Morphological characteristics of Indonesian Rubus flowers

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Abstract. Normasiwi S, Salamah A, Surya MI. 2021. Morphological characteristics of Indonesian Rubus flowers. Biodiversitas 22:1441-1447. Rubus spp. are woody or herbaceous plants that can be used for fruit, ornamental and medicinal purposes. The increasing use of Rubus as a commercial species is highly dependent on the formation of high-quality genetic material. However, the lack of basic biological knowledge is one of the limiting factors in this development. This research aims to describe the morphological characters of Indonesian Rubus flowers at Cibodas Botanical Garden, Indonesian Institute of Sciences, Cianjur, West Java, Indonesia. From January to June 2020, we observed nine Rubus species with five replications, namely R. alceifolius, R. chrysophyllus, R. ellipticus, R. fraxinifolius, R. lineatus, R. molecanus, R. pyrifolius, R. rosifolius, and Rubus sp (Blackberry) in Cibodas Botanical Garden, Indonesia. The results showed variations in the characteristics of the pistil, stamens, torus shape, and duration of flowering stages between species. The mean value of the stamen-pistil ratio for R. pyrifolius was highest among other species (8.27), and R. fraxinifolius was the lowest (0.16). Furthermore, a correlation analysis between stamens and pistils for nine Rubus species was relatively positive (r = 0.598), similarly distinctly positive between pistils and fruits with r = 0.763. Flower development duration takes ranging 10-12 days from initiation to anthesis, depending on the species.

Keywords: Anthesis, floral biology, Rubus, reproductive systems

INTRODUCTION

Plant reproductive systems and morphology have unique relations (Peng et al. 2014). Flowering plants have a different major in pattern sex expression depending on their form characteristics (Endress 2010; Williams 2012; Lu et al. 2015). Diversity species of flowering plants are generally indicated by the diversity of flowers and fruits, and species classification largely depends on flower and fruits idiosyncrasies (Redmond and Stout 2018; Ramírez-Barahona et al. 2020). The diversification of floral structure and function gives a convincing illustration in adaptive evolution and offers convenience for researching the functional association between floral traits and plant mating strategies.

Rubus (Rubus spp.), the genera that belong to Rosaceae, are woody or herbaceous plants with simple, pinnate, or palmate leaves. It is distributed in all continents except Antarctica on cultivable soils ranging from tropical to subarctic regions (Alice and Campbell 1999). In the tropics, Rubus are found in mountainous areas (Setyawan 1999). The prevalence of interspecific hybridization, polyphloidy, and various forms of apomixis in Rubus, made it having a lousy reputation among taxonomists due to taxonomic classification became difficult (Kalkman 1993; Yang et al. 2015; Sochor et al. 2019; Lechowicz et al. 2020). In 1910, Focke grouped Rubus into twelve subgenera, namely Chamaemorus, Cylactis, Dalibarda, Chamaebatus, Comaropits, Orobatus, Dalibardastrum, Malachobatus, Anaplobatus, Idaeobatus, Lampobatus, and Rubus (Eubatus). Furthermore, Zandez and Kalkman (1981) note four subgenera distributed in Malesia, including Chamaebeatus, Idaeobatus, Malachobatus, and Micranthobatus. Moreover, 46 Rubus species were recorded in the Malesia region, 25 spread in Indonesian mountain forests. In general, these genera display similar characters, such as having an inflorescence terminal in 5-merous flowers, being mostly bisexual, rarely unisexual, and being dioecious, with numerous stamen and pistils on various type of torus. These plants most notable characteristic is their collective drupes fruit with or without the torus, rarely loose individually, with a juicy or fleshy mesocarp (Kalkman 1993).

Reproductive biology and flowering observations of some Rubus species (Raspberry, Blackberry) have been reported from America, North American, New Zealand, Norway, and Britain (Williams 1959; Heide and Sonstebry 2011; Nielsen et al. 2017; Hodnefjell et al. 2018; López et al. 2019). Most of these studies focused on single or commercials species. However, floral character morphology studies of wild Rubus are rare in South-east Asia, especially in Indonesia. Furthermore, the Indonesian Rubus flower’s diversity has attracted little attention in the study of the functional association between floral traits and plant mating strategies. Cibodas Botanical Gardens (CBG), an ex-situ conservation institution located at the Cibodas Biosphere Reserve area, has thirteen wild species of Rubus as their garden collections. They namely Rubus...
acuminatissimus, Rubus alpestris, Rubus alcefolius, Rubus chrysophyllus, Rubus ellipticus, Rubus elongatus, Rubus fraxinifolius, Rubus lineatus, Rubus moluccanus, Rubus rosifolius, Rubus sumatranaus, Rubus pyrifolius, and Rubus sp. Besides these species, CBG also has commercials Rubus cultivar such as blackberry and raspberry.

Susandarini (2016) and Moreno-Medina et al. (2018) reported that Rubus is one of the pioneers of forest succession and is potentially used for fruit, ornamental, and medicinal purposes. The development of Rubus as a commercial species depends on the development of high-quality genetic material. In-plant breeding program, the desired characteristics are obtained using existing variability, and the essential process for plant improvement is the understanding of pollination mechanisms (Frankel and Galun 2012; Sedgley and Griffin 2013). Furthermore, such material will require information on the species basic biology, which is currently lacking in some respect. This research aims to describe the morphological character of nine species of Indonesian Rubus flowers at Cibodas Botanical Garden. The information Rubus’s floral morphology may add information on flower characters diversity for pollination knowledge and breeding systems.

**MATERIALS AND METHODS**

**Study species and site**
All observations and experiments were carried out at Cibodas Botanical Garden, Indonesian Institute of Sciences, Cianjur, West Java, Indonesia, from January to June 2020. CBG is located in the Cibodas Biosphere Reserve area with an altitude of 1200-1400 m a.s.l., an average temperature of 20°C, and air humidity up to 96%. Nine species Rubus spp. were investigated, consisting of eight wild species, R. alcefolius, R. chrysophyllus, R. ellipticus, R. fraxinifolius, R. lineatus, R. moluccanus, R. pyrifolius, R. rosifolius, and one commercial species, namely Rubus sp. (Blackberry).

**Flower development**
Observation of the nine Rubus species flowering stage was carried out at each stage of development, starting with a flower bud with a 1 mm diameter until one day before the flower anthesis. Then, the number of days needed for anthesis was also calculated.

**Flower morphology characters**
The variations in the flower morphology traits of Rubus spp were observed in the flower of nine species of Rubus, which were randomly selected with five replications. The flower’s recorded characters were the number of pistils, the number of stamens, the stamen-pistil ratio, the number of fruits, and the inflorescence half flower at anthesis. Some additional characters were the color of the pistil and stamen, flower bud diameters one day before (D-1) anthesis, flower bud growth diameters, and the torus’s size and shape at one day before (D-1) anthesis; the sepal and petal colors also observed. The number of pistils, stamens, and fruits were identified by the naked eye and counting manually. The size and form of flowers were measured by Microdirect 1080P HDMI Handheld digital microscope, then calculated with a mean value of five flowers per species. The torus’s shape was divided into four categories: umbrella, triangular, parallel, and bulge. The Munsell plant tissue color chart measured the flower color. Then, the data were obtained, processed by the MS. Excel and IBM SPSS Statistics 21 software to get the mean value of size parts of flowers, stamens, pistils, and fruits number correlation within species analyzed by Rank Spearman correlation (two-tailed). Some data sets were transformed to a logarithmic scale before analysis to equalize variances. Fisher’s Least Significance Different (LSD) 5% was used as further statistic analysis when ANOVA shows significant differences and all the variable were analyzed by Principal Component Analysis (PCA).

**RESULTS AND DISCUSSION**

**Flower development**
The nine species of Rubus have different flower development (Figure 1). Time from flower initiation to anthesis (fully open flower) ranging from 10-12 days. Among the nine species of Rubus, the faster growth of flowering time is R. lineatus and R. chrysophyllus. Simultaneously, those that take longer to anthesis are R. alcefolius, R. ellipticus, R. fraxinifolius, and Rubus sp (Blackberry).

The appearance of half flower-cuts in Figure 2 illustrates a difference in length of pistils and stamens among the species and the torus variation shape. Anthers of those nine Rubus dehisced from outer to inner, which started at anthesis until D+3, and after D+3, anthers leak and dry out. Similarly, most stigmas were receptive at the anthesis stage. On the other hand, nine Rubus on observation produced nectar at the filaments' base as a reward.

**Flower morphology characters**
Comparing the number of pistils, stamen, and fruit of Rubus (Table 1) showed that R. rosifolius has the highest number of stamen and pistil among the species, with an average of 122 stamens and 404.6 number pistils. Meanwhile, R. lineatus had the lowest number of stamens with 42.67, and R. pyrifolius had the lowest number of pistil with 6.6. The number of fruits from natural-pollinated among the species showed that R. fraxinifolius was the highest number of fruits with 340.00 (93%), and the lowest was R. pyrifolius with 1.33 (20%) fruits. Oppositely, the mean value of the stamen-pistil ratio for R. pyrifolius was highest among other species (8.27), and R. fraxinifolius was the fewest (0.16).

Additionally, the correlation analysis by Spearman rank (Table 2) between stamens and pistils for nine species of Rubus was relatively positive (r = 0.598). Similarly, the correlation analysis between pistils and fruits distinctly positive with r = 0.763. On the other hand, between stamens and fruits was not any significant correlation.
Figure 1. Flower development processes of nine *Rubus* species. A. *R. alceifolius*, B. *R. chrysophyllus*, C. *R. ellipticus*, D. *R. fraxinifolius*, E. *R. lineatus*, F. *R. moluccanus*, G. *R. rosifolius*, H. *R. pyrifolius*, I. *Rubus* sp. (blackberry). Scale bar = 1 cm.

Figure 2. Half flower-cuts of nine species *Rubus*. A. *R. alceifolius*, B. *R. chrysophyllus*, C. *R. ellipticus*, D. *R. fraxinifolius*, E. *R. lineatus*, F. *R. moluccanus*, G. *R. pyrifolius*, H. *R. rosifolius*, I. *Rubus* sp. (blackberry). Scale bar = 1 mm. One day old flower (D1).
Table 1. The number of stamen, pistil, and fruit of nine species *Rubus*

| Species of *Rubus* | Number of stamens | Number of pistils | Stamen-pistil ratio | Number of fruits |
|--------------------|-------------------|-------------------|---------------------|-----------------|
| *R. alceifolius*    | 95.20b            | 73.20d            | 1.30bc              | 17.00c          |
| *R. chrysophyllus*  | 56.25d            | 39.33f            | 1.99b               | 35.67bc         |
| *R. ellipticus*     | 80.00bc           | 138.80c           | 0.58b               | 38.33b          |
| *R. fraxinifolius*  | 61.60cd           | 365.33b           | 0.16c               | 340.00a         |
| *R. lineatus*       | 42.67d            | 67.33de           | 0.65bc              | 36.67bc         |
| *R. moluccanus*     | 95.20b            | 73.20d            | 1.30bc              | 35.67bc         |
| *R. pyrifolius*     | 53.60d            | 6.60g             | 8.27a               | 1.33d           |
| *R. rosifolius*     | 122.00a           | 404.60a           | 0.30c               | 292.00a         |
| *Rubus* sp. (Blackberry) | 64.00cd        | 49.00e            | 1.33bc              | 26.00bc         |

Note: The number followed by similar low case letters in the same column are not significantly different according to the LSD test at 5%.

Table 2. Spearman rank correlation coefficients number of stamen, pistils, and fruits for nine species of *Rubus*

| Correlation coefficient | Number of stamens | Number of pistils | Number of fruits |
|-------------------------|-------------------|-------------------|-----------------|
|                         | 1.00              | 0.598**           | 0.218           |
| Number of stamens       |                   |                   |                 |
| Number of pistils        | 0.598**           | 1.00              | 0.763**         |
| Number of fruits         | 0.218             | 0.763**           | 1.00            |

** Correlation is significant at the 0.01 level

Table 3. Characteristics (length and color) stamen and pistil of nine species *Rubus*. The number followed by similar low case letters in the same column are not significantly different according to the LSD test at 5%.

| Species of *Rubus* | Stamen | Pistil |
|--------------------|--------|--------|
|                    | Length (mm) | Color            | Length (mm) | Color            |
| *R. alceifolius*   | 3.95b | Light-yellow-green | 7.32a | Grayish-yellow-green |
| *R. chrysophyllus* | 2.23de | Pale yellow | 4.25de | Light-yellow-green |
| *R. ellipticus*    | 2.73cd | Light yellow-green | 1.90f | Light-yellow-green |
| *R. fraxinifolius* | 1.91ef | Pale yellow | 1.82f | Grayish-yellow-green |
| *R. lineatus*      | 1.35f | Strong yellow | 4.70cd | Pale yellow |
| *R. moluccanus*    | 3.01c | Pale yellow-green | 5.64b | Light-yellow-green |
| *R. pyrifolius*    | 4.84a | Pale yellow | 5.12bc | Strong-yellow-green |
| *R. rosifolius*    | 2.52de | Light yellow-green | 1.81f | Strong-yellow-green |
| *Rubus* sp. (Blackberry) | 5.59a | Pale yellow | 3.95e | Grayish-yellow-green |

Table 4. Characteristic flower of nine species *Rubus* (mean±s.e). The number followed by similar low case letters in the same column are not significantly different according to the LSD test at 5%.

| Species of *Rubus* | Flower bud diameters D-1 (mm) | Flower bud growth diameters (mm/day) | Torus D-1 | Flower color |
|--------------------|-------------------------------|-------------------------------------|-----------|--------------|
|                    |                              |                                      | Diameters (mm) | Forms | Petal color | Sepal color |
| *R. alceifolius*   | 5.83±0.103                   | 0.41±0.035                          | 1.84±0.248 | Umbrella | Grayish-yellow-green | Strong-yellow-green |
| *R. chrysophyllus* | 3.74±0.182                   | 0.42±0.063                          | 1.35±0.066 | Triangular | Grayish-yellow-green | Strong-yellow-green |
| *R. ellipticus*    | 3.71±0.283                   | 0.34±0.062                          | 0.93±0.071 | Triangular | Pale-yellow-green | Moderate-yellow-green |
| *R. fraxinifolius* | 5.04±0.678                   | 0.43±0.361                          | 1.52±0.176 | Bulge | Grayish-yellow-green | Moderate-yellow-green |
| *R. lineatus*      | 5.05±0.551                   | 0.37±0.037                          | 1.60±0.140 | Parallel | Grayish-yellow-green | Moderate-yellow-green |
| *R. moluccanus*    | 4.32±0.342                   | 0.34±0.053                          | 2.40±0.185 | Umbrella | Grayish-yellow-green | Moderate-yellow-green |
| *R. pyrifolius*    | 3.51±0.105                   | 0.25±0.057                          | 0.99±0.119 | Umbrella | Grayish-yellow-green | Strong-yellow-green |
| *R. rosifolius*    | 5.11±0.439                   | 0.30±0.007                          | 1.85±0.184 | Bulge | Grayish-yellow-green | Strong-yellow-green |
| *Rubus* sp (blackberry) | 5.81±0.132               | 0.48±0.019                          | 1.22±0.557 | Bulge | Grayish-yellow-green | Strong-yellow-green |

Note: *D-1= one day before anthesis, ms = not significant
Furthermore, Table 3 gives information about the stamen and pistil’s characteristics (length and color). The longest stamen and pistil, namely Blackberry (5.59 mm) and R. alceifolius (7.32 mm), respectively. The shortest stamen and pistil, namely R. lineatus and R. rosifolius with 1.35 mm and 1.81 mm in length, respectively.

The highest mean of flower bud diameters (5.83 mm) was found in R. alceifolius, followed by Blackberry with 5.81 mm in widths (Table 4). The R. pyrifolius has the smallest flower bud, with 3.51 mm in diameters. Variations of the floral form of the nine species of Rubus are also shown in the torus character. R. alceifolius, R. moluccanus, and R. pyrifolius have an umbrella-shaped torus; R. chrysophyllus and R. ellipticus are triangular, and R. fraxinifolius, R. rosifolius, and Rubus sp (Blackberry) have similar torus form, namely bulb. On the other hand, the torus of R. lineatus is parallel. Additionally, petal and sepal colors among the nine species look similar in that most petals are greyish yellow-green, while sepals’ colors are moderate to intense yellow-green.

The morphology characteristics of all Rubus species reported in this study are similar and relatively consistent with those described by Kalkman (1993) and Graham and Woodhead (2011). Kalkman (1993) described the Rubus species as hermaphrodites, wherein elaborated thyrsoid with whole ovate sepal formed by five imbricated pieces. Moreover, Graham and Woodhead (2011) reported that Rubus flowers are pentamer flowers with small, white to pink petals, many stamens, and an apocarpous gynoecium of many carpels, a cone-like receptacle. Recent PCA phenetic analysis represents that similar flower forms of Rubus did not indicate they in the same clades (Real and Madulid 2019); instead, the homoplasy of flower forms may be correlated with pollination agents (Van der Niet et al. 2014; Souto-Vilarós et al. 2018). On the other hand, the separation of the structure of reproductive organs from their function is practically impossible because of its associated functionally and indivisible (Dafni 1992).

The development of racemose flower in Rubus not concurrent but alternating; one flower and the others in the raceme have a range of 1-10 days. The onset and duration of stages in the flower development affected by endogenously sources used in the flowering process and strongly influenced by the climatic condition under which the plants were growing (Donders et al. 2014; Hatfield and Prueger 2015; Hodnefjell et al. 2018).

Nine species of Rubus of CBG collection used in this study divided into two subgenera are Ideobatus and Malachobatus. The species included subgenera Ideobatus, namely R. ellipticus, R. fraxinifolius, R. rosifolius, and Blackberry. On the other hand, R. alceifolius, R. chrysophyllus, R. lineatus, R. moluccanus, and R. pyrifolius include subgenera Malachobatus. The characteristics of the subgenera Ideobatus and Malachobatus were showed in the length of stamen and pistils. The length of the stamen of R. alceifolius, R. chrysophyllus, R. lineatus, and R. moluccanus (subgenera of Malachobatus) were longer than their pistils and have elevated torus shape. In otherwise, R. ellipticus, R. fraxinifolius, R. rosifolius, and Rubus sp (Blackberry) (subgenera Ideobatus) have the stamens shorter than the pistils and a more varied torus shape from elevated to flat (Zandee and Kalkman 1981).

The different lengths of pistil and stamen might indicate the divergence of pollination behavior for each species. Jiang et al. (2010) and Kuester et al. (2017) reported that a field investigation validated the stamen-pistil ratio showing

### Table 5. Eigenvalue and percentage of variance values and factor loadings generated by PCA

|            | PC1     | PC2     | PC3     |
|------------|---------|---------|---------|
| Eigenvalue | 2.980   | 2.658   | 1.449   |
| Variability (%) | 37.254% | 20.728% | 18.109% |
| Cumulative (%) | 37.254% | 76.092% | 76.091% |
| Number of petals | 0.817   | 0.488   | -0.164  |
| Number of stamens | 0.297   | 0.784   | -0.31   |
| Number of fruits | 0.89    | 0.192   | 0.036   |
| Length of pistil | -0.884  | 0.163   | 0.053   |
| Length of stamen | -0.606  | 0.041   | -0.074  |
| Flower bud diameter | -0.01   | 0.503   | 0.755   |
| Flower bud growth diameter | 0.044   | -0.222  | 0.89    |
| Torus diameter | -0.116  | 0.789   | 0.187   |

Figure 3. The component plot in rotate space by PCA.
their outcrossing levels of the breeding system. The higher stamen-pistil ratio indicated they a nearly obligate outcrossing species; on the opposite, the getting lower of the stamen-pistil ratio indicates that they are facultative autonomous species. The positive correlation between stamen-pistil and pistil–fruit described that pistils have a significant role in pollination. The average percentage of natural pollinated-fruitfulness of each species was different. Pawar et al. (2017) reported that the self-pollination in *R. ellipticus* resulted in the highest percentage of fruit set (96.66%), followed by natural pollination (76.66%). Hiregoudar et al. (2019a) observed that the initial fruit set percentage of natural pollination was 68.75% in *R. paniculatus*.

Hiregoudar et al. (2019a,b) reported that the appearance of stigma turned to a thoroughly creamy color on the day of anthesis indicates the highest amount of sugary secretion and fresh display. The pollination conducted at this stage led to the highest fruit set. The styles became completely straight and showed peak receptivity. Further analysis of stigma receptivity and morphological change may lead to visible associations between stigma receptivity and flower development stages that could be employed in controlled pollination methods in *Rubus*.

A fundamental comprehension of flower structure, sexuality, and phenology is essential for understanding the floral life cycle needed for any pollination study (Dafni 1992; Feijo et al. 2012). Flower morphology shows the pollination mechanisms of *Rubus* and determines the most pollinator visits often. Likewise, investigation of the fertility behavior of internal verticils (androecium and gynoecium) allows the designing of improvement for breeding programs of the species (López et al. 2019). Our study shows that the variety in the floral morphology of *Rubus* would directly impact the diversity of flower characters for pollination knowledge and breeding systems. On the other hand, information on *Rubus* ssp pollination systems is critical to enhancement breeding programs with controlled pollination.

In conclusion, the nine species of Indonesian *Rubus* have a specific character of floral morphology. The flower characteristics variation shows on the pistil, stamens, torus shape, and duration of flowering stages between species. Flower development duration takes ranging 10-12 days from initiation to anthesis. Furthermore, a positive correlation was presented between the number of stamens and pistil and between the number of pistils and fruits. The variety in *Rubus*’ floral morphology would directly impact flower characteristics’ diversity for pollination knowledge and breeding systems.

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