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Flowering and Fruit Set Under Malaysian Climate of *Jatropha curcas* L.

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Abstract: Problem statement: In recent years *Jatropha curcas* has drawn the world's attention as it has the potential to replace fossil fuel as biodiesel. However, the biggest setback in Jatropha cultivation in Malaysia is the low percentage of fruit set and the high ratio of male to female flowers. In order to further understand the flowering and fruit characteristics of Jatropha, floral and fruit development timeline is needed in order to develop solutions for the problems in low fruit set. **Approach:** This study described the flowering behaviour of *Jatropha curcas* cultivated under Malaysia and 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). **Results:** Jatropha is monoecious and produces individual flowers in a dichasial cyme. Each Jatropha inflorescence has at least six compound cymes. Male flower anthesis started the earliest at 12.00 am and again at 6.10-6.46 am. Female flower anthesis commenced at 6.35-8.25 am. Male flowers opened for a period of 8-11 days, while female flowers opened for only 3-4 days. The reading of the male to 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 bears fruits profusely in January, May and August 2009. Development of the floral meristem consists of three stages that include a vegetative stage, transition from vegetative to floral stage and the development of flower parts. The meristem was in the transition stage at day 6. All Sepals and a petal were developed at day 18 but there was no presence of reproductive organs developing at this stage. Flower and fruit development takes approximately 3 months to complete the cycle from the initiated floral bud stage until fruit maturity. **Conclusion:** Continuous flowering and the incidents where flowering terminated in the middle of the flowering period were factors believed to cause the wide range of fruit ripening times recorded. The problem of small number of fruits produced in *Jatropha curcas* is mainly caused by the small number of pistillate flowers present in each inflorescence that range from 0 to 10 flowers in the same inflorescence. Jatropha could have two to four cyclical fruiting peaks in Malaysia.

Key words: Floral morphology, *Jatropha curcas*, reproductive organs, floral anthesis, pistillate flowers, dichasial cyme, Scanning Electron Microscope (SEM), Formalin Acetic Acid (FAA)

INTRODUCTION

Jatropha curcas L. (Euphorbiaceae) is an introduced plant in Malaysia and is valued for its use as an oilseed crop (Camellia *et al*., 2009; Effendi *et al*., 2010; Ahmadpour *et al*., 2010). In recent years, due to concerns about fossil fuel depletion, this plant has attracted attention as it has potential to partially replace fossil fuel as biodiesel. Currently, 40 million tonnes of diesel are consumed in India annually and so India now grows approximately 7.4 million hectare of Jatropha making it the largest Jatropha producing nation. In Malaysia, Jatropha plantings have 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 differential ripening time of fruits on the same inflorescence. Bhattacharya *et al*. (2005) reported that

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only 50% of 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 1000-1600 mm annually and mean temperatures varying from 20-25°C in winter and 30-32°C in summer (Murthy *et al*., 1982). Although there have been reports on Jathropa cultivation, most work has been carried out in India and due to different climate and soil conditions, information on flowering and fruit set of Jatropha in Malaysia will be required. Malaysia is a country with a tropical, equatorial climate with temperatures varying from 20-36°C and 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 in order to address identified problems with the small number of fruits produced. One approach to address this problem is to study the floral biology, floral ontogenesis, floral anthesis characteristic and 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 floral and fruit development in Malaysia.

MATERIAL AND METHODS

Field observations were carried out from November 2008-June 2009 on three to four year old plants at Field 2, Universiti Putra Malaysia, Serdang (03°00.512N, 101°42.101E). Twenty four years old plants were selected randomly and were used for flower data collection and observation. Flowers were observed for 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. Vegetative shoots were tagged and observed for their developmental changes up to fruiting stage. Floral structures were observed using Scanning Electron Microscopy (SEM). Samples were collected and fixed in 70% Formalin Acetic Acid (FAA) and dehydrated to the critical point using osmium tetraoxide. 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 (Psyquay and Firdaus, 2010). Male to female flower ratio and flower to fruit ratio were recorded based on 10 inflorescences selected randomly from 20 plants. Observations on order of male and female anthesis were carried out to determine their protandry or protogyny characteristics.

RESULTS

Floral biology and flowering behaviour: *Jatropha curcas* is monoecious and 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 with a pedicel measuring 0.6-1.0 cm in length. There are five petals and male flowers measure 0.75-0.9 cm in length and 0.3-0.4 cm in width while female flowers measure 0.7-0.9 cm in length and 0.3-0.4 cm in width. Flowers have five sepals; with each sepal ranging from 0.40-0.60cm in length and 0.20-0.30 cm width in the male flower and approximately 0.45-0.75 cm length and 0.20-0.40 cm width in the female flower.

Staminate flowers have ten functional stamens with stamens varying from 0.6cm to 0.7cm in length where they are arranged in two distinct whorls of five each in a single column and adjacent to each other (Fig. 1a). The anther is dithecal with dehisced pollen longitudinally (Fig. 1b). The ovary is completely absent in staminate flowers but has five nectaries (Fig. 1c). The pistillate flower was devoid of stamens and the style arose at the ovary apex (Fig. 1d) with a distinct ovoid ovary terminating in a three-lobed stigma and surrounded by five nectaries (Fig. 1e). The placenta was present in the apical-axile position with 3 placentae at the top of a septate ovary (Fig. 1f). The pistil measured 0.45-0.68 cm in length and 0.3-0.35 cm in width.

Jatropha inflorescences can either be simple with 6 individual cymes or can be 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 (Fig. 2).

From eight observations, the flowering sequence of female flowers in *Jatropha curcas* begins at 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. 3). This sequence creates only a mature fruits on each bunch. Mature fruits are present at the upper most terminal of each inflorescence (F) and the lowest cyme tier (A), with green fruits in the middle of each inflorescence.

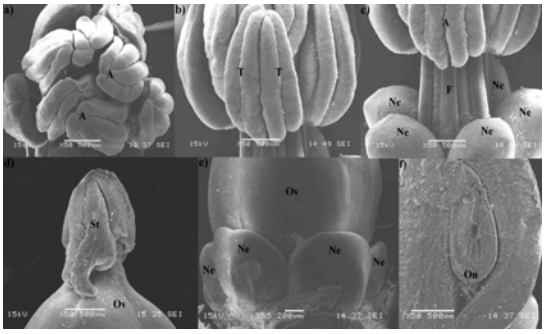

Fig. 1: Scanning electron micrograph of floral reproductive organs of *Jatropha curcas* (×50). a-c. Staminate; anther with two theca and stamens are closed with each other. d-f. Pistillate; three-lobed stigma, ovoid ovary and apical-axile placenta. Abbreviations: T, theca; F, filament; A, anther; Ne, nectaries; St, stigma; Ov, ovary; Ou, ovule

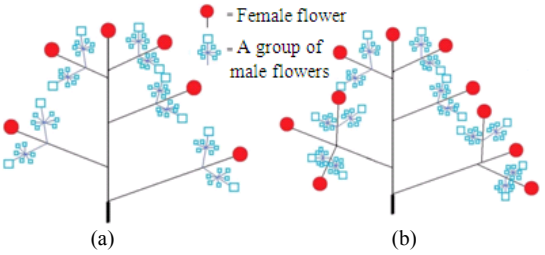

Fig. 2: The Jatropha inflorescence structure with (a) 6 compound cymes; (b) 10 compound cymes

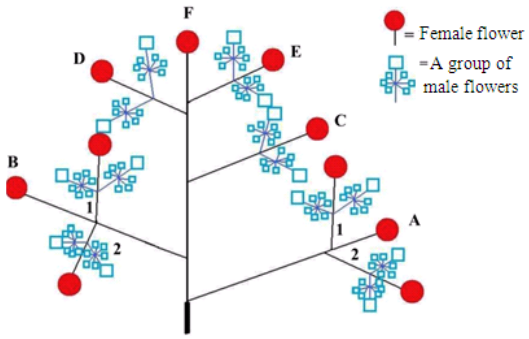

Fig. 3: The flowering sequence of female flowers of *Jatropha curcas* begins at upper most terminal floret (F) in simultaneously with upper most terminal floret in the lowest cyme (A) and followed by B, D, C, A1 and E for the second day of flowering

The timing of floral anthesis for male flowers resulted in two distinct peaks following seven observations. Male flower anthesis was initiated at 12.00 am (24-25°C) and at 6.10-6.46 am (22-23°C) while female flower anthesis commenced at 6.35-8.25 am (22-24°C) (Table 1).

Table 1: Floral anthesis time of male and female flowers

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

Male flowers take about 30-45 min from initiation of a small opening to full bloom and subsequent pollen release while female flower takes approximately 40-55 min from initial opening to full bloom.

Male flowers open for a period of 8-11 days, while female flowers only open for 3-4 days (Table 2). From four inflorescences observed everyday over the period of flowering, the ratio of male to female flowers opened on the same day was sufficient to ensure successful pollination (Table 2). The peak time for female flower opening is on day 2-3 of the flowering period (Table 2). The flowering pattern of female flowers was not consistent throughout the flowering period. On other occasions, flowering of 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 during day 5 and day 6, but then plants recommenced flowering at day 7 (Table 2).

Ratios of male to female flowers were 22: 1 (December 2008) and 27: 1 (April 2009) from data on ten inflorescences. 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.

Floral and fruit development: Jatropha trees produce many leaves when they are in flowering period. Trees then drop their leaves after fruit set. In the current study, terminal stems of Jatropha bear fruits profusely 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 initiation of visible flower buds. From observations taken, development of the floral meristem consists of at least three tages that include; a vegetative stage (Fig. 4a), a transition stage (Fig. 4b) and development of the flower parts (Fig. 4c and d). During the initial day of sampling (day 0), the meristem showed a vegetative dome shape that measured around 150 µm (Fig. 4a). At day 6, the meristem was in transition stage where it started to rise and was ready to differentiate into organs (Fig. 4b). At day 18, All sepals and a petal were developed and there was no presence of reproductive organs developing at this stage (Fig. 4c and d).

Table 2: Flower daily anthesis for four inflorescences

	Inflorescence 1		Inflorescence 2		Inflorescence 3		Inflorescence 4	
Day of anthesis	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	

Table 3: Mean and number of staminate and pistillate flowers in an inflorescence and number of fruits produced from the pistillate flowers for two observations

	Number of staminate		Number of pistillate		Number of fruits	
Inflorescence number	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

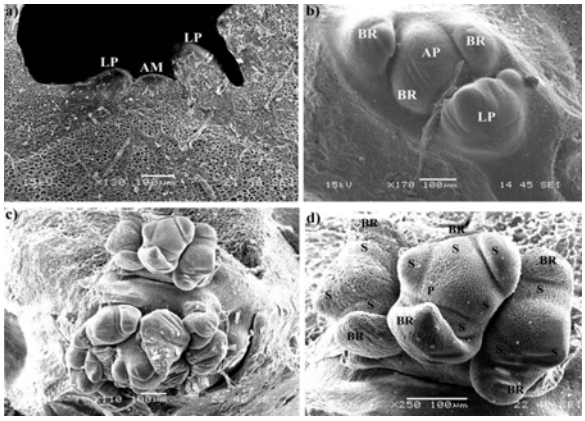

Fig. 4: Micrographs of the vegetative, transition and early floral stages of *Jatropha curcas*. (a) Vegetative dome shape meristem that measures around 150 µm was found on day 0; (b) At day 6, shoot meristem started to rise and ready to differentiate into organs; (c) Two clear branch of inflorescence 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

Fig. 5: 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

The floral bud became visible after 24 days from the first day of observation (Fig. 5a and b). It then took approximately 26 days from the day of visible floral bud to floral anthesis (Fig. 5c). Once floral anthesis had began, flowers then opened daily. Flowering lasted

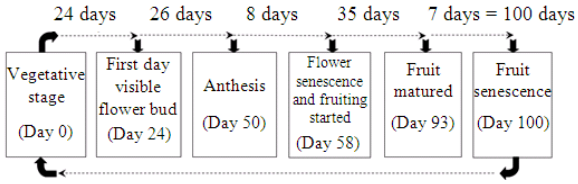

Fig. 6: Timeline of flowering and fruit set showing *Jatropha curcas* took about 3 months to complete a fruiting cycle

approximately 8 days (Fig.5 d) and this was followed by 33 days for fruit to mature (Fig. 5e). Fruit senescence occurred seven days after fruit maturity (Fig. 5f). The complete the cycle of fruit set and development required approximately 100 days (Fig. 6).

DISCUSSION

The compound dichasium cyme is composed of few individual simple cymes. Generally, in the simple cyme, female flowers are produced at the centre surrounded by male flowers. In some cases however, the expected female flower positions are replaced by male flowers making the ratio of female florets lower than that of male florets. The arrangement of individual flowers grouped together into inflorescences also promotes attraction` and foraging rate by foragers (Raju and Ezradanam, 2002) Large number of flowers tends to increase the attraction of pollinators because emission of chemical attractants is more intense and flowers are more visible (Tcherkez, 2004).

From all of the floral anthesis observations, plants showed a protandrous pattern of opening with male flowers opening before female flowers. This observation contrasts that of Heller (1996) and Sunder (2006) but support those of Raju and Ezradanam (2002). This mechanism promotes cross pollination either via geitonogamy or xenogamy but allogamy is also possible given that pollen released from male flowers and anthesis of the female flower occur simultaneously (Firdaus *et al*., 2010). Studies on the Jatropha breeding system, pollination ecology and crop nutrition are suggested to further elucidate this mechanism.

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 leads to a discrete period of flower opening for both male and female flowers

Initial fruit set for Jatropha reached as high as 92% for pistillate flowers. This indicates that individuals do not suffer from under-pollination. Production of pistillate flowers is low and each are surrounded by a large number of staminate flowers with a male to female flower ratio of 22:1 to 27:1 that promotes effective pollination maximally. This result was similar to that achieve by Bhattacharya *et al*. (2005) who recorded 29:1 male to female flower ratio in their studies.

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 (Jamaluddin, 2008). Flowering is usually triggered after a dry and dormant period and 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).

CONCLUSION

The study revealed that female flowers of Jatropha opened for a period of three to four days while 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 cause the wide range of fruit ripening times recorded. Initial fruit set of Jatropha was high, as much as 92% of the pistillate flowers set fruit. The problem of small number of fruits produced in *Jatropha curcas* is mainly caused by the small number of pistillate flowers present in each inflorescence that range from 0 to 10 flowers in the same inflorescence. Details of flower structure and understanding their individual functions during the process of fruit setting would be assist cultivar improvement and can optimise yields and synchronize fruit maturity.

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

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