Variations in the morphology of *Hibiscus rosa-sinensis* crested peach flowers in nature

Saifudin and A Salamah

Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia

Corresponding author’s email: salamah@sci.ui.ac.id

Abstract. *Hibiscus rosa-sinensis* has a wide variety of flowers. The variations even can be observed in one hybrid only. The hybrid is known as crested flower (double type I), one of four categories of *H. rosa-sinensis* flower shapes in nature. This study purpose was to categorize the variation in crested flowers based on morphological observation using 200 samples. The observations showed the form of crested peach could be divided into three groups: crested peach that resembles a single flower (*crested single-like*), which resembles a double flower (*crested double-like*), and a transitional between single-like and double-like flower (*crested intermediate-like*). Groupings are based on the length of the staminal column which measured at the closest distance between the corolla position and the additional stamen or petal. The length of the staminal column on a crested single-like is more than 4 cm (a > 4 cm), whereas the crested double-like is less than 1 cm (a < 1 cm), and the crested intermediate-like is 1–4 cm (1 ≤ a ≤ 4). Groupings are also reinforced by comparing the average number of staminodium petaloid, stamen-petal intermediate, and stamen. The crested single-like has a 1:2:2 ratio, the crested intermediate-like has a 2:1:1 ratio, while the crested double-like has a 5:1:1 ratio. Of the total 200 crested samples used in the study, 89.5 % of them had intermediate-like forms, while 6.5 % were double-like, and 4 % single-like. It can be concluded that the real crested of *H. rosa-sinensis* is an intermediate-like form.

Keywords: *Hibiscus rosa-sinensis*, crested peach flowers, crested flowers morphology

1. Introduction

*Hibiscus rosa-sinensis* L. is a species of tropical *Hibiscus*, a flowering plant from the Hibisceae tribe, family Malvaceae, which is widespread throughout Asia, including Indonesia [1]. In Indonesia, *H. rosa-sinensis* is well-known as “Kembang Sepatu” [2]. *Hibiscus rosa-sinensis* has a wide variety of flowers, not only in size but also colours and shapes [3, 4]. The colours of *H. rosa-sinensis* flowers can be white, pink, red, orange, peach, or yellow [5]. The main shapes of *H. rosa-sinensis* flowers are single and double (figure 1a and figure 1b). There is also a crested, a transitional shape between single and double flowers [6] (figure 1c).
Figure 1. Variety of *H. rosa sinensis* flowers (a) single flower, (b) double flower and (c) crested flower.

The floral organs of *H. rosa-sinensis* form a concentric rings, with sepals positioned at the outermost, followed by petals, stamens, and carpels in the center positions. Below sepals, there is 5 to 8 number of epicalyx [7]. The flower also has a staminal column which holds 60–70 stamens that surrounds an exerted synstylous gynoecium with five fused stigmas [3]. In double type and crested type flower, there are also additional organs resembling petal such as staminodium petaloid and stamen-petal intermediate (figure 2c and figure 2d). Prihatiningsih assumed that both of additional petals allegedly is a modification of the stamen homeosis symptoms in nature [4]. Homeosis is a transformation of a structure full or partially, into another structure [8].

The research of Prihatiningsih tries to connecting homeosis symptoms with the variation of *H. rosa-sinensis* flowers, especially that grows in Universitas Indonesia campus [4]. Besides a staminodium petaloid and stamen-petal intermediate, a symptom of homeosis was observed by the presence of sepaloid that suspected replace the ovary. Prihatiningsih concluded that homeosis symptoms are important for determining the diversity of *H. rosa-sinensis* flowers in nature, especially in the form of double and crested flowers [4].

The understanding of the meaning of double flowers then enhanced by Salamah et al. through the observation of *H. rosa-sinensis* flower parts that grow in Universitas Indonesia campus [9]. Salamah et al. concluded that double type flower is a flower having one or more extra organs resembling petal [9]. Salamah et al. added that the additional petals on *H. rosa-sinensis* having a variety in either its position, size, number, and composition [9]. Based on that, Salamah et al. grouped the flowers of *H. rosa-sinensis* into four new categories which are single, double type I, double type II, and doubles type III [9]. The form of a crested flower then belongs to double type I.

The formation of additional organs on *H. rosa-sinensis* flowers had an impact in the variety of their flowers. Moreover, based on direct observation in nature, variations of *H. rosa-sinensis* L. flowers can also be seen in one hybrid only, especially in crested flower (double type I). There have been no previous studies that discuss diversity in crested flowers in nature. Therefore, the study of variation in crested flower through morphological observations needs to be done.

The variation in *H. rosa-sinensis* flowers shows the phenomenon of polymorphism. Barret et al. interpreted polymorphism as the emergence of more than one form (phenotype) species of plants [10]. A mutation resulting from the changes of gene expression, which one of the processes was known as homeosis can cause polymorphism. Polymorphism due to homeosis can be used as a base for grouping or classifying [11].

2. Method

We collected two hundreds of *Hibiscus rosa-sinensis* flowers of crested peach from a cultivated growing population at Universitas Indonesia campus and surrounding areas. Groupings are based on the length of the staminal column which measured at the closest distance between the corolla position and the
additional stamen or petal. As a standard for comparison, there was also a measurement of the length of staminal column which measured at the closest distance between the corolla position and the stamen on 10 flowers of single peach and 10 double red flowers. Groupings are also reinforced by comparing the average number of other flower organs such as epicalyx, calyx, corolla, stamodium petaloid, stamen-petal intermediate, stamen, and stigma. Tables and figures were used to present all data.

3. Results and discussion

3.1. The main character of grouping

The grouping of *H. rosa-sinensis* crested flowers into three categorized as *single-like*, *intermediate-like*, and *double-like* is based on its resemblance with single and double flowers that are the primary forms of *H. rosa-sinensis* flowers in nature. Furthermore, the main character of the grouping is the length of staminal column, which measured at the closest distance between the corolla position and the additional stamen or petal. Visually, staminal column is the most striking part monitored and readily distinguished.

In single flowers of *H. rosa-sinensis*, there are not any additional petals so that the length of staminal column was measured at the closest distance between the corolla position and the stamen. Based on additional observations against 10 samples of single peach flowers, the average number of the closest distance between the corolla position and the stamen is 4.23 cm. Hence, the standard of the length of staminal column in crested *single-like* flowers must be above 4 cm (a > 4 cm).

In double flowers, there is no distance between corolla and additional petals so that the standard of the length of staminal column in crested *double-like* flowers must be under 1 cm (a < 1 cm). It was discovered that at a distance under 1 cm, the position between corolla and additional petals

![Figure 2. Parts of *H. rosa sinensis* crested flower.](image-url)
seems no distance so as to resemble with double flowers. Meanwhile, to categorize crested intermediate-like flowers, staminal column must have a length of by a range of 1–4 cm (1 ≤ a ≤ 4 cm). In other words, crested intermediate-like flowers having characteristics among the crested single-like dan crested double-like (figure 3).

Based on observations, 89.5 % of H. rosa-sinensis crested flowers are in the category of crested intermediate-like, 6.5 % crested single-like and 4 % crested single-like. The average number of staminal column in crested intermediate-like flowers was 2.4 cm, in crested double-like was 0.5 cm, and in crested single-like was 4.4 cm (table 2).

Among the three types of H. rosa-sinensis crested flowers, there was also a different ratio between the average number of additional petal and stamen, which can support the data due to the process of grouping. The ratio between the average number of staminodium petaloid, stamen-petal intermediate, and stamen in crested single-like flowers was 1:2:2, crested intermediate-like was 2:1:1, while in crested double-like flowers was 5:1:1. However, the ratio between those three kinds of flower organs, cannot be applied to differentiate the three type of crested flowers in nature, especially if it is devoted to one by one individual's flowers. Variation in the number of staminodium petaloid, stamen-petal intermediate, and stamens that found among individual flowers could be the reason why the ratio could not be used to differentiate.

In spite of this fact, it can be ascertained that the number of stamens and stamen-petal intermediate in crested single-like flowers generally always more than the number of staminodium petaloid. While in crested intermediate-like flowers, the number of staminodium petaloid and stamen-petal intermediate generally more than of stamens. While in the case of crested double-like flowers, the number of a staminodium petaloid was always more than the number of stamens and stamen-petal intermediate. In some samples observed, the difference in the number even looked very significant, which can reach 19:0:2 ratio.

Furthermore, from the morphological observation of additional petals, the greater number of staminodium petaloid will determine the appearance of crested flower into double type. Comparing to stamen-petal intermediate (SPI), staminodium petaloid (SP) seem to have more similarities to corolla, either in shape or size. So that, crested flowers that are having more staminodium petaloid seem to have so many petals, resembling to double type flower (figure 4a). In another case, crested flowers that have more stamens (St) will have the appearance of flowers resembling single type (figure 4b).

3.2. Universal characters of H. rosa-sinensis crested flowers

Based on the observation of 200 samples of H. rosa-sinensis flowers of crested peach, there are several parts of the flower which are fairly equal so that morphologically cannot be used as the basis of grouping. Those parts are epicalyx, calyx, corolla, and stigma. Generally, the crested peach flowers of H. rosa-sinensis having 6 epicalyx, 5 calyx, 5 corollas, and 5 stigmas (table 1).

3.3. Flowers' polymorphism

The formation of new structures such as staminodium petaloid and stamen-petal intermediate shows a high level of polymorphism in the flowers of H. rosa-sinensis. According to Barret et al., polymorphism caused by a variety of factors, both internal and external [10]. Internal factors that trigger polymorphism mainly because of the changes of gene expression [12], meanwhile, the external factors that influence the polymorphism are photoperiodism, temperature, water supply, and nutrients [13,14]. Barret et al. added that in plants that male and female genital organs located within one flower, such as H. rosa-sinensis, polymorphism has a role in reproductive factors, particularly in terms of the pollination [10].

Polymorphism in plants can be caused by the changes of gene expression resulting from a mutation which later plays a critical role in the process of evolution [14]. Prihatiningshih concluded those changes as the result of mutations in the homeotic genes in the process of flower formation [4]. By Sattler, this condition called with the term homeosis [8].
There are five classes of \textit{MADS-box} genes (A, B, C, D, and E) that regulate the formation of floral organs. The A-class genes are responsible for calyx formation, C-class gene for carpel formation, and D-class genes for ovule formation. Interaction between A- and B- classes genes responsible for corolla formation. Meanwhile, B- and C-classes genes interaction responsible for stamen formation. The cofactors critical for the development of three flower circles (corolla, stamen, and carpel) is influenced by the E-class genes [15-17].

Besides homeosis, it can also be assumed that transdifferentiation has a role to the polymorphism in \textit{H. rosa-sinensis} crested flowers. Transdifferentiation is the capability of adult cells to change directly into other adult cells that were functional [18]. Transdifferentiation can occur without going through a phase of cell division [19] and without any change of form and cell size [20]. Almeida et al. added that transdifferentiation is part of morphogenesis process in plants, both in \textit{in vitro} and \textit{in vivo} condition [21]. The phenomenon of transdifferentiation in \textit{H. rosa-sinensis} crested flowers especially can be seen morphologically through the process of stamen-petal intermediate formation. As presented in figure 5, the developmental process of stamen-petal intermediate seems to be derived from a stamen, especially in the part of filament. The establishment of a petal-like structure in stamen-petal intermediate was started from the region around anthers and widely to followed the formation of a sheet on the filament. The size of stamen-petal intermediate looks positively correlated with the number of filaments cells that are going to transdifferentiation process into cells that initiate the petal-like structure.

\begin{table}
\centering
\caption{The comparison of three types of \textit{H. rosa-sinensis} crested flowers.}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|}
\hline
Crested flower type & Number of samples & \textit{Epicalyx} & \textit{Calyx} & \textit{Corolla} & \textit{SP*} & \textit{SPI**} & \textit{Stamen} & \textit{Stigma} & Length of Staminal Column (cm) \\
\hline
Single-like & 8 & 6 & 4.6 & 5 & 12.3 & 17 & 16.5 & 5 & 4.4 \\
Intermediate-like & 179 & 6.5 & 4.8 & 5 & 22.2 & 9.5 & 6.2 & 5 & 2.4 \\
Double-like & 13 & 6.2 & 4.8 & 5 & 19.5 & 3.7 & 4.1 & 5 & 0.5 \\
\hline
\end{tabular}
\end{table}

\* \textit{Stamino-dium Petaloid (SP)}

\*\* \textit{Stamen-petal Intermediate (SPI)}

(a) (b) (c) (d)

\textbf{Figure 3.} Three types of \textit{H. rosa-sinensis} crested flower (b, c, d). (a) \textit{Single peach}, (b) \textit{Single-like}, (c) \textit{Intermediate-like}, and (d) \textit{Double-like}.
4. Conclusion
The primary purpose of this study is to group the crested flower into some categories based on morphological observation. The main character of the grouping is the length of the staminal column, which measured at the closest distance between the corolla position and the additional stamen or petal.

There are three types of crested flower in nature, which are crested single-like, double-like, and intermediate-like. The morphologic observation of 200 samples of *Hibiscus rosa-sinensis* flowers showed that 89.5% of them have the form of intermediate-like, while 6.5% double-like, and 4% single-like. The differences among these types of flowers lie on the length of its staminal column. The crested single-like flower has a staminal column with the length above 4 cm (a > 4 cm), whereas the crested double-like is less than 1 cm (a < 1 cm), and the crested intermediate-like is 1–4 cm (1 ≤ a ≤ 4).

The different also can be seen from the ratio of the average number of staminodium petaloid, stamen-
petal intermediate, and stamen. The ratio in crested single-like flower is 1:2:2, crested intermediate-like is 2:1:1, while in crested double-like flowers is 5:1:1. Based on these results, writers concluded that the crested peach flower of *H. rosa-sinensis* in nature is having the form of intermediate-like. Meanwhile, due to the symptoms of homeosis and transdifferentiation in *H. rosa-sinensis*, it is still needed a further study both in anatomy and molecular aspects.

**Acknowledgments**

This work was financially supported by Universitas Indonesia under research grant PITTA on behalf of A S with grant contract number No.2229/UN2.R3.1/HKP.05.00/2018.

**References**

[1] Rao K N V, Geetha K, Raja A and Banji D 2014 *J. Pharmacogn. Phytochem.* 3 29-37

[2] Essiett U A and Iwok E S 2014 *Amer. J. Med. Biol. Res.* 2 101-17

[3] MacIntyre J P and Lacroix C R 1996 *Can. J. Bot.* 74 1871-82

[4] Prihatiningsih R 2011 *Studi Variasi Bentuk Bunga Hibiscus rosa-sinensis L. Secara Morfologi, Anatomi, dan Molekular di Kampus UI, Depok* (Depok: Departemen Biologi, FMIPA Universitas Indonesia)

[5] Gilman E F 1999 *University of Florida Cooperative Extension Service Institute of Food and Agricultural Sciences* **254** 1-3

[6] Beers L and Howie J 1990, available at http://www.hibiscusworld.com/BeersBook/Intro.htm

[7] Bowman J L 1997 *J. Biosci.* **22** 515-27

[8] Sattler R 1988 *Amer. J. Bot.* **75** 1606-17

[9] Salamah A, Prihatiningsih R, Rostina I and Dwiranti A 2017 *AIP Conf. Proc.* **2023** 020136

[10] Barrett S C H, Jesson L K and Baker A M 2000 *Ann. Bot.* **85** 253-65

[11] Lee S J, Jeung J U, Cho S K, Um B Y, Chung W, Bae J M and Shin J S 2002 *Mol. Cells* **13** 362-8

[12] Yamaguchi T, Lee D Y, Miyao A, Hirochika H and An G 2006 *The Plant Cell* **18** 15-28

[13] Meyerowitz E M, Smyth D R and Bowman J L 1989 *Development* **106** 209-17

[14] Wu C A, Streisfeld M A, Nutter L I and Cross K A 2013 *Plos One* **8** e81173

[15] Theissen G, Becker A, Di Rosa A, Kanno A, Kim J T, Munster T, Winter K U and Saedler H 2000 *Plant Mol. Biol.* **42** 115-49

[16] Ferrario S, Immink R G and Angenent G C 2004 *Curr. Opin. Plant Biol.* **7** 84-91

[17] Robles P and Pelaz S 2005 *Int. J. Dev. Biol.* **49** 633-43

[18] McManus M T, Thompson D S, Merriman C, Lyne L and Osborne D J 1998 *Plant Physiol.* **116** 891-9

[19] Shoji Y, Sugiyama M and Komamine A 1996 *Plant Cell Physiol.* **37** 401-403

[20] Krishnamurthy K V, Bahadur B, Adams S J and Venkatasubramanian P 2015 *Plant Biology and Biotechnology* **1** 73-111

[21] Almeida M, Graner E M, Brondani G E, Oliveira L S, Artioli F A, Almeida L F, Leone G F, Baccarin F J B, Antonelli P O, Cordeiro G M, Oberschelp G P J and Batagin-Piotto K D 2015 *Advances in Forestry Science* **2** 13-22