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Phenotypic plasticity in the ovarium of crested flower of *Hibiscus rosa-sinensis*

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Abstract. *Saifudin, Salamah A. 2019. Phenotypic plasticity in the ovarium of crested flower of* Hibiscus rosa-sinensis*. Biodiversitas 20: 1241-1247. Hibiscus rosa-sinensis* L. or known as Kembang Sepatu in Indonesia has a variety that is very diverse for the shape of flowers, sizes, and colors. Variations of *Hibiscus rosa-sinensis* L. flowers not only can be observed among its hybrids but also in one hybrid. The hybrid is known as a crested flower (double type I), one of four categories of *H. rosa-sinensis* flower shapes in nature. This study aims to know the variation of the crested flower through morphological and anatomical observations of its ovaries. The morphological observation was carried out by observing ovarian outer surface under the Dino-Lite microscope. An anatomical observation was done by observing the longitudinal section (l-s) of fresh ovarian samples under a light microscope with 4x magnification. Morphological observation of 200 samples showed three types of external structure of ovaries: *non-twisted ovary*, *twisted ovary*, and *ovary with its wall forming a structure like a petal*. Anatomical observation of 137 samples showed three types of ovaries: *normal ovary*, *ovary with its internal undergo a change or a reduction*, and *ovary with a formation of pistillum-like structure*. Variations that occur indicate plasticity phenomenon in crested flowers. Further research is needed to find out the main factors causing variations in crested flower ovaries.

Keywords: Crested, flower, *Hibiscus rosa-sinensis*, ovary, plasticity.

INTRODUCTION

Hibiscus rosa-sinensis L. is a species of tropical *Hibiscus*, a flowering plant in the Hibisceae tribe of the family Malvaceae, grown throughout Asia, including Indonesia (Rao et al. 2014). In Indonesia, *H. rosa-sinensis* is well-known as "Kembang Sepatu" (Essiett and Iwok 2014). *H. rosa-sinensis* has a wide variety of flowers, not only in size but also colors and shapes (MacIntyre and Lacroix 1996; Prihatiningsih 2011). The colors of *H. rosa-sinensis* flowers can be white, pink, red, orange, peach, or yellow (Gilman 1999). The main shapes of *H. rosa-sinensis* flowers are *single* and *double*. There is also a *crested*, a transitional shape between single and double flowers (Beers and Howie 1990).

The latest study grouped the form of *H. rosa-sinensis* flower into four categories which are single, double type I, double type II, and double type III, whereas the form of a crested flower belongs to double type I (Salamah et al. 2018) (Figure 1). The difference between each of the four conspicuous forms of *H. rosa-sinensis* flowers is the presence of the additional organ resembling petal that we called as staminodium petaloid and stamen-petal intermediate. In single flower, those two additional petals were not found while in double flowers the additional petals were varying in size, number, and composition.

The difference between the four types of *H. rosa-sinensis* flowers is also observed from the anatomical side, especially in the structure of the ovary. Rostina (2017) had seen the differences between the ovary of the four types of flowers in *H. rosa-sinensis*. The single flower has a normal ovary with five carpels, axillary placentation, and locules that is visible. A similar condition is also found in the ovary of double type II with a smaller size in the ovary. Meanwhile, on the observations of the crested flower ovary (double type I), locules cannot be distinguished although the presence of ovules still can be observed. In the ovary of double type III flowers, the chamber in the ovary only contains structure that is resembling petals, without any ovules (Figure 2).

Based on previous studies, the observation of variation in the *H. rosa-sinensis* ovary usually conducted by comparing morphological and anatomical structures among its hybrids. Meanwhile, based on direct observation in nature, variations of *Hibiscus rosa-sinensis* L. flowers can also be seen in one hybrid only, especially in crested flower (double type I). So, the study of variation in crested flower ovaries through morphological and anatomical observations need to be done.

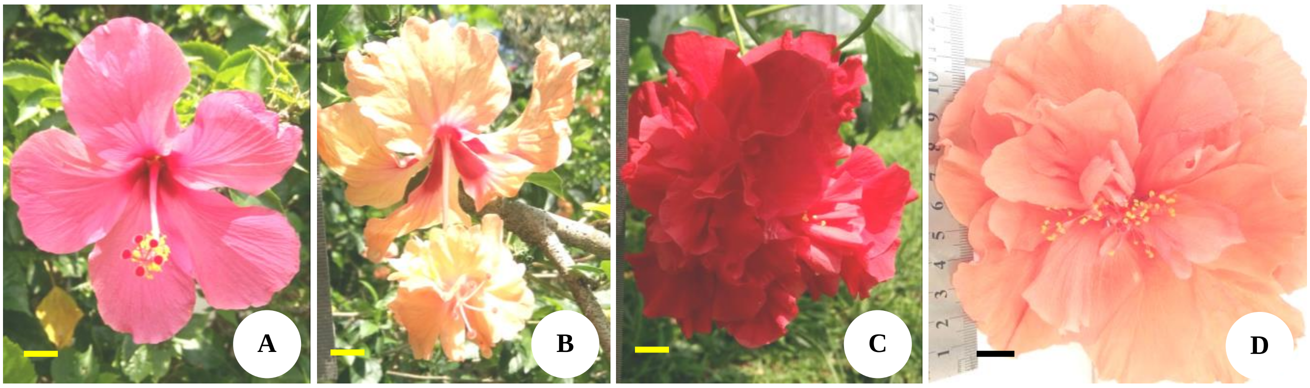

Figure 1. Type of *Hibiscus rosasinensis* flowers in nature. A. Single flower, B. Crested flower (Double type I), C. Double (type II) flower, D. Double (type III) flower. Bar = 1 cm

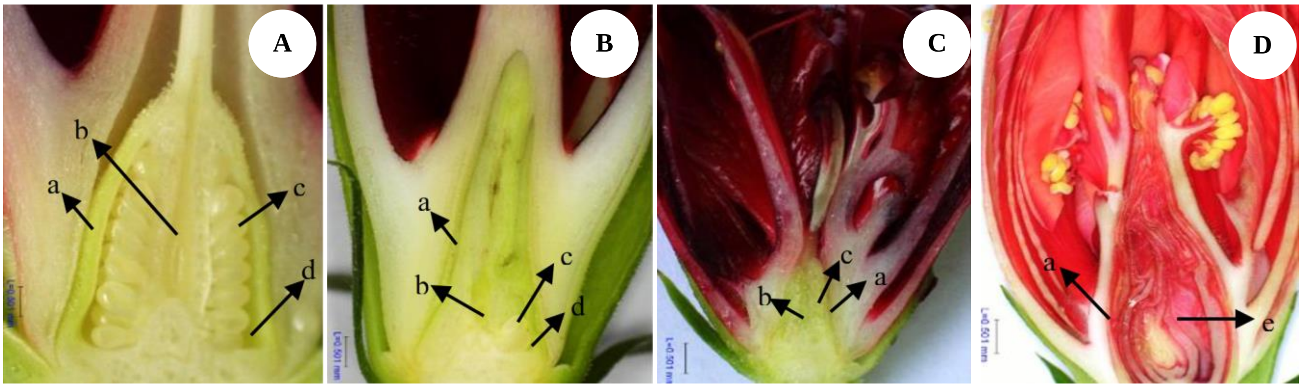

Figure 2. Structure of ovary in four types of *Hibiscus rosasinensis* flower. A. *Ovary of single flower*, B. *Ovary of* crested (*double* type I) flower, C. *Ovary of double* type II flower, D. *Ovary of double* type III flower. a. ovary wall b. placenta c. ovule d. *locule* e. *petal-like structures* (after Rostina 2017)

MATERIALS AND METHODS

About 200 ovaries of *Hibiscus rosa-sinensis* crested anthesis flowers were collected from a cultivated growing population at Universitas Indonesia campus, Depok and Bojong Gede residential, Bogor. Morphological observation of ovaries was carried out by observing its outer surface under Dino-Lite microscope. Anatomical observation was done by making a longitudinal (l-s) section from fresh ovarian samples using a free hand-section method without paraffin. An anatomical section was carried out using a light microscope [Leica] with 4x magnification. The products of anatomical observation were documented in videos due to the size of ovarian samples. The full anatomical ovarian figure was produced by combining several pieces of screenshot figure of sample running videos using screenshot application in the computer. Only representative full ovarian figure then used as anatomical observations data. Observation in transverse section (x-s) of crested anthesis flowers ovaries and longitudinal section (l-s) of 12 stages of flower development before anthesis was also carried out to support the data. All data were presented in tables and figures.

RESULTS AND DISCUSSIONS

Morphological variation in crested ovaries

The ovary of crested flowers shows uniqueness in the pattern of twist. The pattern varied in the form of the level of the depth that is allegedly participating in the change of the ovary structure. In spite of this fact, the normal ovary with no twist that resembles single flowers is still found. Furthermore, there is also the ovary with the wall forming a petal-like structure. Based on morphological observation against 200 ovaries of crested flowers, as many as 168 samples (84%) showed a twisted pattern of the ovary, 19 samples (9,5%) showed a non-twisted or normal ovary, and 13 samples (6,5%) showed the ovary with its wall forming a structure like a petal. Of the 168 twisted ovary samples, 88 twisted into the right direction and 80 towards the left (Figure 3). Based on the longitudinal section (l-s) observation of 12 stages of flower development before anthesis, the twisting process has begun since 3rd stage (Figure 4).

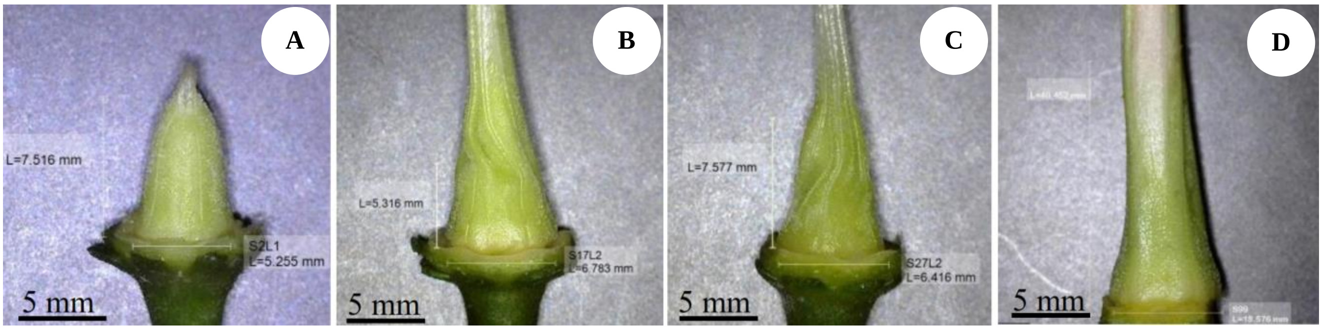

Figure 3. Morphological variation in crested ovaries of *Hibiscus rosa-sinensis*. A. Normal ovary (resemble *single* flower), B. Left-twisted ovary, C. Right-twisted ovary, D. Ovary with a wall that forms petal-like structure

A B C D E

Figure 4. Anatomical features of crested ovaries before anthesis of *Hibiscus rosa-sinensis*. A. 1st stage of floral development before anthesis, B. 2nd stage of floral development before anthesis, C. 3rd stage of floral development before anthesis, D. 4th stage of floral development before anthesis, E. 5th stage of floral development before anthesis, x. dent in ovary wall showed twisting process

Anatomical variations in crested ovaries

Among 200 samples of ovaries used in this study, only 137 ovaries that representative as samples of anatomical study. Anatomical observation showed three types of the ovary which are the normal ovary with interior structure resembles to single flower ovary, the ovary with its internal undergo a change or a reduction, and the ovary with its internal structure showed the formation of a new structure resembling pistillum. Based on anatomical observation of 137 samples of representative crested ovaries, 27 samples (19%) showed normal ovary with interior structure resembles to single flower ovary, 62 samples (45%) showed ovary with its internal undergo a change or a reduction, and 48 samples (35%) showed ovary with its internal structure forming a new structure resembling pistillum.

The ovary with interior structure resembles single flower ovary supposedly associated with its morphology structure that did not have a twist pattern (Figure 5.A) or having a shallow depth of twist (Figure 5.B). This condition indicates that basically, the ovary of *H. rosa-sinensis* crested flowers is designed with a pattern like a single flower ovary. Further research still needed to prove this assumption, especially in the stage of ovarian tissue development before anthesis. Moreover, the same situation also happened on the ovaries which its internal structure undergo a change or a reduction. The level of the depth of twist in ovary correlates with the changing process of internal ovarian structures. The thicker the twist, the more changing happened in ovarian internal structures (Figure 5.C). The ovary with a very high level of the depth of twist, causing the reduction of ovarian internal structures (Figure 5.D). The ovary which its internal structure forming a new structure resembling pistillum (Figure 5.C), having a pattern of twist that was not significantly different with the ovaries which its internal structure undergo a change or reduction (Figure 5.D). Based on that result, it allows us to assume that the depth of twist did not correlate with the formation of a new structure resembling pistillum.

Based on the results of Figure 6, there are two possibilities for the origin of the formation of a pistillum-like structure in the ovary. The pistillum-like structure could be derived from an ovule or funiculus (Figure 6.A) and derived from a placenta or the basal parts of the ovary (Figure 6.B and 6.C). The result of transverse section (x-s) observation (Figure 7.A) support the findings of Figure 6, in which pistillum suspected originating in a part of an ovule or the funiculus that seem occupy the position of the carpel of the ovary. The pistillum-like structure then insists on the position of the ovule found in the carpel and then pushes the other carpels and leads to an irregular arrangement of the internal structure of the ovary (Figure 7.A).

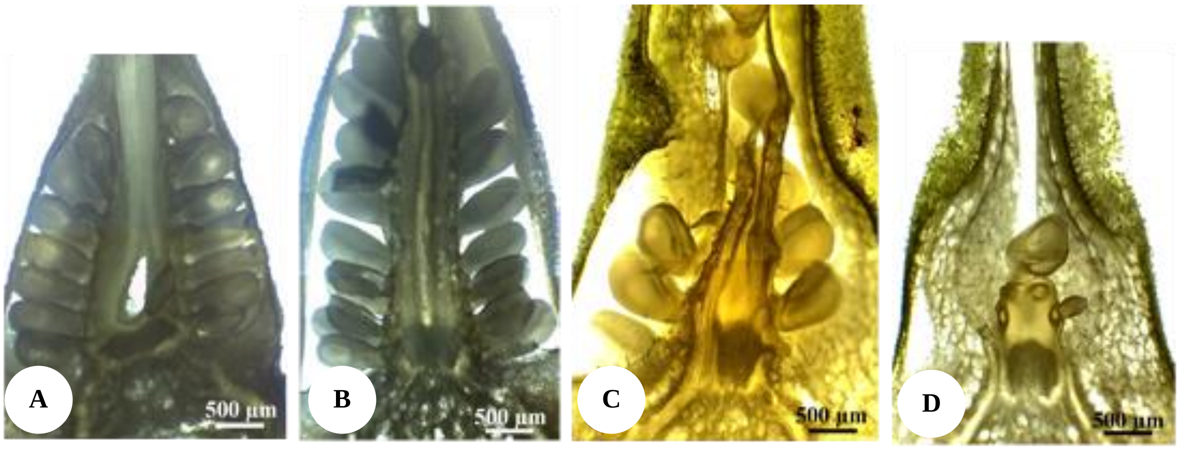

Figure 5. Variation of internal structure of crested flower ovary of *Hibiscus rosa-sinensis*. A. Internal structure of non-twisted ovary, B. Internal structure of ovary with shallow depth of twist, C. Internal structure of ovary with deeper depth of twist, D. Internal structure of ovary with deepest depth of twist

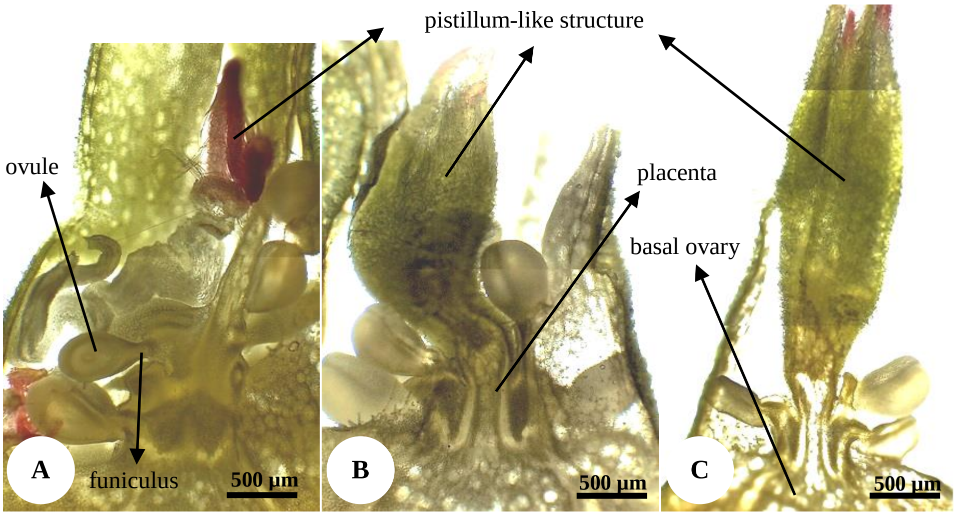

Figure 6. The origin of the formation of a pistillum-like structure in ovary of *Hibiscus rosa-sinensis*. A. Pistillum-like structure derived from ovule or funiculus, B. Pistillum-like structure derived from placenta, C. Pistillum-like structure derived from the basal of ovary

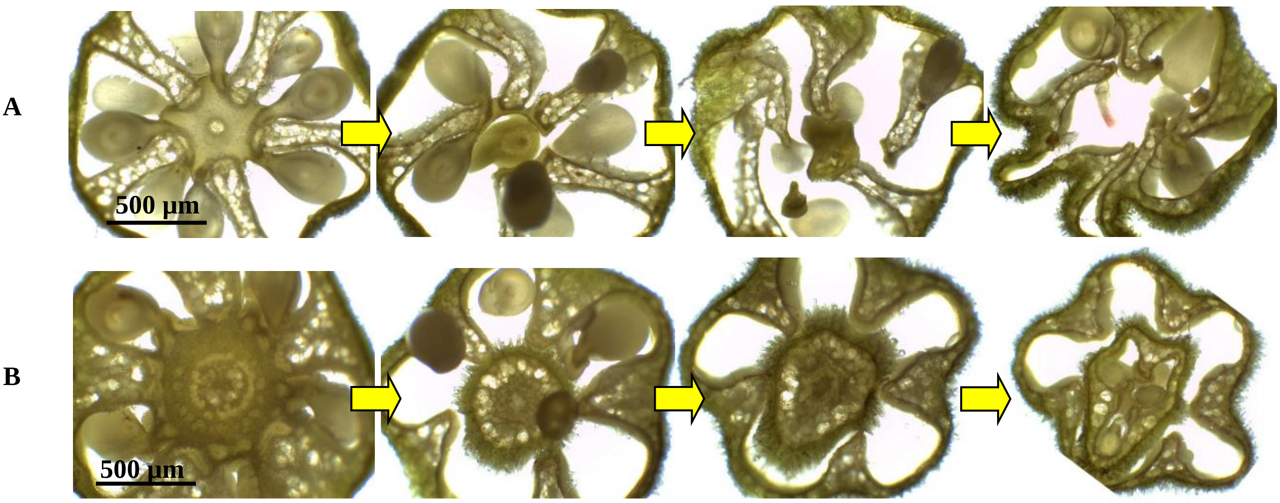

Figure 7. Transverse section (x-s) of ovary of *Hibiscus rosa-sinensis* with pistillum-like structure derived from an ovule or the funiculus (A), and derived from a placenta or the basal parts of the ovary (B)

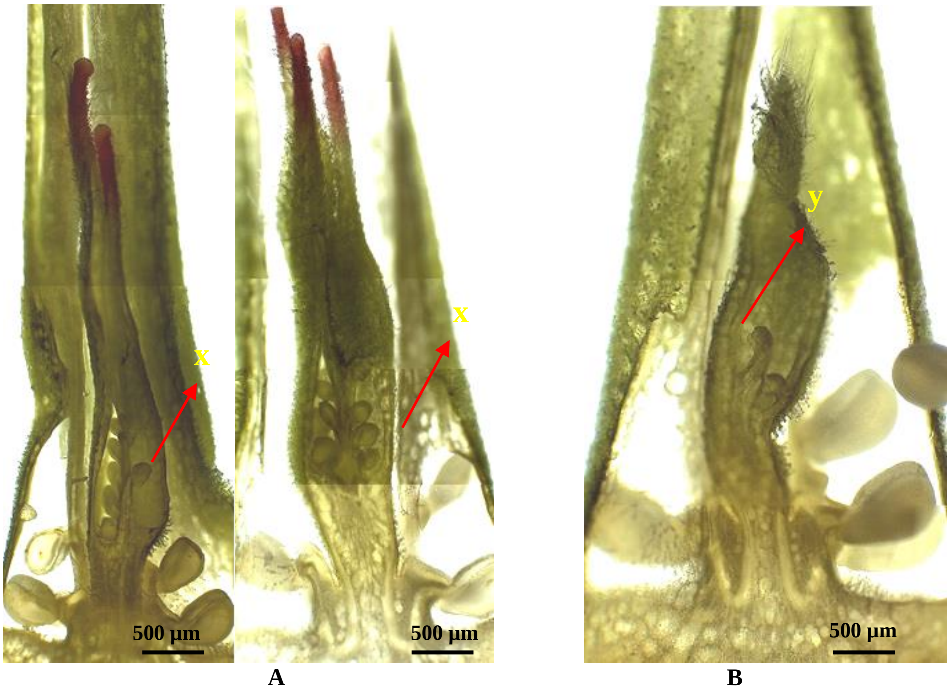

Figure 8. Phenomenon of double flower in *Hibiscus rosa-sinensis* crested flower. A. x. ovule-like structure in the inside of pistillum-like structure, B. y. new pistillum-like structure in the inside of pistillum like-structure

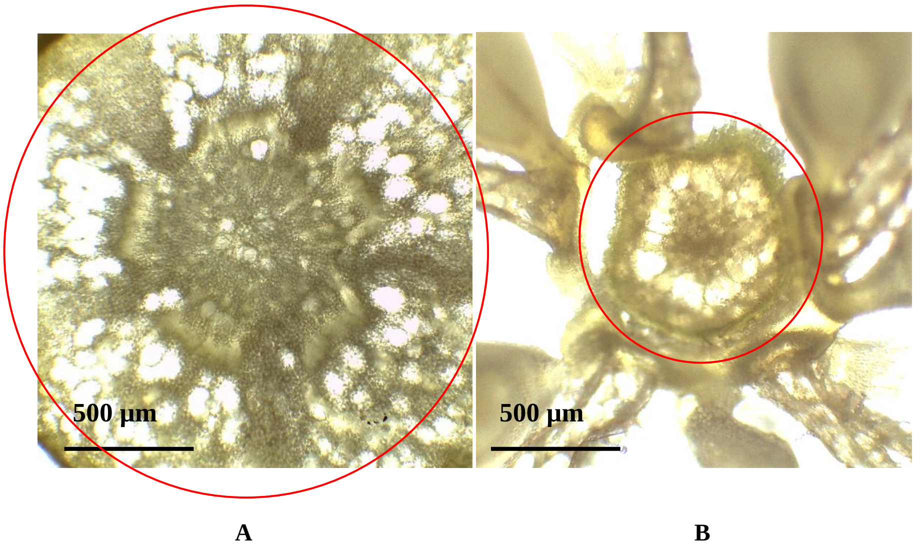

Figure 9. The similarities between basal part of main ovary (A) and basal part of pistillum-like structure (B) of *Hibiscus rosa-sinensis*

Meanwhile, in the ovary that is having a pistillum-like structure derived from a placenta or the basal part of the ovary, the formation of structures are visible originating in a part of the core of ovary. The pistillum-like structure then urges the carpel and then make an impact on the loss of ovules (Figure 7.B). This condition is then allegedly as one of the factors participating in internal structure reduction of crested flower ovary. The formation of a new structure that resembles pistillum strengthened by the existence of an ovule on its inside (Figure 8.A). At one sample, pistillum-like structure was even can bring a new structure that also resembling pistillum (Figure 8.B). This condition showed the phenomenon of double flower, the formation of new flower inside a flower. This phenomenon strengthened by the result of transverse section (x-s) of basal part of ovary with pistillum-like structure that showed similarities with basal part of main ovary (Figure 9). To prove this allegation, Further research, especially through the Scanning Electron Microscopy (SEM) method, is still needed.

Phenotypic plasticity

Variation on the ovaries of *H. rosa-sinensis* crested flower, whether morphology and anatomy, allegedly a symptom of phenotypic plasticity. Turcotte and Levine (2016) said that phenotypic plasticity occurs when a genotype expresses different phenotypes in different environments. Gao et al. (2018) added that the phenotypic plasticity is the form of plant adaptation against the environment. This is supported by the result, which of two growing locations of *H. rosa-sinensis*, the campus of Universitas Indonesia, Depok and the Bojong Gede residential, Bogor, show the different structure of ovary, whether morphology and anatomy.

The difference of environmental factors of the two locations clearly seen from the temperature, light intensity, and humidity (Table 1). Based on measurements, the temperature at the Universitas Indonesia campus was higher than Bojong Gede residential, although they were not too significant. The light intensity measurement showed that *H. rosa-sinensis* crested flowers grew in Universitas Indonesia campus got higher illumination than those that grew in Bojong Gede residential. Of these two environmental factors had an influence on the moisture wherein the location of Bojong Gede residential having higher humidity than in the area of the Universitas Indonesia.

Based on morphological observation, *H. rosa-sinensis* crested flowers that grow at Bojong Gede residential showed only two forms of ovary which are a twisted ovary and ovary with the wall forming petal-like structure. Meanwhile, crested flowers that grow at the campus of Universitas Indonesia showed all three types of ovary. Likewise on anatomical observation that can be concluded that crested flowers that grow at Bojong Gede residential showed only two patterns of ovary which are the ovary with its internal undergo a change or a reduction, and the ovary with its internal structure forming a new structure resembling to pistillum. While crested flowers that grow at the campus of Universitas Indonesia showed all their anatomical types of ovary (Table 2).

Irrespective of the differences in the environmental factors in two locations that bring a different variation in the morphology and anatomy of crested ovary, we still can not conclude yet about the specific factors that affecting those variations. It is due to the fact that each individuals flowers can show variation both in morphology and anatomy aspect, even though they appear in the same plant of *H. rosa-sinensis*. Gao et al. (2018) stated that the ability of plant to response the environmental changes by phenotypic plasticity depend on their genetic characteristics. The characteristic mainly about the ability to adapt to environmental changes. But in order to understand what gene that had played a role in phenotypic plasticity in the ovary of crested flower, including the environmental factor, further research is still needed, both in morphology, anatomy, and molecular aspects.

Phenotypic plasticity can also be assumed as a product of transdifferentiation. Transdifferentiation is capability of adult cells to change directly into other functional adult cells (McManus et al. 1998). Transdifferentiation can occur without going through of cell division phase (Shoji et al. 1996) and without a change of form and size of the cell (Krishnamurthy et al. 2015). Almeida et al. (2015) added that transdifferentiation is part of morphogenesis process in plants, both in *in vitro* and *in vivo* condition. Transdifferentiation events have been observed by Shoji et al. (1996) in *Zinnia elegans* which there has been a change of mesophil leave cells into the elements of trachea. Transdifferentiation has been also observed on the nuts beans (*Phaseolus vulgaris*) and *Sambucus nigra* which its corticoid cells undergo a change into abscission cells (McManus et al. 1998).

Table 1. Environmental factors of two growing locations of *Hibiscus rosa-sinensis* crested flower

Location	Temperature (ºC)			Average Light intensity (Lux)			Humidity (%)		
	7-10 am	12-15 pm	16-18 pm	7-10 am	12-15 pm	16-18 pm	7-10 am	12-15 pm	16-18 pm
Campus Universitas Indonesia, Depok	28	33,5	28,5	6532	9004	4098	75	46	54
Bojong Gede residential, Bogor	25,5	32,5	29,3	4489	2889	684	79	49	58,5

Table 2. Type of ovary of *Hibiscus rosa-sinensis* in two different locations based on morphological and anatomical observation

Location	Number of morphological observation sample	Type of ovary				Number of anatomical observation sample	Type of ovary		
		Non-twisted	Right twisted ovary	Left twisted ovary	Forming petal-like structure		Ovary with interior structure resembles single flower ovary	Ovary with its internal undergo a change or a reduction	Ovary with pistillum-like structure
Campus Universitas Indonesia, Depok	93	19	35	39	0	82	27	48	7
Bojong Gede residential, Bogor	107	0	53	41	13	55	0	14	41

The formation of a new structure resembling pistillum in the ovary allegedly is one of transdifferentiation symptoms on *H. rosa-sinensis* crested flower. Based on observations, it can be seen that the emergence of a structure resembling pistillum in the ovary can be assumed as a transdifferentiation process of the cells of ovule, funiculus, placenta, and basal cells of ovary. Those cells then transformed into cells that form a structure similar to pistillum. To prove all the allegation, further research is needed.

In conclusion, the morphology and anatomy observation against 200 samples of *Hibiscus rosa-sinensis* crested flower shows a variety in the ovary. The variety can be seen not only in the outer feature but also in their interior structure. The variety of morphology shows three main characteristics of the ovary which are non-twisted ovary (normal ovary), twisted ovary, and ovary with a wall forming petal-like structure. The variety of anatomy shows three main characters of the ovary which is the ovary with interior structure resembles single flower ovary, the ovary with its internal undergo a change or a reduction, and the ovary with its internal structure showed the formation of a new structure resembling pistillum. Variations that occur indicate phenotypic plasticity phenomenon in crested flowers. Further research needs to be performed to find out the main factors causing variations in crested *H. rosa-sinensis* flower ovaries.

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REFERENCES

Almeida M, Graner ÉM, Brondani GE, Oliveira LS, Artioli FA, Almeida LV, Leone GF, Baccarin FJB, Antonelli PO, Cordeiro GM, Oberschelp GPJ, Batagin-Piotto KD. 2015. Plant morphogenesis: theoretical bases. Adv For Sci 2 (1): 13-22.

Beers L, Howie J. 1990. Growing *Hibiscus*. http://www.hibiscusworld.com/BeersBook/Intro.htm.

Essiett UA, Iwok ES. 2014. Floral and leaf anatomy of *Hibiscus* species. Amer J Med Biol Res 2 (5): 101-117.

Gao SL, Mo L, Zhang J, Zhang J, Wu J, Wang N, Zhao, Gao Y. 2018. Phenotypic plasticity vs. local adaptation in quantitative traits differences of *Stipa grandis* in semi-arid steppe, China. Scientific Reports 8: 1-8.

Gilman EF. 1999. *Hibiscus rosa-sinensis.* University of Florida Cooperative Extension Service Institute of Food and Agricultural Sciences 254: 1-3.

Krishnamurthy KV, Bahadur B, Adams SJ, Venkatasubramanian P. 2015. Development and organization of cell types and tissues. Plant Divers Organ Function Improv 1: 73-111.

MacIntyre JP, Lacroix CR. 1996. Comparative development of perianth and androecial primordial of the single flower and the homeotic double-flowered mutant in *Hibiscus rosa-sinensis* (Malvaceae). Canada J Bot 74: 1871-1882.

McManus MT, Thompson DS, Merriman C, Lyne L, Osborne DJ. 1998. Transdifferentiation of mature cortical cells to functional abscission cells in bean. Plant Physiol 116: 891-899.

Prihatiningsih R. 2011. Studi variasi bentuk bunga *Hibiscus rosa-sinensis* L. secara morfologi, anatomi, dan molekular di kampus UI, Depok. [Hon. Thesis]. Universitas Indonesia, Depok. [Indonesian]

Rao KNV, Geetha K, Raja A, Banji D. 2014. Quality control study and standardization of *Hibiscus rosa-sinensis* L. flowers and leaves as per WHO guidelines. J Pharmacognosy and Phytochemistry 3 (4): 29-37.

Rostina I. 2017. Studi Homeosis Ovul Pada Ovarium Bunga *Hibiscus rosa-sinensis* L. Secara Anatomi. Universitas Indonesia, Depok. [Indonesian]

Salamah A, Prihatiningsih R, Rostina I, Dwiranti A. 2018. Comparative morphology of single and double flowers in *Hibiscus rosa-sinensis* L. (Malvaceae): a homeosis study; Proceedings of the 3rd International Symposium on Current Progress in Mathematics and Sciences 2017 (ICPMS2017), Bali, 26-27 July 2017. DOI:10.1063/1.5050107

Shoji Y, Sugiyama M, Komamine A. 1996. Suppression by 5-Bromo-2-deoxyuridine of transdifferentiation into tracheary elements of isolated mesophyll cells of *Zinnia elegans*. Plant Cell Physiol 37 (3): 401-403.

Turcotte MM, Levine JM. 2016. Phenotypic plasticity and species coexistence. Trends Ecol Evol 31 (10): 803-813.