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Flower Initiation, Morphology, and Developmental Stages of Flowering-Fruiting of Mindi (*Melia azedarach* L)

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Abstract

The study was aimed to determine flower initiation, floral morphology and to observe the stages of flowering and fruit development of mindi (Melia azedarch L) within a population for one period of time 2008–2009. The methods used were observing directly over the trees and some vegetatives and generatives buds were sampled for dissecting. The observation revealed that the inflorescence type of mindi was panicle, located at the end of a branch. The number of flower varied among inflorescences, ranged between 30–80 that bloomed simultaneously. The flower was hermaphroditic with position of anther was closed to stigma that selfing might be happened. Usually, the ovary contained 5 ovules that developed into seeds. Reproductive cycle was proceeded for 6–7 months within the year, first observation commenced from flower initiation that occur in August, generative buds to flower burst in September–October. Early fruits were formed in October–November and fruits reached physiological-maturity in January–February. Reproductive success was 34%, indicated that the rate of fertilized ovules proportion to be potencially viable seeds were relatively low.

Keywords: indian lily, phenology, reproductive biology, reproductive cycle, seed production

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Introduction

Mindi or indian lily (*Melia azedarach* L) is an introduced species in Indonesia mostly found in West Java as one of agroforestry plants. The species have a good quality timber with high economic value. Moreover, its multipurpose properties make the species is attractive to be developed as its leaf, bark, and seeds are useful. According to Khan *et al.* (2008), such parts of the plant can be utilized as medicines for headache, fever, antiseptics, pesticides, even best for healing of HIV or cancer diseases due to its antibiotics contain.

Ecologically, this species has a wide range of habitat, and grows well at the altitude of 100–1300 m asl. The good environmental adaptability made the species as good candidate for timber estates or land rehabilitation in Java. However, in general the timber productivity of mindi forest stands is low partly due to use of low quality seeds. A seed with a poor genotype will produce a poor progeny regardless of the environment is. The procurement of high quality seeds, therefore could be achieved from the right seed sources (Schmidt 2000).

The seed production of mindi at 3 locations in West Java was about 1.3–5.8 kg tree^{-1} (Pramono *et al*. 2009). The low seed production could be due to low flower initiation, flower abortion (due to climate, abnormality, and disfunction) (Nerd *et al.* 1998; Berjano *et al.* 2006), periodicity (irregularity), dichogamy (protandy and protoginy), pollen viability periods, short period of stigma receptivity, lack of pollen recources and pollination (Campbell 1987), and adversed environmental condition (climate and pest/diseases attack) (Jaeger *et al*. 2006; Kameyama & Kudo 2009).

Flower initiation, morphology as well as flower and fruit development need to be studied in order to determine the limiting factors of seed production. The time of flower initiation is an important sequence to determine the right time for flower induction to enchance fruiting. Unusual flower structures or asynchrony in male and female reproductive organs (dichogamy) will cause difficulty to pollinate in nature. Even though, such characteristics for sometime would support the management of seed production naturally in term of reducing the occurence of selfing (Bawa & Hadley 1990).

The observation on development cycle of flowering to fruiting might be a guide to predict the harvesting time properly, so that the efficiency can be achieved. By calculating the number of flowers, fruits and seeds produced during the cycle, the reproductive potential can be identified, thus the potency of seed production would be able to be estimated. In addition, it would be an important information to decide the exact time of hand pollination in a seed orchard based on the knowledge of floral structures and their pollination characteristics (Willson 1997). The study was aimed to determine floral morphology and initiation, and to observe the stages of flowering and fruiting development including their reproductive success of mindi within a population for one period of time (2008–2009).

Methods

The material plants consisted of 10 selected trees of mindi used for the observation of flower and fruit morphology, as well as flowering to fruiting development cycle. The study was carried out at the experimental station of Research Center for Tea and Quinine Gambung, Bandung, West Java, Indonesia, with the altitude of 1,340 m asl, latitude of 07^{O}14' S and 107^{O}514'E, rainfall of 1,200–1,600 mm year^{-1}, temperature of 15–28 ^{O}C, and RH 40–50%.

Flower initiation The time of flower initiation was observed by sampling several buds monthly for 3 months (July–October) to be processed using paraffin technique. Ten buds were taken from the selected 10 trees. Buds were dissected longitudinally at both sides and put in a vial filled with 10 ml formalin-acetic acid-alcohol (FAA) solution. The samples were brought to a laboratory for paraffin embedding then sectioned using microtome at 6–10 µm thickness. Staining was done using safranin and anillin blue and mounted with Entellan (Merk). The specimens then were observed under light microscope with magnification of 50 times (Syamsuwida & Owens 1997). Observation was emphasized on the presence of flower primordia.

Flower morphology and fruit development The samples were taken from 3 inflorescences per branch, 5 branches per tree of the 10 selected trees. The inflorescences were observed individually for flower morphology and developmental stage including flower initiation, flower buds development, flower stucture, number of ovul per pistil, and fruit development (from fruit initiation until maturation). Each branch was tagged with colored ribbon and every developed inflorescens was labelled. Any changes of flowering to fruiting structures were recorded by taking notes of time (date and period), shape and color (Owens *et al*. 1996). During developmental stage observation, the number of flower and fruit per inflorescence, number of ovul per pistil, and seed per fruit were counted for calculating the reproductive success of the plant.

Reproductive success Pre-emergent reproductive success (PERS) that was defined by Wiens *et al.* (1987) as the number of egg cells that complete development and survive to enter the ambient environment, refers to the number of viable seeds released into the environment. PERS is calculated by multiplying the fruit to flower and seed to ovule ratios using the follow formulas:

Flower to fruit ratio (*FF*):

R: Fr/Fl = number of fruits/number of flowers *[1]*

Ovule to seed ratio (*OS*):

R: S/O = number of seeds/number of ovules *[2]*

Pre-emergent reproductive success (*PERS*):

PERS = (fruit/flower ratio) × (seed/ovule ratio) *[3]*

Data analysis Fruit set, seed set, and reproductive success (PERS) were compared statistically by one way analysis. Data were arcsin transformed to achive normality.

Results and Discussion

Floral initiation Floral initiation is the transition of vegetative meristem (leaf primordium) to be apical reproductive (flower primordium) that eventually develop to be flower (Owens & Blake 1985). The changes might be several days, weeks or months before the appearance of flower buds. The observation of floral initiation of mindi at Gambung revealed that it was lasted for several months. Initiation of the flowering occured at a number of month before flower buds appeared and continued when some inflourescences had been developing.

The present of vegetative meristem (leaf primordium) and apical reproductive (flower primordium) in dissected samples collected every month from 10 trees for 3 months were shown in Figure 1. The observation showed that most buds collected in late July/early August were still in vegetative developmental stage of about 70% (Figure 1). Thus, generally dissection on the slide glass collected in July–August indicated the present of leaf primordia (Figure 2a).

Most of bud samples i.e 70% (Figure 1) collected from 10 trees in early September showed that apical reproductive had been formed as indicated in a micro ultra section of floral spike bud (Figure 2b). Therefore, it could be said that in general, floral initiation of mindi at Gambung might be occur in early September, even though in the late of August floral initiation had been determined (30%). Floral initiation continued until October as 60% of apical reproductive were found. In this month, some flowers were also blossomed on several

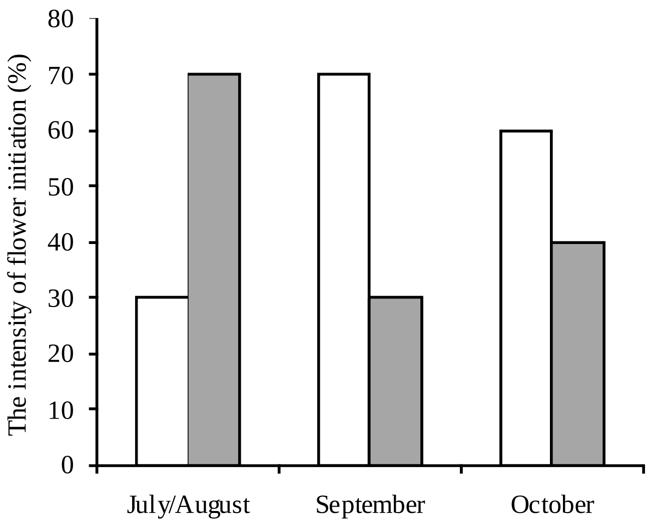

Figure 1 The graph of apical reproductive and meristem vegetative present in dissected samples of buds. ☐ Apical reproductive, ■ meristem vegetative.

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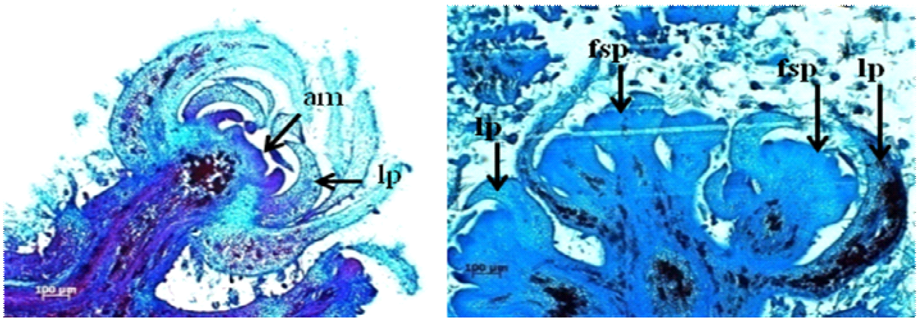

(a) (b)

Figure 2 (a) Median longitudinal section of a vegetative bud showing apical meristem (am), leaf primordium (lp); (b) section of floral spike buds in the axil of leaf showing floral spike primordia (fsp), leaf primordia (lp) of mindi.

branches and by the end of the month small young fruits were developed. In the following month (November), flower buds and vegetative shoots were still developed.

The onset of floral initiation of mindi observed in Gambung began in the late of August/early September and proceeded to the end of October. It was observed that the period from leaves flushed to the emergence of generative buds was relatively short (4–5 days). The observation also showed that development of reproductive organs were simultaneous in that each individual bud was initiated and develop into flower bud, bloomed, and after pollination developed into fruits with several seeds inside. This continues development of reproductive organs was more common in the tropics as compared to those in the temperate zone that underwent flower bud dormancy during the winter.

Inflorescences at all stages of development were apparent within an inflorescence. Therefore, a single time of floral initiation does not exist in mindi. As a result, it is difficult to determine particular climatic factors causing flowering. Unlikely, in most temperate trees of which floral initiation occur at the same time, time of initiation is more precise due to seasonal climatic condition (Owens & Blake 1985). However, several factors might be involved to affect and stimulate floral initiation (van Schaik *et al*. 1993).

Flower and fruit morphology of mindi The fragrant flowers of mindi were arranged in a panicle (Esau 1976). The erected inflorescences appeared at the tip of a branch. The flower was 0.8–1.0 cm long with 5 white petals. The purple pistil enclosed a stylus and 8 yelow anthers were positioned at the top closed to the stigma. An inflorescence consisted of about 30–80 flowers that bloomed simultaneously.

Styles (1972) categorized mindi as an andromonoecious species in which the tree produces 2 types of flowers i.e. hermaphrodite and male flowers. Observation in this research revealed that the trees planted in Gambung produced only hermaphrodite flowers, means male and female organs are born in the same flower. In this case, it might be happened when the trees grow at different sites as it was affirmed by Ambruster *et al.* (2007) that the structure of plant reproduction could be changed evolutionary by either genetical or environmental factors. Some factors may affect the size and morphology of flower. Armbruster *et al.* (2007) stated that annual species of self-compatible collinsia and tonella showed a wide range of variation on size and flower morphology, as well as the pattern of stamen and stylus lengthen during anthesis.

The position of the stigma within the flower influences the efficiency of pollen transfer (Waites & Agren 2006). Within mindi flower anthers and stigma are positioned close each other makes it possible for self-pollination (Figure 3c). Nevertheless, it has not been understood the self-compatibility of mindi that may cause either selfing or outcrossing. The flower bloomed at 09.00 pm and remained opened for 4–5 days. Petals aborted 3–4 days after blooming.

The structure of the flower was somewhat like dish or bowl with 5 white petals (Figure 3a) indicated that bees were the potential pollinator of the species (Faegri & van der Pijl 1971). Pistil (Figure 3b) was thick and purple color with light green mucous stigmatic surface that dominated the center of the flower. Eight yellow and hairy anthers were found surrounding the stigma in each flower (Figure 3c). According to Kittelson and Maron (2000) the proportion of selfing and outcrossing in a population is affected by self-sterility, flowering behaviour and availability/activity of pollen agents. Usually, flowers have natural mechanism to reduce selfing, i.e temporally and spatially. Temporally, the receptivity of male and female occur at a different time, meanwhile spatially the position of males are separated from the females.

Eight to ten days after flower bloomed, the ovary started to enlarge and turned to green color (Figure 4a). In general 5 ovules were found in an ovary that will develop into seeds (Figure 4b and Figure 4c). However, only 3 seeds were commonly found in a fruit. Cross section of ovary showing several layers i.e exocarp, mesocarp that will develop into a fleshy part, endocarp will develop into a hard seed coat, and carpels where ovules located (Figure 4b).

Young fruits of mindi were light green 0.5–1.0 cm length,

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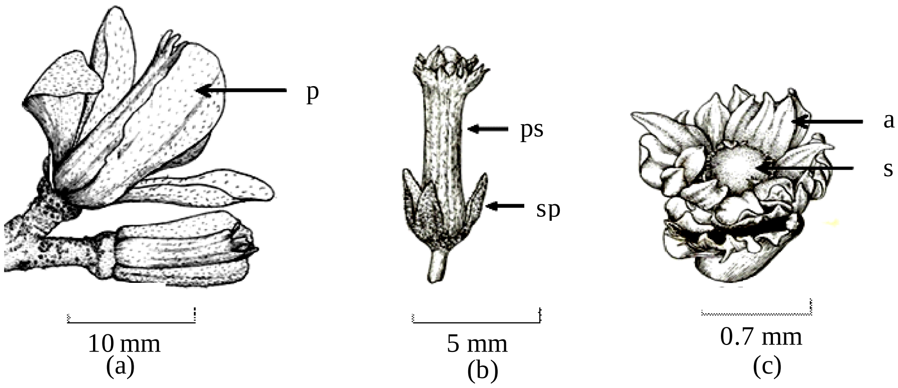

Figure 3 (a) petal (p); (b) pistill (ps) and sepal (sp); (c) anther (a) and stigma (s).

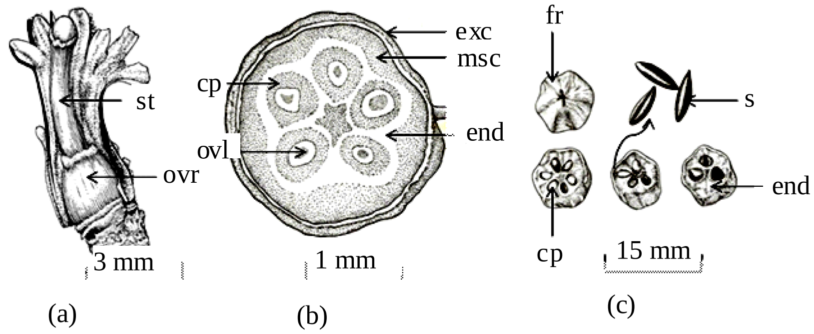

Figure 4 (a) Swollen ovarium (ovr) and stylus (st); (b) cross section of ovarium showing 5 ovules (ovl),carpels (cp), exocarp (exc), mesocarp (msc), and endocarp (end); (c) cross cutting of matured fruits (fr) showing carpels (cp), endocarp (end), and seeds (s).

then enlarged and when matured (physiologically) is distinguished by the fleshy yellow color and reach the size of 12 mm in diameter, 16 mm in length. The seeds were of 0.6–0.8 cm length with very hard seed coat. The structure of fruit consist of some layers including the outer layer of exocarp, the fleshy part of mesocarp, the hard part of endocarp, and carpels that each carpel contain of 1 seed .

In general, the structure of flower can be recognized in the mature fruit or seed-bearing organs. However, sometime the same floral structure may give rise to a different mode of dispersal, as fruit develops according to dispersal pattern which is independent of floral morphology (Schmidt 2000).

The development stage of flowering and fruiting of mindi

Flowering and fruiting development process was observed initially from the appearance of axillary buds that developed into an enclosed floral buds. The floral buds then grew became completed flower structures with enclosed petals. The individual flower burst with white sepals and purple enfolded pistil, the surface of stigma was exposed in the middle of pistil (receptive stigma). When pollination happen, the petals would abort and ovarium would get swollen. The ovarium would then enlarged and formed young fruit with light green color. The young fruit developed to be a bigger grown fruit (dark green) and when it matured, the size relatively similar but the color changed to yellow green and eventually the skin was wrinkle before shedding (Figure 5 and Table 1).

Reproductive cycle of mindi at Gambung proceeded for 6–7 months in a year (2008–2009), commenced from the occurence of axillary buds, floral buds, flower blossom, early/young fruits, matured fruits, and seeds (Figure 5). Flower initiation was observed separately by cutting some branches from several trees every month to excise the appearance of buds.

The period and time of developmental stages of flowering and fruiting would be different for every location. Djam'an *et al*. (2008) reported that the flowering time of mindi in Bogor occurred in August. This was 1 month earlier than those observed at Gambung. According to Gibblin (2005) floral longevity of *Campanula rotundifolia* had greater longevity when fitness growth rate were lower and floral maintenance costs were less. In addition, geographic variation in total longevity was mediated through gender-specific differences in the conditional response to fitness growth rate. Failures might be taken place at any stage of development during the reproductive cycle from flowering to fruiting. Such failures will affect both quality and quantity of the seed, therefore a good management at any stages of plant development is necessary.

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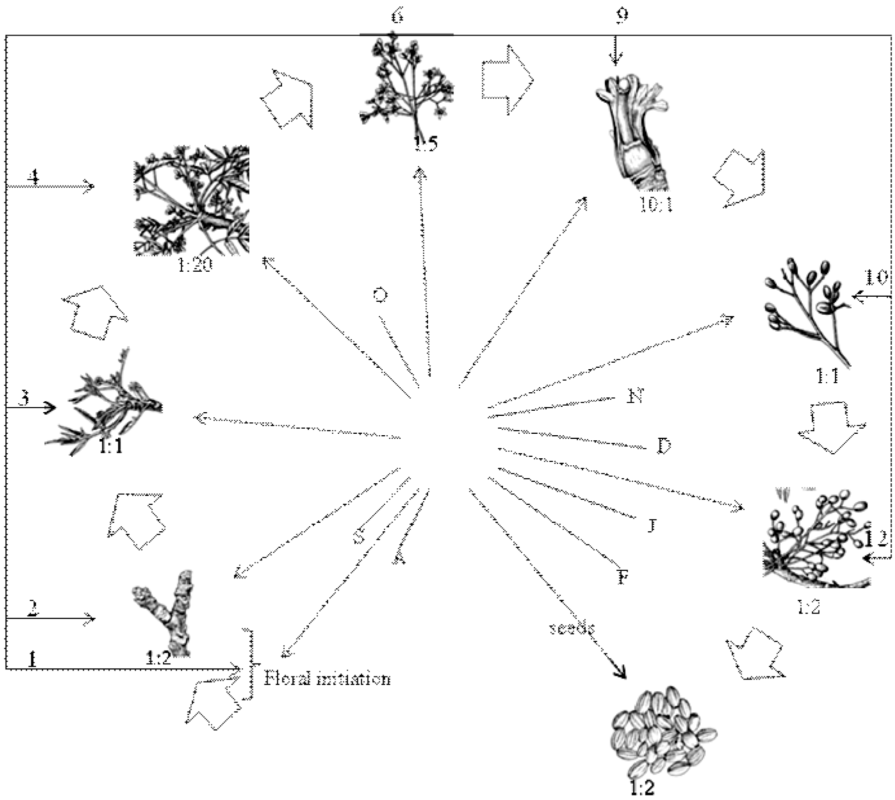

Figure 5 Reproductive cycle of mindi in South Bandung, West Java.

Table 1 The period and time of developmental stage of flowering and fruiting

Number of rotation	Reproductive stages	Periods (days)	Time	Notes
1	Flower initiation	60	Late August–September	
2	Generative buds	5–6	September	
3	Floral buds	7–9	September	Floral were congested
4	The opening of floral buds, individual flowers were still congested	4–5	September	
5	Flower buds were enlarged	10–12	September	Flowers shoots at the base had been exposed but did not burst yet
6	Individual flowers were blossomed	4–5	October	Mostly the flowers were blossomed
7	Reseptive periods	1	October	The stigma was mucous, anthers were opened and pollen grains attached on the surface of stigma
8	Petals were aborted	3–4	October	
9	Ovarium enlargement	6–7	October	The bottom of pistil was swollen , light green in color
10	Early/young fruits	12–16	October–November	Ovarium tube getting bigger, oval, light green
11	Grown fruits	20–25	November–December	Fruit structure has firmed, bigger, dark green
12	Physiologically matured fruits	30–40	January–February	The size relatively the same as grown fruits, yellow in color
13	Fruits shed	30–50	March–April	Fruits were shrieked, wrinkle, yellow brown

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Table 2 The results of ANOVA for the effect of trees on the number of observed flower and fruit per inflorescence, ovule per flower, seed per fruit, flower to fruit ratio, ovule to seed ratio, and pre-emergent reproductive success (*PERS*) of mindi

Variables	df	SS	MS	F	Sigf
Sum of flower per inflorescence	4	281.406	70.352	1.990	0.151
Sum of fruit per inflorescence	4	4.710	1.177	0.162	0.954
Sum of ovule per flower	4	2.584	0.646	1.464	0.266
Sum of seed per fruit	4	2.640	0.660	1.474	0.263
flower to fruit ratio (*FF*)	4	0.376	0.094	2.194	0.074
ovule to seed ratio (*OS*)	4	0.050	0.120	1.225	0.345
PERS	4	0.306	0.076	3.269	0.043

Table 3 Significant differences test of reproductive biology variables in relation to trees

Variables	Tree numbers				
	1	2	3	4	5
Sum of flower per inflorescence	8.134 a	8.220 a	12.557 a	17.000 a	6.750 a
Sum of fruit per inflorescence	3.600 a	4.780 a	3.220 a	4.082 a	4.252 a
Sum of ovule per flower	3.338 a	3.317 a	2.983 a	2.765 a	2.382 a
Sum of seed per fruit	2.746 a	3.053 a	2.360 a	2.400 a	1.925 a
Flower/fruit ratio (*FF*)	0.404 ab	0.577 a	0.263 ab	0.232 b	0.570 a
Ovule/seed ratio (*OS*)	0.824 a	0.917 a	0.763 a	0.867 a	0.785 a
PERS	0.326 ab	0.537 a	0.190 b	0.205 b	0.462 a

Pre-emergent reproductive success (PERS) assessment The ANOVA results for the effect of trees on the number of fruits per inflorescence, ovule per flower, seed per fruit, flower to fruit ratio, ovule to seed ratio and pre-emergent reproductive success (PERS) of mindi at Megamendung (Bogor) are shown in Table 2. It was revealed from the ANOVA that the trees did not give significant effects on the number of flower and fruit per inflorescence, number of seed per fruit and ovule per flower as well as ovule to seed ratio of the plant. However, the values of FF and OS were significantly influenced by the trees (Table 2). The differences for FF were occured between tree number 2, 5, and 4. Meanwhile, PERS values were significantly different between tree number 2, 5, and tree number 3, 4. The distinction of PERS values might be happened among trees. According to Liao *et al.* (2009) several explanation can be involved for this case, including firstly, large trees with abundance flowers exhibition may recieve pollinator visits, however there may be less number of visits per flower and low of stigmatic pollen load due to a large number of flowers reducing reciept of pollen. Secondly, resource competition among trees perhaps more intense in large than small trees, and reproductive success per flower is then might be reduced with flower number. Thirdly, individual flower in a large tree seemed to be surrounded by inflorescences of the same genetic, resulting a proportionally more geitonogamous pollination, this would be a higher risk of producing selfed zygotics and reducing maternal fitness through inbreeding depression.

It was revealed that the highest FF was counted at tree number 2 (0.58 ± 0.09), mostly the value of FF in this study was higher (0.41 ± 0.17 in average) than those fruit set observed in different site in Bogor (0.16) (Djam'an *et al.* 2008). In this case might be due to different time and site gave different results.

The average value of OS reached a rate of 0.83 ± 0.06. The high rate of OS was probably caused by the ratio of ovules to be potentially viable seeds were high. However, research on seed germination of mindi showed that the germination capacity of the seeds collected from Megamendung (Bogor) were low (23%). This could be happened because of the thicknesses and hardnesses of the seed coat (endocarp) that need to be broken before sowing. The hardnesses of endocarp was due to the high content of lignin (22–26%) that needed chemical solution (sulphuric acid) to softened the cell wall (Yulianti 2011).

The average PERS value of mindi was 0.34. Thus, the proportion of the ovules which were succeeded to be fertilized and developed into viable seeds were 34%, predominantly. To increase the value of PERS, FF must be enhanced and this would be realized by improving silvicultural techniques. There was an hypothesis that reproductive success is limited by resources of pollen (wether hermaproditic or andromonoceous flowers) (Liao *et al.* 2006). In the case of mindi flowers, the sexual expression of hermaprodit tended to reduce the PERS values. It seemed that pollen resources for mindi to do pollination were insufficient. However, it needed to be clarified.

Common phenomenal of hermaproditic plants are produced a low ratio of fruit/flower (Ariesta *et al*. 1999; Holland *et al*. 2004). In fact, fruit set is influenced by many factors of which related to reproductive biology including pollination and flowering behaviour. According to Ariesta *et al* (1999) pollination was the main limitation to seed production. Abundance of inflorescences will attract pollinators to visit the flowers, moreover the availability of pollen resources were also an important factor in fruit production (Bawa & Webb 1984). The variation of PERS among trees were high (0.19–0.52). This might be happened when the phenotypic performances of the trees such as total height, stem diameter, and length of canopy were varied. However, this might have to be proven by determining the corellation between PERS and phenotypic variables.

Conclusion

Reproductive structure of mindi that consists of male and female gamets in the same flower could imply the type of pollination of the plant. The development cycle of flowering and furiting of mindi proceeded for 6–7 months, starting from the appearance of generative buds in September, flower burst, receptive and petal flushing in October up to matured fruits ready to harvest in January–February. Rate of fertilized ovules proportion to be potencially viable seeds were relatively low and it was predicted that the potency of seed production would be reduced. To increase the value of PERS, it is recommended to enhance the FF ratio by applying silvicultural techniques. Floral initiation of mindi was recorded in late August/early September and continued until late October. Thus, the right time of adding treatments to induce flowering and enhance fruiting might be carried out 1–2 months beforehand.

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