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Floral Biology and Flowering Phenology of *Jatropha Curcas*

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ABSTRACT : *Jatropha curcas* is an oil bearing species with multiple uses and considerable economic potential as a biofuel plant. Plant flowering and breeding characteristics are important for us to understand the reproduction of plant populations. The present study describes the floral biology and flowering phenology of *J. curcas* which is a prerequisite for hybridization program for genetic improvement through conventional breeding. The plant produces flowers in dichasial inflorescences. Normally, the flowers are unisexual, and male and female flowers are produced in the same inflorescence. Only a few male flowers are produced in an inflorescence, and fruits are produced only through pollination between different flowers from the same or different plants. This study includes a description of the inflorescence, flower anatomy of both male and female flowers, female : male ratio, pollen : ovule ratio, flowering phenology, pollen viability, stigma receptivity, comparison of selfing methods and a comparison of geitonogamy and xenogamy. This information may be useful in *J. curcas* breeding programmes.

Keywords : *Jatropha curcas*, Floral biology, Flower anatomy, Flowering phenology, Crossing technique

INTRODUCTION

Global energy supply is based mainly on fossil fuels, which have many disadvantages, besides, their fast depletion. It is widely agreed that a more sustainable alternative energy source needs to be developed in near or distant future. One promising option consists of bio-fuels, which are renewable in nature and do not contribute to the green house effect (Openshaw, 2000; Mandpe et al., 2005). Many oil producing crops and plants have been considered for the purpose, among which *Jatropha curcas,* a member of Euphorbiaceae family, with several desirable attributes has evoked interest all over the tropics as a potential biofuel crop (Martin and Mayeux, 1985; Jones and Miller, 1991). Since, *J. curcas* doesn't compete with conventional crops for cultivation, dilemma of 'food versus fuel' will not arise (Fairless, 2007). *J. curcas* can also form an important component of silviculture and has medicinal / therapeutic uses too (Duke and Wain, 1991).

Genetic improvement of any crop depends on the availability of variability in that crop and variability in any crop depends mainly on the natural pollination mechanisms of that crop. The knowledge of floral biology is a prerequisite for any hybridization program for genetic improvement of that crop through conventional breeding (Wei et al., 2007). Despite the extensive *J. curcas* planting programmes world wide, little is known about its floral biology. Earlier studies made on the floral biology of *J. curcas* (Solomon Raju and Ezradanam, 2002; Bhattacharaya et al., 2005; Wei et al., 2007), concentrate mainly on insect foraging behavior on *J. curcas* and a little attention is paid to development of suitable hybridization technique or floral biology.

Keeping the economical importance of *J. curcas* and critical analysis of earlier reports, the present study mainly describes the floral biology and flowering behaviors of *J.curcas* which can be directly used in any conventional breeding program of *J. curcas*.

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Material and Methods

Study site

The present study was conducted during October, 2007 to February, 2008 (Oct-Feb is the flowering season of *J. curcas*) at Anand Agricultural University, Anand (22° 35' N and 72° 55' E with an altitude of 45.1 meters above the mean sea level). The soil of experimental site is sandy loam alluvial in origin and poor in organic matter content (Coarse sand 1.3%, fine sand 63 %, silt 15% and clay 20%).

Inflorescence and Floral morphology

Inflorescences were carefully observed for their position on the plants, type and opening of flowers. The flowers (both male and female) in an inflorescence were further observed for their position in the inflorescence, shape and size of the flower buds before and after opening and anthesis.

Female : Male ratio

It is difficult to distinguish between male and female flowers in the inflorescence at very early stages of its development and at the same time it is also difficult to count the number of flowers at very later stages of inflorescence development (after flower opening) as many flowers drop (especially male) from the inflorescence just after blooming. Hence, inflorescences of appropriate stage (just before opening of flowers) were tagged for counting the number of male and female flowers. Eight genotypes (two plants per replication, three replications) were selected from the *J. curcas* plantation for the present study. All the inflorescences on the plant were observed from initiation of the flowering to last inflorescence on the plant during the season. (Table 1)

Pollen : Ovule ratio

Undehisced mature anthers from different plants were individually immersed in a drop of water on a glass slide to release pollens from the anthers by using a pointer under a stereoscopic microscope (Carl, Zeiss). The drop was then covered with a cover slip and total number of pollens per anther was counted using a compound microscope (Olympus). The pollen grains were separately counted from both upper and lower tier of stamens of the male flowers. Based on the pollen production from both tiers of stamens, the total pollen production per flower was calculated. The pollen ovule ratio was determined by dividing the number of pollen grains per male flower by the number of ovules per female flower, which is normally three.

Phenology

100 inflorescences (before flower opening) from 10 healthy growing plants were randomly selected and tagged from the *J. curcas* plantation for the present study. Every day the selected inflorescences were observed. As the inflorescences started blooming (flower opening started), all the opened male and female flowers were counted and opened male flowers were removed.

Pollen viability and germination

The pollen grains were stained with acetocarmine (1%) to test the pollen viability (Pearson and Harney, 1984) and were observed under a compound microscope (Olympus). The number of stained and unstained pollen grains were counted separately and percent pollen viability was calculated, considering the stained pollen grains as viable.

Stigma receptivity

To study the stigma receptivity, approximately 125 inflorescences (25 each for pollination on 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} day) from healthy growing plants were selected and tagged (indicating pollination day) before flower opening. All male flowers were removed from the inflorescences and these inflorescences were then bagged to avoid natural pollination. Bags of all these inflorescences were opened

daily and observed for any opened female flowers and then placed again. Buds from approximately 25 inflorescences were pollinated on the 1st day and a white thread was tied at the base of each pollinated flower to mark it as pollinated, remaining flowers in these inflorescences were also pollinated on the day these flowers opened (1st day). Inflorescences in which pollination was to be done on the 2nd day, a coloured thread was tied on the base of opened flowers on the 1st day and a white thread was tied to these flowers after pollination on the 2nd day. Similarly, flowers were marked for pollination till 3rd, 4th and 5th days in separate inflorescences and pollinations were carried out only once in all cases. Number of fruits set was recorded from each of these selected inflorescences separately and per cent fruit set was calculated, which indicated the stigma receptivity (%).

Per cent fruit and seed set

Natural fruit set rate (%) was studied using 436 female flowers from 47 inflorescence of 39 plants. Per cent fruit set was calculated by dividing the no. of capsules or Fruit set per inflorescence by the no. of female flowers per inflorescence. Whereas, percentage seed set was calculated as no. of seeds obtained per inflorescence divided by thrice the no. of female flowers in the inflorescence (as each female flower has three ovaries).

Comparison of selfing methods

For comparison of selfing methods *viz*., only bagging and bagging of the intact inflorescences followed by manual pollination (selfing), selected inflorescences were bagged till all male flowers withered or the stigma of all female flowers in the inflorescence dried completely. Alternatively, in the second method, the inflorescences were opened daily and the opened male flowers from the same inflorescence or other inflorescence of the same plant / genotype were used for pollination. This comparison was attempted in 4 genotypes *viz*. *SKN Big*, *Urulikanchan*, *Chhatrapati* and *Hansraj* and per cent fruit set was calculated for both the methods of selfing.

Comparison of geitonogamy / xenogamy

153 female flowers from 26 plants were used for geitonogamy study and 183 female flowers from 35 plants for xenogamy study. The inflorescences were emasculated before opening of any female flower and then bagged. Pollination was done on the 1st day of the flower opening. Hand pollination of female flowers with male flowers of the same plant or genotype was done for geitonogamy and pollination of the female flowers with male flowers of another plant (not of same genotype) in case of xenogamy. Bagging of inflorescence before flower opening and after pollination was done to ensure desired pollination only. Per cent fruit set was observed in geitonogamy and xenogamy separately.

Results and Discussion

Inflorescences in J. curcas are borne terminally on branches. Flowers in the inflorescences are produced in racemose pattern in a dichasial/ biparous cyme pattern i.e., in the inflorescence, main axis ends in a flower and at the same time it produces two lateral younger flowers. The lateral and succeeding flowers develop in the same manner. The results also indicate that J. curcas is a monoecious plant, the male and female flowers are separate but are borne in the same inflorescence. Normally the inflorescence produces a central female flower surrounded by a group of male flowers (Fig. 1). However, in some cases, the position where a female flower was expected was found substituted with a male flower. Also only male flowers are produced in few inflorescences. The inflorescence takes about 1 to 1½ month from the initiation of floral bud to complete opening of the flower. The male and female flowers can be distinguished from each other only 10-15 days before flower opening (Fig. 2).

In the flower morphology of J. curcas it was observed that (i) female flowers (and buds) are relatively larger than the male flowers (and buds), (ii) the petals and sepals of

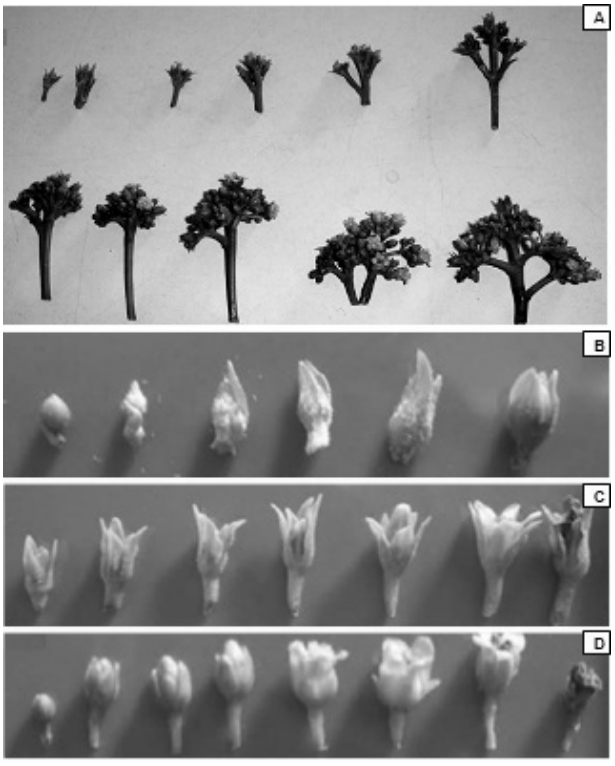

Fig. 1. Growth stages of inflorescence and flowers. A. Growth stages of an inflorescence (racemose) which bears female and male flowers both in the same inflorescence. B. Growth stages of undifferentiated flowers. C. Growth stages of female flowers. D. Growth stages of male flowers.

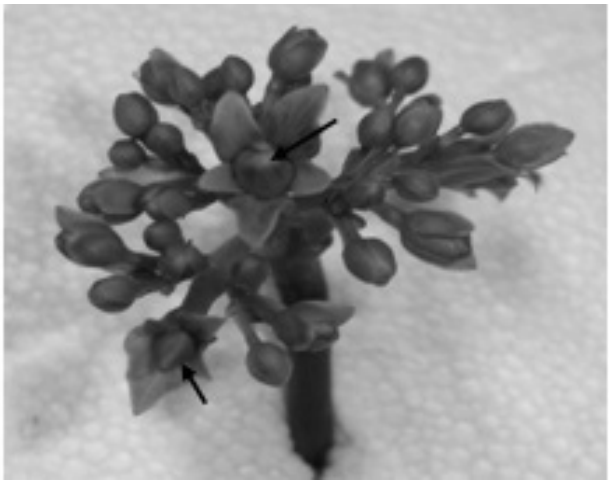

Fig. 2. Dichasial cyme (biparous) of *J. curcas*. The flowers are unisexual and in the same inflorescence. Typically female flower/bud is located in the centre (position pointed by arrows) surrounded by male flowers/buds.

the female flower are also larger as compared to those of male flower, (iii) tip of the female bud is pointed, whereas,

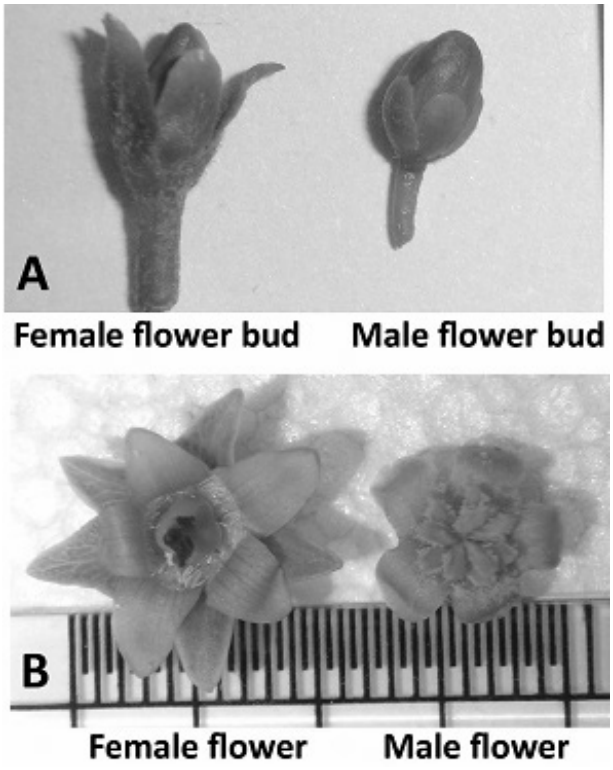

Fig. 3. External flower morphology. A. Female and male flower bud. B. Female and male flower

in male flowers it is blunt, (iv) the female flowers are centrally located in the inflorescence while the male flowers are usually surrounding the female flowers, however, the male flowers may be sometimes centrally located (Fig. 3).

Male flowers are odourless and tray shaped, sepals and petals are five each and free, petals are conivent at the flower base forming a short tube, stamens are ten, diadelphous arranged in two tiers, lower five stamens are free, while the upper tier of stamens is united. Anthers and pollen grains are yellow in colour, anthers are dithecous and dorsifixed with the filament (Fig. 4).

The female flowers also bear five sepals and five petals which form a small tube. The ovary is tricarpellary, with three styles and three bifurcated stigmas (Fig. 5).

The female flowers were found to open in synchrony with male flowers. All the opened male flowers drop off within a day, while the unpollinated / unfertilized female flowers only may fall off. Sepals and petals of fertilized flower gradually enlarge and the growing fruit reaches its full size approximately by 90 days.

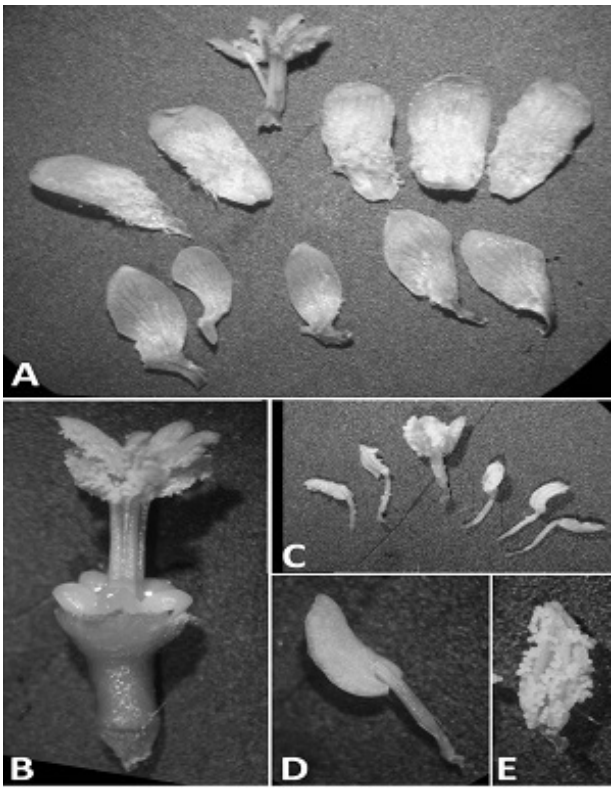

Fig. 4. Male flower anatomy of *J. curcas*. A. Dissected male flower showing-androcium (center top) with five petals (center) and calyx with five sepals (bottom). B. Close view of a male flower without petals and sepals, showing the arrangement of androcium in two tier of five stamens each. C. Diadelphous (5+5) anthers-upper five united and lower five free stamens. D. Magnified view of dithecus and dorsified undehiced anther. E. Magnified view of dehiced anther showing yellow pollen grains.

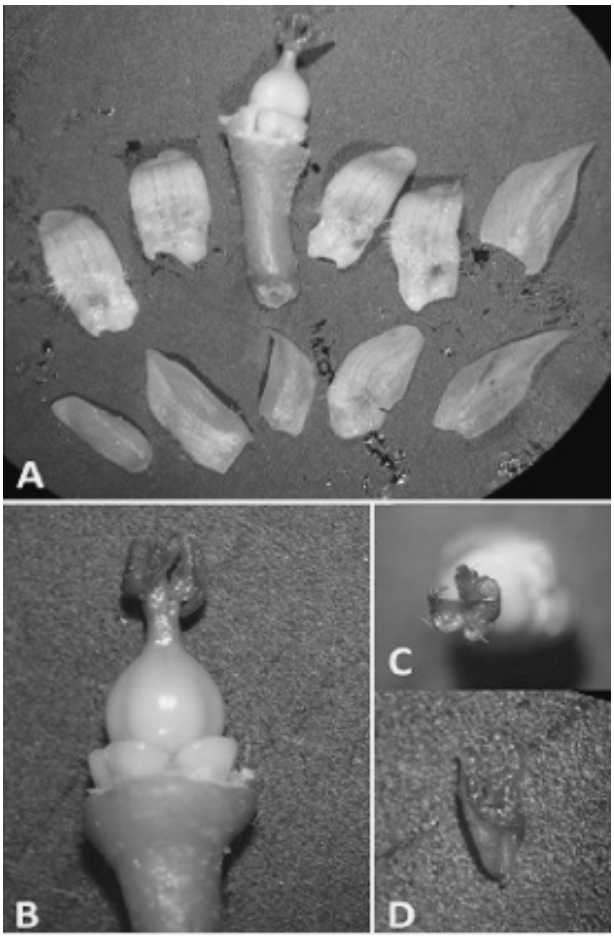

Fig. 5. Female flower anatomy of *J. curcas*. A. Dissected female flower showing - gynoecium (center top), corolla with five petals (center top) and calyx with five sepals (Bottom). B. Close view of a female flower without petals and sepals, showing the arrangement of carpel (stigma, style and tricarpellary ovary). C. Top view of the stigma (three bifurcated stigmas). D. Magnified view of a single bifurcated stigma.

It was observed that the average female : male flower ratio ranged from 1: 15.15 in CSMCRI-GUJ-Banas-1205-C2 to 1:28.12 in NBPGR-GUJ-SKN-0605-Chhatrapati. The total number of female flowers ranged from 78 in CSMCRI-GUJ-Panch-0106-C3 to 499 in NBPGR-GUJ-SKN-0605-Chhatrapati. This also indicated that the female : male ratios in J. curcas, however, are not a conservative index of yield potential, the total number of female flowers borne by the plant could be a better indication of its yielding potential (Table 1).

In the present investigation it was also observed that the pollen production in the lower tier in the stamen 360, while that in upper tier of the stamen was 370. Hence, the total pollen production per flower was 3650. Considering these values, the average pollen ovule ratio for the selected eight genotypes was 27278:1. The availability of pollens in this case does not appear to be a limiting factor in presence of natural pollinators. Flower opening was normally between 7:30 am and 10:00 a.m. Occasionally flowers may open at noon or in the afternoon, when the temperature is very low during the winter. This was uniform in all the accessions studied. Ambient temperature influences anthesis and on cold (< 15℃), overcast, or misty days flowers may not open completely the entire day or the normal time of opening may be delayed until conditions are more favorable.

Table 1. Floral character of eight genotype of *J. curcas* during flowering season (October 2007-February 2008).

Sr. No	Genotype	Number of inflorescences bloomed	Number of Female Flowers	Number of male flowers	Number of flowers	Female : Male ratio
1	CSMCRI-GUJ-Banas-1205-C1	76	390	6644	7034	1:17.04
2	CSMCRI-GUJ-Banas-1205-C2	38	177.	2681	2858	1:15.15
3	CSMCRI-GUJ-Panch-0106-C3	27	78	1975	2053	1:25.32
4	CSMCRI-OR-Ganj-1205-C5	52	186	4002	4188	1:21.52
5	NBPGR-GUJ-SKN-0605-Chhatrapati	158	499	14031	1453	1:28.12
6	NBPGR-GUJ-SKN-0605-Hansraj	105	343	8029	8372	1:23.41
7	NBPGR-GUJ-SKN-0605-SKN-Big	105	376	8245	8621	1:21.93
8	NBPGR-GUJ-SKN-0605-Urlikanchan	113	341	9182	9523	1:26.93
	Min	27	78	1975	2053	1:15.15
	Max	158	499	14031	14530	1:28.12
	Mean	84.25	298.75	6848.63	7147.38	1:22.42

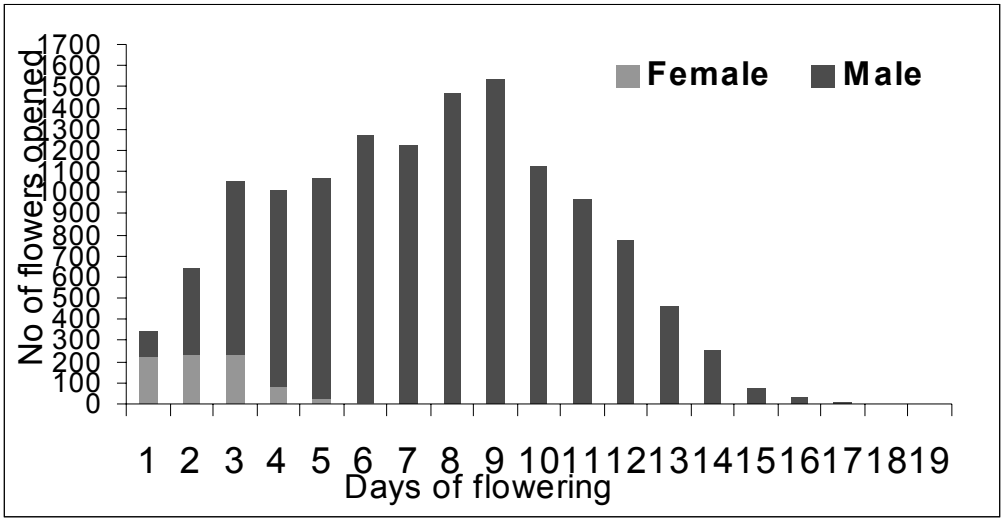

Fig. 6. Phenology of flower opening in inflorescences of *J. curcas*

The flowering phenology observations from the selected inflorescences indicated that flower opening was continuous once blooming started. The flowering duration ranged from 6 to 19 days with an average of 13.24 days. Male and female flowers both bloomed on 1st day of inflorescence blooming. More number of female flowers opened than male flowers on 1st day of inflorescence blooming and the female flowers opened from the 1st to 7th day of inflorescence blooming with maximum frequency on 2nd day. The male flowers however continued opening from 1st to 17th day of inflorescence blooming with maximum frequency on 9th day. Flowers of both sexes open synchronously (Fig. 6). The male flowers continue to bloom even after the female flower blooming in an inflorescence is completed, favouring conditions for cross pollination.

The pollen viability from the freshly collected pollens indicated more than 87.6% viability. No significant differences were observed in viability of pollens from the upper and lower tier of anthers. Stigma receptivity was found maximum on 1st day of flower opening (82.9% fruit set). However, it remained receptive on 2nd, 3rd, and 4th day of flower opening with 66.7%, 78.9%, and 41.1% fruit set, respectively. Stigma was found completely withered on 5th day. Highest fruit set was observed in flowers

Table 2. Stigma receptivity of *J. curcas* based on percent fruit set.

Days of Pollination	Total no. of female flowers pollinated	No. of capsules formed	Fruit set (%)
1st day	111	92	82.88
2nd day	102	68	66.67
3rd day	166	131	78.92
4th day	128	53	41.41
5th day	Stigma completely withered		

Total 125 inflorescences were taken for this study (25 for each treatment).

Table 3. Comparison of selfing techniques based on per cent fruit (capsule) set in four genotypes.

Genotype	Selfing technique			
	Only bagging		Bagging + Manual pollination	
	Total buds	no. of capsule set	Total buds	no. of capsule set
SKN Big	108	56	61	50
	51.85%		81.96%	
	Cal 't' @32df=2.68 *			
Chhatrapati	74	38	63	40
	51.35%		63.49%	
	Cal 't' @28df=1.62 NS			
Hansraj	72	42	55	42
	58.33 %		76.36 %	
	Cal 't' @27df=2.52 *			
Urulikanchan	56	31	47	40
	55.35 %		85.11 %	
	Cal 't' @20df=2.65 *			

* Significant at 5% probability level (F test); NS, not significant.

Table 4. Comparison of geitonogamy and xenogamy based on percent fruit (capsule) set.

Geitonogamy			Xenogamy		
Number of flowers selfed	Number of fruits set	Fruit set (%)	Number of flowers crossed	Number of fruits set	Fruit set (%)
153	62	40.52	183	90	49.18

pollinated on the 1st day (Table 2).

The observation on the fruiting behaviour indicated that the natural fruit set varied from 37.50% to 100.00% with an average of 87.61% and the average seed set percentage ranged from 54.78 to 92.14% with an average of 78.93%. Hand pollination gave higher seed set as compared to selfing in all four genotypes. Differences were significant in SKN Big, Hansraj & Urulikanchan but were non-significant in Chhatrapati only (Table 3). It was also observed that pollination with the same genotype and well as with a different genotype, both reduced the per cent fruit set as compared to the natural fruit set. However, higher fruit set was observed in case of xenogamy. The findings reveal that both the systems functional in J. curcas, with xenogamy being predominant (Table 4).

Overall the present study indicates that J. curcas is a monoecious plant, the male and female flowers are separate but are borne in the same inflorescence. The flowering phenology showed that female flowers open in the initial phases of the inflorescence blooming in synchrony with

the male flowers however the later continue to open long after all female flowers in the inflorescence are pollinated. The male flowers drop off very early after opening but the centrally located female flowers have a thick peduncle and it continues to get nourishment for fruit development. A comparison of xenogamy and geitonogamy indicated that xenogamy is predominant and this behavior favours cross pollination in J. curcas. The results obtained in this study are in general in concurrence with the reported results (Solomon Raju and Ezradanam, 2002). While selecting the plants for higher yielding potential, the total number of female flowers borne by the plant may be given more weightage rather than selecting plants with better female : male ratio and number of inflorescences borne by the plant. There is scanty information in literature on most of the aspects of floral biology of J. curcas like percent fruit and seed set, pollen viability and stigma receptivity even in the unpublished literature. Further studies are needed to clearly elucidate the role of weather and genetics on the number of female flowers produced in an inflorescence, pollen viability and pollen germination.

In conclusion, present study has done complete floral biology and flowering phenology of J. curcas which is useful for any hybridization programs for genetic improvement through conventional breeding method.

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