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The Influence of Season on Phenotypic Plasticity Symptoms in *Hibiscus rosa-sinensis* Crested Peach Flowers

(Pengaruh Musim terhadap Gejala Keplastikan Fenotip pada Bunga *Crest* Pic *Hibiscus rosa-sinensis*)

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ABSTRACT

The variation of *Hibiscus rosa-sinensis* flower in the form of a crested peach is thought to show phenotypic plasticity symptoms in nature. Phenotypic plasticity is a condition in which genotypes give rise to different phenotypes in different environments. The aim of this study was to determine the plasticity response of *H. rosa-sinensis* crested peach flowers to seasonal changes, both morphologically and anatomically. Flower samples were taken from the residential area of Bojong Gede, Bogor, in two different seasons: the dry season in 2018 and the rainy season in 2021. Morphological observations were made by calculating the number and size of each flower section using measuring instruments and a Dino-Lite microscope. Anatomical observations were made by observing the internal structure of the ovaries using a 4× magnification light microscope. Measurement of environmental parameters, such as temperature, humidity, and light intensity, was also carried out in this study to determine the symptoms of phenotypic plasticity. The phenotypic plasticity responses of *H. rosa-sinensis* crested peach flower are clearly observed in the number and composition of the stamen, staminodium petaloid, intermediate stamen-petal, and external-internal structure of the ovary. *H. rosa-sinensis,* in the form of crested flowers, showed a different phenotypic plasticity response in different seasons. The light intensity and temperature factors play an important role in phenotypic plasticity. Research with various observation times is still needed to determine the range of phenotypic plasticity responses of the *H. rosa-sinensis* flower form of crested peach in nature.

Keywords: Crested; flower; *Hibiscus rosa-sinensis*; phenotypic; plasticity

ABSTRAK

Variasi bunga *Hibiscus rosa-sinensis* berbentuk *crest* pic dianggap menunjukkan gejala keplastikan fenotip secara semula jadi. Keplastikan fenotip ialah keadaan apabila genotip menimbulkan fenotip yang berbeza dalam persekitaran yang berbeza. Matlamat kajian ini adalah untuk menentukan tindak balas keplastikan bunga *crest* pic *H. rosa-sinensis* terhadap perubahan musim secara morfologi dan anatomi. Sampel bunga diambil daripada kawasan perumahan Bojong Gede, Bogor, dalam dua musim berbeza: musim kering pada tahun 2018 dan musim hujan pada tahun 2021. Pemerhatian morfologi dilakukan dengan mengira bilangan dan saiz setiap bahagian bunga menggunakan alat pengukur dan Mikroskop Dino-Lite. Pemerhatian anatomi dibuat dengan memerhati struktur dalaman ovari menggunakan mikroskop cahaya pembesaran 4×. Pengukuran parameter persekitaran, seperti suhu, kelembapan dan keamatan cahaya, juga dijalankan dalam kajian ini untuk menentukan gejala keplastikan fenotip. Tindak balas keplastikan fenotip bunga *crest* pic *H. rosa-sinensis* diperhatikan dengan jelas dalam bilangan dan komposisi stamen, staminodium petaloid, stamen-kelopak perantaraan dan struktur luar-dalaman ovari. *H. rosa-sinensis*, dalam bentuk *crest*, menunjukkan tindak balas keplastikan fenotip yang berbeza pada musim yang berbeza. Faktor keamatan cahaya dan suhu memainkan peranan penting dalam keplastikan fenotip. Penyelidikan dengan pelbagai masa pemerhatian masih diperlukan untuk menentukan julat tindak balas keplastikan fenotip bagi bentuk bunga *H. rosa-sinensis crest* pic dalam persekitaran semula jadi.

Kata kunci: Bunga; *Crest*; fenotip; *Hibiscus rosa-sinensis*; keplastikan

Introduction

The *H. rosa-sinensis* flowers mainly have two shapes that are single and double. There is also a crested, a transitional shape between single and double flowers (Beers & Howie 1990). The difference between the forms of *H. rosa-sinensis* flowers is the presence of the additional organ resembling a petal called staminodium petaloid and stamen-petal intermediate. Those two additional petals were not found in a single flower, while in double and crested flowers, the additional petals varied in size, number and composition (Saifudin & Salamah 2019). The form of the *Hibiscus* flower used in our research samples is crested. According to Salamah et al. (2017), crested peach flowers are categorized into double type I, with the number of staminodium petaloid ranging from 7 to 28 and intermediate stamen-petal totaling 1-21 (Figure 1).

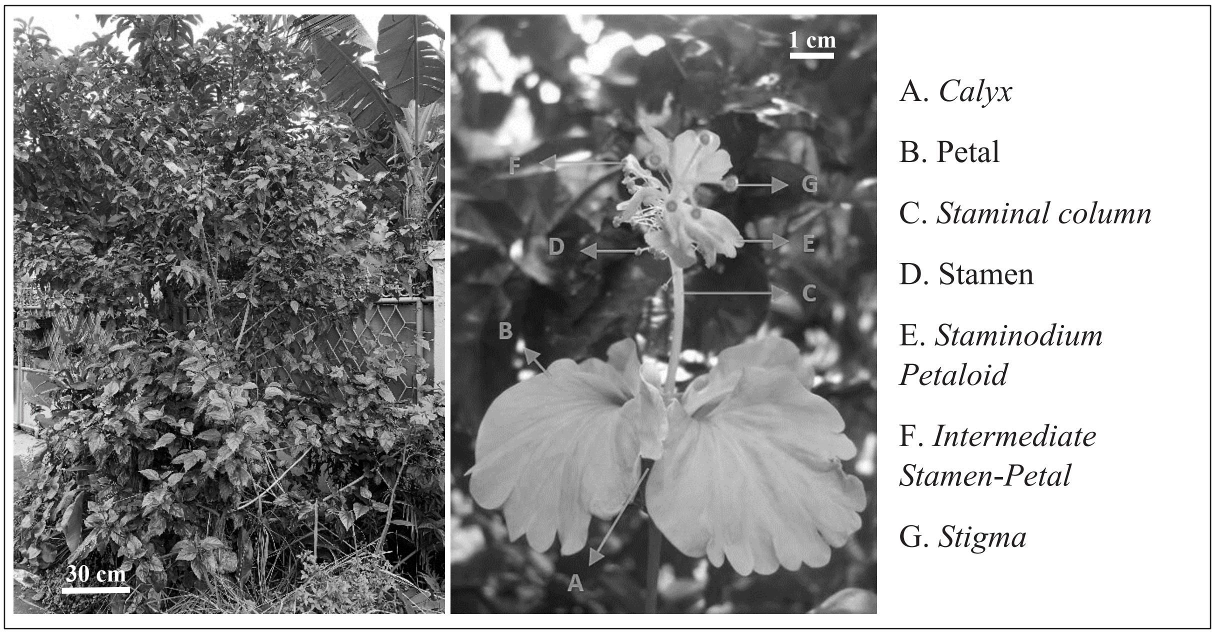

FIGURE 1. Crested peach form of *H. rosa-sinensis* flower

Hibiscus rosa-sinensis, a crested peach flower form, is a shrub 2-4 m in height (Figure 1). It has simple ovate-shaped leaves with a glossy dark green color. Leaf tips of *H. rosa-sinensis* are tapered (acuminatus) with sawed (serratus) or ringed (crenatus) leaf margins. The composition of the leaves of *H. rosa-sinensis* is alternate, where leaves are arranged alternately in a spiral pattern around the twigs. Each branch's node has only one leaf (Gilman 1999) with flowers in the leaf axils (flos lateralis or flos axillaris) (Tjitrosoepomo 2000).

Variations in the shape, color and size of *H. rosa-sinensis* flowers can occur in one individual plant. Individuals who usually produce crested peach flowers can produce single peach, single red and double peach flowers (Prihatiningsih 2011). Saifudin and Salamah (2019) have proven that the *H. rosa-sinensis* flower, in the form of crested peach, has morphological and anatomical variations, especially in the additional organs of flowers and ovaries. This variation is thought to be closely related to the phenomenon of phenotypic plasticity in nature, which is a form of plant adaptation to environmental changes (Gao et al. 2018). Phenotypic plasticity occurs when a genotype gives rise to different phenotypes in different environments (Turcotte & Levine 2016).

Research on phenotypic plasticity in plants is generally carried out on vegetative organs, mainly in leaves. Epidermal thickness, palisade, number of stomata and chlorophyll content were the parameters observed for plasticity responses (Gratani 2014). Research on phenotypic plasticity in flowers has been conducted by Arnold et al. (2019), proving that extreme temperature changes affect a plant's flowering time in the population. In addition, seasonal changes affect the size and colour of flowers in *Moricandia arvensis,* which determines the number of pollinators (Gómez et al. 2020). Meanwhile, Sobral et al. (2021) proved that the presence of herbivores affects *Raphanus sativus* flower colour plasticity.

There are also studies on changes in flower structure in *Hibiscus rosa-sinensis* due to environmental factors (Rostina 2017; Saifudin 2019; Saifudin & Salamah 2021). However, these studies are generally viewed from the molecular aspect related to the symptoms of homeosis. Based on our previous study (Saifudin & Salamah 2019), homeosis is considered one of the symptoms of phenotypic plasticity in *Hibiscus rosa-sinensis* flowers. This study aimed to determine the plasticity response of *H. rosa-sinensis* crested peach flowers to environmental changes, both morphologically and anatomically. So far, no report has discussed this matter, so the implementation of this research is important. Understanding the symptoms of phenotypic plasticity in nature is essential to anticipate environmental changes, especially related to global warming. This research is also needed as a basis for further research, especially from the genetic and molecular aspects.

Materials and Methods

STUDY SPECIES, SITE, AND PERIOD

Research on phenotypic plasticity in *Hibiscus rosa-sinensis* crested peach flowers was carried out in residential area of Bojong Gede, Bogor, Western Indonesia. The location determination was based on a field survey which showed that the phenomenon of phenotypic plasticity of *H. rosa-sinensis* in Bojong Gede tends to be high. Previous studies have shown a high variety of crested flowers both morphologically and anatomically (Saifudin & Salamah 2021, 2019).

The residential area of Bojong Gede, Bogor, is located at coordinates 06°28.072' S and 106°48.667' E with an altitude of 135 above mean sea level (AMSL). According to data from the Meteorological, Climatological, and Geophysical Agency (BMKG), the Bojong Gede area during September 2018 had low rainfall or was in the dry season, while in February 2021, it had high rainfall or was in the rainy season (Figure 2).

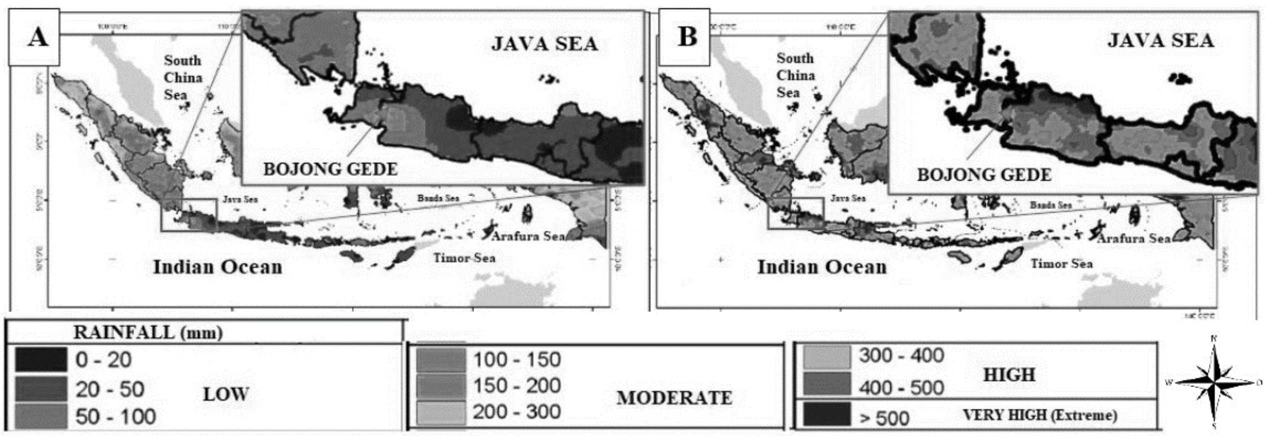

FIGURE 2. Rainfall in the Bojong Gede area in September 2018 (A) and February 2021 (B) (Source: BMKG, 2021)

A total of 200 samples of *H. rosa-sinensis* anthesis flower in the form of crested peach were taken from the residential area of Bojong Gede, Bogor, with different sampling times: September 2018 (100 flowers) and February 2021 (100 flowers). The flower sampling in 2018 represents phenotype plasticity data in the dry season period, while the flower sampling in 2021 represents phenotype plasticity data in the rainy season period.

The flower samples were taken every day from one plant during September 2018 and February 2021 until they reached the desired number. Generally, the number of flowers ranges from 1 to 28 anthesis flowers from different branches per day. Flowers with defects due to disease or insects were not used as samples to prevent data from being biased. Not all samples of flowers obtained have a representative appearance. There is a possibility of human error that makes the sample damaged. Thus, only representative samples were used as 100 flower samples in each observation period in this study.

OBSERVATION AND DATA COLLECTION

Observation and data collection were carried out qualitatively and quantitatively. Qualitative data was collected by morphological and anatomical observation with the same sample sources. The qualitative data includes the process of flower development before anthesis (pre-anthesis), the shape of the flower during anthesis, the phenotype of additional organs of the flower, and the external–internal structures of the ovaries. Morphological observations of the shape of the flower, the additional organs and the external structure of the ovary were carried out by direct observation or by using a Dino-Lite microscope, while anatomical observations of the internal structure of the flower ovaries, both at the pre-anthesis and anthesis stages, were carried out using a 4× magnification light microscope.

Quantitative data was collected by counting the number of stamens and additional organs of flowers. The calculation of the number of epicalyx, calyx, corolla, and stigma was not carried out because, based on preliminary studies, there were no significant differences in each part of the flower between individual flowers. Quantitative data collection was also done by measuring the length of the petiole and staminal column. Measurement of the length of the staminal column is carried out by measuring the closest distance between the position of the petal growth and the first appearance of the stamen or additional organ.

Quantitative data collection was also carried out on environmental parameters, such as temperature, air humidity, and light intensity. Measurement of temperature and humidity was carried out using a Thermo hygrometer, while light intensity measurement was carried out using a 3 in 1 multipurpose soil meter. Measurements of environmental parameters were carried out three times a day: morning (09:00-10:00, Western Indonesia Time [WIB]), afternoon (12:00-13:00, WIB) and evening (15:00-16:00, WIB). Subsequent observation data is presented in the form of figures and tables.

DATA ANALYSIS

Qualitative data is presented in the form of pictures, while quantitative data is presented in tabular form. The data obtained was then tested by chi-square and analyzed descriptively. The chi-square test was carried out to compare the observed data for 2021 with the observations in 2018. Descriptive analysis of phenotypic plasticity symptoms begins by comparing the variation in crested flowers in the two observation periods to determine which flower organs have the most potential to show a plasticity response. The plasticity response can be determined based on changes in the shape (morphogenesis) of flower organs and the number and composition of each part of the flower. Based on the results of morphological and anatomical observations, as well as the measurement of environmental parameter data equipped with literature studies, a reaction norm curve can be designed to describe the level of phenotypic plasticity in crested flowers and the factors that influence it.

RESULTS AND DISCUSSION

ENVIRONMENTAL PARAMETERS

The results of measurements of environmental parameters at different periods, September 2018 and February 2021, are shown in Table 1. Chi-square tests on the parameters of temperature, air humidity and light intensity show a difference between the observed data for September 2018 and February 2021 (df = 2, α = 0,05,

TABLE 1. Average values of environmental parameters at Bojong Gede Residence in 2018 and 2021

Observation time	Average values of environmental parameters								
	Temperature (℃)			Air humidity (%)			Light intensity (Lux)		
	Morning	Afternoon	Evening	Morning	Afternoon	Evening	Morning	Afternoon	Evening
2018	25.5	32.5	29.3	75	46	54	2532	3004	2098
2021	29.3	31.3	30.2	80.2	73.8	74.3	1858	1983	1285

X2 > 5,9915). Measurements of temperature, air humidity, and light intensity show that the Bojong Gede area in September 2018 has a warmer or drier climate than in February 2021. During the observation period in 2021, no significant changes in each environmental parameter at each observation time (morning–afternoon–evening) were observed compared with the observation period in 2018, which shows significant change. This condition is thought to make an important contribution to each symptom of the plasticity of the *H. rosa-sinensis* phenotype of crested peach flowers. Environmental factors affect the number of flowers formed on *H. rosa-sinensis*. It is known that in the 2018 observation period, the number of flowers produced ranged from 0-23 with an average appearance of 15 flowers per day, while in the 2021 observation period, it ranged from 0-28 with an average appearance of 17 flowers per day.

PHENOTYPIC PLASTICITY OF STAMEN

The average number of stamens formed in the single peach flower, which appears in the upper third of the staminal column is 38-54, while the number of stamens formed in the crested peach flower was 0-50. These observations indicated a reduction in the number of stamens in crested peach flowers compared with the number of stamens in single peach flowers. The reduced number of stamens in crested peach flowers is due to the transdifferentiation of the stamens into new, petal-like structures called intermediate stamen-petal (ISP) and staminodium petaloid (SP).

Transdifferentiation is the ability of an adult cell to transform directly into another functional adult cell (McManus et al. 1998). Transdifferentiation can occur without going through the cell division phase (Shoji et al. 1996) and without any change in the shape or size of the cells (Krishnamurthy et al. 2015). Transdifferentiation is part of the morphogenesis process in plants and can occur both *in vitro* and *in vivo* (Almeida et al. 2015). The transdifferentiation process that leads to morphogenesis is thought to make an important contribution to the phenotypic plasticity symptoms of the crested peach form of *H. rosa-sinensis* flowers in nature.

The formation of a petal-like sheet on the stamen, starting from the area around the anthers and then widening to follow the formation of sheets throughout the filament (Figure 3). The new structure resembles petals but still contains a part of the stamen, which is then known as the intermediate stamen-petal (ISP). The process of transdifferentiation of stamen to form sheets is thought not to stop with the formation of ISP only but continues to develop until no more stamen components are found. The new structure resembles a petal that no longer contains a stamen component, known as the staminodium petaloid (SP).

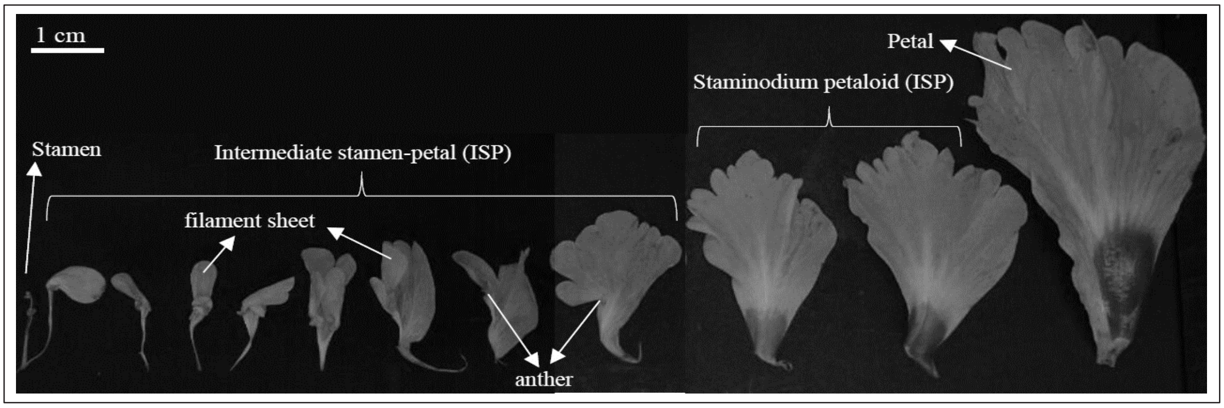

FIGURE 3. Stages of stamen morphogenesis suspected of forming ISP and SP

Based on the results of these morphological observations, it is suspected that the formation of ISP and SP is a manifestation of phenotypic plasticity symptoms in the stamen. The assumption that ISP and SP are new structures as a result of the stamen transdifferentiation process is strengthened by the composition of the number of stamens, ISP, and SP, which, when accumulated, have the same number range as the total number of stamens in single peach flowers (Table 2). Chi-square test on the number and composition of ISP, SP and stamen shows a difference between the observed data for September 2018 and February 2021 (df

= 2, α = 0.05, X2 > 5.9915). The phenotypic plasticity symptoms of the stamen forming ISP and SP in the two observation periods showed that the emergence of ISP in the observation period in 2021 is more prominent than in the observation period in 2018. This condition is inversely proportional to the range of SP that are more numerous in 2018 than in 2021 (Table 3).

TABLE 2. Data for the calculation of crested and single forms of flower perianths

		Single peach flowers								Crested peach flower							
Sample Number		15	16	19	33	50	51	52	65	9	24	39	40	66	67	68	70
Number of flower parts	SP	0	0	0	0	0	0	0	0	25	1	11	20	10	22	15	8
	ISP	0	0	0	0	0	0	0	0	5	4	24	9	24	6	17	24
	Stamen	45	43	38	54	46	43	44	40	0	50	6	6	13	7	22	8
Total		45	43	38	54	46	43	44	40	30	55	41	35	47	35	54	40

TABLE 3. Average number of ISP and SP in 2018 and 2021

Observation Period	Amount range			Average number		
	ISP	SP	Stamen	ISP	SP	Stamen
2018	1-19	6-33	0-28	8.5	23,8	4.65
2021	2-24	0-30	0-50	11,2	17,3	10,2

ISP: intermediate stamen-petal; SP: staminodium petaloid

Based on the entire observation samples, the accumulated number of ISP, SP, and stamen in crested peach flowers was in the range of 30-55. This is not much different from the range of the number of stamens in single peach flowers (38-54) observed in this study and is also almost similar to that observed by Salamah et al. (2017), that is 39-54. The similarity in the range numbers of stamens in single peach flower, as well as the total number of ISP, SP, and stamen in crested peach flower, could lead to the conclusion that both ISP and SP are derived from the stamen. This result supports Prihatiningsih's (2011) assumption, which states the ISP and SP are included as stamen modification because they appear to replace stamens in a single crested flower. However, regarding whether ISP and SP originate from stamen, molecular observations still have to be done.

The results of stamen number observation show that the range of stamens in 2021 is higher than in 2018. Therefore, it can be concluded that the stamen is more triggered to perform phenotypic plasticity into ISP or SP in the dry season, which is suggested that the dry season will more easily trigger plasticity symptoms in *H. rosa-sinensis* crested peach flower by forming more strand-like organs. According to Gritani (2014), climate change and other environmental pressures will trigger plants to show heterogeneity, and phenotypic plasticity is a plant's effort to overcome these environmental pressures. Baranov et al. (2019) added that the level of phenotype plasticity is closely related to climatic conditions, particularly changes in light intensity and temperature.

Environmental stress that triggers the phenotypic plasticity of crested flowers can be seen in temperature, air humidity and light intensity data (Table 1). It is known that in 2018 observations, there was a significant temperature change, especially from morning to noon. If in 2021, the observed temperature increase was only 2 ℃, then in 2018, the increase would reach 7 ℃. This condition also applies to the parameters of air humidity and light intensity. The humidity measurement results show that from morning to noon, there is a 6.4% decrease in 2021 and 29% in 2018. Meanwhile, from morning to noon, the light intensity measurement results show an increase of 125 Lux in 2021 and 472 Lux in 2018. Changes in temperature, humidity, and light intensity from morning to afternoon are thought to be factors that influence the phenotypic plasticity process of crested peach flowers in nature.

Changes in temperature, humidity, and light intensity are thought to be closely related to the various physiological activities of plants that tend to be actively carried out in the morning before noon, such as photosynthesis, hormone biosynthesis and transportation of nutrients to every part of the plant body, including flowers. According to Trivellini et al. (2011), photosynthetic carbohydrates play an essential role in plant metabolism. Apart from being a source of energy, carbohydrates play a role in the osmoregulatory function and act as signal molecules involved in important processes of plant life, one of which is flower death.

PHENOTYPIC PLASTICITY OF OVARY

The symptom of phenotypic plasticity found in the outer structure of the ovary is the twisted outer wall of the ovary. The depth and direction of the twisting pattern are thought to influence the presence or absence of the changes in the internal structure of the ovaries. Although generally showing a twisted ovary pattern, an ovary with a non-twisting outward appearance, which resembles the morphology of a single-flowered ovary, is still found. In addition, there is also a symptom of phenotypic plasticity in the form of the character of the ovary wall forming a sheet (Figure 4).

Table 4 shows that the ovarian wall of flower samples observed in the period 2018 differs from 2021. This data is confirmed by the results of the chi-square test, which shows that the null hypothesis is rejected, which means there are differences in ovarian wall morphology between the observation periods of 2018 and 2021 (df = 2, $\alpha = 0.05$, $X2 > 5.9915$). Most of the ovaries from the period of 2018 showed a twisted pattern in the wall, while most ovaries of the 2021 samples showed a non-twisted wall pattern. Another striking difference is that the modification of the walls to form sheets was only found in the 2018 observation period, while the ovarian wall pattern without twisting was only found in the 2021 observation period.

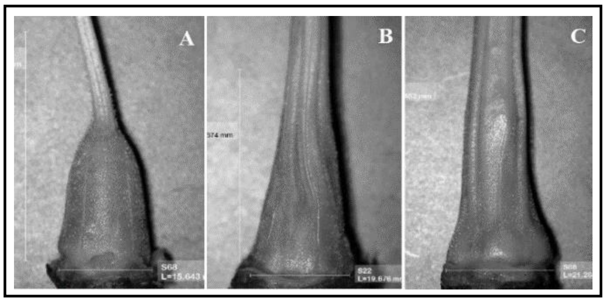

FIGURE 4. Ovarian wall morphological variations of crested peach flowers: without twisting (A), twisting (B), and forming sheet (C)

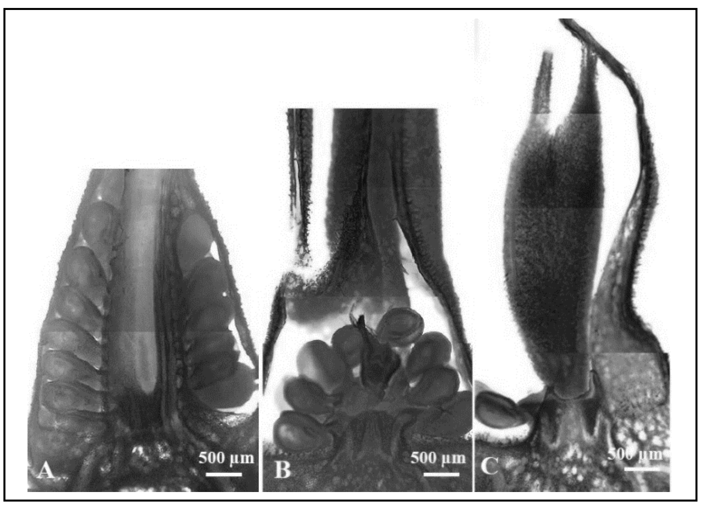

FIGURE 5. Three types of internal structure of the ovary of a crested flower: resembles a single flower (A), with changes or reduction (B), and form a new structure resembling pistil (C)

TABLE 4. Morphological data of the outer wall of the ovary of the crested peach flower

Observation period	Ovarian wall morphology		
	Not twisted	Twisted	Forming sheets
2018	0	87	13
2021	75	25	0

The twisting pattern and modification of the ovary wall to form a sheet are thought to be part of the phenotypic plasticity symptoms of the crested peach *H. rosa-sinensis* flower. Based on anatomical observations of flower development, it is known that the twisting pattern in the ovary wall has occurred since the bud, to be precise, in the third stage of pre-anthesis (Saifudin & Salamah 2019). This finding shows that the response of plants to environmental changes that lead to phenotypic plasticity symptoms started since the beginning of the flower formation process.

Meanwhile, based on the results of anatomical observations done previously (Saifudin & Salamah 2019), it is known that the internal structure of the ovary of the crested peach flower can display three symptoms of phenotypic plasticity: 1) a character such as a single flower ovary whose internal structure does not change, 2) a character that indicates a change in the internal structure of the ovary, either in the form of an arrangement of ovules that appear random or is experiencing reduction, 3) a character indicating the formation of a new structure resembling a pistil (Figure 5). Most of the samples in the observation period in 2018 showed changes in the internal structures of the ovaries, while the samples in the observation period in 2021 mostly showed the structure of the ovaries that did not change (resembles single flower ovaries) (Table 5).

TABLE 5. Comparison of the number of samples from crested flower ovary anatomy observations

Observation period	The internal structure of the ovaries		
	does not change (resembles a single flower)	undergoes a change or reduction	forms a new structure resembling a pistil
2018	0	59	41
2021	75	25	0

Chi-square test on the internal structure of the ovaries showed a difference between the observed data for September 2018 and February 2021 (df = 2, α = 0.05, X2 > 5.9915). In the form of an internal ovarian structure, the symptoms of phenotypic plasticity forming a new structure resembling a pistil found in 2018 were not found in 2021. Ovaries, whose internal structure resembles a single flower ovary, were not found in 2018 observations but became the dominant character found in 2021 observations.

The ovary, whose internal structure forms a new structure resembling the pistil, and the ovary, whose internal structure is reduced and undergoes changes, both have a twisting pattern that is not much different. Thus, it can be assumed that the depth of the twist pattern is not associated with the formation of new structures resembling the pistil. The formation of a new pistil-like structure is reinforced by the presence of an internal ovule-like structure. In one sample, the pistil-like structure can even give rise to another new structure that also resembles the pistil-form inside the pistil (Saifudin & Salamah 2019).

Saifudin and Salamah (2021) made morphological observations and categorized crested peach flowers into three main types based on the length of the staminal column. The first one is a single-like crested flower, which resembled single flowers and had a staminal column length of more than 4 cm. The second and third ones were double-like crested flowers (crested flowers that resemble double flowers, with staminal columns less than 1 cm) and crested intermediate-like flowers (crested flowers that have the appearance of single-like and double-like flowers that have staminal columns less than 1 cm and less than 4 cm).

When the results of morphological observations in 2018 and 2021 were compared, the frequency of appearance of single flowers in one individual *H. rosa-sinensis* crested form was higher than double flowers. Even double flowers were not found in the two observation periods of 2018 and 2021. Different conditions were observed in single flowers: in the 2018 period, only one single crested flower occurred, while in 2021, there were 15. The single flowers tend to be found more frequently in the rainy season (2021), and this reinforces the assumption of a range of phenotypic plasticity levels in crested flowers.

Symptoms of phenotypic plasticity in the ovaries of crested peach blossoms can be observed in the external and internal structures of the ovaries. The outer structure of the ovary showed a twisting pattern of the outer wall, while the internal ovary showed a change in the number of ovules or in the formation of a new structure that resembles a pistil. Most samples in the observation period in 2018 showed a change in the internal structure of the ovaries, while the samples in the observation period in 2021 mostly showed the structure of the ovaries that did not change (resembling single-flowered ovaries). Based on these conditions, in general, it can be concluded that the phenotypic plasticity that encourages the formation of new structures in crested peach blossoms is triggered, especially in the dry season, where the light intensity tends to be high, which increases the temperature and decreases the humidity around the plant. The drastic change in the three environmental parameters is thought to be an important factor in the appearance of phenotypic plasticity symptoms. According to Callaway et al. (2003), in addition to competition factors and the presence of herbivores, variations in the abiotic environment that triggers plant stress are one of the factors that increase the plasticity expression of plant phenotypes.

The observation results on 41 ovaries samples from the 2018 period showed the formation of a new structure resembling a pistil, indicating as if there had been a phenomenon of a new flower formation in the flower, and so on, which showed the pistil flower phenomenon that leads to the growth of multiple flowers. Therefore, it can be assumed that the peak symptom of phenotypic plasticity in *H. rosa-sinensis* crested peach flower, which is depressed by environmental pressure, will cause double flowers. This assumption is further strengthened by the results of morphological observations in the dry season or the observation period in 2018, where the size and number of each part of the crested peach flower are more similar to the double flower of *H. rosa-sinensis*.

NORM OF REACTION

So far, studies related to flower variation in *H. rosa-sinensis* species are still limited to its external appearance. Beers and Howie (1990), for example, categorized *H. rosa-sinensis* flowers into two main forms, namely, single and double forms, and one transitional form in the form of crested flowers. Through their study, Salamah et al. (2018) further developed this categorization and divided the form of flowers into four types: Single, double type I, double type II and double type III. The crested flower itself is included in the double type I category.

Based on the results of this study, it is assumed that the range of symptoms of the phenotypic plasticity of the crested peach form of *H. rosa-sinensis* is between single and double flowers. The wetter (rainy season) an environment is, the more the character of the crested flower is thought to be like a single flower, while the drier (summer) an environment is, the more the character it has is thought to be like double flowers. Therefore, the crested flower is evidence of the plasticity process of gene expression of a hybrid towards the phenotype of its parent, both single and double parents, which are the natural formation of *Hibiscus* flowers in nature. Environmental factors are the main factor determining the plasticity of the gene expression so that if a norm of reaction curve is made, it will look like the curve in Figure 6.

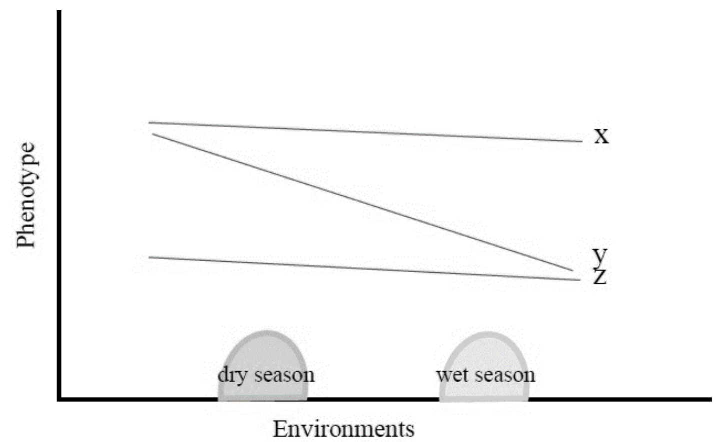

FIGURE 6. Illustration of norm reaction of double-flower (x), crested peach–flower (y), and single-flower (z) genotypes

Based on this curve, the norm of reaction lines in the genotype of single and double flowers appears a little non-horizontal and parallel (line x, z), which means that although environmental factors influence the plasticity of the phenotype, the gene factor determines the phenotypic character more. Meanwhile, the norm of the reaction line on the crested flower genotype is not horizontal at all and will intersect with the norm of the reaction lines of double and single flowers (line y), which indicates that environmental factors have more influence on genotype to bring up the phenotypic characters of crested flowers. Under certain conditions, environmental factors can even trigger the formation of a crested flower phenotype that resembles single and double flowers. The double flower is a wild type of *H. rosa-sinensis* in nature, so it is possible that under extreme environmental conditions (observation of the dry season in 2018), the phenotypic character of the crested flower is more likely to bring out the double flower character.

When referring to environmental factors, single, double, and crested flowers can co-exist. That is, genetic factors remain an important factor in determining the character of crested forms in nature. If we look at the fact that the twisting pattern has been formed since the early stages of preanthesis, then the 'cell fate' in forming the new structure has been specified since the beginning of flower development. According to Schiefelbein (1994), the determination of cell fate will trigger cell differentiation towards morphogenesis through genetic control that is influenced by the environment.

In this study, the crested flower showed no plasticity in forming a double flower as a whole. Regarding this finding, several assumptions were made. First, Indonesia's climate is still below the maximum temperature tolerance limit for *H. rosa-sinensis* crested peach flower to survive without having to make maximum plasticity into a new form. Additionally, crested flowers need a short time to flower (ephemeral flower) so that the plasticity process of crested flowers forming a double flower as a whole becomes difficult. In other words, it takes longer with more extreme temperatures and light intensities to change the shape of a crested flower into a double flower.

According to Trivellini et al. (2011), *H. rosa-sinensis* has a bloom time of 12 hours to die. The short flowering time is thought to be the reason the process of transdifferentiation and morphogenesis of the stamen into a petal-like structure is imperfect so that only intermediate structures appear in nature, in this case, the ISP and SP. If various external and internal factors make it possible for the crested form of *H. rosa-sinensis* to plasticize, the morphogenesis process sequence should occur from stamen to ISP, then ISP to SP, and then SP to a petal structure, thus forming double flowers. In other words, crested flowers are authentic evidence of phenotypic plasticity events that are still or have been taking place in nature. In contrast, single flowers are the only variety in *H. rosa-sinensis* where most of the individual flowers are unable to complete the plasticity journey.

The composition of the additional organs in the double-like type, where the number of SP is far greater than the number of SPI and stamen, also supports the 'journey' of single peach flowers to double flowers as a form of phenotypic plasticity. Even in some samples, the stamen was no longer found. In addition, in double-like flowers, morphologically, a staminal column is found, which gets shorter in size and begins to flatten into sheets, as if to form a double flower-like appearance. If the flowering time is extended, the SP size may continue to widen until it looks more like a petal and adds to the appearance of a double flower.

It is suspected that there are still many symptoms of phenotypic plasticity in the crested form of *H. rosa-sinensis,* which are influenced by various factors other than abiotic environmental factors, particularly those related to the competition or presence of neighboring plants, as well as the presence of herbivores. These factors are connected to affect the level of expression of plant genes towards their plasticity character.

Conclusions

Hibiscus rosa-sinensis in the form of crested flowers showed a different phenotypic plasticity response in different seasons. The light intensity and temperature factors play an important role in the phenotypic plasticity norm reaction. Light intensity and high temperature in summer will direct the phenotypic plasticity of the crested flower to the double flower shape. It is suspected that this is related to the dominance of double parent genes, which are more resistant to summer than single flower parents. The imperfection of the crested flower to form a single flower is thought to be due to the Indonesian climate, which is still below the tolerance limit for crested flower life and the short inflorescence time.

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