Pak. J. Bot., 47(4): 1501-1510, 2015.

FLOWER CHARACTERISTICS AND PHENOLOGY OF ANDROMONOECIOUS *JATROPHA CURCAS*

DASUMIATI[1,3], MIFTAHUDIN[2]*, TRIADIATI[2] AND ALEX HARTANA[2]

[1]*Graduate Program in Plant Biology, Graduate School, Bogor Agricultural University, Kampus IPB Darmaga, Bogor 16680, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Kampus IPB Darmaga, Bogor 16680, Indonesia*
[3]*Department of Biology, Faculty of Sciences and Technology, Syarif Hidayatullah State Islamic University, Jakarta 15412, Indonesia*
**Corresponding authors e-mail: miftahudin@ipb.ac.id, Tel.: +62 251 862283, fax: +62 251 8622833*

Abstract

Andromonoecious *Jatropha curcas* (Jatropha) has male and hermaphrodite flowers in one inflorescence. Jatropha is a potential source for biofuel raw material. The development of this plant has various constraints, such as lower fruit set that causes low seed productivity. The existence of andromonoecious Jatropha with high fruit set provides a great opportunity to overcome the obstacles. Understanding floral biology is a requirement for developing a high fruit set andromonoecious Jatropha. This study was aimed at understanding the flower's characteristics and phenology. The andromonoecious Jatropha flower's characteristics, number and viability of pollen, and phenology were observed and compared to those of monoecious Jatropha. Andromonoecious Jatropha had inflorescence with a dichasial cyme pattern. An inflorescence produced two main branches and several secondary and tertiary branches, where a hermaphrodite flower was found at whose ends. The number of inflorescence branches in andromonoecious Jatropha was higher than that of monoecious Jatropha. The diameter and size of the sepal and stalk in hermaphrodite flowers were found to be larger than those in male and female flowers. The development of andromonoecious Jatropha flowers was divided into 4 phases, namely initiation (0-3 dai = days after initiation), small bud (3-7 dai), large bud (7-20 dai), and flower blooming (20-39 dai) phases. The anthesis and anther dehiscence of hermaphrodite flowers occurred at the same time between 06:00 to 06:59 am, facilitating self-pollination of the hermaphrodite flowers. The average pollen viability during the anthesis phase was 96.26%, and 88.79% of them survived until the next day.

Key words: Flower, Jatropha, Phenology.

Introduction

Jatropha (*Jatropha curcas*) is a very potential and important member of the *Euphorbiaceae* family for producing biodiesel because of its high seed lipid content (Lapola *et al*., 2008; Wang & Ding, 2012; Zahra *et al*., 2014). Oil obtained after trans-esterification of Jatropha seed lipid can be used as biofuel (Achten *et al*., 2008; Sunil *et al*., 2008). Production of Jatropha oil, which is mostly extracted from monoecious Jatropha seeds, indirectly depends on the number and quality of the female flowers. The low ratio of female flowers to male flowers is considered as a limiting factor for seed and oil production (Wu *et al*., 2011), and the tendency to experience cross-pollination led to a variety of seeds produced.

Besides having a monoecious sexual type, Jatropha also has an andromonoecious sexual type, which consists of male and hermaphrodite flowers in the same inflorescence. Andromonoecious Jatropha has a great potential to produce more fruits (Miller & Diggle, 2007) and more seeds, which indirectly will increase oil yield. Therefore, it is necessary to characterize the flower and study the phenology of andromonoecious Jatropha.

Several studies on the biology of monoecious Jatropha flowers have been conducted, including characteristics of its reproductive biology (Wang & Ding, 2012), microsporogenesis and gametogenesis (Liu *et al*., 2007), flower biology and breeding system (Kaur *et al*., 2011), flowering and fruit set (Alam *et al*., 2011), structure and development of flower (Wu *et al*., 2011), biology and genetics improvement (Divakara *et al*., 2009), duration of the life cycle (Tambunan *et al*., 2010), analysis of the embryo and seed proteome (Liu *et al*., 2009), and viability and germination of pollen (Abdelgadir *et al*., 2012). Pollination of Jatropha has also been reported on the aspect of ecology of pollination and fertilization (Raju & Erzadanam, 2002), and the contribution of diurnal and nocturnal insects in pollination (Luo *et al*., 2011). However, the biology of the flower in andromonoecious Jatropha has not been reported so far. It is expected that andromonoecious Jatropha with hermaphrodite flowers will have a different pattern of inflorescence and fruit formation compared to monoecious Jatropha.

The study of flower biology of andromonoecious Jatropha is very important because of the essential role of flowers in reproduction and trait inheritance. Problems, such as low number of fruit set and seed production, can be solved through comprehensive understanding of the biology of the flower. In addition, understanding its flower biology is one of the requirements for the development of high yielding Jatropha varieties through breeding programs. The objective of this research was to study the characteristics of flowers, phenology, and pollen viability in andromonoecious Jatropha. Flowers and flowering behavior of monoecious Jatropha from the same Jatropha accession were used as a comparison.

Materials and Methods

Plant materials: The plant materials used in this research were one-year-old andromonoecious and monoecious Jatropha acc. Dompu grown on latosol soil in Dramaga, Bogor, Indonesia. Three inflorescences per plant for a total of 10 plants per each flower sex were used for flower characteristics, phenology, and pollen viability observation.

Flower characteristics: Flower characteristics were observed on male and hermaphrodite flowers of andromonoecious Jatropha, as well as on male and female flowers of monoecious Jatropha. The observed characters were the branching pattern of inflorescences, the number of hermaphrodite, female, and male flowers per inflorescence, the ratio of hermaphrodite or female to male flowers per inflorescence, the length of flower stalk, the size of stamens and carpel, the diameter of flower, and the size of sepals.

Phenology: The development of Jatropha flowers were observed from initiation phase to anthesis phase. During the development phases, flower phenology was observed, especially during the initiation, blooming, and pollination phases. The process of flower blooming was observed continuously during the period between 04:00 to 09:00 am, while pollination and pollinators observations were carried out starting at 6:00 am to 6:00 pm for five days per inflorescence. Development of flowers was divided by the size of the flower. Mechanism of flowers blooming was divided by processes that occur at the time of blooming.

The observation of flower anatomy is performed on 3 dai (days after initiation) flower bud, hermaphrodite flowers before anthesis and female flowers after anthesis. Those observations were aimed at proving the time of pollination in hermaphrodite flowers. Flowers were fixed immediately in Formaldehyde Acetic Acid (FAA) solution (50 ml absolute ethanol, 5 ml glacial acetic acid, 10 ml 40% formaldehyde, and 35 ml aquadest) and then dehydrated with a Johansen series solution. The flowers or tissues were embedded in paraffin, then sectioned and stained with safranin-fast green, and were examined under light microscope.

The number and viability of pollen: During anthesis, the number of pollen per anther and the viability were observed in each male and hermaphrodite flower in two consecutive mornings (06:00 to 07:00 am) and in the afternoon (04:00 to 06:00 pm). Pollen viability was observed using the method developed by Johansen (1940). Undehiscent anthers were removed from the flower, placed on a glass slide, and added with one drop of 2% I_2KI solution. The anthers were then chopped, incubated for one minute at room temperature, and observed under a light microscope. Viable pollen turned to dark brown due to I_2KI staining, and stayed light brown when not viable.

Results

Flower characteristics: Andromonoecious Jatropha had flowers in inflorescence (*inflorescentia racemosa*) with *dichasial cyme* pattern. Flowers grow at the end of the stem or branches (*flos terminalis*), and axillaries (*flos lateralis*). This inflorescence consisted of two main (primary) branches. The main branch produced secondary branches and the secondary branches produced tertiary branches. Generally, each branch type (main, secondary, and tertiary branches) ended with a hermaphrodite flower and was flanked by a cluster of male flowers (Figs. 1A and B). Consequently, there were less hermaphrodite flowers than male flowers, with a ratio of 1:17-1:8 (hermaphrodite flowers to male flowers) (Table 1).

Hermaphrodite and male flowers can be distinguished starting from the bud phase. The size of hermaphrodite flower buds was larger than that of male flower buds. Hermaphrodite flower buds were taper-shaped with large sepals supported by a long and large stalk. Male flower buds were round-shaped with small sepals supported by a short and small stalk (Table 1). All Jatrophas had light green flower bud (Figs. 1A and C).

The hermaphrodite flower was cup-shaped and bilaterally symmetrical, and hypogynous. The hermaphrodite flower was a complete flower (*flos completus*) that had a perianth and reproductive organs (female and male). The perianth consisted of 5 green calyxes (sepals) and 5 green corollas (petals). The sepal consisted of three large and two small sepals, i.e. polysepalous. The petals have the same size with their edges overlapping (*eutopus*), rotating in one direction either to the left (*sinistrosum contortus*) or right (*dextrosum contortus*) and disjointed (*polypetalous*). This arrangement of petals was apparent at the flower bud phase. In general, a hermaphrodite flower had 10 stamens arranged in two circles of 5 stamens each (Figs. 2A and D). However, several hermaphrodite flowers in Dompu accession had 5-10 stamens. The stamen of male and hermaphrodite flowers consisted of green filament and bright yellow anther. The anther was divided into 2 longitudinal lobes. The hermaphrodite flower had one carpel located in the center. Carpel was rounded, green, ovary superior, orbicular ovate, and equipped with 3-5 loci (generally 3 loci) with each locus containing a single ovule. The number of pistil stalks (stylus) was equal to the number of loci. The stylus was fused at the base and separated at the top to form a stigma that was divided into 2-4 lobes. Generally, the stigma consisted of 3 unequal lobes in length and all lobes were arranged to form a V- or horn shape. Five yellow nectarous glands can be found at the basal part of the flower and inside of the petals (Fig. 2).

The male and hermaphrodite flower had the same shape, symmetry, and perianth. Both flowers were different in their reproductive organs. The male flower had 10 stamens that were arranged in two circles of 5 stamens each without a carpel (Figs. 2C and F). The shape, stamen position, stylus, and anther of the male flower were also the same as those of the hermaphrodite flower.

Based on the structure and arrangement, the flower formula for the hermaphrodite flower was different from that of the male flower. The flower formula for the hermaphrodite flower was ↑⚥ K(5), C(5), A5+5, G(1), while the formula for the male flower was ↑♂ K(5), C(5), A5+5.

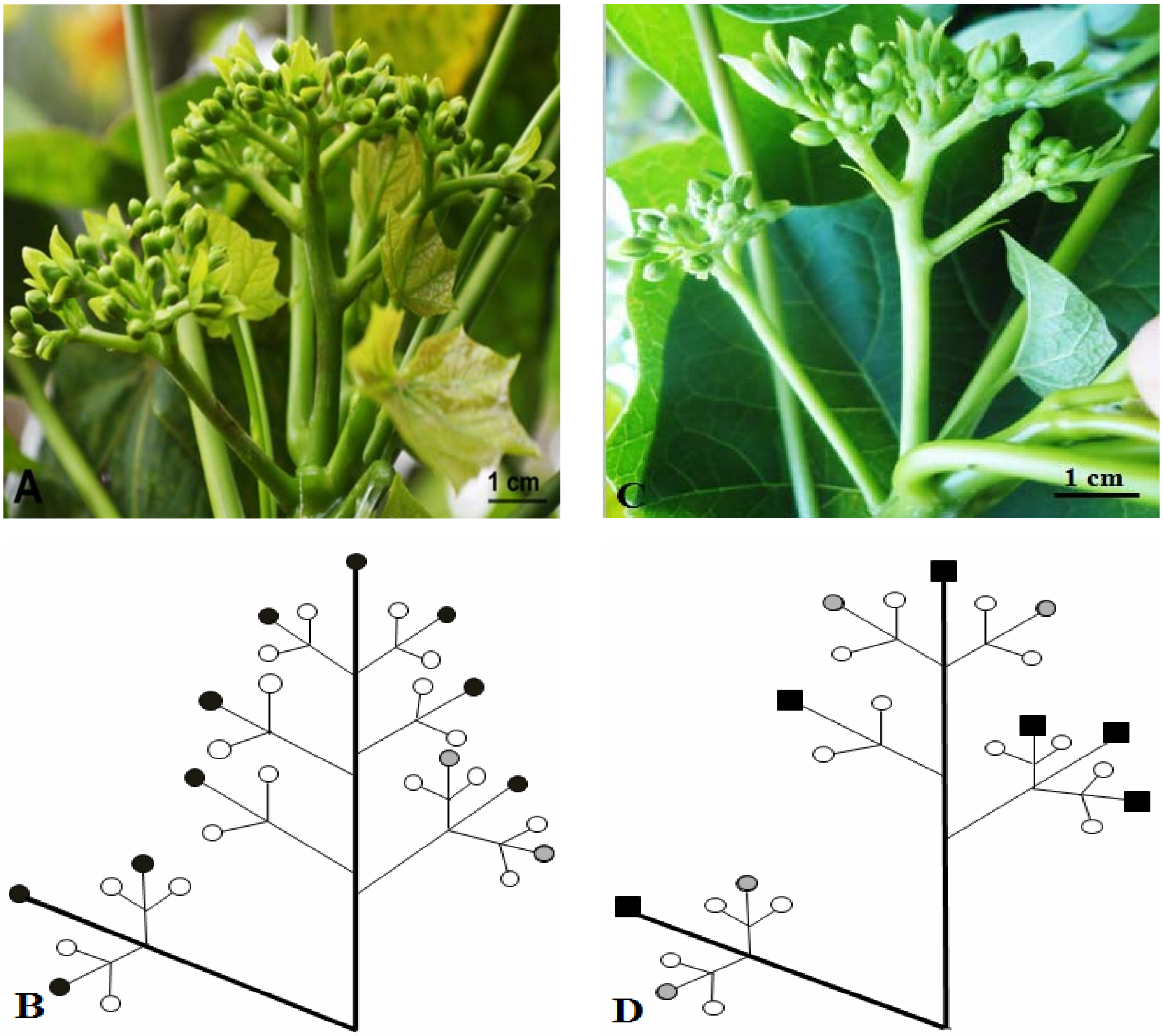

Fig. 1. Flower inflorescence of andromonoecious and monoecious Jatropha. Andromonoecious inflorescence (A), structure of andromonoecious inflorescence (B), monoecious inflorescence (C), structure of monoecious inflorescence (D) (● = hermaphrodite flowers, ○ = cluster of male flowers, ◍ = male flower, ■ = female flowers).

Table 1. Characteristics of andromonoecious and monoecious Jatropha flowers.

Character	Andromonoecious	Monoecious
Number of hermaphrodite flower per inflorescence (pcs)	9 ± 2.90	-
Number of female flower per inflorescence (pcs)	-	6 ± 2.80
Number of male flower per inflorescence (pcs)	116 ± 47.20	103 ± 49.40
Ratio of hermaphrodite or female to male flower	0.08 ± 0.04	0.06 ± 0.03
Flower diameter (hermaphrodite or female) (cm)	0.89 ± 0.04	0.73 ± 0.04
Length of flower stalk (hermaphrodite or female) (cm)	1.19 ± 0.40	0.81 ± 0.12
Sepal size (hermaphrodite or female):		
Length (cm)	0.83 ± 0.10	0.50 ± 0.07
Width (cm)	0.53 ± 0.05	0.34 ± 0.05
Length of carpel (hermaphrodite or female) (cm)	0.65 ± 0.04	0.64 ± 0.04
Length of stamen in hermaphrodite flower (cm)	0.60 ± 0.04	
Male flower diameter (cm)	0.73 ± 0.03	0.62 ± 0.07
Length of male flower stalk (cm)	0.40 ± 0.10	0.38 ± 0.1
Sepal size of male flower:		
Length (cm)	0.41 ± 0.07	0.42 ± 0.06
Width (cm)	0.25 ± 0.04	0.24 ± 0.04
Length of stamen in male flower (cm)	0.60 ± 0.04	0.61 ± 0.04

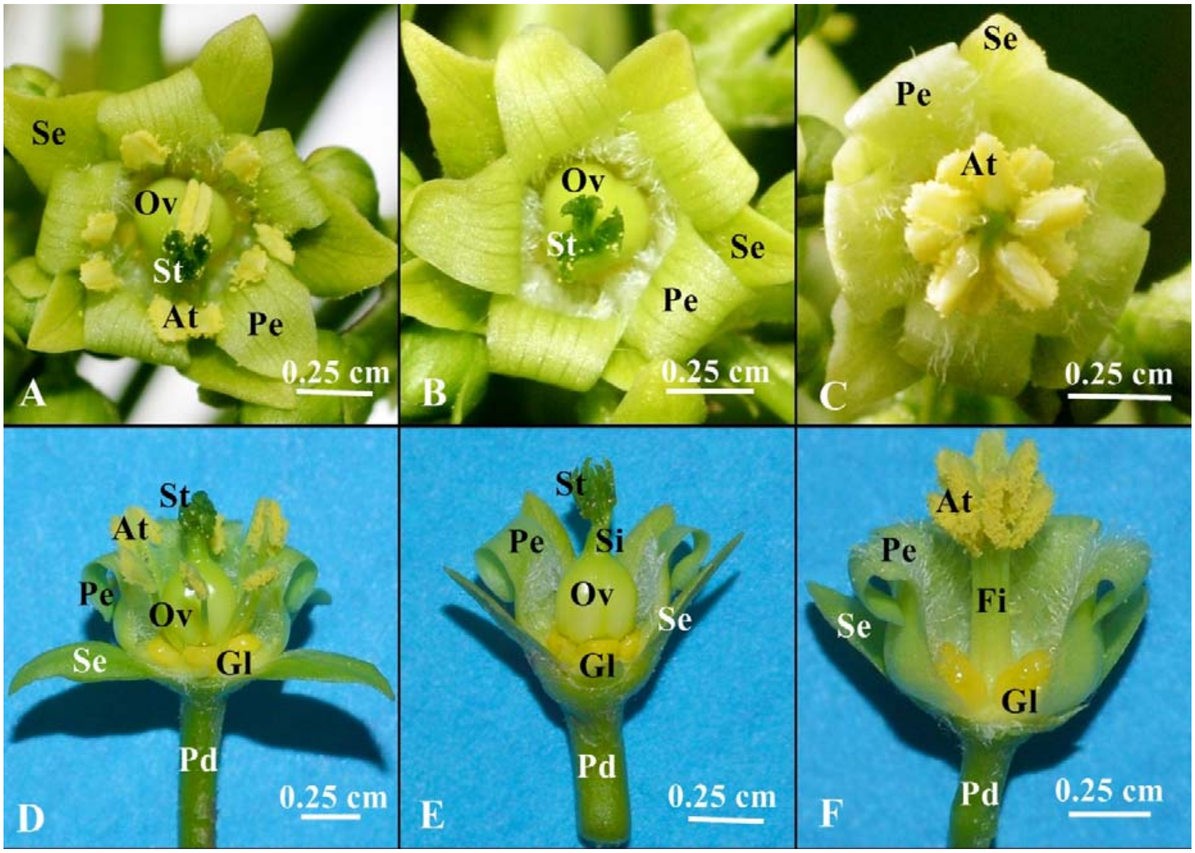

Fig. 2. The structure of Jatropha flowers. Hermaphrodite flowers (A, D), female flowers (B, E), male flowers (C, F), sepals (Se), petals (Pe), ovarioum (Ov), stigma (St), stylus (Si), anther (At), filaments (Fi), or nectarous glands (Gl).

Phenology: Phenology of andromonoecious Jatropha was observed based on the flower development. Environmental factors that were considered to influence phenology were recorded during the observation, i.e.: monthly average light intensity (245 Cal/cm^2/min), monthly average temperature (26.1°C), monthly average relative humidity (84%), and monthly average rainfall (361.7 mm). In this study, flower phenology was divided into four phases; which were flower initiation, small bud, large bud, and flower blooming phases. Flower initiation phase occurred at 0-3 dai, which was characterized by enlargement of apical stem tissues and forming a hump-shaped bud flower (Fig. 3A). Small bud phase occurred at 3-7 dai with the characteristic of the development of flower buds forming inflorescence. At the end of this phase, hermaphrodite flower buds appeared larger than the male flower buds (Fig. 3B). Large bud phase occurred at 7-20 dai with the characteristics of flower buds enlarged and secondary and tertiary inflorescence branches formed. After the development of inflorescence branches, the number of hermaphrodite flowers and male flowers per inflorescence can be determined (Fig. 3C). Flower blooming phase occurred at 20-39 dai, which was characterized by blossoming flowers and ended by all the flowers bloom in the inflorescence (Fig. 3D). The later phase took 15-19 days.

In early flower development, two main inflorescence branches were formed. One of the main branches grew faster than the other one, which was able to be anatomically observed at 3 dai (Figs. 3E and F). The main branches then formed secondary and tertiary branches. Development of every branch in the inflorescence stopped with the formation of final flowers at the end of the branches. This development of branch was started from the main branch, and then followed by the secondary and tertiary branches (Fig. 1).

The blooming sequence of hermaphrodite flowers in andromonoecious Jatropha was in accordance with its position on the inflorescence branches, the order of the formation and the development. Hermaphrodite flowers located in the main branch will bloom first, followed by the flowers located in the secondary and tertiary branches, respectively. Generally, in the same inflorescence, hermaphrodite flowers bloomed one day earlier than male flowers. However, male flowers bloomed first or even earlier than hermaphrodite ones in the same day (Table 2). Hermaphrodite flowers began to bloom after 05:00 am, while male flowers bloomed before 05:00 am. All hermaphrodite flowers on one inflorescence took 1-5 days to bloom, whereas male flowers took 4-19 days depending on the amount of flowers. The blooming process of hermaphrodite flowers began with the formation of a small hole in the tip of the flower buds (Figs. 4A and B). This hole enlarged as the petals open. Completely open petals signified that the flower was in full bloom (anthesis) (Fig. 4C). In some cases, hermaphrodite flowers did not experience anthesis.

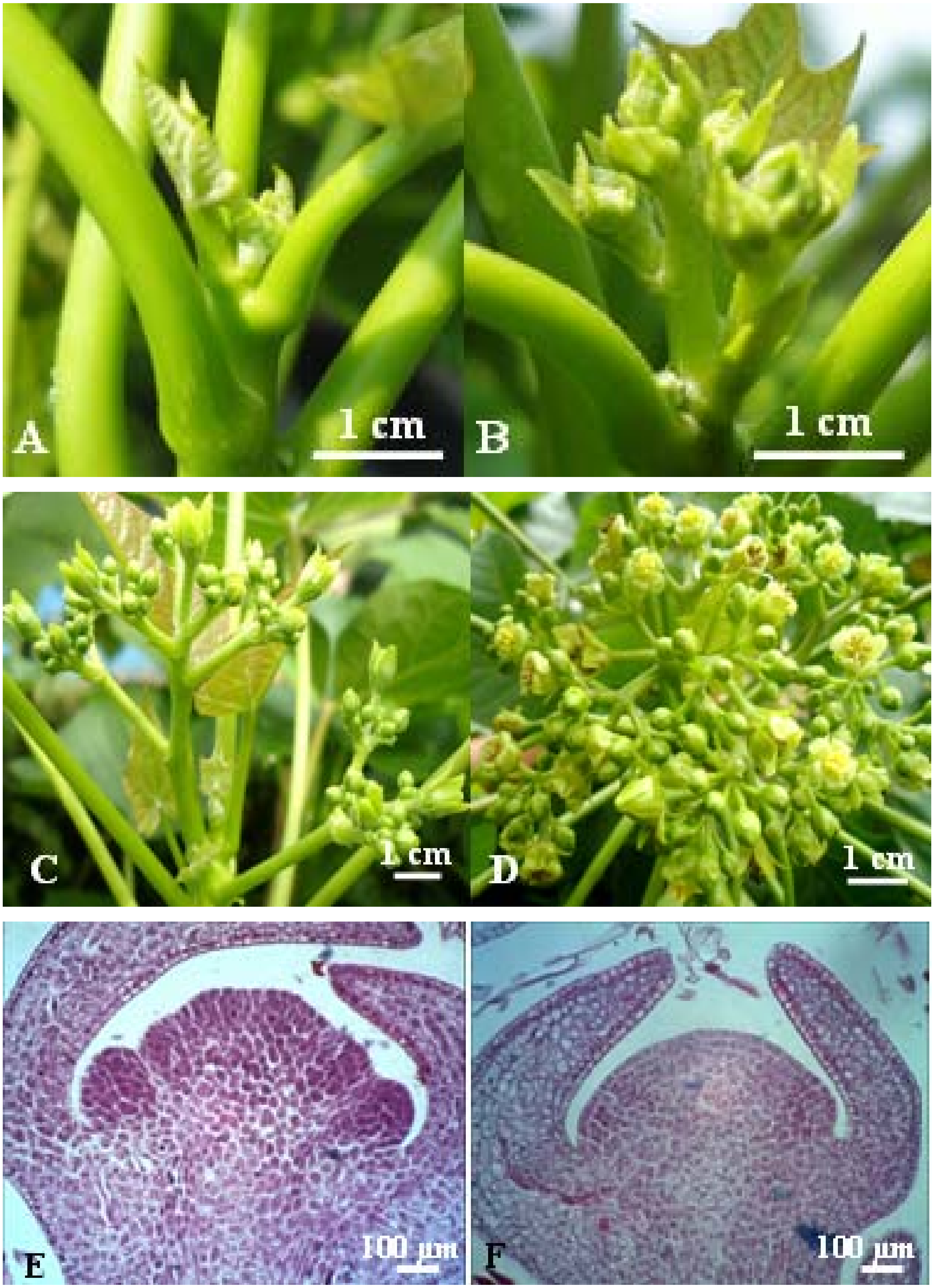

Fig. 3. Development phase of andromonoecious Jatropha flowers and anatomy of flower bud 3 days after initiation. Initiation of flowers (A), small bud phase (B), large bud phase (C), flower blooming phase (D), the anatomy of the first main branch (E), and the second main branch (F).

Table 2. Percentage of blooming flowers at time intervals based blooming process on andromonoecious and monoecious Jatropha.

Flowering process	Andromonoecious		Monoecious	
	Hermaphrodite	Male	Female	Male
	-------------------------- % --------------------------			
Begin to blooming				
< 05.00 am	0.00	8.19	0.00	3.59
05.00-05.59 am	63.93	90.16	65.25	92.37
06.00-06.59 am	33.88	1.64	34.75	1.69
07.00-07.59 am	1.64	0.00	0.00	0.00
>08.00 pm	0.55	0.00	0.00	0.00
Anthesis				
05.00-05.59 am	0.00	1.64	48.27	0.84
06.00-06.59 am	61.75	95.08	50.86	94.91
07.00-07.59 am	34.97	3.28	0.86	4.23
08.00-08.59 am	0.55	0.00	0.00	0.00
No anthesis	2.73	0.00	0.00	0.00
Anther dehiscence				
Before blooming	2.73	0.00	-	0.00
05.00-05.59 am	0.00	0.00	-	0.00
06.00-06.59 am	58.47	0.00	-	91. 93
07.00-07.59 am	36.61	92.35	-	8.47
08.00-08.59 am	2.19	7.65	-	0.00
Pollination				
Before anthesis	2.73	-	0.00	-
05.00-05.59 am	0.00	-	0.00	-
06.00-06.59 am	57.38	-	27.10	-
07.00-07.59 am	37.16	-	49.00	-
08.00-08.59 am	2.73	-	23.00	-
No pollination	0.00	-	0.90	-

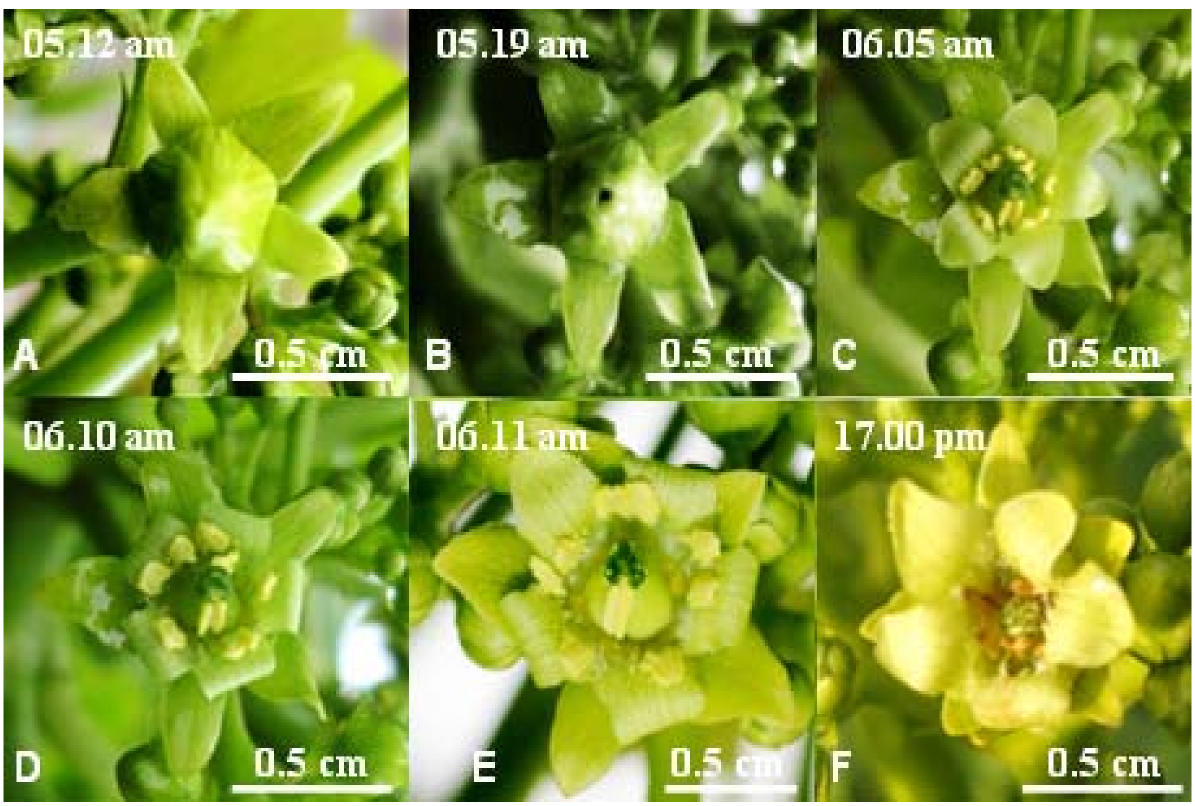

Fig. 4. Blooming process of hermaphrodite flowers. Before blooming (A), Beginning of anthesis (B), Anthesis (C), Anthers dehiscent (D), Pollination (E), and Fruit initiation (F).

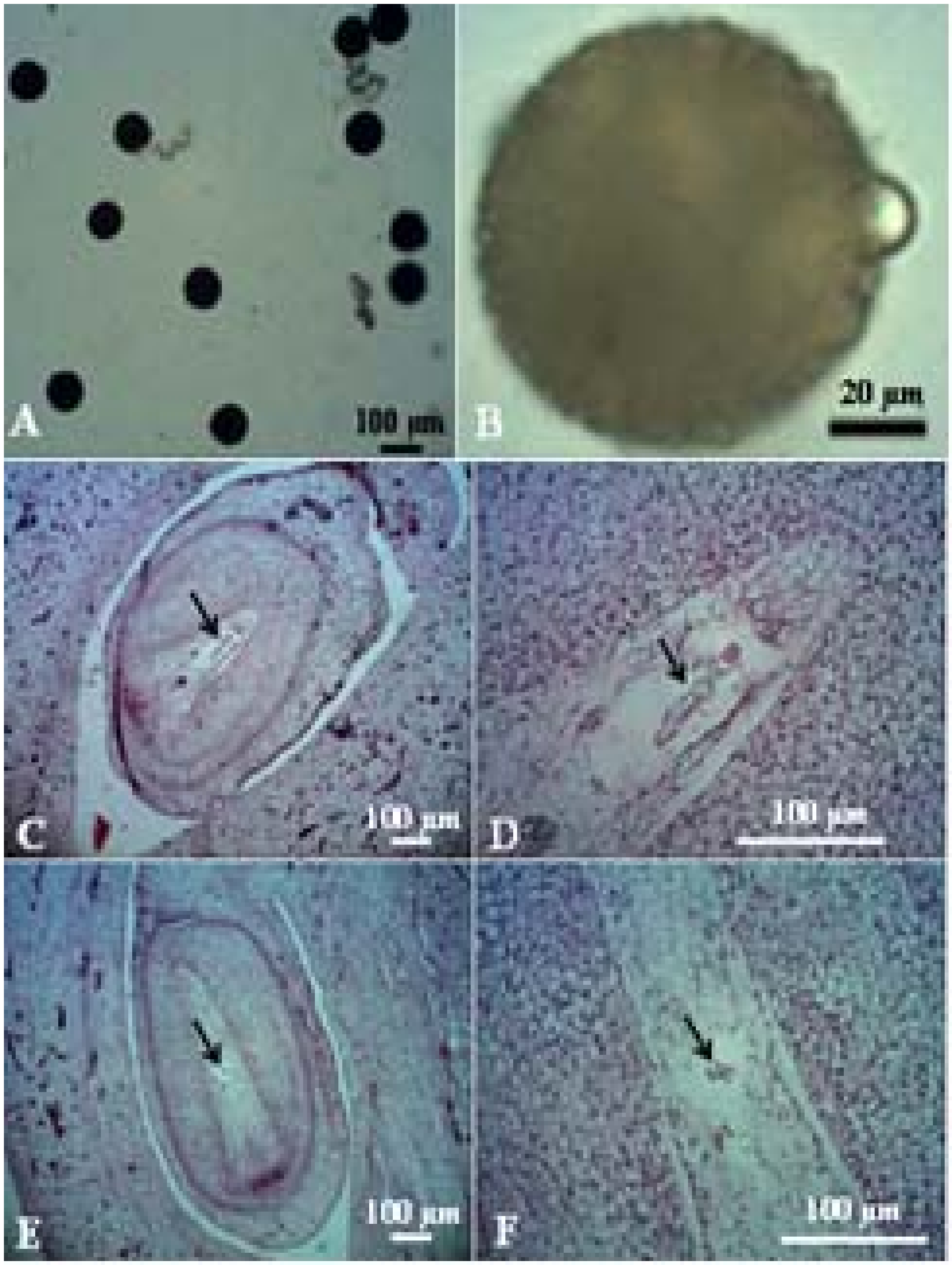

Fig. 5. Viable pollen and germinated pollen in hermaphrodite flowers (A-B). Anatomy of a hermaphrodite flower before anthesis (C-D) and a female flower after anthesis (E-F). The arrows indicated the embryo.

This research observed that most of the Jatropha flowers began to bloom at 05:00 to 05:59 am. At that time, light intensity was 1.80-7.50 lux, air temperature was 28.40-29.60^{o}C, and relative humidity was 74.10-79.40%. Generally, anthesis of hermaphrodite flower occurred in the same period of time between 06:00 to 06:59 am (Table 2) when light intensity ranging from 22.80 to 46.80 lux, air temperature was 27.50-28.80^{o}C, relative humidity was 79.80-86.10%, and the air with no wind. To achieve complete anthesis it would take approximately one hour.

Anthers dehiscence and flower anthesis in the hermaphrodite flowers occurred at the same time. Clumps of bright yellow powder (pollen) move out from the anther, then the anther move away from the carpel. However, some or all anthers still attached to the stigma facilitated self pollination (Figs. 4D and E). The observation also showed that in hermaphrodite flower, pollen germination occurred before blooming (Figs. 5A and B), while the development of embryo occurred before anthesis (Figs. 5C and D). Thus, pollination in hermaphrodite flowers occurred during anthesis. Generally, pollination of hermaphrodite flowers occurred in between 06:00 to 06:59 am. After pollination, petals would wither and close in the afternoon (Fig. 4F). Petals would fall on the next day, but sepals and stamens remained attached to the base of the flower until the fruit was ripe. Fruit development initiated after the petals turned to brown or fell off. All hermaphrodite flowers developed to fruit in the early fruit set, but only 67 to 100% with an average of 89% of the fruit becoming ripe.

The number and viability of pollen: The number and viability of pollen was observed in both hermaphrodite and male flowers 24 hours after dehiscence. The number of pollen in hermaphrodite flowers pollen at any observation time (morning, evening, and the following morning) was less than that of male flowers. The number of pollen per anther in the hermaphrodite flower ranged between 205-369 pollens with an average 267 pollens, whereas the male flower ranged between 250-399 pollens with an average of 298 pollens. Before anther dehiscence in the afternoon, pollen in the hermaphrodite flower anther was approximately 39.99% of total pollen, whereas in the male flower anther it was approximately 51.60%. The percentage of viable pollen in the hermaphrodite flowers in morning and afternoon was 96.26% and 90.54%, respectively. This percentage decreased to 88.79% 24 hours after anthesis. The percentage of viable pollen in the male flowers in morning and afternoon was 96.32% and 89.68%, and decreased to 88.01% within 24 hours after anthesis.

Discussion

Two types of sex are found in Jatropha, i.e.: andromonoecious and monoecious. Andromonoecious Jatropha flower has specific characteristics that distinguish it from monoecious Jatropha flower, i.e.: the number of inflorescence branches, the number of hermaphrodite flowers per inflorescence, and the ratio of hermaphrodite to male flowers.

Andromonoecious Jatropha flower have larger number of inflorescence branches than that of monoecious Jatropha flower (Fig. 1). Each inflorescence branch was ended by a hermaphrodite flower in andromonoecious Jatropha and a female flower in monoecious Jatropha. The number of hermaphrodite flowers in andromonoecious is higher than the number of female flowers in monoecious (Table 1). The differences of the number of inflorescence branches and the number of hermaphrodite or female flowers in one inflorescence between andromonoecious and monoecious Jatropha can be influenced by internal and external factors. Plant growth regulators (PGR), such as cytokinin, auxin, and gibberellin, are one of the internal factors that affect those characteristics. Application of exogenous PGR, especially cytokinin, could influence the formation of inflorescence branches and hermaphrodite flowers on Jatropha. For example, applications of benzyladenine (BA) (Pan & Xu, 2011) or benzyl amino purine (BAP) treatment (Palupi *et al*., 2009), can increase the number of inflorescence branches and caused the flowers to undergo sex transformation from female to hermaphrodite flowers in some inflorescence branches.

The differences in number of male flowers between andromonoecious and monoecious Jatropha did not follow the same pattern as the other types of flowers. It was suggested that those differences were influenced by plant population, climate, and nutrient conditions (Luo *et al*., 2007). This research showed that the ratio of hermaphrodite to male flowers in andromonoecious Jatropha (1:13) was higher than the ratio of female to male flowers in monoecious Jatropha (1:17) (Table 1). The later was higher than the ratio of female to male flowers in monoecious plant as reported by Raju & Ezradanam (2002) and Alam *et al*. (2011), which were 1:29, and 1:22 to 1:27, respectively.

In addition to the characteristics of andromonoecious Jatropha inflorescence, this study also observed the characteristics of hermaphrodite, male, and female flowers. The hermaphrodite flowers had larger diameters and larger sepal than those of the female and male flowers (Table 1). The differences can be seen at each flower developmental phase starting from bud to anthesis phases. Different sepal sizes between male and female flowers were also reported by Alam *et al*. (2011). The sepals in female flowers were 0.20-0.40 cm in length and 0.45-0.75 cm in width, while the sepals in male flowers were 0.40-0.60 cm in length and 0.20-0.30 cm in width. The length of the sepals of female flowers can reach up to 1.80 cm (Jones & Scurhes, 2008). In contrast to the size of the sepals, the size of the stamens and carpels in hermaphrodite, female, and male flowers were similar (Table 1). Similar results were also reported by Wang & Ding (2012) and Alam *et al*. (2011) on monoecious Jatropha flowers.

The phenology of andromonoecious Jatropha flowers is divided into 4 phases: flower initiation, small buds, large buds, and flower blooming phases. The division of flower phase development refers to the division made by Wu *et al*. (2011). They divided the development of monoecious Jatropha flowers into 12 phases (divided into three groups) in accordance with the development of petals, glands, stamens, and pistils until the flower is fully formed. In this study, flower development phases were divided based on flower size and inflorescence branching starting from initiation until all flowers completely bloom, therefore we come to the suggestion that the flower development was divided into 4 phases. The first to third phases have common characteristics with the first to twelve flower development phases reported by Wu *et al*. (2011), while the fourth phase was a blooming phase on one inflorescence. The relationship between the flower development phases proposed in this research and the flower development phases reported by Wu *et al*. (2011) is explained below:

1. The initiation phase is similar to the phases 1-2. There is a transformation from vegetative to generative, stem apical surface and an enlarged or dilated bulge containing the young inflorescence that cannot be seen from the surface of the plant, as they are covered by the young leaves.
2. The small buds phase is similar to the phases 3-6. The formation of sepals, petals, stamens, gland and carpel on hermaphrodite flower can be distinguished from the male flower parts.
3. The large buds phase is similar to the phases 7-12. Flower buds swell and form secondary and tertiary inflorescence. The number of hermaphrodite and male flowers for each inflorescence can already be

differentiated. The developments of male and female flowers reach a completion and maturity.

4. The flower blooming phase. This phase begins with the first flower bloom and ended with all flowers have bloomed.

The blooming time of hermaphrodite and male flowers varies in one inflorescence. The hermaphrodite flowers bloom before, after, or at the same time as male flowers bloom. Kaur *et al*. (2011) found two different blooming times in monoecious Jatropha. The male flowers bloom before (protandri) or after (protogini) female flowers bloom.

The blooming sequence of hermaphrodite flowers was in accordance with their position on the inflorescence branch. Hermaphrodite flowers bloomed successively starting from the main, secondary, and tertiary branches. The same blooming sequence was also reported by Alam *et al*. (2011) in the blooming of female flowers in monoecious Jatropha. The blooming sequence is influenced by the time of flower formation and development. Flowers that had been formed and developed earlier bloomed earlier.

Blooming time was also influenced by environmental factors, including light intensity and relative humidity. Flowers would bloom late when the light intensity is low due to cloudy or rainy weather. The blooming time of flowers on andromonoecious and monoecious Jatropha were almost the same between 05:00 to 07:59 am with mostly the flowers bloom at 05:00 to 5:59 am. Flower anthesis occurred between 5:00 to 8:59, and most flowers anthesis occurred between 6:00-6:59. (Table 2). Another study on monoecious Jatropha in Malaysia showed female flowers bloom at 06:35 to 08:25 am, while the male flowers bloomed at 06:10 to 12:05 am (Alam *et al*., 2011).

There was a different time of anther dehiscence in hermaphrodite flowers and male flowers of andromonoecious Jatropha. The hermaphrodite anther dehisced faster than the anther in male flowers. Therefore stigma of hermaphrodite flowers mostly obtained pollen from its anthers, and self-pollination was more likely occur in the hermaphrodite flower. Female flowers in monoecious Jatropha obtained pollen from male flowers with the help of pollinators. Therefore, the female flowers of monoecious Jatropha tended to be cross-pollinated. In some cases, the female flowers were not pollinated (Table 2). Kaur *et al*. (2011) found anther of male flowers on monoecious Jatropha dehisced one hour after flower anthesis at about 08:00 to 9:00 am (India time).

The number of pollen per anther varied in andromonoecious Jatropha flowers. The number of pollen per anther of male flowers was 298 pollens, which was higher than that of hermaphrodite flowers (267 pollens per anther). Kaur *et al*. (2011) found 61.90-195.10 pollens with an average of 122.30 pollens per anther on monoecious Jatropha. Pollen viability in hermaphrodite and male flowers almost the same. In hermaphrodite flowers, pollen viability on blooming time ranged between 94.44-97.73%, while male flowers ranged between 92.78-98.94%. Viable pollen remained stuck on the anther or survived until the next day (24 hours) and was able to pollinate the stigma. Bhattacharya *et al*. (2005) found that pollen viability is highest during 0-6 hours after anthesis. Abdelgadir *et al*. (2012) found that the viability of pollen in hermaphrodite flowers on Jatropha is lower than that of male related. Kaur *et al*. (2011) obtained low pollen viability in monoecious Jatropha ranged between 58.20-79.40% with an average of 71.60%.

Pollination in Jatropha flowers occurs through self- and/or cross-pollinations. The hermaphrodite flowers on andromonoecious Jatropha experience self-pollination. There were several factors that support this phenomenon, such as the carpel and stamens position, blooming process, stigma receptivity, anther dehiscence, anatomy of hermaphrodite flowers before anthesis, formation of pollen tube before blooming, and the presence of insect (ants) visitors or pollinators. Position of the carpel and stamen was very close with each other in a certain position, making the anthers attach to the stigma. The receptive part of the stigma was enveloped by nectar, facilitating viable pollen to directly attach to the stigma during anther dehiscence. Anatomy of the hermaphrodite flowers of andromonoecious Jatropha showed the development of embryo had occurred before anthesis (Figs. 5C and D), while the female flowers of monoecious Jatropha showed the development of embryo occurred after anthesis (Figs. 5E and F). The evident indicated that the pistil in hermaphrodite flowers was self-pollinated before anthesis. Pollination occurred before flower anthesis in hermaphrodite flower was also supported by the germination of pollen occurred before the flowers bloom. In addition, the anther of the hermaphrodite flower attached to the stigma causing pollen hydration and facilitating the growth of pollen tube. Furthermore, many ants had been already inside the hermaphrodite flower before flower bloom, supporting the suggestion that self-pollination was likely to occur in the hermaphrodite flowers.

Ants were the most common insects visiting the Jatropha flowers. Raju & Ezradanam (2002) reported 70% of insect visitors to female flowers were ants. Ants open the possibility of self-pollination because ants move from male to female flowers on the same flower inflorescence. The ants carry pollen on their legs and body, then the pollen sticks to the receptive part of the stigma at the same inflorescence. Wang & Ding (2012) also found that the presence of ants could cause autogamy, while winged insects, such as flies, caused cross-pollination.

Pollination is the starting point for fruit development. The fruit set on andromonoecious Jatropha was higher than that of monoecious Jatropha. The percentage of hermaphrodite flowers that develop into fruit (fruit set) on andromonoecious Jatropha (67-100% with an average of 89%) was higher than that on monoecious Jatropha (57-100% with an average of 79%). Comparing to the fruit set in monoecious Jatropha from other research reports, Kaur *et al*. (2011) reported 61.60% fruit set, while Alam *et al*. (2011) reported only 37% and 61.60% from two observation seasons. The difference in the fruit set was due to population variation, plant age, and climate as well as soil condition (Luo *et al*., 2007). Jatropha develops flowers starting from 5-8 months after planting. The older the plant, the less fruit it produces (Heller, 1996). Climate affects the presence of pollinators and the availability of water, and both are important factors in pollination and fruit development. The fruit set did not occur in monoecious Jatropha when there were no pollinators. The availability of sufficient water is critical in the development of the fruit. The nutrient requirements of plants for fruit set and development can be sufficiently supplied when the plants are grown in fertile soil.

Based on the characteristics, phenology, pollination, and fruit set of hermaphrodite flowers, andromonoecious Jatropha has a good chance to be developed. Andromonoecious Jatropha has more hermaphrodite flowers per inflorescence than monoecious Jatropha. The hermaphrodite flowers undergo self-pollination, supporting high fruit set. The fruit set of hermaphrodite flowers reached 100%, resulting in the high production of fruit and seeds.

Conclusions

The flower type of andromonoecious Jatropha is *inflorescentia racemosa*. Each inflorescence produces two main branches that continuously branch into secondary and tertiary branches. Each branch is ended by hermaphrodite flowers. Hermaphrodite flower size, flower diameter, stalk length, and sepal size are larger than that of the male and female flowers. Flower development is divided into 4 phases, namely initiation (0-3 dai), small bud (3-7 dai), large bud (7-25 dai), and flower blooming phases. Blooming of hermaphrodite flowers is in accordance with the position and formation of the flower in the inflorescence branch, starting from hermaphrodite flowers in the main branch tip, and then followed by the flowers in the secondary and tertiary branch. The hermaphrodite and female flowers begin to bloom after 05:00 am. The male flowers begin to bloom earlier than that of the hermaphrodite and female flowers, but generally, flower anthesis occurs between 05:00 to 05:59 am. Anthers of hermaphrodite flowers dehisce earlier than that of the anther in male flowers. High viability pollen produced from both hermaphrodite and male flowers still survive until the next day after anther dehiscence.

Acknowledgements

The authors are indebted to Mrs. Walid Rumlat and Mr. Abdul Hakim Hidayatullah for their excellent technical assistance.

References

Abdelgadir, H.A., S.D. Johnson and J.V. Staden. 2012. Pollen viability, pollen germination and pollen tube growth in the biofuel seed crop *Jatropha curcas* (Euphorbiaceae). *South Afr. J. Bot.*, 7: 132-139.

Achten, W.M.J., L. Verchot, Y.J. Franken, E. Mathijs, V.P. Singh, R. Aerts and B. Muys. 2008. Jatropha bio-diesel production and use. *Biomass Bioenerg.*, 32: 1063-1084.

Alam, N.C.N., T.L. Abdullah and A.P.A. Nur. 2011. Flowering and fruit set under Malaysian climate of *Jatropha curcas* L. *Am. J. Agri. & Biol. Sci.*, 6 (1): 142-147.

Bhattacharya, A., K. Datta and S.K. Datta. 2005. Floral biology, floral resource constraints and pollination limitation in *J. curcas* L. *Pakistan J. Biol. Sci.*, 8: 456-460.

Divakara, B.N., H.D. Upadhyaya, S.P. Wani and C.L.L. Gowda. 2009. Biology and genetic improvement of *Jatropha curcas* L.: a review. *Appl. Energ.*, 87(3): 732-742.

Heller, J. 1996. *Physic nut. Jatropha curcas* L. Promoting the Conservation and Use of Underutilitized and Negleted Crop 1. IPGRI., Rome, pp: 36.

Johansen, D.A. 1940. *Plant Microtechnique*. 5th Ed. Mc Graw Hill Book Company, Inc. New York, pp: 49-64.

Jones, M.H. and S. Scurhes. 2008. *Pest Plant Risk Assessment Physic Nut (Jatropha curcas).* Bio Security Queensland Departement of Primary Industries and Fisheries, Queensland.

Kaur, K., G.P.S. Dhillon and R.I.S. Gill. 2011. Floral biology and breeding system of *Jatropha curcas* in North-Western India. *J. Trop. Forest Sci*., 23(1): 4-9.

Lapola, D.M., J.A. Priess and A. Bondeau. 2008. Modeling the land requirements and potential productivity of sugarcane and jatropha in Brazil and India using the LPjmL dynamic global vegetation model. *Biomass Bioenerg*, 33: 1087-1095.

Liu, H., B.K. Kirchoff, G.J. Wu and J.P. Liao. 2007. Microsporogenesis and male gametogenesis in *Jatropha curcas* L. (Euphorbiaceae). *J. Torrey Bot. Soc.*, 134: 335-343.

Liu, H., Y.J. Liu, M.F. Yang and S.H. Shen. 2009. A comparative analysis of embryo and endosperm proteome from seeds of *Jatropha curcas*. *J. Integr. Plant Biol.*, 51(9): 850-857.

Luo, C.W., L. Kun, C. You and Y.Y. Sun. 2007. Floral display and breeding system of *Jatropha curcas* L. *Forest Studies China*, 9: 114-119.

Luo, C.W., Z.Y. Huang, X.M. Chen, K. Li, Y. Chen and Y.Y. Sun. 2011. Contribution of diurnal and nocturnal insects to the pollination of *Jatropha curcas* (*Euphorbiaceae*) in southwestern China. *J. Econ. Ent.*, 104(1): 149-54.

Miller, J.S. and P.K. Diggle. 2007. Correlated evolution of fruit size and sexual expression in andromonoecious Solanum sections Acanthophora and Lasiocarpa (*Solanaceae*). *Am. J. Bot.*, 94(10): 1706-1715.

Palupi E.R., M. Surahman and K. Warid. 2009. Aplikasi ZPT untuk keserempakan pemasakan buah jarak pagar (*Jatropha curcas* L.). Prosiding Seminar Hasil-Hasil Penelitian. *IPB. Bogor*, pp: 146-157.

Pan, B.Z. and Z.F. Xu. 2011. Benzyladenine treatment significantly increases the seed yield of the biofuel plant *Jatropha curcas*. *J. Plant Growth Regul.*, 30: 166-174.

Raju, A.J.S. and V. Ezradanam. 2002. Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (*Euphorbiaceae*). *Current Sci.*, 83: 1395-1398.

Sunil, N., K.S. Varaprasad, N. Siraraj, T.S. Kumar, B. Abraham and R.B.N. Prasad. 2008. Assessing *Jatropha curcas* L. germplasm *in-situ* : A case study. *Biomass Bioenerg.*, 32: 198-202.

Tambunan, A.H., H. Hambali, Sutrisno and M. Surahman. 2010. Lifecycle duration and maturity heterogeneity of *Jatropha curcas* Linn. *J. Sustain. Dev.*, 3(2): 291-295.

Wang, X.R. and G.J. Ding. 2012. Reproductive biology characteristic of *Jatropha curcas* (*Euphorbiaceae*). *Rev. de biol. Trop.*, 60(4): 1525-1533.

Wu, J., Y. Liu, L. Tang, F. Zhang and F. Chen. 2011. A study on structural features in early flower development of *Jatropha curcas* L. and the classification of its inflorescences. *Afr. J. Agric. Res.*, 6(2): 275-284.

Zahra, N.B., M. Ahmed, Z.K. Shinwari, M. Zafar and S. Sultana. 2014. Systematic significance of anatomical characterization in some euphorbiaceous species. *Pak. J. Bot*., 46(5):1653-1661.

(Received for publication 20 March, 2014)