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Reproductive Biology and Pollinator Studies in *Jatropha curcas* L. : An Important Bio-Diesel Plant

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Abstract

To understand the barriers to fruit and seed set and to know the factors regulating the genetic structure of populations, the present study on floral biology and pollinators was carried out in *Jatropha curcas*. Ten plants were selected to investigate the objectives. The inflorescence is monoecious with protandry and is racemose with dichasial cyme pattern. The average numbers of male flowers and female flowers per inflorescence were 136.4 ± 10.82 and 8 ± 0.71 respectively. The inflorescence is having two tiers viz., large tier and small tier. The average length and spread were recorded as 7.624 ± 0.84 cm and 6.373 ± 0.55 cm in large tier, and 6.101 ± 0.99 cm and 4.357 ± 0.36 cm in small tier respectively. In an average, inflorescence has taken 18.9 ± 0.67 days for its development from visual stage of initiation and the time taken for the full bloom of inflorescence was 14.2 ± 0.75 days. Anthesis started from 0650 h and lasted up to 1150 h. Anthers dehisced at a mean time of 1.28 h after flower opening. Stigma attained receptivity between 01.20 to 2.25 h after anthesis and remained receptive for 2—3 days. The estimated average number of pollen grains per flower was 1601 ± 70. The pollen exhibited 91.06 ± 2.42% fertility. The highest percentage (4.95%) of fruit set in relation to total number of flower buds established was noticed in open pollination. Flowers exhibited both entomophilous way of pollination and wind pollination. Honey bees were observed as the predominant insect pollinators.
Key words : Reproductive biology, Pollinators, *Jatropha curcas.*

Jatropha curcas is a shrub or small tree, belonging to the family Euphorbiaceae and is thus closely related to other important cultivated plants like rubber, castor. It is commonly known as dravanti, janglirandi, rattanjat and jamalghota in different states of India. It is known as physic nut and purging nut in English. Plant reaches a height of 3—8 m with stem up to 20 cm diameter. It is cultivated in Central and South America, South-East Asia, India and Africa (1). The genus *Jatropha* has 176 species distributed throughout the world. Among them, 12 species are recorded in India. Two species of *Jatropha* that are cultivated commercially include *Jatropha curcas* and *J. glandulifera* R. Br. *Jatropha curcas* is mainly promoted for bio-diesel because of higher oil content (up to 48%), whereas *J. glandulifera* is known for its beautiful flowers and oil content (up to 27%). Studies on reproductive biology of forest trees and other important plants is important in understanding barriers to fruit and seed set in natural habitats and to understand factors regulating the genetic structure of populations. It is an important pre-requisite for the effective exploitation of the economic potential of a species (2). The present study was aimed to assess the floral biology and related aspects of *Jatropha curcas*—a tree-bom-oil (TBOs) seed plant growing at Vellanikkara to know its potential for biodiesel production.

Methods

The present study was carried out in Kerala Agricultural University, Thrissur. The inflorescence was studied for its type, length and breadth, number of buds and flowers, and number of fruits. Time of flowering was studied by observing 10 plants selected randomly from the population. Observations on number of inflorescence per plant and number of flowers per inflorescence were recorded. To study the time taken for full development of inflorescence from vi-

Table 1. Time of anthesis, anther dehiscence and stigma receptivity.

	Anthesis	Anther dehiscence	Time taken after anthesis (hrs)	Stigma receptivity	Time taken after anthesis (h)
1	08.35 am	09.20 am	0.45	10.05 am	1.30
2	11.50 am	12.45 pm	0.55	01.10 pm	1.20
3	06.50 am	07.55 am	1.05	08.30 am	1.40
4	09.55 am	10.50 am	0.55	11.25 am	1.30
5	09.20 am	10.35 am	1.05	11.00 am	1.40
6	08.40 am	09.50 am	1.10	10.45 am	2.25
7	10.25 am	11.20 am	0.55	12.10 pm	1.45
8	10.10 am	11.30 am	1.20	12.05 pm	1.55
9	09.15 am	10.25 am	1.10	11.30 am	2.15
10	08.40 am	10.00 am	01.20	10.50 am	02.10
Mean ± SE	09.14 ± 0.429	10.23 ± 0.42	0.88 ± 0.098	09.18 ± 1.027	2.1 ± 0.125

sual stage of formation, ten young flowering shoots from five different plants was tagged soon after appearance of flower bud in the leaf axil. Time taken for the full bloom of inflorescence from opening of first flower to the last flower in a inflorescence were recorded. The shoots were periodically observed during the flowering season to find out the exact time of visual emergence of flower buds. Progressive stages of flower bud development were studied by labeling and closely watching flower buds randomly selected on each plant.

Buds were tagged soon after their emergence. Observations were made on the time duration between emergence of flower bud to flower opening, bud to flower ratio and bud to fruit ratio. Fifty fresh flower buds and flowers were collected from ten plants in such a way that five each from each plant. Hand sections (both LS and TS) were taken and examined under occular microscope and description of morphological features like size, color, and number of floral parts ; sepals, petals, androecium and gynoecium were recorded. Floral formula and floral diagrams were drawn. Fifty inflorescences were marked on ten plants (5 each), and diameter of flower, length of style and diameter of stigma were measured. The number of pistilate and staminate flowers in an inflorescence were separately counted and recorded.

Anthesis, Anther Dehiscence and Stigma Receptivity

With the objective of understanding the exact time of flower opening, 10 mature flower buds (one on each plant) were marked and the time at which the buds started to split was noted at 1 hour interval. The observations were repeated over a period of 4 days. Anther sacs were examined at every 15 minutes interval to arrive at the time of anther dehiscence. The exact time of stigma receptivity was determined through peroxidase test using H_2O_2 (3).

Pollen Studies

Anther sacs collected from the fully opened flowers just prior to the time of anther dehiscence were allowed to dehisce in water taken in a petri dish. A drop of pollen suspension was transferred to acetocarmine solution kept on a clean glass slide. After covering with a clean cover slip, the slides were kept

Table 2. Number and percentage of fruit set and fruit drop in different modes of pollination. *Significant at 0.05 level.

Treatment	No. of buds	No. of fruits	Fruit (%)	Fruit maturity	Fruit maturity (%)	Fruit drop (%)
Cross pollination	167.1	8.2	4.95	6.7	85.96	14.04
Self pollination	165.9	6.2	3.70	5.3	81.62	18.38
Wind pollination	145.1	4.8	3.28	3.5	73.12	26.88
F Test		*	*	*	*	*
CD		2.41	1.21	2.26	9.23	9.23

Table 3. Number and frequency of insect visits in *Jatropha* flower. P : Pollen, N : Nectar.

	Floral visitors	Frequency (5 min)	Per cent	Foraging type
1	*Apis indica*	9	32	P, N
2	*A. dorsata*	4	14	P, N
3	*A. florae*	6	21	P, N
4	*Vespa* sp.	3	11	P
5	*Sphex* sp.	2	7	P
6	*Thrips* sp.	2	7	P, N
7	*Pithitis binghami*	1	4	P
8	*Ceratina simillima*	1	4	P

as such for 10 minutes for the pollen grains to get stained and were examined under a microscope. Staining technique to study the fertility of pollen grains was adopted from Zirkle (4). Pollen grains normally shaped and stained in acetocarmine were treated as viable and fertile whereas unstained and irregular shaped were regarded as non-viable and sterile.

Mode of Pollination

To ascertain the precise mode of pollination, fruit set was observed by tagging 10 inflorescences from ten plants. Observations on number of buds initiated and fruit set was recorded. The extent of open pollination under natural conditions was determined by tagging 10 inflorescences (one in each plant). Ten inflorescences (of matured flower buds) were covered with butter paper to prevent pollen contamination from outside (self pollination). To know the extent of wind pollination, ten inflorescences were covered with nylon mesh to prevent pollen from insect pollinators. These were later examined for fruit set and percentage was calculated in each mode of pollination.

Flower Pollinators

To study the role of insects in natural pollination of *Jatropha*, close observations were made at 1 hour interval to see if any insects visited the flowers. This was repeated for 3 days during peak flowering period. The insects visiting the flowers were trapped and identified.

Results and Discussion

Inflorescence Characters and Floral Morphology

In the present investigation, *Jatropha curcas* flowered twice in a year. While in the first season, flowering started from March second week and continued till third week of May, and in second season it was from August second week to October third week. The inflorescence is racemose with dichasial cyme pattern. The average number of inflorescence per plant is 12.90 ± 1.13. Flowers are unisexual, and male and female flowers are produced in the same inflorescence. Normally, the inflorescence produces a central female flower surrounded by a group of male flowers. These observations were confirmed with other earlier researchers (5—7). The inflorescence is having two tiers, large tier and small tier. The average length and spread of the large tier were recorded as 7.62 ± 0.84 cm and 6.37 ± 0.55 cm and 6.10 ± 0.99 cm and 4.35 ± 0.36 cm in small tier respectively. On an average, an inflorescence took 18.9 ± 0.67 days for its development from visual stage of initiation. Average time taken for the full bloom of inflorescence was 14.2 ± 0.75 days. The average number of male flowers and female flowers per inflorescence were 136.4 ± 10.82 and 8 ± 0.71 respectively (5). The average male to female flower ratio was 17.3 ± 1.37.

The male flower is greenish white, odourless and salvar shaped with an average length and spread of 6.95 ± 0.19 mm and 5.54 ± 0.23 mm respectively. The flower is actinomorphic and incomplete. The sepals and petals are five (pentamerous) and free. The sepals are arranged in imbricate aestivation. The petals are valvate and connitent at the flower base forming a short tube. Stamens are ten, diadelphous, arranged in two tiers of five each. The outer tier is free, while the inner tier is united. The average length of stamen was 5.07 ± 0.24 mm. The anthers are yellow, dithecous and dorsifixed. The pollen grains are yellow, globular and inaperturate. The floral base contains nectar in trace amount. Female flower is quite similar to the male flower in shape, color and slightly fragrant, but is relatively larger showing a length and spread of 7.25 ± 0.19 mm and 6.02 ± 0.28 mm respectively. Sepals are free and in imbricate aestivation. The petals

are valvate, forming a small tube at the flower base. The styles and stigmas are both three and free. The average length of style was 6.46 ± 0.16 mm. The stigmas are green, darker than petals and are bifid. The ovary has three carpels. The floral base is villose, and contains five elliptical glands under the ovary. The villose flower base secretes nectar in trace amount. These findings are in concurrence with the earlier observations (5—7).

Anthesis, Anther Dehiscence and Stigma Receptivity

Anthesis started from 06.50 am and lasted up to 11.50 am i.e., forenoon pattern of anthesis was noticed and is similar to earlier observations made (5, 6). The mean time of flower opening was 09.14 ± 0.42 am. Maximum number of flowers opened between 08.30 am and 11.00 am. The same flowers used for anthesis were observed for anther dehiscence. Anther dehiscence was noticed through longitudinal slits. Anthers dehisced at the mean time of 1.28 ± 0.09 hr (0.88 minutes) after flower opening (anthesis). Anther dehiscence occurred between 07.55 am to 12.45 pm and maximum flowers dehisced during 9.30 am to 11.30 am. Researchers reported that the anthers in *J. curcas* dehisce an hour later by longitudinal slits (5). The time at which stigma become receptive and also time period between anthesis and stigma receptivity is given in the Table 1. Stigma attained receptivity between 01.20 to 2.25 hours after anthesis and remained receptive for 2—3 days. On an average, stigma becomes receptive 2.1 ± 0.12 h after anthesis. The pollen grains are yellow in colour and globular or spheroidal in shape which is in confirmation to the observations made by others (5). The estimated average number of pollen grains per flower was found to be 1601 ± 70 with 91.06 ± 2.42% fertility (6).

Mode of Pollination

Earlier studies showed that the mode of pollination in *Jatropha curcas* is out-crossing. In the present study the inflorescence were observed for fruit set per cent in different modes of pollination viz., cross pollination, self pollination and wind pollination (6). The average number and percentage of fruits produced in each mode of pollination is presented in the Table 2. In open pollination, the percentage of fruit set was 4.95 (highest among different modes) in relation to total number of flower buds established. Whereas the percentage of fruit set in self pollination and wind pollination was 3.7 and 3.28 respectively. Scientists reported that *Jatropha curcas* is predominantly cross pollinating. They also stated that seed quality depends mostly on cross-pollination in *J. curcas* but selfing is also compatible (5).

Pollinators

The flowers exhibited both entomophilous way of pollination and wind pollination. The insects associated with pollination of *Jatropha* flowers and their per cent of visits are given in Table 3. Honeybees (*Apis indica* Fabr., *A. dorsata* Fabr., and *A. florae* Fabr.) were observed as the predominant insect pollinators. A scientist reported that honeybees (*Apis dorsata, A. florea* and *A. mellifera*) were most common and effective pollinators in *J. curcas* (6). The bees mentioned above were found to move between flowers, fast, spending a small amount of time in search of more floral rewards. In the pollination of *Jatropha curcas*, *Apis indica* alone accounted 32%. The bees when they visited a flower, moved towards the location of the anthers and stigma. In doing so, they invariably contacted the stigma effecting pollination. Among bees, only long-tongued ones such as *Apis dorsata,* and *A. indica*, are found to have access to nectar. Wasps (*Sphex* sp. and *Vespa* sp.) also caused similar effect in flowers but, their visits were occasional. *Thrips* sp. being very small, moved inside the flowers and have easy access to both nectar and pollen.

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