

## Natural Variability in Some Functional Traits of Hermaphrodite *Jatropha*

Aruna R Prakash\*, Ravi Prakash CH, Sarnam Singh, Arup Ghosh and Pradeep K Agarwal

Department of Plant Omics, CSIR-Central Salt and Marine Chemicals Research Institute, GB Marg, Bhavnagar, Gujarat, India

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#### \*For Correspondence

Aruna R Prakash,  
Department of Plant Omics,  
CSIR-Central Salt and Marine  
Chemicals Research Institute,  
GB Marg, Bhavnagar-364002,  
Gujarat, India. Tel: +91 9909941408.

**E-mail:** arunaprakash@csmcri.res.in

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#### ABSTRACT

To assess the genotypic and phenotypic diversity present in *Jatropha curcas*, a large number of Candidate Plus Trees (CPTs) were collected from various locations of different agricultural zones of India. *J. curcas* is normally a monoecious with inflorescence of unisexual flowers but one CPT from the existing pool showed hermaphroditic nature bearing bisexual flowers. Hermaphrodite plants showed significant increase in various functional traits like plant height, number of branches, leaf length, leaf width, leaf thickness, fresh weight of leaf, dry weight of leaf, leaf dry mass content, guard cell length, chlorophyll contents, stomatal conductance, net photosynthetic rate and leaf transpiration rate as compared to normal plants. Based on the results obtained from the present study, it is suggested that hermaphrodite plants may be more useful for production of increased biomass as compared to monoecious plants of *J. curcas*. Flow cytometry analysis revealed that genome size of plants of hermaphrodite was higher than monoecious *Jatropha* and the ratio was close to 2:3 of monoecious and hermaphrodite plants.

### INTRODUCTION

*Jatropha curcas* L. is getting global priority and gaining attention in the recent past due to biodiesel production, soil quality improvement and soil carbon sequestration. Apart from its seed oil as a source of bio-diesel, other plant parts also have several industrial and medicinal importances<sup>[1-3]</sup>. In addition, plants of *J. curcas* are widely planted in the tropics as a 'living fence' around fields as well as for reforestation of eroded wasteland by improving the soil quality of such lands<sup>[4-7]</sup>. On the other hand, if *Jatropha* is cultivated on arable lands, it does not interfere other crops which are grown for food production<sup>[8-10]</sup>. In addition to this, being an excellent and eco-friendly renewable energy source of biodiesel *Jatropha* exhibits more biodegradable biomass as compared to other identified non-edible oil producing plants viz, *Madhuca logifolia*, *Madhuca indica*, *Moringa oleifera*, *Ricinus communis*, *Pongamia pinnata* etc.<sup>[11,12]</sup>.

Generally, most of the plants have a natural survival instinct to ensure successful reproduction and continuation of the species. Sometimes when the growing conditions become harsh, their survival mechanism triggers in response. Under such conditions, plants will produce inflorescences with male and female flowers so that they can self-pollinate and ensure their survival. Sometimes, plant will produce flowers consisting of both the sexes simultaneously resulting in hermaphroditism.

*J. curcas* is predominantly monoecious producing both male and female flowers on the same plant but hermaphrodite flowers bearing plants also occur occasionally<sup>[13]</sup>. The general type of inflorescence of *J. curcas* (monoecious) is actually unlimited compound i.e., racemose with a dichasial branch pattern, where female flowers are located in the center surrounded by male flowers<sup>[14]</sup>. In monoecious *Jatropha*, there are about five female flowers in one inflorescence<sup>[15]</sup>. The ratio of female to male flowers varies from 1:20<sup>[16]</sup> to 1:29<sup>[15]</sup>. The low number of female flowers in monoecious *J. curcas* limits its fruit production that results not only in low seed production but also causes lack of seed continuity.

Although several studies on biology of monoecious *J. curcas* have been conducted including variability among genotypes

[17,18], characteristics of its reproductive biology [19], and breeding system [20] but any broad study related to morphology, physiology and genetic constituents of hermaphrodite *Jatropha* plant has not been reported so far.

Incidentally, during one of the study conducted at our experimental field, a large number of candidate plus trees (CPTs) were collected from various locations of different agricultural zones of India to assess the existing genotypic and phenotypic diversity present in *Jatropha curcas*. Out of these CPTs, one CPT was identified as hermaphrodite that showed a remarkable difference in its growth and morphological traits over the monoecious *J. curcas*. The present study concerned with the detailed analysis of monoecious and hermaphrodite *J. curcas* that display different floral types with considerable variations in morphological as well as physiological traits.

## MATERIALS AND METHODS

### Plant Materials and Experimental Site Location

*T. cacao* seeds from mature pods of ♀SNK64 × ♂UPA143 obtained by manual pollination at Barombi Kang (South-West Region, Cameroon) seeding farm were used to set up a nursery. Leaves of four months old plants of full sib progeny from ♀SNK64 × ♂UPA143 (of the above nursery) were used for leaf disc test, total and individual flavones families' analysis.

### Agronomical Practices and Study of Plant Characters

The planting density was 1111 plants ha<sup>-1</sup> (3 m × 3 m planting distance). Normal agronomical practices were followed during the transplanting and establishment in field. For the present study, five plants of six years old were randomly selected from each three replications of monoecious and hermaphrodite field for morphological observations, Scanning Electron Microscopy (SEM) studies, physiological estimations and Flow Cytometry Analysis (FCA).

### Morphological Characters

Important morphological traits were selected for phenotypic assessment and their variability estimations for monoecious and hermaphrodite types of candidate plus trees (CPTs). Observations were taken on plant height, number of branches, canopy circumference, leaf area, leaf length, leaf width, leaf thickness, fresh leaf weight, dry leaf weight, leaf dry mass content (LDMC), specific leaf area (SLA), stomata density, stomata frequency, stomata index, guard cell length, guard cell width, epidermal cell frequency and epidermal cell density.

Ten mature leaves (two from each plant) of almost similar age, stage and size were taken from each replication for recording of leaf characters. Leaf area was recorded using portable leaf area meter CI-202 (CID, INC. USA). Specific leaf area (SLA i.e., the ratio of leaf area to leaf dry mass) and leaf dry mass content (LDMC i.e., the ratio of leaf dry mass to fresh mass) were calculated by the standard protocol [21]. Stomatal density was calculated as the number of stomata per unit area and stomatal index was calculated as ratio between the number of stomata in a given area and the total number of stomata and other epidermal cells in the same area.

### Physiological Characters

Fully expanded 5<sup>th</sup> leaf from the top of randomly selected 5 plants in three replications was collected from both monoecious and hermaphrodite types of plants to estimate the chlorophyll contents [22]. Leaves were washed with distilled water to remove leaf adhered impurities and 1.0 g leaf material was grounded in liquid nitrogen using a mortar and pestle. The chlorophyll pigments were extracted and re-extracted with 80% chilled acetone in dark. The homogenates were centrifuged at 3000 rpm at 4 °C for 3 minutes and the absorbance was read at wavelengths 645 nm, 652 nm and 663 nm in Epoch Micro-Plate Spectrophotometer (BioTek, US).

Leaf gas exchange parameters namely net photosynthesis rate ( $P_N$ ), stomata conductance ( $g_s$ ), and transpiration rate ( $T_r$ ) were recorded from a total of 10 leaves of same age from 5 selected plants (two leaves from each plant) in three replications of monoecious and hermaphrodite plants using a Portable Licor XT-6400 (Licor Instruments, USA). All the measurements were taken between 10.30 A.M. and 12.30 A.M. under cloud free conditions. Water use efficiency (WUE) was calculated as net productivity or net photosynthetic rate by per unit water transpiration rate ( $P_N/T_r$ ). Intrinsic water use efficiency was estimated as the ratio of net photosynthesis rate to stomatal conductance ( $P_N/g_s$ ). Analysis of variance (ANOVA) of replicated data for morphological, physiological and anatomical characters was recorded by the standard procedures [23].

### Flow Cytometry Analysis

Flow cytometry analysis was performed as per the methods outlined by earlier researchers [24,25]. Approximately, 50 g mixed leaf samples from both types of plants were chopped with sharp razor blade to liberate intact nuclei into Tris - MgCl<sub>2</sub> buffer, pH 7.5 (0.2 M Tris, 4 mM MgCl<sub>2</sub>·6H<sub>2</sub>O, 0.5% v/v TritonX 100, 0.3% β-mercaptoethanol and 3.0% PVP). After incubating the samples for 30 minutes at 4 °C in dark, samples were passed through a 50 μm nylon filter. The intact nuclei were stained in dark at 4 °C for 6 hours with 1.4 ml of 4, 6-diamidino 2-phenylindole (DAPI). The stained intact nuclei were analyzed on a Partech PA-II Flow Cytometer (Partech, Munster, Germany). Chicken red blood cells (CRBC) were used as a reference standard to develop internal standards of *J. curcas* which in turn served for the identification of ploidy level or absolute genome size. An internal standard

for diploid was developed from a monoecious *Jatropha* plant. In order to identify the genome size difference in hermaphrodite *Jatropha*, the Gaussian Peak of a diploid *J. curcas* was assigned channel-100.

**Scanning Electron Microscopy (SEM)**

For Scanning Electron Microscopy (SEM) study of leaf epidermal cells and stomata, ethanol fixed small sections (approximately 6 mm diameter) of mature leaf pieces were prepared from 5 leaves of each three replication of both plant types [26] and the images were obtained on SEM instrument (Model: LEO 1430 VP). Stomata density, stomata frequency, stomata index (%), epidermal cell frequency and epidermal cell density were calculated as per the method outlined by few researchers [27]. Flower details were investigated by light microscopy and Scanning Electron Microscopy (SEM).

**RESULTS**

The initial identification of hermaphrodite was made from visual observations on *Jatropha curcas* plants. From the existing germplasm pool of *Jatropha*, a morphologically and reproductively dissimilar plant population was observed among monoecious CPTs. These dissimilar plants have some gain characters like expanded leaves, dark green canopy, profusely branched stem and hermaphrodite flowers over monoecious plant (Table 1).

**Table 1.** Sources and geographical location of different genotypes of *Jatropha curcas*.

S.N.	Candidate plus trees	Source of location	Latitude	Longitude
1	Ranpur (IC-565730)	Ranpur, Banaskantha, Gujarat	24° 17'56.74"N	72° 55'40.90"E
2	PCM (IC-565731)	Panchmahal, Gujarat	22° 50'54.74"N	73° 55'2.28"E
3	Chikhali (IC-565732)	Chikhla, Banaskantha, Gujarat	24° 19'33.71"N	72° 53'31.69"E
4	Shyamlaaji (IC-565733)	Shyamlaaji, Gujarat	23° 41'38.65"N	73° 22'42.28"E
5	KGR (IC-565734)	Banaskantha, Gujarat	24° 17'53.23"N	72° 44'44.65"E
6	CP-9 (IC-565735)	Gopalpur, Ganjam, Odisha	19° 27'18.43"N	85° 2'3.09"E
7	CP-13	Humma, Ganjam, Odisha	19° 26'28.25"N	85° 4'10.74"E
8	CP-17	Berhampur, Odisha	19° 17'46.72"N	85° 52'51.61"E
9	Akola (IC-565738)	Akola, Maharashtra	20° 42'31.92"N	77° 2'57.92"E
10	Zanjmer (IC-565739)	Zanjmer, Bhavnagar, Gujarat	21° 11'36.36"N	72° 3'46.37"E
11	CSM-11	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
12	CSM-12	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
13	CSM-13	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
14	CSM-14	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
15	CSM-15	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
16	CSM-16	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
17	CSM-17	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
18	CSM-18	Dantiwada, Gujarat	24° 19'28.61"N	72° 19'37.60"E
19	CSM-19	Koteshwar-F*, Kutch, Gujarat	24° 30'53.59"N	72° 52'56.79"E
20	CSM-20	Ambaji-F*, Gujarat	24° 18'42.22"N	72° 50'30.56"E
21	CSM-21	Panchmahal-F*, Gujarat	22° 48'11.72"N	73° 34'26.67"E
22	CSM-22	Ranpur-F*, Gujarat	22° 19'18.32"N	71° 44'6.47"E
23	CSM-23	Kumbhariya-F*, Gujarat	24° 19'43.69"N	72° 51'52.14"E

F\* genotypes collected from forest location.

**Morphological Variability**

Growth data obtained in the present study showed differences in basic architecture and morphological dissimilarity between monoecious and hermaphrodite *J. curcas*. It was further observed that hermaphrodite plants showed better establishment and vigorous growth rate than normal (monoecious) *J. curcas* plants. Plant height, number of branches plant<sup>-1</sup> and plant canopy circumference were significantly higher (p<0.001) in hermaphrodite plants (Table 2).

**Table 2.** Growth and leaf characters in monoecious and hermaphrodite *J. curcas* plants.

Traits	Plant Type	
	Monoecious	Hermaphrodite
Plant height (cm)	275.28 ± 2.67 (250.3-295)	294.0*** ± 2.59 (268.3-313)
Number of branches plant <sup>-1</sup>	50.5 ± 1.42 (40-57)	63.0*** ± 1.63 (52-75)
Plant canopy circumference (cm)	288.30 ± 5.26 (250.3-295)	333.83*** ± 6.16 (268.3-313.0)
Leaf area (cm <sup>2</sup> )	110.68 ± 1.85 (104.36-115.36)	161.72** ± 2.08 (155.50-168.00)
Leaf length (cm)	10.93 ± 0.21 (10.00-13.00)	12.67** ± 0.21 (11.00-14.00)
Leaf width (cm)	11.80 ± 0.41 (10.00-13.50)	13.88** ± 0.41 (13.20-15.50)
Leaf thickness (LT) (µm)	171.66 ± 1.31 (166.73-174.41)	175.64* ± 1.02 (172.87-178.13)

Fresh leaf weight (g)	1.90 ± 0.08 (1.74-1.99)	2.84** ± 0.08 (2.70-2.99)
Dry Leaf weight (mg)	356.03 ± 8.14 (325.85-370.25)	542.83** ± 10.18 (515.48-575.58)
Leaf Dry Mass Content (LDMC) mg g <sup>-1</sup>	187.31 ± 0.35 (186.06-188.14)	191.05** ± 0.44 (190.00-192.34)
Specific Leaf Area (SLA) (m <sup>2</sup> kg <sup>-1</sup> )	31.11* ± 0.26 (30.54-32.03)	29.81 ± 0.19 (29.19-30.17)

(Mean ± SE), SE-Standard error, \*: significance ≤ 0.05, \*\*: significance ≤ 0.01, \*\*\*: significance ≤ 0.001. Ranges of traits in bracket are given below the mean ± SE.

The leaf size, leaf structure, leaf colour and leaf margin were differed in hermaphrodite as compared to monoecious plants. The floral and fruit morphology of monoecious and hermaphrodite plants are presented in **(Figure 1)**. The inflorescence bears large number of flowers (25-200) with dichasial cyme in both monoecious as well as hermaphrodite plants. However, hermaphrodite flowers were slightly bigger and larger (ca.2.5 cm) when fully expanded as compared to monoecious female flowers (ca. 2.0 cm). In monoecious *J. curcas* male and female flowers occur separately within the same inflorescences is a prominent feature.



**Figure 1.** Plant habit, flowers and fruit of monoecious and hermaphrodite *Jatropha curcas* (a) Monoecious plant with penta-lobed leaves (b) Hermaphrodite plant with tri-lobed leaves (c) Inflorescence showing male and female flowers separately (d) Inflorescence with hermaphrodite flowers (e) Single female flower showing pistils (f) Close up of hermaphrodite flower with androecium and gynoecium (g) L.S. Of fruit with fully developed seeds (h) L.S. of fruits with undeveloped seeds.

### Physiological Variability

Physiological parameters were measured to observe the variations for physiological traits in monoecious and hermaphrodite plants **(Table 3)**. Results revealed that the physiological traits in both monoecious and hermaphrodite plants of *J. curcas* varied considerably. The length of guard cells in hermaphrodite plant leaves was found to be more. Total chlorophyll, chlorophyll-a and chlorophyll-b were significantly higher ( $p < 0.05$ ) in hermaphrodite leaves. Total chlorophyll in hermaphrodites ranged between 1.90 mg g<sup>-1</sup> to 2.30 mg g<sup>-1</sup> with a mean of 2.13 ± 0.03 mg g<sup>-1</sup> as compared to the range between 1.82 mg g<sup>-1</sup> to 2.15 mg g<sup>-1</sup> in monoecious plants (mean of 2.05 ± 0.02 mg g<sup>-1</sup>).

**Table 3.** Physiological parameters in monoecious and hermaphrodite *J. curcas* plants.

Traits	Monoecious Hermaphrodite	
Total chlorophyll (mg g <sup>-1</sup> )	2.05 ± 0.02 (1.82-2.15)	2.13* ± 0.03 (1.90-2.30)
Chlorophyll a (mg g <sup>-1</sup> )	1.61 ± 0.02 (1.41-1.75)	1.66* ± 0.02 (1.52-1.81)
Chlorophyll b (mg g <sup>-1</sup> )	0.44 ± 0.01 (0.40-0.51)	0.47* ± 0.01 (0.35-0.52)
Net photosynthesis rate (P <sub>N</sub> )-(μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> )	15.8 ± 0.16 (14.7-16.8)	18.22*** ± 0.16 (15.10-19.5)
Stomatal conductance (g <sub>s</sub> ) (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	0.27 ± 0.01 (0.20-0.30)	0.32** ± 0.01 (0.30-0.40)
Leaf transpiration rate (T <sub>r</sub> ) (mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> )	4.59 ± 0.11 (4.10-5.22)	5.56** ± 0.10 (4.6-6.8)
Water use efficiency (WUE=P <sub>N</sub> /T <sub>r</sub> ) (μmol CO <sub>2</sub> /mmol H <sub>2</sub> O)	2.02 ± 0.06 (1.65-2.31)	1.93 ± 0.02 (1.85-2.03)
Intrinsic water use efficiency (IWUE=P <sub>N</sub> /g <sub>s</sub> ) (mmol CO <sub>2</sub> mol <sup>-1</sup> H <sub>2</sub> O)	51.30 ± 0.60 (49.14-54.97)	49.17 ± 1.26 (39.48-53.45)

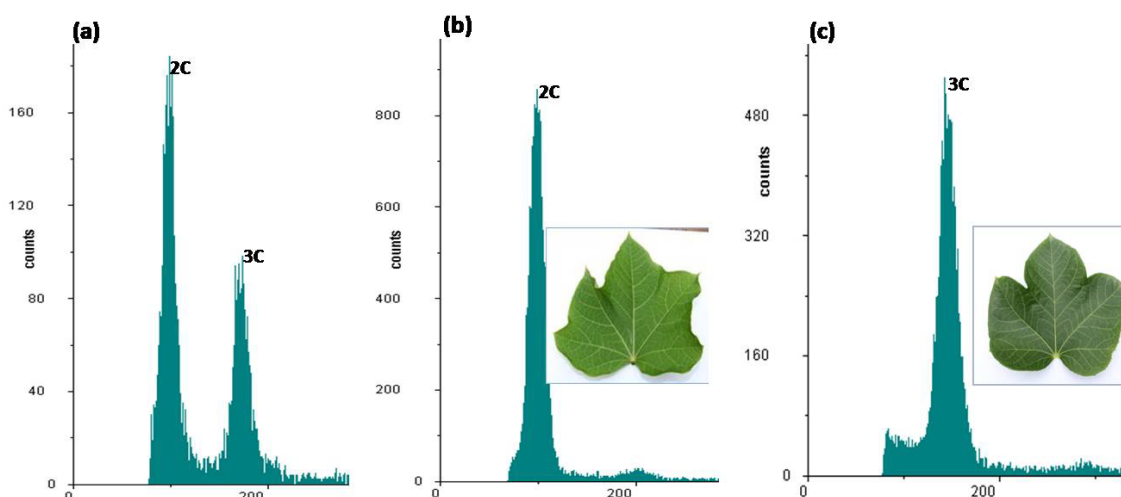
(Mean ± SE), SE-Standard error, \*: significance ≤ 0.05, \*\*: significance ≤ 0.01, \*\*\*: significance ≤ 0.001.  
Ranges of traits in bracket are given below the mean ± SE.

In hermaphrodite plants, the average chlorophyll-a and chlorophyll-b contents were observed as 1.66 ± 0.02 mg g<sup>-1</sup> and 0.47 ± 0.01 mg g<sup>-1</sup> respectively while in monoecious plants, chlorophyll-a and chlorophyll-b contents were 1.61 ± 0.02 mg g<sup>-1</sup> and 0.44 ± 0.01 mg g<sup>-1</sup> respectively. Net photosynthetic rate, stomatal conductance and leaf transpiration rates were also significantly higher in hermaphrodite plants. Average Net photosynthetic rate was 18.22 ± 0.16 μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> in hermaphrodites and 15.8 ± 0.16 μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup> in monoecious plants. Though water use efficiency and intrinsic water use efficiency was slightly more in hermaphrodites but not significantly higher than monoecious plants.

### Genome Size Variability

After undertaking morphological and physiological studies, distinct changes were found in monoecious and hermaphrodite plants. In order to differentiate the plants at ploidy level, monoecious and hermaphrodite flowers bearing plants were subjected to a basic study on ploidy and genome size status through flow cytometry application. As the nuclear DNA content reflects the ploidy of a cell, estimation of DNA content is frequently used for ploidy determination.

Ploidy level was determined for *Jatropha* plants. High-resolution flow cytometry of nuclear DNA was performed on macerated plant tissue. Based on genome size, all plants bearing monoecious flowers were diploid (2n=22) with the exception of hermaphrodite ones which were proved to be triploid (3n=33). The genome size of hermaphrodite plants was found higher than the monoecious plants and DNA ratio was almost close to 2:3 ratio of monoecious (2C) and hermaphrodite (3C) confirming that hermaphrodite plants were triploid in nature (**Figure 2**). It is evident from the figure that the comparison of relative positions of G<sub>1</sub> peaks corresponding to the sample nuclei and the nuclei isolated from a plant with known DNA content permit accurate determination of the unknown DNA content.



**Figure 2.** Flow cytometry analysis of monoecious and hermaphrodite plants (a) Confirmation analysis of hermaphrodite with more genome size (3C) *J. curcas* along with diploid monoecious internal standard-peaks distributed at channel 100 and 165 channels (b) Flow crypto graph showing distribution of 2C Gaussian peak for monoecious plant at channel 100 (c) Flow cytograph showing 3C peak for hermaphrodite plant at channel 148.

### Anatomical Variability

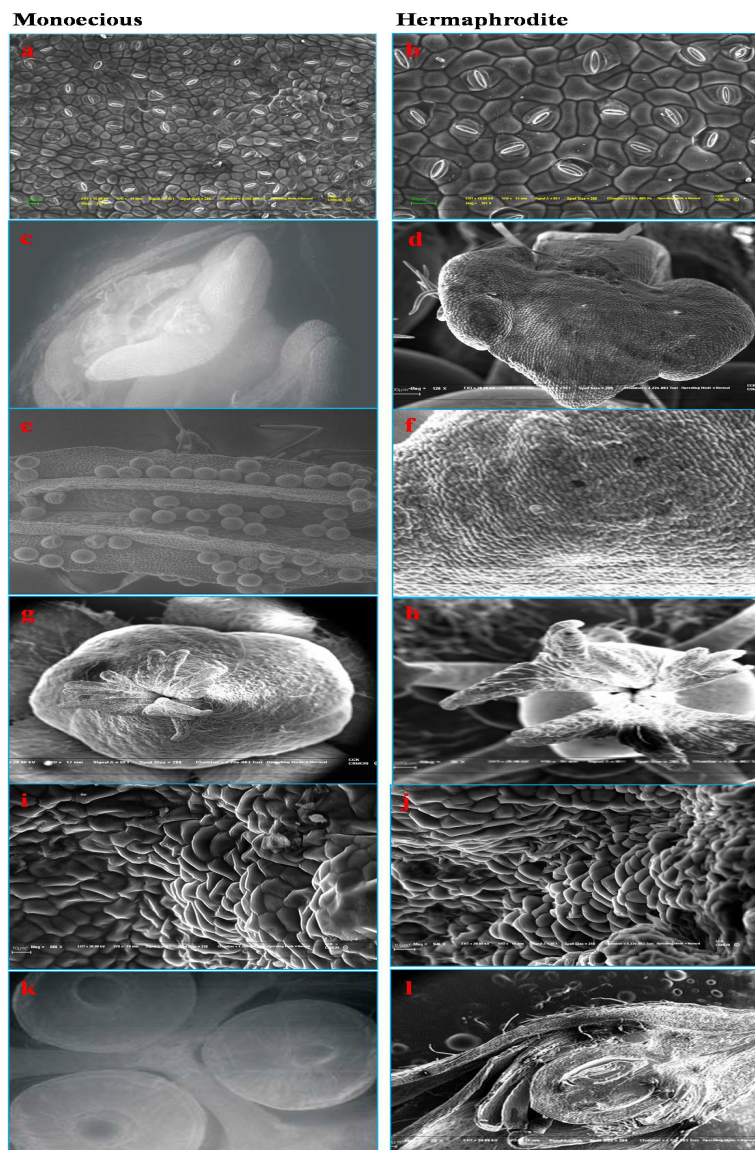
SEM images of leaves and reproductive structures were obtained from monoecious and hermaphrodite *J. curcas* (**Figure 3**). The leaf epidermal cells showed pentagonal to polygonal types and were compactly arranged in both monoecious as well as hermaphrodite plants. The epidermal cells in hermaphrodite plants were seen slightly larger in size. Stomata were

anomocytic and anomotetracytic in both the types of plant leaves. In per unit area, hermaphrodite plant leaves have lower stomata density (0.516) and stomata frequency (8.06) but higher stomata index (5.40) and guard cell length (29.17  $\mu\text{m}$ ). In contrast to this, monoecious plants showed higher stomata density (0.552); stomata frequency (8.62) and guard cell width (8.31  $\mu\text{m}$ ). The mean epidermal cell frequency and stomata density were found to be more in monoecious plants when compared to hermaphrodite *J. curcas* (Table 4).

**Table 4.** Scanning electron microscopy of leaf and stomatal analysis of monoecious and hermaphrodite plants of *Jatropha curcas*.

Leaf traits	Monoecious plants	Hermaphrodite plants
Stomata density	0.552 $\pm$ 0.004 (0.51-0.53)	0.516 $\pm$ 0.004 (0.51-0.53)
Stomata frequency	8.62 $\pm$ 0.26 (8.1-9.4)	8.06 $\pm$ 0.01 (7.9-8.12)
Stomata index (%)	5.31 $\pm$ 0.11 (5.31-6.21)	5.40 $\pm$ 0.05 (5.40-5.71)
Guard cell length ( $\mu\text{m}$ )	19.02 $\pm$ 0.32 (17.0-20.8)	29.17** $\pm$ 0.45 26.4-30.7
Guard cell width ( $\mu\text{m}$ )	8.31** $\pm$ 0.31 (7.17-10.7)	7.59 $\pm$ 0.14 (7.05-8.18)
Epidermal cell frequency	142.36 $\pm$ 1.95 (135.4-147.0)	139.80 $\pm$ 1.69 (134.0-144.20)
Epidermal cell density	9.12 $\pm$ 0.125 (8.68-9.42)	8.96 $\pm$ 0.11 (8.59-9.24)

(Mean  $\pm$  SE), SE-Standard error, \*\*: significance  $\leq$  0.01  
Ranges of traits in bracket are given below the mean  $\pm$  SE.



**Figure 3.** Scanning electronic micrographs of monoecious and hermaphrodite plants (a) Epidermal cells showing stomatal distribution in monoecious plant leaf (b) Epidermal cells showing stomatal distribution in hermaphrodite plant leaf (c) Anther with pollen sacs (d) Aerial view of anther with intact pollen sacs (e) Anther dehiscence releasing pollen grains (f) Anther showing undeveloped pollen sacs (g) Pistil with trifid stigma from female flower (h) Pistil along with stamen in a hermaphrodite flower (i) Unisexual flower stigma-cells are elongated and loosely packed (j) Bisexual flower stigma-cells are compactly arranged (k) Tri-locular ovary showing three well developed ovules (l) Fruit showing undeveloped ovule.

In hermaphroditic flowers, stamens were situated below the long styles and short in length with small anthers arranged in one tier where as in monoecious plants, the anthers were arranged in two tiers with opened pollen sacs. Sepals and petals of hermaphrodite flowers were larger as compared to unisexual flowers. Anthers with developed pollen sacs were observed in monoecious and the anther dehiscence and the pollen grains coming out from were seen while anther dehiscence was not observed in hermaphrodite flowers as the flowers were self-sterile.

The stigmas of monoecious as well as hermaphrodite plants were trifid with distinctly divided lobes and large styles (**Figure 3**). However, the cells of hermaphrodite flower were tightly bound as compared to the female flower monoecious plants. The under developed ovules were observed in ovaries of hermaphrodite fruits. Fruits developed from both the types of flowers were same in size, ellipsoid and tri-lobed but the seed set was not observed in hermaphrodite plants.

## DISCUSSION

In present study hermaphrodite plants showed that all flowers are hermaphroditic nature (male and female characters in the same flower) however, no complete fruiting was observed. The plant did show fruit bearing but the seeds are underdeveloped as a characteristic of true hermaphrodites. For understanding the developmental issues hermaphrodite *J. curcas* is non-functional sterile rudiment of sex organs of the inappropriate gender.

The morphology of hermaphrodite flowers can be viewed as a result of modifications within a multifaceted developmental framework and loss of reproductive organ function may be due to the maturation status of individual sex organs and also irregularities in the fruit development due to the presence of uneven number of chromosome sets, meiotic irregularities and high frequency of aneuploidy.

The change in morphological characters to be bigger size as consequent of polyploidy was also well known in earlier studies [28-30]. Ploidy level affects many morphological and fitness traits, including stomatal size, flower size and seed weight in *Arabidopsis thaliana* (L.) as reported earlier by researchers [31].

The architectural position of the stomata and the epidermal cells on leaf surface is an important factor which determines the rates of photosynthesis and water use efficiency in plant systems. In the present study, observations have clearly showed the epidermal cells per unit area and their frequency was much lower in hermaphrodite plants when compared to the monoecious plants of *J. curcas*. The length of the guard cells in hermaphrodite plant leaves was found to be more. The length of the guard cells has a good indicator for the possible identification of increased ploidy in the plants as reported by earlier researchers [32-34]. A lot of variations in physiological traits were observed in same age plants of different *J. curcas* genotypes when growing in different or same agro climatic zone and showed different canopy spread (canopy volume) of the plant [16,35].

The higher rates of growth and increased physiological traits that has been observed in the present study can be attributed to large size of stomata with improved rates of photosynthesis which in turn increased growth rate and biomass production as reported by some earlier researchers [36-39]. Adaptation of a plant species to an environment, its growth and yield strategies depends upon the rates of photosynthesis, transpiration and stomatal conductance [40]. The previous studies reveal that genome size and ploidy influence plant biomass [41,42].

Earlier researchers observed that the hermaphrodite plants occur relatively commonly in wild plant populations as a consequence of production of unreduced diploid gametes [43]. This was also stated that hermaphrodite individuals have low levels of fertility caused by chromosome-pairing irregularities at meiosis and most gametes produced by a triploid individual are likely to be haploid [44].

Increase in certain morphological as well as physiological traits recorded in our study are the results of a consequence of increase in genome size or ploidy level also suggested by authors [45]. Similar observations were made from other plant species like *Populus* species, *Citrus clementine* and *Salix* species [34,46,47].

The present study finds that hermaphrodite plants of *Jatropha* have enormous plant growth with increased genome size and plant physiology. The high plant growth characters would be helping to diminish global warming gases and will make more greenish wasteland field. Furthermore, the hermaphrodite plants may use as ornamental, road fences, fuels and development of hexaploids.

## CONCLUSION

In the past few decades many polyploid ornamental flowers and fruit trees have gained a lot of commercial value. More height, increased number of branches and enhanced levels of photosynthesis that has been observed in the hermaphrodite genotype from same age group of *J. curcas* can be attributed to the polyploidy nature of the plant. Even with drawback on yield, long-term studies on flowering period, pollen formation, fruit abortiveness and undertaking crossing experiments with normal diploid plants after doubling their chromosomes artificially with Colchicine, will produce improved varieties for higher economic yields.

Besides the hermaphrodite accession of *J. curcas* would be valuable alternatives as bio-shield for protection from wild animals, landscape developments and for the induction of hexaploids through modern biotechnological tools i.e., induction of

micro nuclei and micro protoplast fusion techniques<sup>[48]</sup>, which in turn can yield seeds with increased size and seed oil content for the production of biodiesel in future.

This indicated that hermaphrodite *Jatropha* will also be additional useful in live fence, reforestation of eroded wastelands, mitigation of global warming and medicinal value and thus raising of hermaphrodite plantation is highly economical and eco-friendly and it can be easily propagated through stem cuttings. The results obtained from this investigation will serve as preliminary information and important clues on hermaphrodite *Jatropha* to be utilized for future germplasm improvement programs.

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