

Floral Biology, Flowering Behaviour and Fruit Set Development of *Jatropha curcas* L. in Malaysia

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ABSTRACT

This paper describes the flowering behaviour of *Jatropha curcas* cultivated under Malaysian tropical climate. Investigation was carried out by observing the floral morphology, flowering sequence of pistillates, floral anthesis time, flower daily anthesis, flowering and fruiting plant behaviour, flower sex and fruit set ratio. Floral reproductive organs were examined using Scanning Electron Microscope (SEM). *Jatropha* is monoecious and it produces individual flowers in a dichasial cyme. Each *Jatropha* inflorescence has at least six compound cymes. The male flower anthesis started the earliest at 12.00 am and once again at 6.10 am to 6.46 am. The female flower anthesis commenced at 6.35 am to 8.25 am. The male flowers opened for a period of 8 to 11 days, while the female flowers opened for only 3 to 4 days. The reading of the male to the female flower ratio was taken twice, 22:1 in December 2008 and 27:1 in April 2009. The flower to fruit ratios were 6:5 (January 2009) and 2:1 (May 2009). Numerically, 0-10 female flowers and 25-215 male flowers are produced in the same inflorescence. In this study, the terminal stem of *Jatropha* bore fruits profusely in January, May and August 2009. Meanwhile, the development of the floral meristem consists of three stages which include a vegetative stage, a transition from vegetative to floral stage and development of flower parts. The meristem was in the transition stage at day 6. Although all sepals and a petal were developed at day 18, the presence of reproductive organs developing at this particular stage was not detected. Flower and fruit development took approximately 3 months to complete the full cycle, i.e. from the initiated floral bud stage until fruit maturity.

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INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) was introduced as a plant in Malaysia and it is valued for its use as an oilseed crop (Heller, 1996; Openshaw, 2000). Due to concerns over depletion of fossil fuel in the recent years, this plant has attracted attention because of its potential to partially replace fossil fuel as biodiesel. Currently, 40 million tonnes of diesel are annually consumed in India, and for this reason, India is now growing approximately 7.4 million hectares of *Jatropha*, making it the largest *Jatropha* producing nation. In Malaysia, *Jatropha* planting has been initiated at a trial scale. From this initial start, the biggest constraint on *Jatropha* identified to date has been the small number of fruits produced per inflorescence and also the differential ripening time of fruits on the same inflorescence. Bhattacharya *et al.* (2005) reported that only 50% of the female flowers set to fruit in Lucknow, India. Fruiting behaviour and pollination ecology of *Jatropha curcas* have also been studied by Raju and Ezradanam (2002). Their studies were carried out in the Eastern Ghats, India, at an altitude of 900 m where the climate is tropical monsoonal with an average rainfall of 1000 to 1600 mm annually and the mean temperatures varying from 20 to 25°C in the winter and 30 to 32°C in the summer (Murthy *et al.*, 1982).

Although there have been reports on *Jatropha* cultivation, most work has been carried out in India and due to the different climatic and soil conditions, information on the flowering and fruit set of *Jatropha* in

Malaysia is apparently required. Malaysia is a country that enjoys a tropical, equatorial climate, with temperatures varying from 20 to 36°C and an average annual rainfall of 2300 mm. In order to further understand the flowering and fruit characteristics of *Jatropha*, a floral and fruit development timeline is needed to address the identified problems with the small number of fruit produced. One approach to address this particular problem is to study the floral biology, floral ontogenesis, floral anthesis characteristic, as well as pollination ecology and pollen-style interactions. The objectives of the present study were to describe the floral biology and flowering behaviour of *Jatropha curcas* and to determine the timeline of the floral and fruit development in Malaysia.

MATERIAL AND METHODS

Field observations were carried out from November 2008 to June 2009 on three- to four-year old plants at Field 2, Universiti Putra Malaysia in Serdang (03°00.512N, 101°42.101E). Twenty four-year old plants were selected randomly and used for flower data collection and observation. Flowers were observed for their floral morphology, flowering sequence of pistillates, floral anthesis time, flower daily anthesis, flowering and fruiting plant behaviour, flower sex and fruit set ratio. These data were used to construct a timeline for flower development. In addition, vegetative shoots were tagged and observed for their developmental changes up to fruiting stage. Floral structures were observed using

Scanning Electron Microscopy (SEM). The samples were collected and fixed in 70% formalin acetic acid (FAA) and dehydrated to the critical point using osmium tetroxide. Dehydrated samples were then mounted on aluminium stubs and sputter coated with gold and viewed under a JEOL JSM-5610LV scanning electron microscope at an accelerating voltage of 15 kv (Spence, 2001). The male to female flower ratio and flower to fruit ratio were also recorded based on 10 inflorescences which were randomly selected from 20 plants. Observations on the order of male and female anthesis were carried out to determine their protandry or protogyny characteristics.

RESULTS AND DISCUSSION

Floral Biology and Flowering Behaviour

Jatropha curcas is monoecious and its flowers are unisexual. The plant produces individual male and female flowers in a

compound dichasium cyme pattern. Several dichasial cymes are clustered at the main inflorescence. Inflorescences are formed at the terminal of branches. *Jatropha* flowers are pale green in colour (see Fig.1a and Fig.1b), with a pedicel measuring 0.6cm to 1.0cm in length. There are five petals and the male flowers (Fig.1a) measure around 0.75cm to 0.9cm in length and 0.3cm to 0.4cm in width, while the female flowers (Fig.1b) measure about 0.7cm to 0.9cm in length and 0.3cm to 0.4cm in width. Flowers have five sepals; with each sepal ranging from 0.40cm to 0.60cm in length and 0.20cm to 0.30cm width in the male flower (Fig.1a), and approximately 0.45cm to 0.75cm in length and 0.20cm to 0.40cm in width in the female flower (Fig.1b).

Staminate flowers have ten functional stamens which are varying from 0.6cm to 0.7cm in length, and are arranged in two distinct whorls of five each in a single

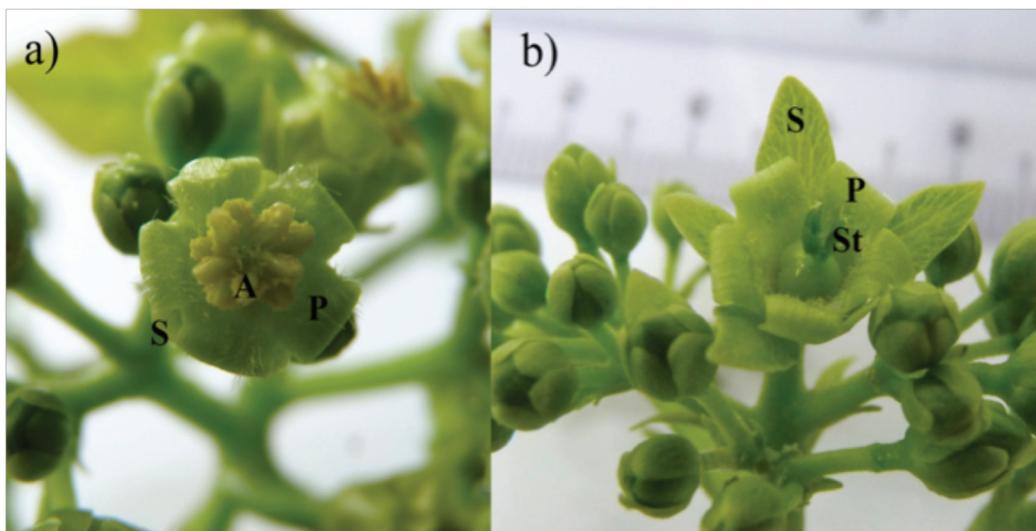


Fig.1: *Jatropha curcas*; (a) male and (b) female flower. Abbreviations: P, petal; S, sepal; A, anther and St, stigma

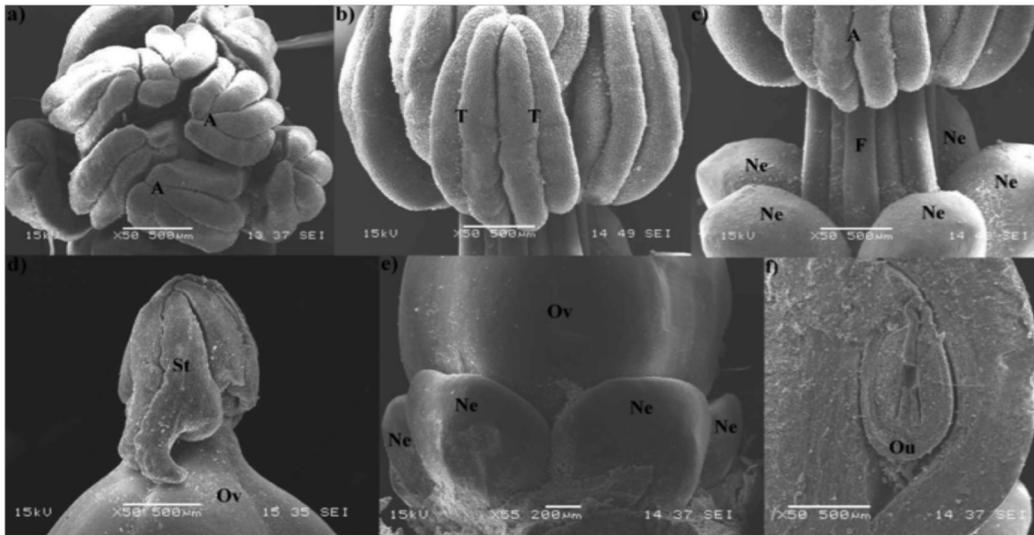


Fig.2: Scanning electron micrograph of floral reproductive organs of *Jatropha curcas* (x50). a-c. Staminate; anther with two theca and stamens are closed with each other. d-f. Pistillate; three-lobed stigma, ovoid ovary and apical- axile placenta. Abbreviations: T, theca; F, filament; A, anther; Ne, nectaries; St, stigma; Ov, ovary; Ou, ovule

column and adjacent to each other (Fig.2a). The anther is dithecal with longitudinal dehisced pollen (Fig.2b). The ovary is completely absent in staminate flowers but has five nectaries (Fig.2c). The pistillate flower was devoid of stamens and the style arose at the ovary apex (Fig.2d), with a distinct ovoid ovary terminating in a three-lobed stigma and surrounded by five nectaries (Fig.2e). The placenta was present in the apical-axile position with 3 placentae at the top of a septate ovary (Fig.2f). The pistil measured around 0.45cm to 0.68cm in length and 0.3cm to 0.35cm in width.

The compound dichasium cyme is composed of few individual simple cymes. Generally in the simple cyme, the female flowers are produced at the centre surrounded by the male flowers (Fig.3). In some cases, however, the expected female flower positions are replaced by the male

flowers, making the ratio of the female florets lower than that of the male florets. The arrangement of the individual flowers grouped together into inflorescences also promotes attraction and foraging rate by foragers (Solomon & Ezradanam, 2002). Large number of flowers tends to increase the attraction of pollinators because the emission of chemical attractants is more intense and flowers are more visible (Tcherkez, 2004).

Jatropha inflorescences can either be simple with 6 individual cymes or more complicated with up to 10 individual cymes. Normally, when showing a complicated structure, the secondary inflorescence located at the base of the main inflorescence will have more tertiary inflorescences attached to it (see Fig.4). Based on eight observations, the flowering sequence of the female flowers in *Jatropha curcas* begins at

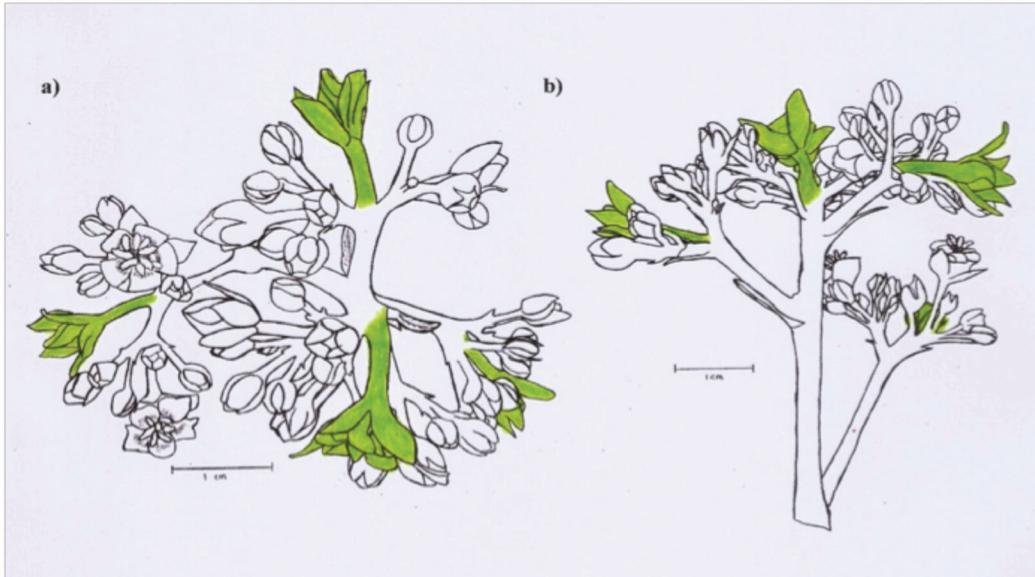


Fig.3: *Jatropha curcas*; inflorescence (a) Upper view (b) Side view. The female flowers were located at the centre of cyme which coloured with green colour. Abbreviations: cm, centimeter

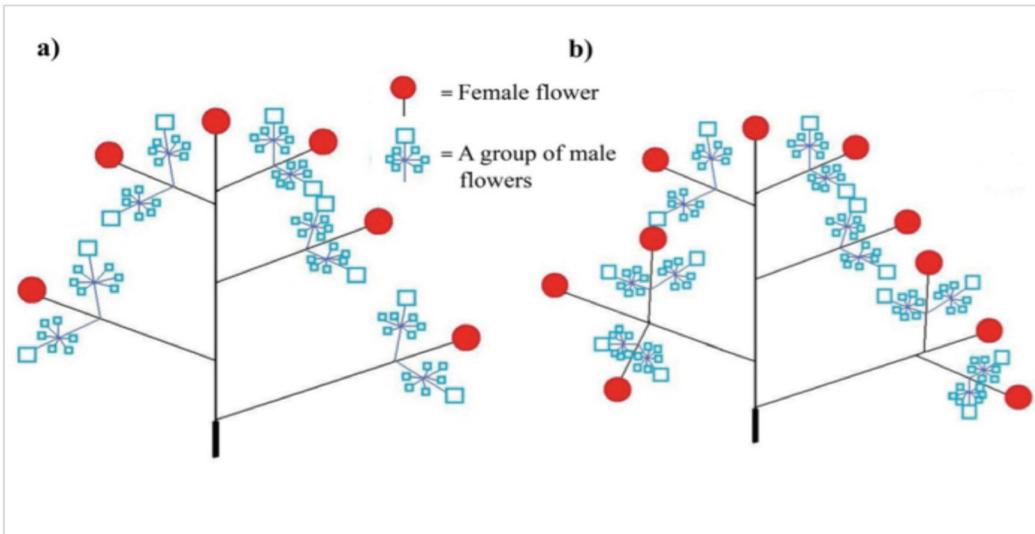


Fig.4: The *Jatropha* inflorescence structure with (a) 6 compound cymes (b) 10 compound cymes

the upper most terminal of the inflorescence (F) and simultaneously on the upper most terminal of the lowest cyme tier (A) and this is followed by B, D, C, A1 and E for the second day of flowering (Fig.5). This

sequence creates only mature fruit on each bunch. Mature fruit are present at the upper most terminal of each inflorescence (F) and the lowest cyme tier (A), with green fruit in the middle of each inflorescence.

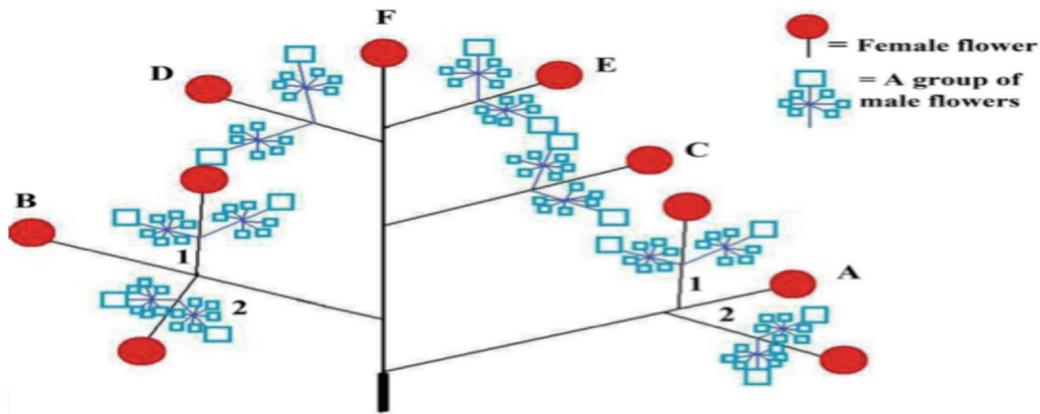


Fig.5: The flowering sequence of female flowers of *Jatropha curcas* begins at upper most terminal floret (F) in simultaneously with upper most terminal floret in the lowest cyme (A) and followed by B, D, C, A1 and E for the second day of flowering

The timing of the floral anthesis for the male flowers resulted in two distinct peaks following seven observations. The male flower anthesis was initiated at 12.00 am (24-25°C) and at 6.10 am to 6.46 am (22-23°C), while the female flower anthesis commenced at 6.35 am to 8.25 am (22-24°C), as shown in Table 1. The male flowers take about 30 to 45 minutes from the initiation of a small opening to full bloom and the subsequent pollen release, while the female flower takes approximately 40 to 55 minutes from the initial opening to full bloom. From all the observations, the plants showed a protandrous pattern of opening, with male flowers opening before the female flowers. This observation contrasts with that of Heller (1996) and Sunder (2006), but supports that of Solomon and Ezradanam (2002). This mechanism promotes cross pollination either via geitonogamy or xenogamy but allogamy is also possible given the fact that pollen is released from the

male flowers and the anthesis of the female flower occurs simultaneously. Nonetheless, studies on the *Jatropha* breeding system, pollination ecology and crop nutrition are suggested to further elucidate this mechanism.

TABLE 1
Floral anthesis time of the male and female flowers

Number of Observation	Flower Anthesis Time	
	Male	Female
1	12.05 am	8.25 am
2	6.29 am	7.30am
3	6.15 am	6.43am
4	6.25 am	6.40am
5	6.10 am	6.35 am
6	6.27 am	6.39 am
7	6.46 am	7.45 am

The male flowers open for a period of 8 to 11 days, while the female flowers open for only 3 to 4 days (Table 2). From the four inflorescences observed everyday over the period of flowering, the ratio of the male to

TABLE 2
Flower daily anthesis for four inflorescence

Day of Anthesis	Inflorescence 1		Inflorescence 2		Inflorescence 3		Inflorescence 4	
	Male	Female	Male	Female	Male	Female	Male	Female
Day 1	6	-	7	2	1	-	5	2
Day 2	8	2	32	6	6	-	10	2
Day 3	29	5	26	2	14	3	20	4
Day 4	23	1	43		24	2	20	
Day 5	17	0	23		17	1	24	
Day 6	8	0	21		29		29	
Day 7	7	1	22		10		14	
Day 8	9		3		9		24	
Day 9			1		3		30	
Day 10			3				5	
Day 11							2	

the female flowers opened on the same day was sufficient enough to ensure successful pollination (Table 2). The peak time for the female flower opening is on days 2 to 3 of the flowering period (Table 2). However, the flowering pattern of the female flowers was not consistent throughout the flowering period. On other occasions, the flowering of the female flowers terminated in the middle of the flowering period and then resumed the following day. There was a situation at Inflorescence 1 where no female flowers were open on days 5 and day 6, but then the plants recommenced flowering on day 7 (Table 2).

Cymose inflorescences lack a main axis. The main shoot terminates in a flower, while growth continues through lateral axes produced below the terminal flower. These lateral axes again form terminal flowers and this process is repeated several times. The

basal flower matured first with subsequent maturation occurring from apex to base (Simpson, 2006). This pattern will cause flower maturity to occur at different times and lead to a discrete period of flower opening for both the male and female flowers.

Meanwhile, the ratios of the male to female flowers were 22:1 (December 2008) and 27:1 (April 2009) from the data on ten inflorescences. The flower to fruit ratios were 6:5 (January 2008) and 2:1 (May 2009) (Table 3), respectively. Numerically, 0-10 female flowers and 25-215 male flowers were produced in the same inflorescence. The initial fruit set for *Jatropha* reached as high as 92% for the pistillate flowers. This indicates that individuals do not suffer from under-pollination. The production of pistillate flowers is low and each is surrounded by a large number of staminate

TABLE 3

Mean and number of staminate and pistillate flowers in an inflorescence and the number of fruits produced from the pistillate flowers for two observations

Inflorescence no.	No. of staminate		No. of pistillate		No. of fruits	
	Obs. 1	Obs.2	Obs. 1	Obs.2	Obs. 1	Obs.2
1	78	80	3	4	3	4
2	98	38	4	0	4	0
3	183	25	5	0	4	0
4	105	36	8	0	7	0
5	112	48	6	3	6	2
6	95	39	8	0	8	0
7	109	69	3	0	3	0
8	215	39	10	0	10	0
9	155	96	3	5	2	4
10	143	67	6	3	6	3
Mean	129	54	6	2	5	1

Notes: (Obs.) Observation

flowers with a male to female flower ratios of 22:1 to 27:1 that promote effective pollination maximally. This result is similar to that achieved by Bhattacharya *et al.* (2005) who recorded a 29:1 male to female flower ratio in their studies.

Floral and Fruit Development

Jatropha trees produce many leaves when they are in flowering period (Fig.6a). Trees then drop their leaves after fruit has set (Fig.6b). In the current study, the terminal stems of *Jatropha* profusely bore fruit in January, May and August 2009. In March, June, and October, flowering took place after the vegetative stage. It then took about a month from the vegetative flush to the initiation of visible flower buds. From the observations carried out, the development of the floral meristem consisted of at least three stages which included a vegetative

stage (Fig.7a), a transition stage (Fig.7b) and the development of the flower parts (Fig.7c and Fig.7d). During the initial day of the sampling (day 0), the meristem showed a vegetative dome shape that measured around 150µm (Fig.7a). At day 6, the meristem was in the transition stage where it started to rise and was ready to differentiate into organs (Fig.7b). At day 18, all the sepals and petal were developed, but there was no presence of reproductive organs developing at this particular stage (Fig.7c and Fig.7d). Meanwhile, the floral bud became visible after 24 days from the first day of observation (Fig.8a and Fig.8b). It then took approximately 26 days from the day of visible floral bud to floral anthesis (Fig.8c). Once the floral anthesis had began, flowers then opened daily. Flowering lasted approximately 8 days (Fig.8d) and this was followed by 33 days for the fruit to

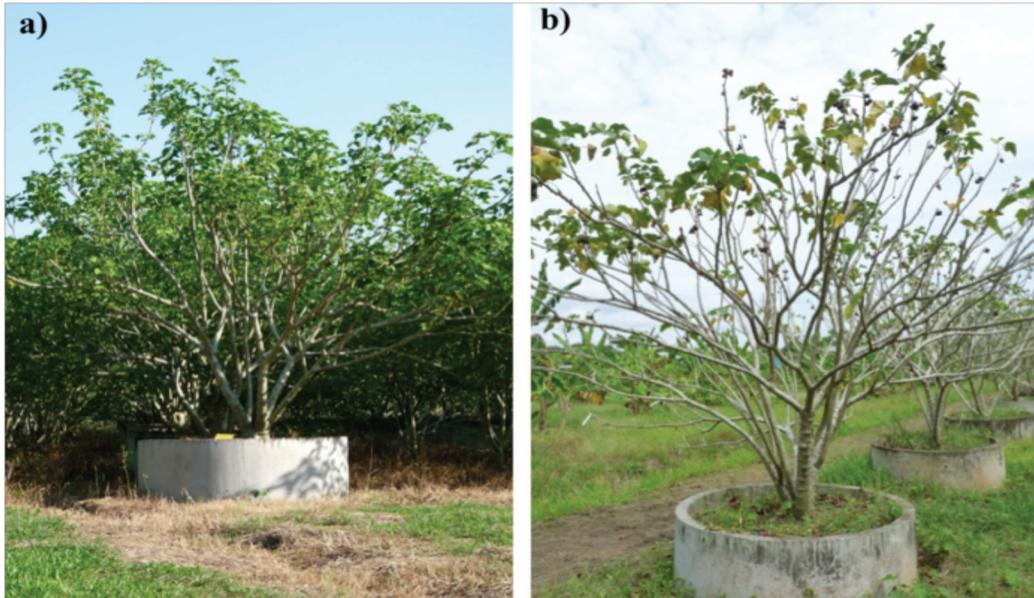


Fig.6: *Jatropha* trees approximately three to four years old are (a) full of leaves when it was in flowering period, (b) drop their leaves abundantly when it was in the fruiting period

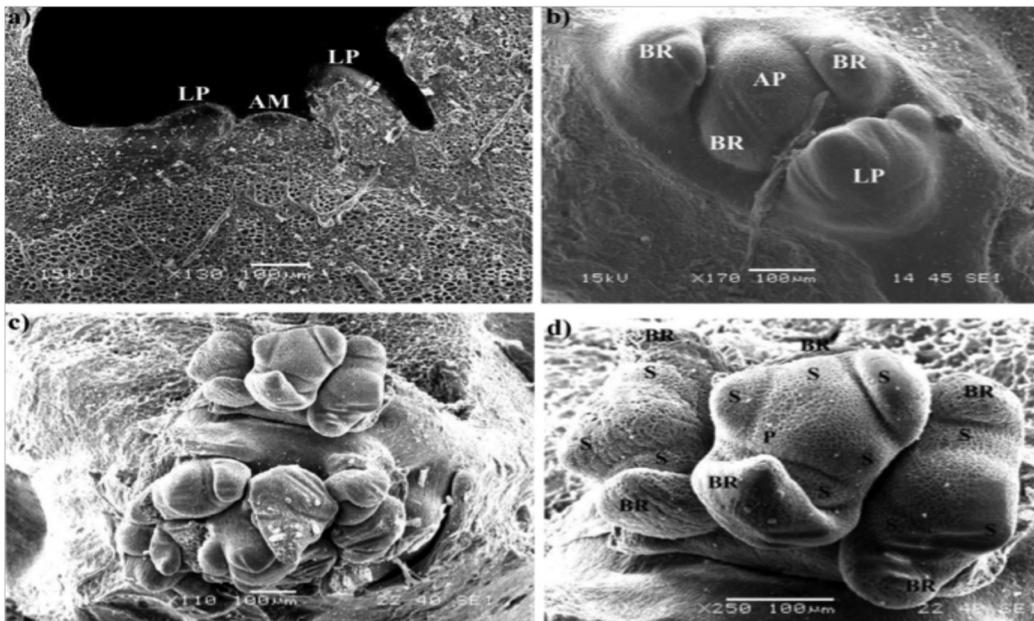


Fig.7: Micrographs of the vegetative, transition and early floral stages of *Jatropha curcas*. (a) Vegetative dome shape meristem that measures around 150 μ m was found on day 0. (b) At day 6, shoot meristem started to rise and ready to differentiate into organs. (c) Two clear branch of inflorescence are presence at day 18. (d) Focus image of day 18 showed the sepals and petal have been developed at this stage. Abbreviations: LP, Leaf Primordia; AM, Apical meristem; AP, Apex; BR, Bract; P, Petal; S, Sepal

mature (Fig.8e). Fruit senescence occurred seven days after fruit maturity (Fig.8f). A complete cycle of fruit set and development required approximately 100 days (Fig.9).

Jatropha in Malaysia shows a characteristic year round free bearing habit combined with multiple cyclical fruiting peaks. Natural peaks can be altered by weather conditions and by culture manipulations in plantations (Milan, 2008).

Flowering is usually triggered after a dry and a dormant period and it is induced by prolonged periods of raised soil water availability (Jongschaap *et al.*, 2007). Flower formation could be influenced by the weather conditions at the time of bud differentiation. Dry weather induces flower bud formation and heavy rainfall promotes formation of vegetative buds (Heller, 1996).

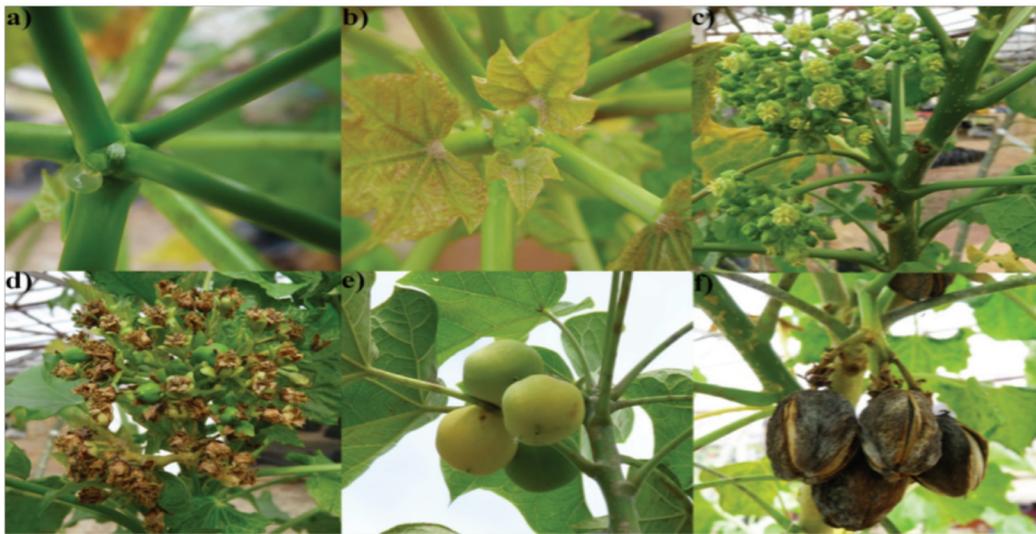


Fig.8: Macrophotographs of flower and fruit development showing (a) Vegetative stage at Day 0, (b) First visible flower bud at Day 24, (c) Anthesis at Day 50, (d) Flower senescence and fruiting at Day 58, (e) Mature fruit at Day 93 and (f) Fruit senescence at 100 days.

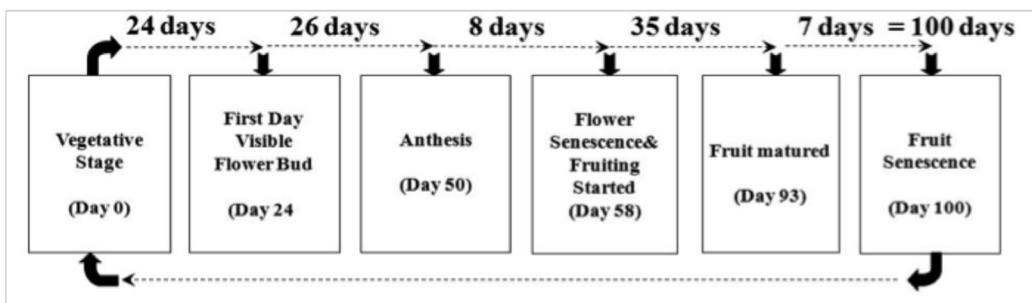


Fig.9: Timeline of flowering and fruit set showing *Jatropha curcas* took about 3 months to complete a fruiting cycle

CONCLUSION

The study revealed that the female flowers of *Jatropha* opened for a period of three to four days, while the male flowers opened for longer periods varying in duration from eight to eleven days. Continuous flowering and the incidents where flowering terminated in the middle of the flowering period were factors believed to have caused the wide range of fruit ripening times recorded. The initial fruit set of *Jatropha* was high, i.e. as much as 92% of the pistillate flowers set fruit. The low fruit set problems in *Jatropha curcas* is mainly caused by a small number of pistillate flowers present in each inflorescence that range from 0 to 10 flowers in the same inflorescence. Details of the flower structure and understanding their individual functions during the process of fruit setting will assist cultivar improvement and can optimise yields and synchronize fruit maturity.

Floral and fruit development takes approximately 3 months to complete the cycle from the initiated floral bud stage until fruit maturity. This indicates that *Jatropha* could have two to four cyclical fruiting peaks in Malaysia, depending on the weather conditions and cultivation practices.

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