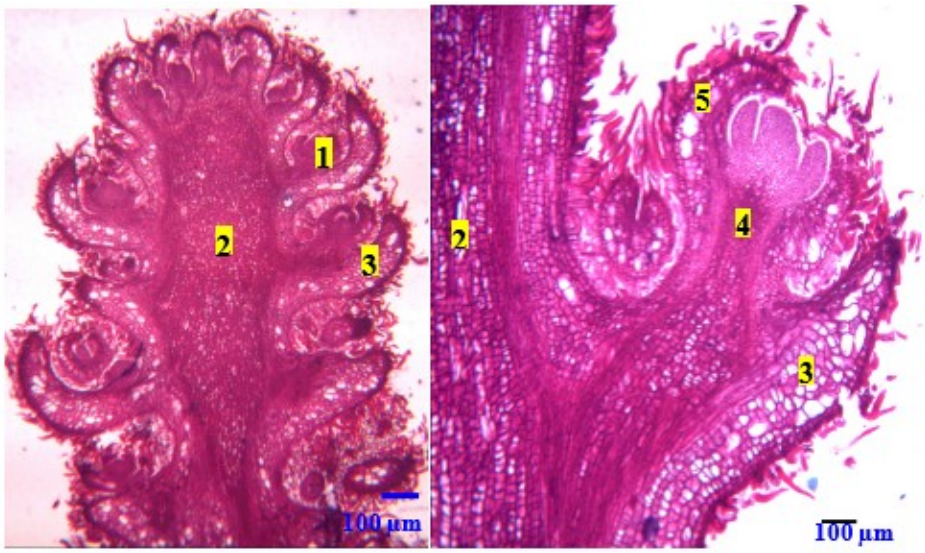

Figure 1. Longitudinal section of pulasan inflorescence 1. Flower Primordia, 2. Floral axis, 3. Bracteole, 4. Flower stalk, 5. Sepal.

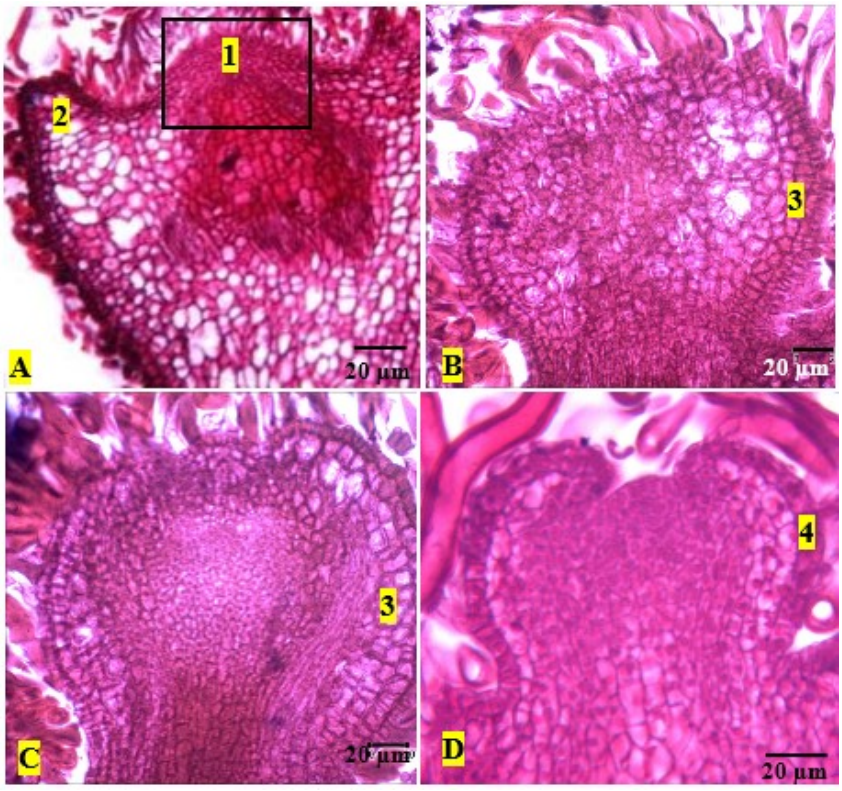

Figure 2. Formation of the sepals of hermaphrodite pulasan flower A. Meristems which will develop into a primordia sepals, B-D. The stages of sepal formation, 1. Meristem dome, 2. Bracteole, 3. Sepal, 4. Epidermis of sepal.

Stamen development started from the anther followed by the formation of the filament (Figure 4). While the filament is still short, the anther will form tetrasporangium. There are four types of attachment of the anther to the filament: basifixed, dorsifixed, versatile, or adnate. Adnate is found in pulasan, where both thecae are attached to the filament while the anther is facing to the flower center (introrse). The position of the stamens on the petals varies, some are facing each other and the others are interspersed.

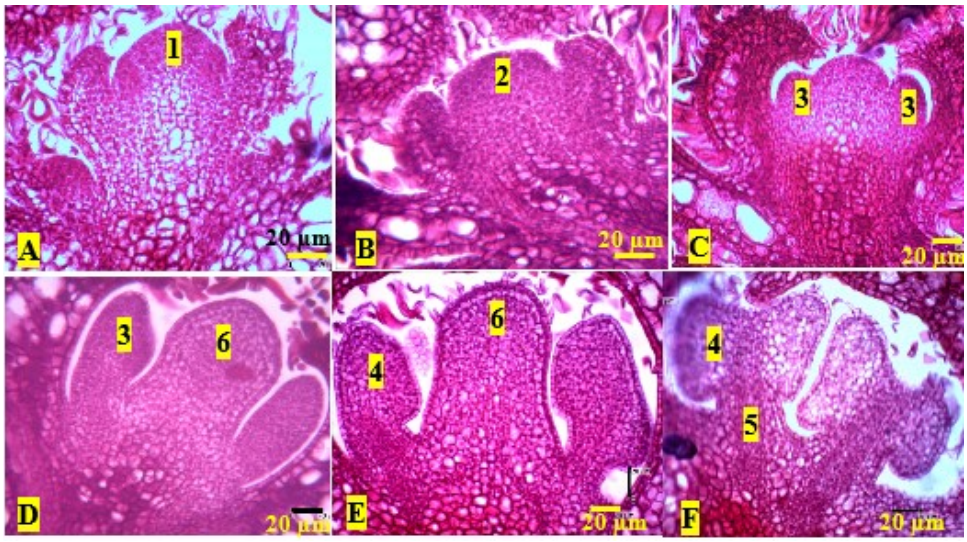

Figure 3. Development of stamens in hermaphrodite pulasan flowers A-F. primordia stamens, 1. Meristem dome, 2. Levelling of meristem, 3. Primordia stamen, 4. Primordia anther, 5. Primordia filament, 6. Primordia pistil.

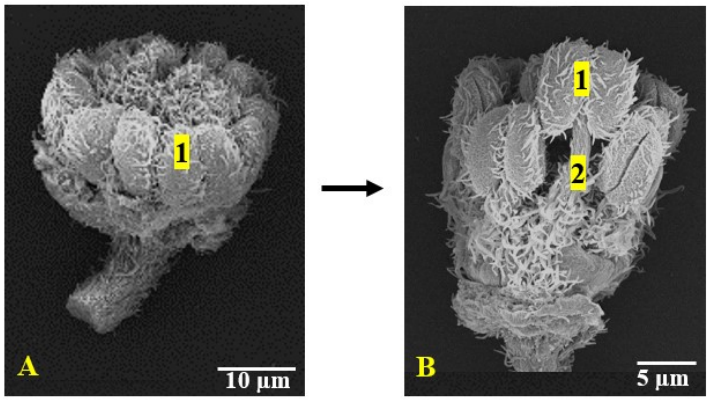

Figure 4. Stamen development on hermaphrodite pulasan flower A. Early stage, B. Advanced stage, 1. Anther 2. Filament.

All the stamens were seen to be in the same position, lower than the pistil, in young flower (Figure 4A). However, in the next stage, all the filaments were elongated individually exceeding the pistil (Figure 4B). This phenomenon was predicted to be related to the formation of stamens that occur gradually. Therefore, the elongation of the filament follows simultaneously. In the later stages, the pistil is elevated. Thus in mature hermaphrodite flower the position of the pistil is higher than that of the anthers.

The pulasan anthers consisted of several layers, the outermost was papillary epidermis followed by endothecium cells in the inner part. Fibrous bands were found within the endothecium cells (Figure 5). These fibrous bands were originated from the inner part of the tangential wall (Bhojwani & Bhatnagar 1996). In the earlier, more primitive plants relative, the endothecium consisted of many layers. Whereas in the latest, more advanced relative, the endothecium tissues tend to be reduced (Bhandari 1984).

Endothecium cells have the role to open the anther. The next layer is the middle layer. The thickness of this layer varies from 2–3 layers or more depending on the type of plant. The innermost part of the anther is tapetum. Tapetum provides nutrition for microspores development. Microspores develop further into pollen. Tapetum was not present in the mature tetrasporangia.

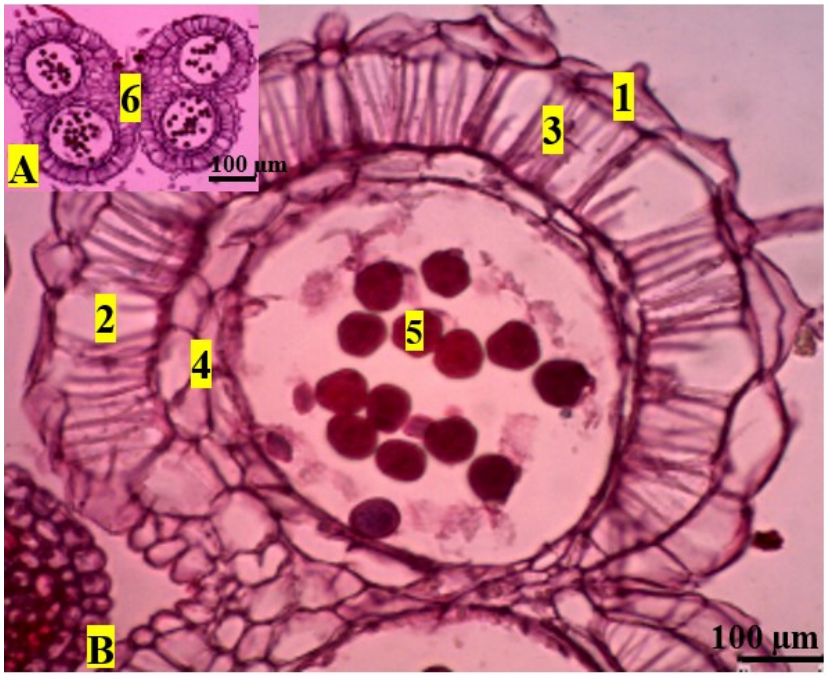

Figure 5. Cross section of the anther of pulasan male flower A. Tetrasporangia, B. Magnified sporangium, 1. Epidermis, 2. Endothecium, 3. Fibrous/Callose bands, 4. Middle layer, 5. Pollen, 6. Filament.

In pulasan relatives, *Dodonaea*, the anther consists of several layers. The outermost layer is the epidermis followed by one layer of endothecium cells, a middle layer consisting of three layers, and a tapetum in the innermost. There is no middle layer and tapetum found in the mature *Dodonaea* anthers because the two structures have been absorbed (Karkare-Khushalani & Mulay 1964). In the adult anther in pulasan, tapetum was not found as well, however, the middle layer still existed.

Pollen on pulasan flowers is formed along with the early development stages of the very young pistils. When a new pistil forms the ovary, pollen has been developed in the anther (Figure 6). Pollen development can occur at different periods even in the same flower (Figure 7), and pollen has matured when the pistil has not matured yet (Figure 6). Thus, it is suspected that protandry transpires in pulasan. Protandry complicates self-pollination, although the possibility of occurrence is small.

Male and hermaphrodites pulasan flowers have triangular pollen when observed in the equatorial plane. Whereas when viewed in a polar plane, the surface is convex, radially symmetrical, has an aperture in the form of three pores and three elongated slits, so-called tricolporate type. It also has striata on the surface as ornament (Figure 8).

Tricolporate pollen, other than in Sapindaceae family members, can also be found in other families such as *Vitaceae* e.g *Vitis vinifera* (Gallardo *et al.* 2009), *Lamiaceae* family, for example, *Lantana indica* and *Clerodendrum phlomides* (Perveen & Qaiser 2007), and the *Scrophulariaceae* family for example *Verbascum nigrum* (Furness & Rudall 2004). However, the same family can have different pollen types, for example *Sapindaceae* has tricolpate pollen on *Acer obtusatum* (Furness & Rudall 2004) and tricolporate type on *D. viscosa* (Karkare-Khushalani & Mulay 1964). The same type of plant can even have a different type of pollen. Male flower of *Vitis vinifera* subsp. *sylvestris* has tricolporate pollen, whereas the female flower has oval to rounded pollen without aperture.

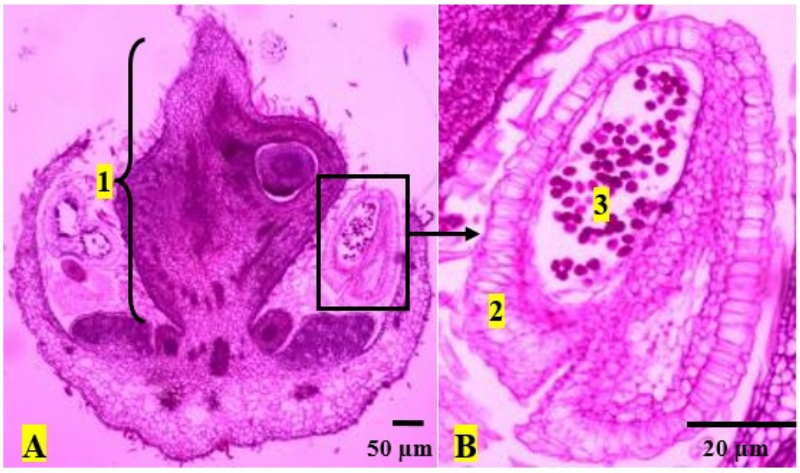

Figure 6. Longitudinal section of young hermaphrodite pulasan flower, it can be seen that the pollen ripens earlier than the pistil A. Flower bud, B. Longitudinal section of tetrasporangium, 1. Pistil, 2. Sporangium wall, 3. Pollen.

Pollen apertures are related to germination, directly or indirectly. In general, sprouts grow from one aperture, but can also appear in more than one aperture. Pollen aperture is an important taxonomic character to classify a plant (Bhojwani & Bhatnagar 1996). Pollen of pulasan has three pores and three slits, however, the sprouts appears from one pore.

Pistil develops the last. The process of pistil development (Figure 9) started from the expansion of meristem cells in the center of the flower, then the cells undergo differentiation. Firstly, the ovary is formed. Next, the tip of the pistil is slightly narrowing, over time the end part is split into two parts. This part will be the stigma. After this process, then the pistil style is elongated. In mature pistil, the development ends with the stigma curving outward.

Figure 7. Different stage of maturation of pollen in one pulasan male flower 1. Immature pollen, 2. Mature Pollen, 3. Sepal.

There are several types of cells discovered on the stigma. The epidermis in the upper part and the lower part of the stigma had a different shape. The upper epidermis was made up of a layer of the rectangular epidermal cell which was larger than the lower epidermis. The lower epidermis consisted of 1–2 layers of smaller rectangular cells. Trichomes found in the upper epidermis were shorter than those in the lower epidermis. Rectangular multiseriate parenchymal cells were observed under the epidermis. However, the cells were shorter the closer they are to the pistil. The vascular bundle is located inside the parenchyma. It is the extension of the vascular bundle in style.

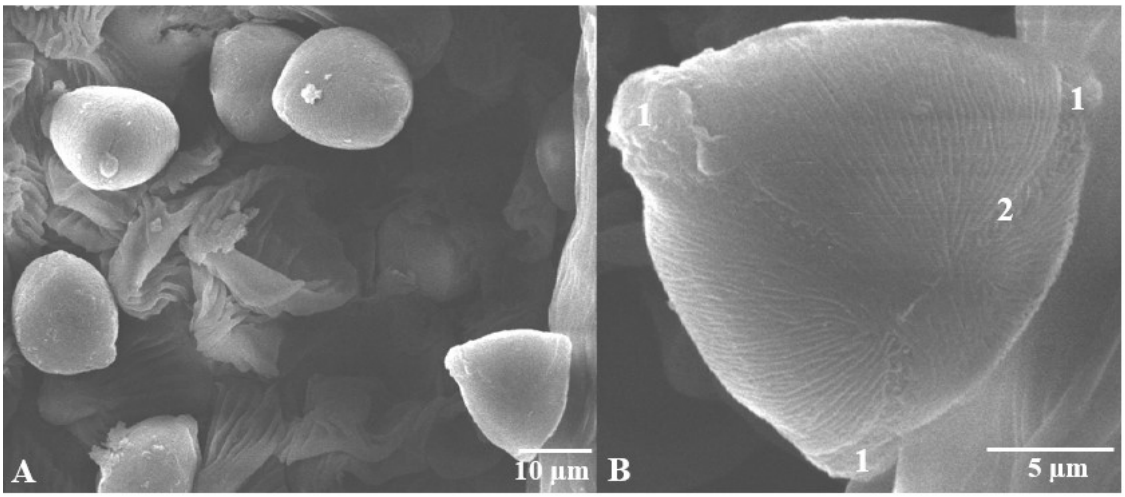

Figure 8. Pollen from pulasan flower A. Pollens, B. Parts of pollen. 1. Pore, 2. Slit.

The stigma was connected to the style. The outermost part was made up of 1–2 layers of rectangular and nearly identical epidermal cells. In some parts, the epidermis was modified into unicellular trichomes. Rectangular-celled parenchymal tissues were observed under the epidermis. Among these tissues, the vascular bundle has thickened secondary walls like a spiral and scalariform. Spiral thickening was seen on protoxylem while scalariform form was found in metaxylem (Fahn 1991). At the innermost part, there are parenchymal cells which were generally rectangular.

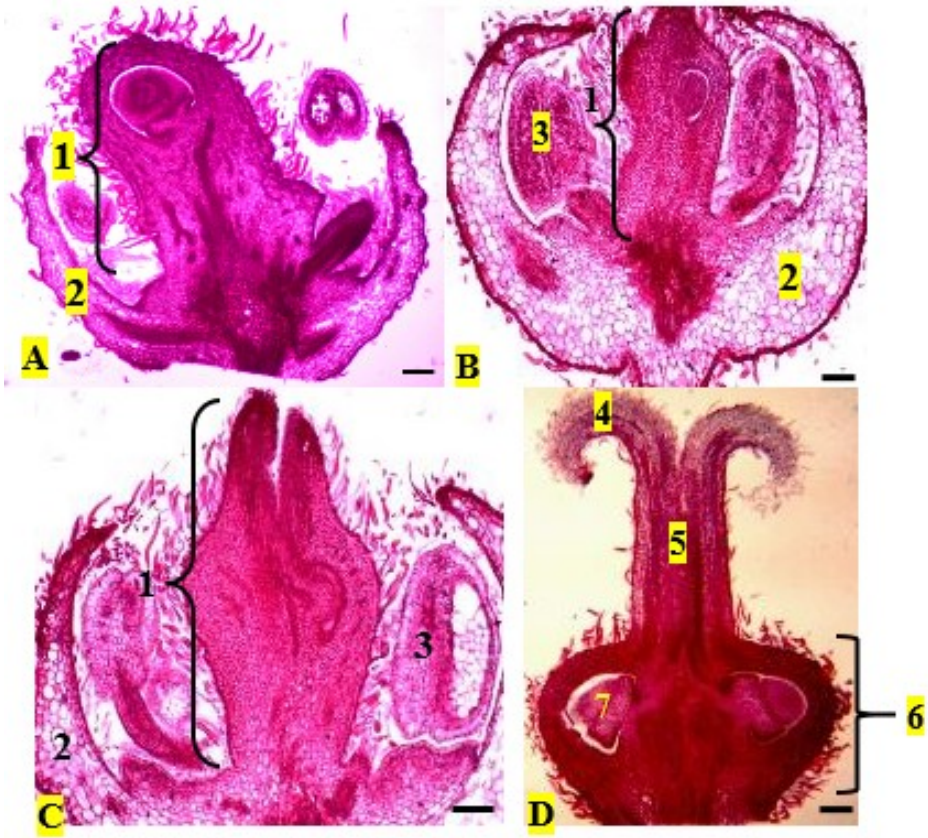

Figure 9. Development of the pistil of pulasan flower A.Pistil at early development stage, B,C. Pistil at advance development stage, D. Mature Pistil, 1.Pistil, 2.Sepal, 3.Stamen, 4.Stigma, 5.Style, 6.Ovary, 7.Ovule. Scale bar = 100 µm.

The other part of the pistil, the ovary is located above the perianth. Ovary of pulasan consisted of 2–3–(5) carpels. Each carpel contained one ovule. The ovule has two integuments (bitegmic). This integument type is commonly found in angiosperms (Wang 2010). In bitegmic ovules, the inner integument was differentiated earlier than the outer integument (Bhojwani & Bhatnagar 1996). Pulasan micropyle is an endostome type that is limited to the inner integument, while the outer integument does not contribute to micropyle formation (Figure 10).

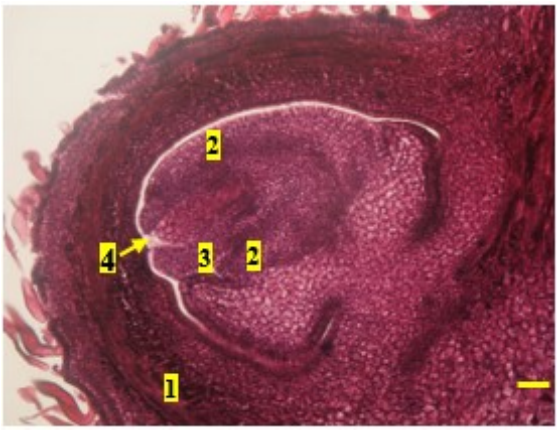

Figure 10. Longitudinal section of pulasan ovule and its parts 1. Wall of ovary, 2. Outer Integument, 3. Inner Integumen , 4. Microphyle. Scale bar = 200 µm.

CONCLUSION

The flowers of pulasan inflorescence have different stages of development. Large flower buds were interspersed with small flower buds. Each bud has one bracteole. The sepals were made up of uniseriate epidermis, and many layers of parenchymal tissue. Vascular bundles in different sizes were found among the parenchymal tissues. The stamen had the introrse anther. The pulasan anther consisted of several layers. The outermost was the papillary epidermis and followed by the endothecium cells with fibrous bands. The pollen of pulasan flower was matured prior to the maturation of the pistil, thus pulasan flower was protandry. The development of the ovary prior to the formation of the stigma. The ovule of pulasan has a bitegmic integument and the micropyle was an endostome type.

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