Flower and Fruit Development Phenology and Generative Reproduction Success of *Hibiscus rosa-sinensis* spp.

การพัฒนาชีพลักษณ์ของดอกและผลและความสำเร็จของการสืบพันธุ์ของ *Hibiscus rosa-sinensis* spp.

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Abstract

Information on flowers and fruits development (phenology) of the species *Hibiscus rosa-sinensis* spp is needed to support the potential for genetic improvement programs or hybridization, however, is still limited. Thus research on phenology of the flowers and fruits development is important to do. The aims of the research is to investigate the phenological development of flowers and fruits, and generative reproduction success. The design of the research was explorative descriptive with observation method. The study was conducted in the experiment garden of Biology Education Department of UNY. Ten samples of developing flowers taken from one plant were labeled for observation. The observation of developing flowers were done since the very begining of flowering or the initiation phase. Fruit development was observed from the fall of perianth and anther until the fruit maturation. Results show that the average length of flowering period and fruit development is 61 days, with the details as follows: initiation phase is 26 days; preanthesis is 5 days; anthesis is 1 day; pollination and fertilization is 3 days; and fruit development phase is 27 days. Morphologically, the young fruit is green, capsule-shaped, the dimension is 3.2 cm long and 1.3 cm across, the ripe fruit dehisces with many small heart-shaped and brownish-black seeds produced. Moreover, the seed shell is hairy. The generative reproduction success was indicated by the production of viable seeds which are able to germinate and produce offspring showing various morphological juvenile leaves, flowers, and fruits.

Keywords: *Hibiscus rosa-sinensis* ssp, Phenology, Generative reproduction.

บทคัดย่อ

ข้อมูลการพัฒนาของดอกและผล (ชีพลักษณ์) ของสายพันธุ์ *Hibiscus rosa-sinensis spp.* เป็นสิ่งจำเป็นในการสร้างศักยภาพการปรับปรุงพันธุกรรมหรือการผสมข้ามพันธุ์ อย่างไรก็ตาม ข้อมูลดังกล่าวยังมีข้อจำกัด ดังนั้น งานวิจัยเกี่ยวกับการพัฒนาชีพลักษณ์ของดอกและผล จึงมีความสำคัญอย่างยิ่ง การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์การพัฒนาชีพลักษณ์ของดอกและผล รวมถึงความสำเร็จของการสืบพันธุ์ โดยใช้การวิจัยเชิงพรรณนาด้วยวิธีการสังเกตที่ดำเนินการในสวนของภาควิชาชีววิทยาแห่ง UNY มีการเก็บตัวอย่างดอกที่กำลังเติบโตของพืชชนิดเดียวกัน 10 ตัวอย่าง เพื่อดำเนินการสังเกตตั้งแต่พืชเริ่มออกดอกหรือในขั้นการเริ่มต้น สำหรับการสังเกตการพัฒนาของผลจะดำเนินการตั้งแต่วงกลีบรวมและอับละอองเรณูร่วง จนกระทั่งผลเริ่มสุก ผลการสังเกตพบว่า ความยาวเฉลี่ยของระยะเวลาการออกดอกและการเจริญเติบโตของผล คือ 61 วัน โดยมีรายละเอียดดังต่อไปนี้ ขั้นการเริ่มต้น 26 วัน ระยะ preanthesis 5 วัน ระยะ anthesis (การออกดอก) 1 วัน ระยะการผสมเกสรและการปฏิสนธิ 3 วัน และขั้นตอนการเจริญเติบโตของผล 27 วัน ในด้านโครงสร้าง ผลอ่อนจะมีสีเขียว รูปร่างคล้ายแคปซูล ยาว 3.2 เซนติเมตร กว้าง 1.3 เซนติเมตร ผลสุกจะแตกออกเป็นเมล็ดรูปหัวใจขนาดเล็กและมีสีน้ำตาลดำ นอกจากนั้น เปลือกเมล็ดยังมีขน โดยการสืบพันธุ์จะสำเร็จเมื่อมีเมล็ดที่สมบูรณ์ที่สามารถแตกหน่อและผลิตดอกผลรุ่นต่อไป ซึ่งมีโครงสร้างใบ ดอก และผลอ่อนที่แตกต่างกันไปได้

คำสำคัญ : *Hibiscus rosa-sinensis spp*. ชีพลักษณ์ การสืบพันธุ์

Introduction

Phenology is the study of the timing or periodicity of recurring biological phenomena. *Hibiscus rosa-sinensis* including in Malvaceae family has a very diverse color, size and shape of the flowers. Flowers appear throughout the year, has abundant species and varieties grow in lowland or in highland, in tropical and subtropical regions, that are reasonable to cultivated as ornamental plants (Warren, 1997; Kumar & Singh, 2012). To increase the genetic potency of this plant some efforts through hybridization is necessary. Hybridization aims to obtain the desired genetic combinations via crossing two or more different plant genotypes (Damaiyani & Metusala, 2011). Success in plant hybridization, particularly in the manufacture of high yielding varieties, which can not be separated from the phases of information development of flower and fruit is termed phenology.

Flowering, fruiting, leaf flushing, and seed germination are the examples of phenology phenomena in plants (Roubik et al., 2005). Generally, according to Ratnaningrum (2004) flower and fruit development are divided into 6 phases so called (1) flower induction phase, (2) flower initiation phase, (3) pre-anthesis phase, (4) anthesis phase, (5) pollination and fertilization phase, and (6) fruit formation, fruit ripening, and seed formation (Fruit set development). However, time and identification marks of flower and fruit development phases are different between spesies, depending on the internal and external factor interactions (Heddy, *et al*, 1994). Interaction between internal and external factors give an impact to flowering process, such as flowering periods, irregular bearing or irregular fruiting time, dormancy, and juvenility at the same period (Ashari, 1998).

Information about generative reproduction aspects in *Hibiscus* rosa-*sinensis* is still limited, so research on phenology of the flowers and fruits development is important to do. The aims of the research is to investigate the phenological development of flowers and fruits, and generative reproduction success The information obtained is expected to be a basic reference for further research related to this species especially for genetic improvement programs or hybridization.

Objective

The aims of the research is to investigate the phenological development of flowers and fruits, and generative reproduction success on *Hibiscus rosa-sinensis* spp.

Materials and Methods

The design of the research was explorative descriptive with observation method. The study of phenology flower and fruit development was conducted in the experiment garden of the Biology Education Department of UNY on January-April 2012. The observation of flower developing was done since the very begining of flowering or initiation phase until the flowers perfectly open, also when the pollination occured. Ten flower samples in the initiation phase were taken from one plant of *Hibiscus rosa-sinensis* having pink flowers was labeled in January for observation of the flower development. Fruit development was observed since the fall of perianths and anthers until fruit maturation. The samples of fruit development

taken from all flowers were pollinated by hand and labeled in January-March 2012. The hand pollination was done in two methods of pollination namely: 1) self pollination (Pollens are taken from similar individual pink flowers of *Hibiscus rosa-sinensis*) and 2) cross pollination (Pollens are taken from different individual pink flowers of *Hibiscus rosa-sinensis*). All samples were observed every morning at 08.00 o'clock. The seeds obtained after fruits maturation were germinated in soil media to investigate the success of generative reproduction. The tools using in this research were, ruler, plastic labels, and digital camera.

Results and Discussion

A. Flower and Fruit Development Phenology

Results show that the flower development of *Hibiscus rosa-sinensis* needs 30-32 days to complete all flower development phases and 27 days to complete fruit development. Results show different time required to complete all phases in several species, such as *Uncaria gambir* needs 112 days (Jamsari et al., 2007), *Syzygium pycnanthum* needs 89 days (Mudiana & Ariyanti, 2010) *Centella asiatica* needs 11-14 days (Damaiyani & Metusala, 2011) and 182-192 days are needed in *Paphiopedilum glaucophyllum* var. *glaucophyllum* (Yulia, 2006). Different periods in flowers and fruit development, according to Ashari (1998), depend on internal and external factor interactions.

The flower development phase of *Hibiscus rosa-sinensis* divided into four phases. The first is flower initiation including three-stage development namely: a) 9 day early initiation stage; b) 9 day small flower bud stage; and c) 8 day large flower bud stage. The second is pre-anthesis phase needs 5 days, including two stage flower development that is: a) 3 day early pre-anthesis stage and b) 2 day last pre-anthesis stage. The third is anthesis phase or blooming stage requiring one day. The fourth is pollination and fertilization phases which occurs in the blooming stage and one day after. Visualization of flower development phases is shown in Figures 1.

Figure 1 Flower development phases from flowers initiation phase until polination and fertilization phases in Hibiscus rosa sinensis spp. First phase is flower initiation including 9 day early initiation stage (a-d. 32 DBA - 24 DBA), 9 day small flower bud stage (e-h. 23 DBA – 15 DBA), and large flower bud stage (i-l. 14 DBA – 6 DBA); Second phase is pre-anthesis including 3 day early pre-anthesis stage (m-0. 5DBA – 3DBA) and 2 day last pre-anthesis stage (p-q. 2DBA- 1DBA); The third phase is 1 day anthesis or fully blooming stage (r.); Fourth phase is pollination and fertilizasion phases (s. Day of polination; t. 1 DAP; u. 2 DAP). ec: epicalyx, ca: calyx, co: corola, ov: ovary, pe: pedicel, clm: column, stg: stygma, st: stamen, pln: pollen, DBA: days before anthesis, DAP: days after pollination.

Initiation phase categorized into three stages based on morphology and length ratio between epicalyx and calyx. Early initiation stage (Figures 1. a-d) is the first time that the buds can be identified as a generative bud which will grow into flower. This stage is characterized by the flower parts which can clearly be seen from outside is just the epicalyx that covers other flower parts. Small flower bud stage (Figures 1. e-h) is a continuation of the early initiation phase. This stage characterized by the epicalyx covering calyx and other flower parts has opened. Calyx that covers corolla and sexual flower organs inside clearly appears. Buds grow and develop, at the end of this stage the length ratio between epicalyx and calyx is one. The last stage of initiation phase is the large flower bud stage (Figures 1. i-l) and the identification mark in this stage is the length ratio between bud and epicalyx is one. The buds continue growing until maximum length and occupy the calyx space. The mark of the final stage is calyx spliting, because corolla and sexual organs inside grow and push the calyx.

Pre-antesis phase is the phase before fully blooming flowes happen, two stages of this phase must be completed before flowers prefectly blooming. The first stage is early preanthesis (Figures 1. m-o) characterized by the corolla buds which begin to appear from outside, because they are not covered anymore by calyx. Corolla bud size is not longer than that of calyx. The second stage is the last pre-anthesis (Figures 1. p-q). In this stage corolla is increasingly large and growing quickly until maximum size just in two days. The last stage is marked by the length of corolla bud which surpasses the calyx length.

Anthesis (Figure 1. r) is the phase in which flowers have been in full bloom, at this stage all flower parts visible from the outside except the ovary indicating that all of the flower parts have been fully formed. The parts of flower are pedicle; epicalyx consisting of 7-8 units, green in color; calyx consisting of 5 sepals which fuses in the basal region, green in color; corolla consists of 5 petals which are separated each other but fuses and forms a column in basal area, pink in color; stamens in great quantities fuse each other and form a column or monadelphus; 5 stigmas are separated each other, the styles, however, fuse each other inside the column. Anther dehisces several times after perfectly blooming, in the morning

between 08.00 until 09.00 o'clock. Flowers keep blooming only for 1 day, wilting a day after, and falling two days after blooming. It indicates that this flower only has a short time of pollination stage.

Fertilizasion is the last stage in flower development which is marked by corolla, column, and pistil falling. After this stage the ovary continues to develop into fruit. The phenology of fruit development was observed in 31 flowers after pollination, but only four fruits well develop and reach maturation and produce seeds. The fruits yielded from human assisted self pollination is one of 15 flowers, from human assisted pollination is 3 of 9 flowers, and none of 7 not pollinated flowers.

Results show that fruit development needs 27 days to complete the whole development phase. The fruit development phase is divided into four stages of development. The first stage is the development of young fruits, the characteristics are at the beginning of this stage the ovary color is yellow after corolla, column and pistil fall, then the ovary enlarges and fill the space of calyx, then the fruit color slwoly turn to green, 5 days need to complete this stage. This stage is crucial since usually the fruit will be aborted at this stage. The second stage is the growth and development of young fruit to maximum size, characterized by the continuation of growing to a maximum size, capsule-like shape, green color, with calyx and epicalyx accessories, 1 -1.3 cm in diameter, 2.4 to 3.2 cm in length, 9 days needed to complete this stage. The third stage is fruit ripening, characteristics of this stage is fruits stop growing, perikarp starts shrinking and hardening, fruit and accesory colors change to yellowish, yellow or brownish, this stage lasts 10 days. In the fourth stage, the fruits are dehisced, the characteristic of most Malvaceae fruits (Loveless, 1986) that capsules dehisce by five longitudinal slit suitable with numbers of carpels. It also happens in Malvales like *Durio* sp (Tjitrosoepomo, 2007). The early stage is characterized by spliting of slit from the fruit tips and then it continues spliting to the base of the fruit to five parts at the last stage. Three days are needed to complete this stage. Dehicense is a mechanism of seed dispersal, when seeds receive appropriate environment they will be able to germinate, grow and develop to new individuals (Scagel et al., 1969). Visualization of fruit development phase was shown in Figures 2.

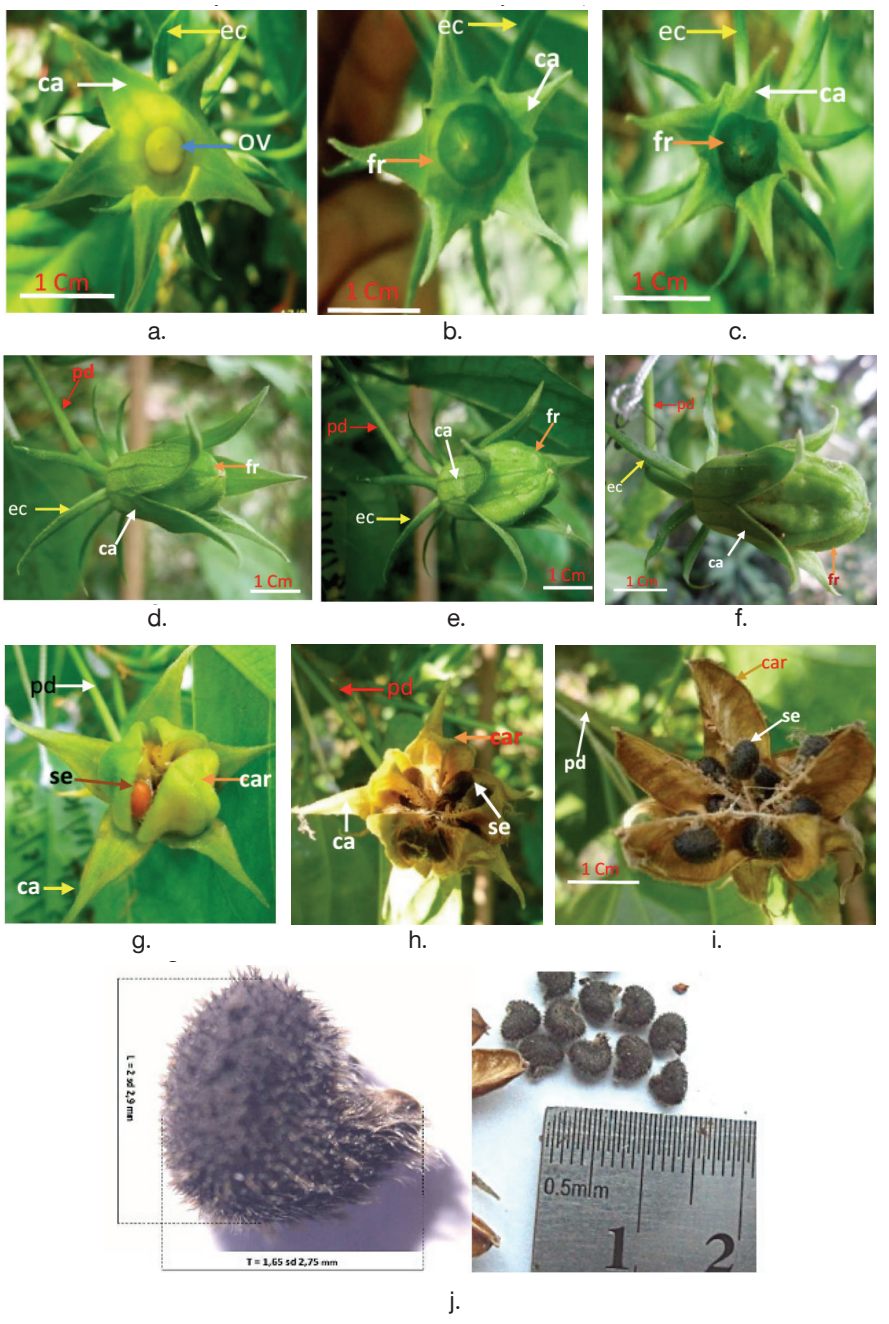


Figure 2 Fruit development phase in Hibiscus rosa-sinensis. a-c. development of young fruit stage needs 5 days to complete (1 DAP - 5 DAP); d-e. growing and developing of young fruit to maximum size stage needs 9 days to complete (6DAP - 14 DAP); f-g. fruit ripening stage needs 10 days to complete (15 DAP - 24 DAP); h-i. dehiscent stage needs 3 days to complete (25 DAP -27 DAP); j. mature seed. pd: pedicel, ec: epicalyx, ca: calyx, car: carpel, se: seed, DAP: day after pollination.

B. Generative Reproduction Success

Generative reproduction or sexual reproduction according to Enger (2000) is organism reproduction which produces new individuals through fertilitation occured between ovum and sperm. In Angiosperm generative reprodution succes is show by yielding viable seeds which are able to germinate (Bhojwani & Bathnagar, 1999). According to this reseach results seeds of *Hibiscus rosa-sinensis* obtained from fruit development are able to germinate with high viability when they are planted in soil media. The offsprings are able to grow and develop and show various morphological juvenile leaves, flowers, and fruits indicating generative reproduction success (Shown in Figure 3). Only four fruits obtained from 31 flowers pollinated in January-March indicate that the yield of fruits is low. It indicates that *Hibiscus rosa-sinensis* with pink flowers is hard to bear fruit, this is in accordance with Ajhitadoss (2006) statement that the fruit of *Hibiscus rosa-sinensis* is usually abortive.

The number of abortive fruits in 2- 8 days (Figure 4.) after pollination giving evidence that the distance between stigma and anther inhibits pollination to happen. Possible factors causing aborted fruits are the internal factors (Genetic) which is expressed by hormone system in fruit and seed development, compatibility or incompatibility in pollination and fertilitation processes. It thus is important to investigate the abortive before mature fruit in further researches.

Conclusion

Flower and fruit development of pink-flowered *Hibiscus rosa-sinensis* spp needs 61 days to complete all phases and stages of development, the details are as follows: Initiation phase needs 26 days includes three stages : a) 9 day- early initiation stage; b) 9 day-small flower bud stage; and c) 8 day-large flower bud stage; Pre-anthesis phase which 5 days needed includes two stage flower development namely: a) 3 day-early pre-anthesis stage and b) 2 day- last pre-anthesis stage; anthesis phase or blooming stage which 1 day period needed; pollination and fertilization is 3 days; and fruit development phase is 27 days including: a) development of young fruit stage needs 5 days to complete, b) growing and developing of young fruit to maximum size stage needs 9 days to complete, c) fruit ripening stage needs 10 days to complete, and d) dehiscent stage needs 3 days

to complete. The generative reproduction success is indicated by the production of viable seeds which are able to germinate and produce offsprings showing various morphological juvenile leaves, flowers, and fruits.

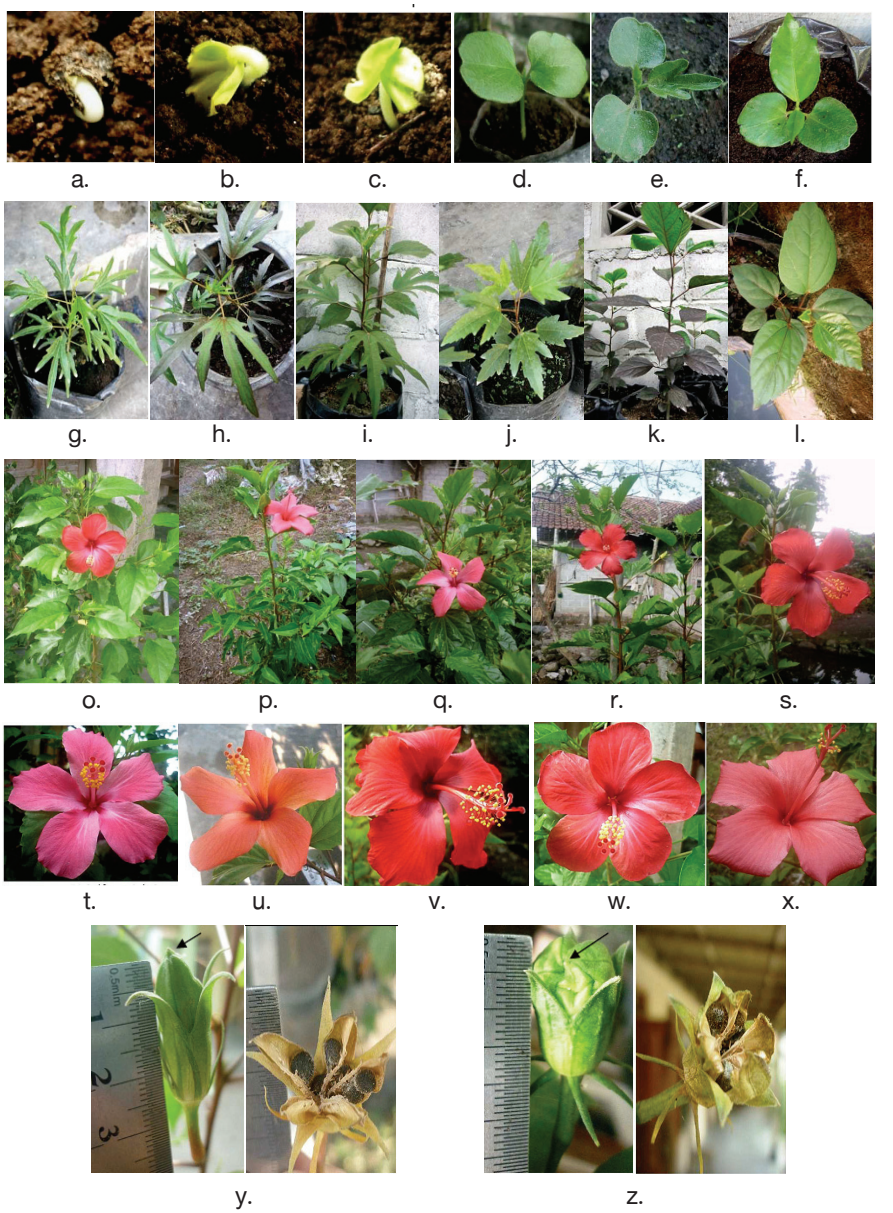
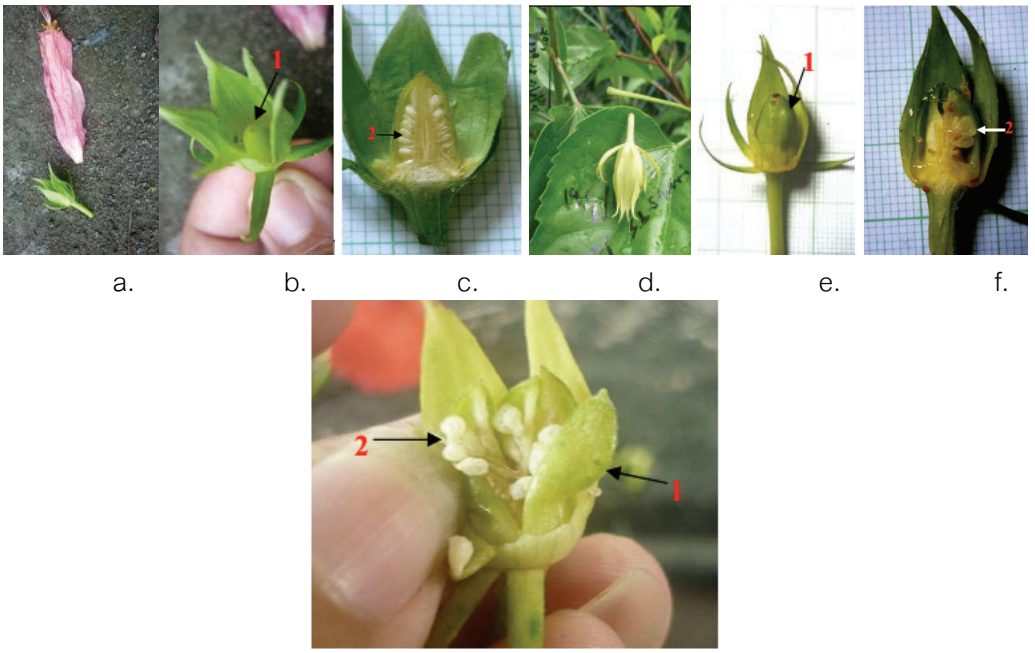


Figure 3 Generatif reproduction success of Hibiscus rosa-sinensis spp showed by seedling, various morphology of juvenil leaves, flower shapes and colors, and the fruit shapes of the offspings. a-d. seedling; c-d. different shape margin in the first leaf; g-h.juvenil phase morphology variation of the offspings; o-p. mature phase; t-x. variation of the flower shapes and colors; y-z. variation of the fruit shapes.

The success of generative reproduction depends on the internal factors (genetic) which are expressing by hormone system in fruit and seed development, compatibility or incompatibility in pollination and fertilitation process or external factors like nutrition, temperature, humidity in fruit and seed development. Thus future reseach needs to investigate factors causing the occurrence of abortive fruits.



a. b. c. d. e. f.

g.

Figure 4 Fruit falling after human assisted pollination. a-c the fruit aborted two days after pollination, fruit fall with corolla, collumn and pastille, the ovules have not yet grown to seeds (Figure c) ; d-f fruit fall six days after pollination. Figure e show the ovary grown into young fruits, and several ovules have grown into seeds (Figure f); Figure g. fruit fall eight days after polination show numerous enlarged ovules failing to be mature seeds. 1: carpells, 2: ovules

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