Pulasan [(*Nephelium ramboutan-ake* (Labill.) Leenh.] fruit trees: variations in flower morphology, and associated differences in pollination type

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Abstract

Pulasan (*Nephelium ramboutan-ake*) is a species of *Sapindaceae* which has hermaphrodite and staminate flowers on different trees. Morphological flower structures and pollination types of the pulasan fruit tree have not previously been reported thoroughly. This study was undertaken to observe the reproductive structure of flowers, and to examine its correlation with pulasan pollination type. A total of 67 hermaphrodite and 14 male trees of pulasan were observed. Some hermaphrodite inflorescences were covered using cloth bags for between four and six weeks to calculate number of fruit, and to determine their pollination system. The number of individual flowers per inflorescence on male pulasan trees was higher than for hermaphrodite ones. Panicles and spikes were found on both types of pulasan inflorescence. Flowers having four sepals was the commonest structure in both flower types. However, five stamens were commonly found in hermaphrodite flowers, while male flowers often had six. In general, stigma were curved in shape, with modification in some flowers. Anther of hermaphrodite pulasan flowers naturally never open, although their pollen is viable, so there is a mechanical barrier for self-pollination. It was concluded that pulasan tends to be an allogamous plant.

Introduction

Sapindaceous plants have various kinds of reproduction structure. Some of them have paniculate inflorescences, (*e.g. Litchi chinensis* and *Pometia pinnata*),[1,2] some have compound dichasia (*e.g. Dimocarpus longan*)[3] and some, thyrses (*e.g. Cupania ludowigii*).[4] Like other members of *Sapindaceae*, plants of the genus *Nephelium* vary in reproductive structure, in terms of flower position (axillar, terminal, or pseudoterminal) and the number of flower parts.[5] It was known that in *N. lappaceum*, male trees produce male flowers, but there are also hermaphrodite trees producing hermaphrodite flowers, some of which function as male, some as female.[6] With a hermaphrodite tree of that type, pollination can take place without the existence of a male tree. *Nephelium ramboutan-ake* also has male and hermaphrodite trees. However, it was not known whether hermaphrodite flowers of this species can function like those of *N. lappaceum*.

The flower morphology of *N. ramboutan-ake* has been described briefly in several academic texts.[5-7] However, research on morphological characteristics and pollination type in respect of pulasan flowers, was still very limited. The hypothesis of this study was that if young inflorescences covered with cloth bags produce fruits, then the pollination was autogamous; if not, then the pollination was allogamous. To determine whether a plant is reproducing via autogamy or allogamy, observation of the flower structure is needed. This study was undertaken to observe those characteristics of pulasan flowers which correlate with pollination type.

Materials and Methods

Plant material and research locations

The plant material used in this research involved 81 pulasan trees, consisting of 67 hermaphrodite trees and 14 male trees. From each tree, 100 flowers were taken, giving a total number of 8100 flowers observed. Flowers were taken from pulasan trees in the districts of Bogor, Sukabumi, and Semarang, in Java, Indonesia (Figure 1).

Flower morphology

Morphological data observed were generative characteristics, including inflorescence type, inflorescence position, and the average number of flowers per inflorescence, as well as sepal number, stamen number, stamen size, ovary number, and stigma type. Numeration of each flower part was carried out to know the incidence of different sepal, stamen and ovary counts. Flower structures were observed under binocular microscope and scanning electron microscope (SEM) type JSM-5310LV (JEOL Ltd., Tokyo, Japan).

Pollen viability

Pollen was given one or two drops of 1% aniline blue,[8] for between 15 and 30 minutes, and was then observed under a compound microscope. The color of viable pollen is blue.

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Key words: Flower structure; allogamy; viable pollen.

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Pollination type

The hermaphrodite inflorescences of six trees in Mekarsari Fruit Garden, and of two trees in Cipaku Experimental Garden (Bogor), were observed, (giving a total of eight observed trees). The experimental design used in this study was the posttest-only control design. Two kinds of inflorescences were used in this observation: treated and control. In total, 146 young inflorescences from the eight trees were covered with cloth bags to prevent allogamy. As a control, 53 young inflorescences not encased in cloth bags, were used. Every two weeks, all of the inflorescences were observed to see whether or not fruits were being produced. After four to six weeks, the bags were opened and the percentage of inflorescences which had produced fruit were counted. These results were compared to the formation of fruit on the control flowers.

Results

Flower morphology

The pulasan flower types were raceme and panicle, located on axillary or pseudo-terminal stems. Each inflorescence consisted of male flowers only, or hermaphrodite flowers only, and these were found separately, on different trees. The generative structure, and flower number in each inflorescence, were different for hermaphrodite and male trees. Male trees had a higher average number of individual flowers per inflorescence, when compared with hermaphrodite trees (Table 1).

Hermaphrodite flowers consisted of sepal, stamen, pistil, and nectar disc (Figure 2A). Male flowers consisted of sepal, stamen, pistillode and nectar disc (Figures 2B,C). Except for the nectar disc, the surfaces of all parts of pulasan flowers were hairy. Neither male nor hermaphrodite flowers have petals. There are several dents on the edge of the nectar disc where the stamen adhere to it, such that the number of nectar dents corresponds to the number of stamen.

The symmetry of pulasan flowers is varied, being either actinomorphic or zygomorphic (Figures 2D-H). Pulasan sepals stick to each other at their base, while the edge is free. On both sides of the surface and the edge of sepals, are found yellowish green hairs. The number of sepals varied in both flower types; being between four and eight in hermaphrodite flowers, and between four and seven in male flowers (Table 1).

The length of the stamen in pulasan hermaphrodite flowers was 2.5±0.6 mm (n=20), while in the staminate flowers, it was 3.6±0.4 (n=20). The length of the filament in hermaphrodite flowers, at 1.7±0.4 mm, was shorter than that in staminate flowers, at 3.1±0.4. The anthers in hermaphrodite flowers are longer (0.8±0.3) than those in staminate flowers (0.5±0.0). Trichome coverage on stamens is different for the two flower types (Figures 3A-D); trichomes cover the whole surface of the stamen in hermaphrodite flowers, but only cover three-quarters of the length of filament in staminate flowers, whose anthers are relatively glabrous. The attachment between the anther and filament is adnate in both flower types.

The anthers of staminate flowers open during anthesis, but those of the hermaphrodite flowers are not opened. Generally, stamens of hermaphrodite flowers attach to the flower until the flower has ripened for one week, at which time the stamen generally falls out. However, with several flowers that developed

Table 1. The morphological characteristics of flowers on hermaphrodite and male pulasan trees.

Characters	Hermaphrodite trees (n=67)	Male trees (n=14)
Inflorescence type	Panicle and raceme	Panicle and raceme
Inflorescence site	Axillary and pseudoterminal	Axillary and pseudoterminal
Flower number per inflorescence	2-986	6-1265
Average flower number per inflorescence	58±30	120±72
Flower parts*		
Sepal	Present	Present
Sepal number	4-8	4-7
Petal	Absent	Absent
Stamen	Present	Present
Stamen number	5-9	5-9
Filament	All covered by trichomes	Part covered by trichomes
Anther	Covered by trichomes	Glabrous
Pistil	Present	Absent
Ovary number	2,3,5	Absent
Pistillode	Absent	Present
Pistillode surface	Absent	Covered with trichomes
Nectar disc	Present	Present

*N, observed hermaphrodite flower number = 6700 flowers from 67 trees; observed male flower number = 1400 flowers from 14 tree.

Figure 1. Sampling sites in Java island: green, Bogor; red, Sukabumi; violet, Semarang (insert = Indonesia).

into fruit, the stamens were still *in situ* until the flower developed into a young fruit, at the age of four weeks (Figures 3E,F).

The anther position within hermaphrodite flowers was lower than that of the stigma (Figure 2A). The anther had not opened by the time the pistil had ripened. Observation of hermaphrodite flower pollen showed that its color turned dark blue after test treatment, and was able to germinate (Figures 3G,H), indicating that the pollen of hermaphrodite pulasan flowers is viable. However, since the anther could not open naturally, the pollen could not play a role in pollination. It was inferred that natural autogamy in pulasan plants was unlikely to occur.

Pistils vary in terms of four characteristics: stigma number, stigma shape, presence of sty-

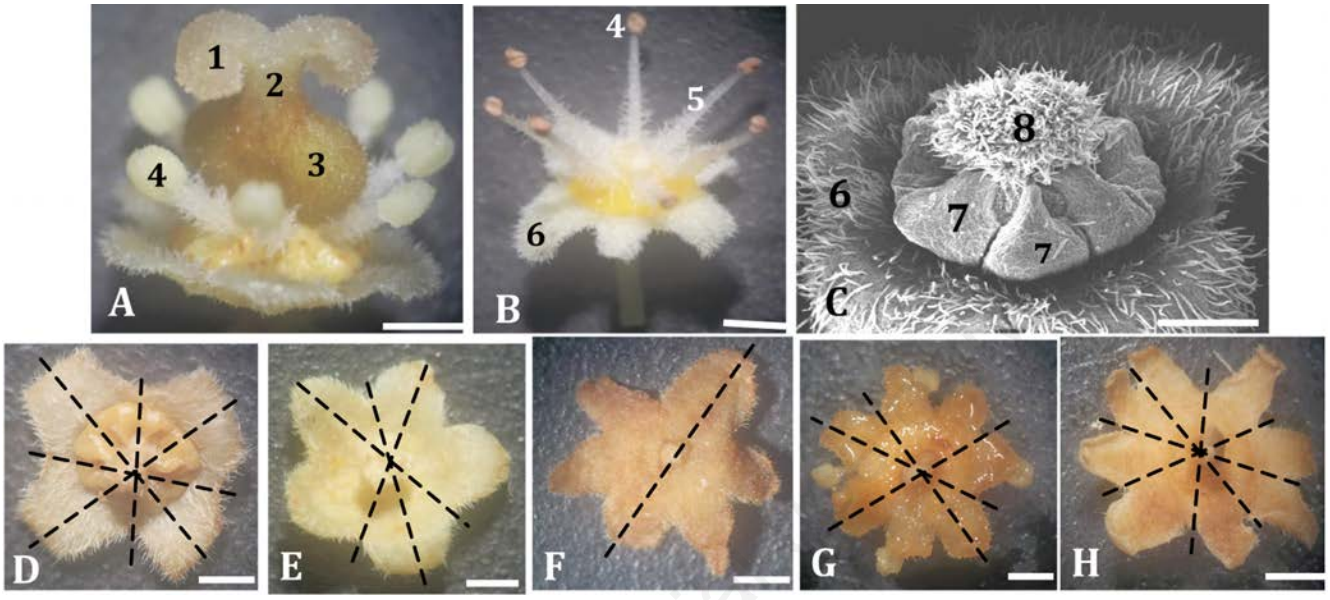

Figure 2. Pulasan flower: A) hermaphrodite; B) staminate; C) staminate, with stamen removed; D,E,G), and H) actinomorphic; F) zigomorphic; 1: stigma 2: stylus 3: ovary 4: anther 5: filament 6: sepal 7: nectar disc 8: pistillode. Scale bar = 1 mm. A,B,D,E,F,G), and H) under binocular microscope; C) under *scanning electron microscope.*

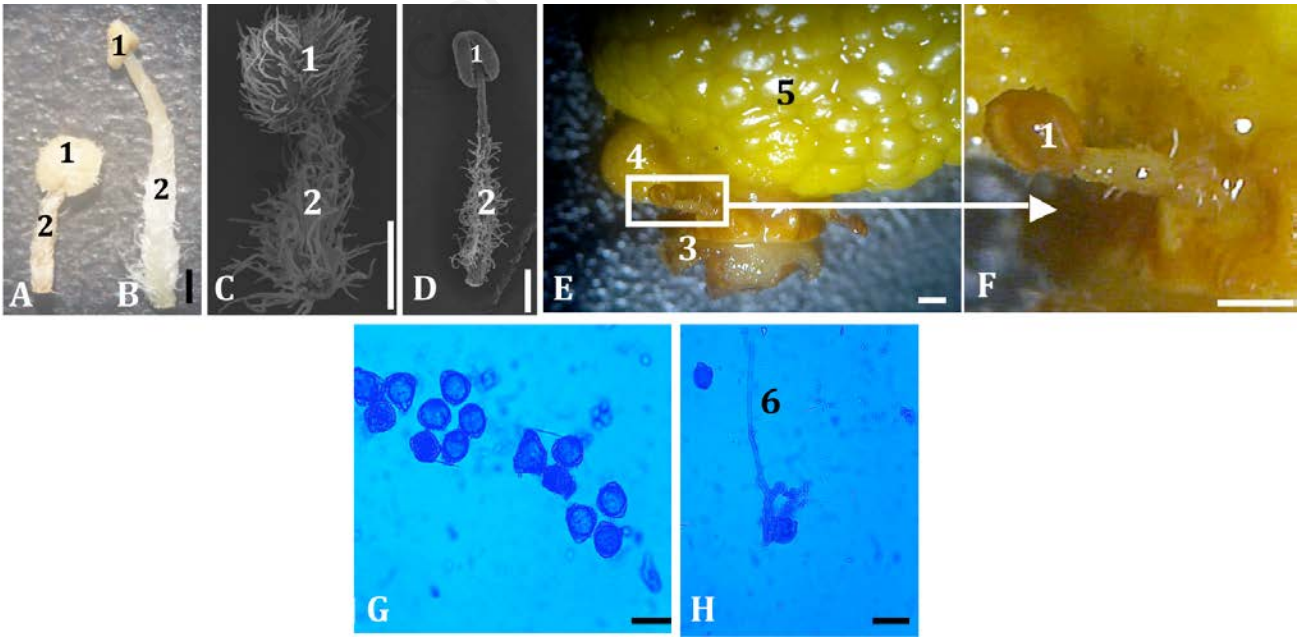

Figure 3. Stamen and pollen of pulasan: A,C) hermaphrodite stamen; B,D) staminate stamen; E,F) stamen on four-week-old fruit; G,H) viable and germinated pollen on hermaphrodite flower. Scale bar A-D=0.5 mm, E,F=1 mm, G,H=20 µm. 1: Anther; 2: filament; 3: stamen; 4: reduced ovary; 5: developed ovary, young fruit 6: pollen tube.

lus, and ovary number. Stigmas observed were bifid, trifid, or five-pronged (Figure 4).The shape of the stigma also varied: letter-m, furcate, curve, and spiral were all observed. Several flowers had no stylus (Figures 4A-D), but most flowers had obvious styli (Figures 4E-I). The number of ovaries varied: two, three, or five (Figures 4J-L).

Percentage of flower parts

The number of flowers with four sepals was higher than for other structures: more than 50% in hermaphrodite flowers, and more than 47% in staminate flowers. Both flower types showed a similar pattern in terms of percentage occurrence of sepal number: the more sepals, the less the percentage occurrence of that structure (Table 2). Stamens in pulasan flowers varied in number, from five to nine. The percentage occurrence of stamen numbers in the two flower types was different (Table 2): for hermaphrodite flowers, having five stamens was the commonest arrangement, whereas for staminate flowers, six stamens occurred most frequently. The number of ovaries varied between two, three, or (rarely) five (Table 2). Ovaries were obviously only found in hermaphrodite flowers, and having two ovaries was overwhelmingly the commonest structure, at more than 30 times more frequently found than the next commonest, three ovaries. No flowers were found with four ovaries, and only one in 6700 flowers had five ovaries.

Type of pollination

The experiment which involved covering bud inflorescences, showed that out of 146 covered inflorescences, 98.6% failed to develop fruit entirely; the other 1.4% of observed flowers only developing fruit for between two and four weeks, at which time the immature fruit fell out. Therefore, at the end of the research, no mature fruit had formed. During six weeks' observation, of the young inflorescences used as a control, 100% produced fruit. This experiment supports the contention that autogamy in pulasan has a very small chance of occurrence, and that allogamy is likely to be the reproduction method used.

Table 2. Percentage of pulasan flower parts.

Part of flower	Number	Percentage of	
		Hermaphrodite flowers*	Staminate flowers*
Sepal	4	51.19	47.65
	5	43.87	44.43
	6	4.82	7.71
	7	0.09	0.21
	8	0.03	0
Stamen	5	55.03	25.00
	6	35.91	42.36
	7	7.27	23.71
	8	1.61	7.14
	9	0.18	1.79
Ovary	2	97.13	0
	3	2.86	0
	4	0	0
	5	0.01	0

*N, observed hermaphrodite flower number = 6700 flowers from 67 trees, observed staminate flower number = 1400 flowers from 14 trees.

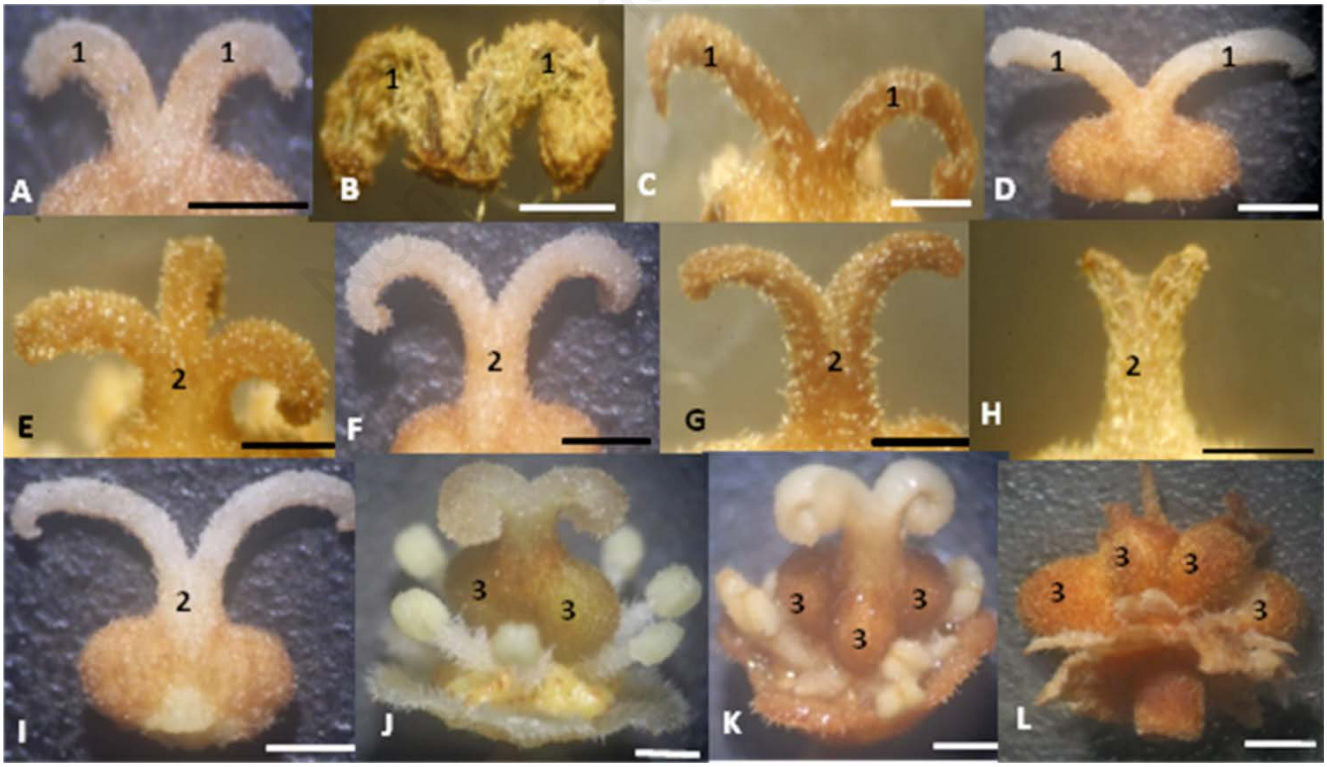

Figure 4. Variation of stigma and ovary. A-D) With no stylus; E-I) with obvious stylus; J)-L) with differing number of ovaries. 1: Stigma, 2: stylus, 3: ovary. Scale bar = 1 mm.

Discussion

The pulasan has hermaphrodite and staminate flowers, on different trees that have exclusively one or other flower type. Hermaphrodite flowers are *perfect* flowers (having both stamens and carpels), while staminate flowers are *imperfect* (having only, in this case, male organs). Both types of flowers are *incomplete*, as neither has any petals or corollas. Even though male and hermaphrodite trees had the same types of inflorescence, the panicles of the male trees were more ramiform, producing more individual flowers.

Pulasan flowers exhibit either zygomorphic or actinomorphic symmetry; the latter being more frequently found. Based on known tendencies of flower evolution, zygomorphic flowers are more advanced than actinomorphic ones.[9] Since pulasan (*N. ramboutan-ake*) has both types of flower symmetry, this tends to support the previous study that located *Nephelium* between the bottom and middle of a dendrogram showing Sapindaceae evolution.[10] Other Sapindaceous plants, such as *Paullinia,*[11] *Cardiospermum grandiflorum* and *Urvillea chacoensis* have zygomorphic flowers,[12] while *Allophylastrum frutescens*[13] has actinomorphic flowers.

The number of pulasan flower parts varies. Hermaphrodite flowers have from four to eight sepals, while staminate flowers have from four to seven (Figure 2D-H). Our result was different from Pauli and Duarte,[14] who reported from four to five sepals on each pulasan flower. The use of more samples (81 trees, 8100 flowers) in this research apparently enabled us to reveal more variations in sepal number per flower.

Stamen number per pulasan flower was between five and nine. Previous research reported that pulasan had from five to eight stamens per flower,[5,6] or from five to nine on hermaphrodite flowers.[7] Stamen numbers of six to nine per flower, occurred more frequently in staminate than hermaphrodite flowers (Table 2). This condition is more beneficial for an androdioecious plant like pulasan, a more stamens per male flower would mean more pollen produced by that flower, increasing male-plant opportunity to pollinate hermaphrodite flowers. Large numbers of stamen (with more pollen) is assumed to be one of the characteristics of cross-pollinating plants.

Variations in stamen number have also been found in other members of Sapindaceae. Lychee flowers have between six and nine stamens, and even (rarely) 11 stamens in staminate flowers. Rambutan flowers may have between four and nine (commonly five to eight) stamens, *N. maingayi* has four to six, and *N. juglandifolium,* seven to eight.[5] Nevertheless, a fixed stamen number (eight) has been found in *Cardiospermum grandiflorum,*[12] and *Cupania ludowigii*.[4]

The stamens of pulasan hermaphrodite and staminate flowers have different morphologies. This was also found to be the case in other Sapindaceous plants, such as *Tina striata*.[15] The stamen of the staminate flower of this species was longer than that of hermaphrodite flower and more than half of the filament length was covered with trichomes, while the entire filament of the hermaphrodite flower was covered with trichomes. Glabrous anthers were found in both flower types of *T. striata*. The stamen of the pulasan is different from that of *T. striata;* the entire anther of the former's hermaphrodite flower being covered with trichomes.

Stigma shape and ovary number were varied (Figure 4). Expanded stigmas were able to receive more pollen, increasing the chances of pollination and fertilization compared to narrow stigma surfaces, as with a furcate shape (Figure 4H). There were either two, three or five ovaries (Figure 4J-L). However, only one ovary developed into a fruit (Figure 3E), while other ovaries did not develop until the fruit matured, (or they simply fell out). This is also characteristic of lychees; even though a lychee may have two or three ovaries, only one will develop and form a fruit.[16]

The anther position in pulasan hermaphrodite flowers was lower than that of the stigma, physically inhibiting the occurrence of autogamy. The most likely pollination in pulasan flowers is allogamy, with pollen originating from a staminate flower and carried by an insect. In the case of pulasan's close relative, the rambutan, allogamy happens in this way, with bees such as *Apis* and *Trigona* as pollinators.[17] With experimental covering of the hermaphrodite flowers to prevent cross-pollination, only 1.4% produced fruit, which was then shed. This suggested that pulasan potency, if attempting autogamy was very small, and that the pulasan tends to be an allogamous plant, with the hermaphrodite plant functioning as a female.

In a case in the subdistrict of Bawen, Semarang, Central Java, Indonesia, a pulasan farm planted 30 pulasan hermaphrodite (only) trees, but these could not produce normal fruit. All fruits had very small arils, and were without seeds. From field observations, it was known that the anthers of the hermaphrodite flowers did not open until the receptive pistil time was over, so that self-pollination did not occur. Consequently, seed formation could not happen. There is a mechanical barrier to the occurrence of autogamy, even though the (unreleased) pollen is viable (Figure 3G,H) and has the potential to pollinate an ovule of the same flower.

Many plant species which are known to be androdioecious plants morphologically, and evidently function as dioecious, are known as cryptic dioecious.[18] Most species of Sapindaceae have hermaphrodite flowers that have a unisexual function;[15] for example, rambutan hermaphrodite flowers function as female flowers.[19] On the hermaphrodite plant, the anther of the hermaphrodite flower is not open, and pollen cannot be released. Other plants, such as *Sapindus emarginatus*,[20] and *T. striata*,[15] exhibit the same characteristic in terms of the anther of the hermaphrodite flower is not open. *S. emarginatus* is a monoecious plant.[20] In general, *T. striata* is a monoecious plant too, but a few plant have only male flowers.[15]

In summary, the key morphological characteristics of pulasan flowers were: anther position in hermaphrodite flowers was lower than that of their stigma (Figure 2A), and anthers of hermaphrodite flowers could not naturally open (Figure 3E,F), so that they could not carry out self-pollination. In total, 98.6% of all covered hermaphrodite inflorescences did not develop fruit, suggesting autogamy was difficult. Pulasan flowers produce nectar (Figure 2A-C) that attracts pollinators. Thus, pulasan tends to be an allogamous plant, with hermaphrodite examples acting as females. The pulasan varies in terms of flower type, flower number per inflorescence, and number of flower parts in each flower. The existence of such great variety in pulasan flowers is assumed to be a genetic consequence of cross-pollination.

Conclusions

Pulasan flowers exhibit much variation in reproductive structure. The inflorescence types were panicle and raceme. Flowers were located on axillary and pseudo-terminal stems. Pulasan trees had two types of flower: staminate and hermaphrodite. The flower symmetries were either zigomorphic or actinomorphic. The morphology of hermaphrodite flower stamens is different to that of staminate flower stamens. The anthers of hermaphrodite flowers were lower than the stigmas. The anthers of hermaphrodite flowers did not open because of a mechanical obstacle. Hermaphrodite flowers, when encased in cloth bags, only produced a few fruit (1.4%). Thus, it was concluded that pulasan tends to be an allogamous plant.

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