Comparative morphological analysis of ovaries in 
*Hibiscus rosa-sinensis* L. single pink, crested peach and 
double orange flowers

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Abstract. *Hibiscus rosa-sinensis* L. generally has a 5-loculed ovary, with each locule consist of numerous ovules. Observations have been made on the ovarian morphology of *H. rosa-sinensis* L. single pink, crested peach, and double orange flowers. The specimens of ovaries were prepared by a free-hand section technique and observed under the Dino-Lite digital microscope. The results show that a single pink flower has a normal ovary that consists of 5 locules. There are two ovule rows in each locule, with each row consists of 6 ovules. The observation of the ovaries of the crested peach flowers shows that one locule with another is indistinguishable, but the ovules are still present. Even though ovule still exists in ovarium, the number and the arrangement of the ovules are difficult to determine. In the longitudinal and cross-sections of the double-orange variation, ovules are not found in the ovary. Instead, petal-like structures seem to replace the ovule position. Based on the results of morphological observations, homeosis events are suspected to occur in the ovaries of crested peach and double orange flowers. Presumably, there is a difference in class C genes AGAMOUS expression of the three types. The obtained morphological data is the basis for further research on the molecular analysis of the three variations of the flower.

Keywords: *Hibiscus rosa-sinensis* L., single pink flowers, crested peach flowers, double orange flowers, morphological analysis

1. Introduction

*Hibiscus rosa-sinensis* L. is known as ornamental flower that widely distributed in the tropics and subtropics, including Indonesia [1]. The *H. rosa-sinensis* L. plant is adored for its floral beauty; so that in some countries *H. rosa-sinensis* L. is called the queen of flowers and is also used as a national flower in Malaysia and Hawaii [2]. In addition to its beautiful flowers, *H. rosa-sinensis* L. also varied in size, shape, and color [3]. The size of the flowers varies from small to large, while common color variations are yellow, orange, pink, red, and white [4]. Shape flower variations commonly found in nature are single, double [5], and there is also a transitional form called crested [2].

Based on preliminary studies conducted on single pink, crested peach, and double orange flowers, it was found that crested peach and double orange flowers have additional petals in the stamen whorl unlike in the single pink flower. Because additional petals are formed in stamen whorls, they are thought to be derived from stamen modification. Based on a molecular analysis in *Arabidopsis thaliana* [6],...
Oryza sativa [7], Lilium longiflorum [8], and many other flowers, the modification occurs in stamen is related to a failure in homeotic gene expression. Class C homeotic gene plays a role in the formation of stamen and pistil [9].

The additional petals observed in the stamen whorl has been performed in previous studies. In this study, we observe and compare ovarian morphology of the three flower types with the aid of DINO capture 2.0 digital microscope. This study aims to support the hypothesis of whether homeosis events also cause changes in the ovaries and ovules of H. rosa-sinensis L. flowers other than in stamen. The results of this study can be used as primary data for further molecular analysis of homeosis phenomenon occur in the ovary of H. rosa-sinensis L.

2. Materials and method

Hibiscus rosa-sinensis flowers were collected from camous of Universitas Indonesia in Depok and from Citayam, Bogor. The variations used in this observation are single pink, crested peach, and double orange flowers (figure 1). At least 3 samples were used for each flowers type. The cross (X-S) and longitudinal (L-S) section of ovary specimens from those three types of flowers were prepared from early to the late stage by a free-hand section technique and observed under the Dino-Lite digital microscope. The scale used for measuring ovaries with the microscope is a millimeter scale with 50x magnification.

3. Results and discussion

The observation of H. rosa-sinensis L. single pink ovary showed normal ovarian development, in which ovary consists of 5 locules (space) (figure 2a) [10]. Each locule is filled by 2 ovule rows (figure 2a and figure 2d), with each row consists of 7 ovules (figure 3a and figure 3d). Therefore, the total ovules in a single pink flower ovary are approximately 70. The number of ovules obtained is different from it’s relative’s, Hibiscus moscheutos, whose having an average of 139 ovules [11]. From the cross-section, it is also known that H. rosa-sinensis L. has axillary placentation type, that is the ovules attached to the inner corner of the locules [12]. The shape and number of ovules from the early stage (figure 2a and figure 3a) is not different from the later stage (figure 2d and figure 3d). The difference is only the size of the ovules, in which the ovules in the later stage are completely developed.

Longitudinal (L-S) and cross (X-S) section observation in the crested peach flower showed different results from the single pink flower. In a crested peach variation, the early stage of flower shows the ovary consisting of 5 locules that cannot be observed clearly because the ovary seems to be twisted (figure 2b). However, the locules in the ovary are indistinguishable from one another in the later stage (figure 2e). The ovules in the early stage seem to develop normally like in single pink flower, but in the later stage the ovules are disorganized, and its number seems to be reduced (figure 3b and figure 3f).

Figure 1. Three H. rosa-sinensis L. flower variation, (a) single pink, (b) crested peach and (c) double orange flowers.
The observation of the double orange flower shows the unique result in which there are no ovules present in the ovary, both in the early and later stage (figure 2c and figure 3c, figure 2f and figure 3f). As compensation, the ovary is filled with petal-like structures instead of ovules. The existence of petaloid in the ovary has never been reported in any flowers.

![Figure 2](image1.png)

(a) (b) (c) (d) (e) (f)

**Figure 2.** Cross section of (a, d) ovary of single pink, (b, e) crested peach and (c, f) double orange flowers. a, b, c shows flowers in the early stage; d, e, f shows flowers in the later stage.

![Figure 3](image2.png)

(a) (b) (c) (d) (e) (f)

**Figure 3.** Longitudinal section of (a, d) ovary of single pink, (b, e) crested peach and (c, f) double orange flowers. a, b, c shows flowers in the early stage; d, e, f shows flowers in the later stage.
The transformation of one structure into another is called homeosis. Loss of homeotic gene expression is assumed to be the cause of homeosis occur in reproductive organs [7]. Homeotic genes play a role in the development of the four whorls of flowers (calyx, corolla, androecium, and gynoecium), which became known as the ABC gene model [13]. Class A gene regulates the formation of sepals and petals, the class of B gene regulates the formation of petals and stamen, while the class C genes regulate stamen and pistil formation [14]. The failure of class C gene expression is thought to be the cause of stamen and pistil modification into petal-like structures in both crested and double flowers [6].

Ovules in the crested peach flowers ovary are still present but have experienced anomaly, while ovules in double orange flower ovary have completely disappeared. The abnormalities observed in both types of flower raises a surmise that in the crested peach flower, the class C genes are still expressed in a lower level relative to single pink flowers, whereas in the double orange flower C class genes are not expressed at all. Therefore, research needs to be done on the expression of class C genes quantitatively to see the different levels of expression in those three flower variations.

4. Conclusion
According to a morphological study done, the single pink flower shows normal ovary condition while the crested peach and double orange flower shows an anomaly in the form of the ovary. The ovary in crested peach flower seems to be twisted, and the ovules are reduced, while the ovary in double orange flower is replaced by petal-like structure. This finding has never been reported before in any flowers.

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References
[1] Ross I A 2003 Medicinal Plants of the World volume 1 (New York: Humana Press)
[2] Beers L and Howie J 1990, available at http://www.hibiscusworld.com/BeersBook/Intro.htm
[3] Pounders C T and Sakhanokho H 2012 HortScience 47 1819-20
[4] Gilman E F 1999, available at http://hort.ifas.ufl.edu/shrubs/HIBROSA.PDF
[5] Purseglove J W 1987 Tropical Crops Dicotyledons (Singapore: Longman Singapore Publishers)
[6] Bowman J L 1997 J. Biosci. 22 515-27
[7] Yamaguchi T, Lee D Y, Miyao A, Hirochika H and An G 2006 The Plant Cell 18 15-28
[8] Benedito, V A, Visser P B, Tuyl J M, Angenent G C, DeVries S C and Krens F A 2004 Journal of Experimental Botany 55 1391-99
[9] Haughn, G W and Somerville C R 1988 Developmental Genetics 9 73-89
[10] Van Steenis C G G J, den Hoed G, Bloembergen S and Eyma P J 2006 Flora untuk Sekolah di Indonesia (Jakarta: PT. Pradnya Paramita)
[11] T. P. Spira, A. A Snow, D. F. Whigham and J. Leak, Amer. J. Bot. 79 428-233 (1992).
[12] Tjitrosoepomo G 2009 Morfologi Tumbuhan (Yogyakarta: Gadjah Mada University Press)
[13] Coen E S and Meyerowitz E M 1991 Nature 353 31-7
[14] O’Maolleidigh D S, Graclet E and Wellmer F 2014 New Phytol. 201 16-30