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Hybrid progenies in *Jatropha* – a new development

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The existing *Jatropha curcas* in the country exhibit varying degrees of success in terms of seed oil yield and susceptiblity to pest and diseases. Hence, an intensive hybridization programme has been attempted between *Jatropha curcas* and other *Jatropha* species to develop new hybrids with higher yield potential and resistance to diseases. Among the interspecific crosses, the cross between *J. curcas* and *J. integerrima* produced successful hybrids with more seed set, while the other crosses failed to produce seeds due to existence of crossability barriers. The F1 hybrids exhibited vigorous growth, but the fruit was small in size indicating *J. integerrima* characters. Hence backcross was attempted and the progenies showed unique characteristics of fruit, seed and oil yield.

Keywords: Interspecific hybrids, *Jatropha*, oil yield, pollen and pistil interaction.

THE suitability of vegetable oils for the production of biodiesel is gaining national and international importance. Tree-borne oilseeds are the best and potential alternative to mitigate the current and future energy crisis and also to transform the vast stretches of wasteland into green oil fields. The potential sources identified so far include *Jatropha curcas*, *Pongamia pinnata*, *Madhuca latifolia*, *Azadirachta indica*, *Calophyllum inophyllum* and *Simarouba glauca*. Among these, *J. curcas* emerges as the most promising tree-borne oilseed on the basis of its

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adaptability to a wide range of edapho-climatic conditions coupled with the suitability of *Jatropha* oil as a source of biodiesel[1,2].

J. curcas L. is a multipurpose plant with several attributes and has evoked interest all over the tropics as a potential biofuel crop[1,3,4]. Such a multiple-utility biofuel crop needs genetic improvement. Currently, crop improvement work in this species is limited; interspecific hybridization has been attempted between different species of *Jatropha* with limited success[5,6]. The interspecific hybridization in *Jatropha* species plays a significant role in crop improvement by transferring useful traits such as high oil content, maximum number of seeds, more femaleness and hard stem for promotion of *Jatropha* as a biofuel crop. The wide crosses among these species resulted in limited success, which might be due to the existence of either pre- or post-zygotic barriers. An understanding of the biological nature of this crossability barrier will provide a way to successful production of new hybrids. However, such studies are dismally modest in this species.

Against this backdrop, the Forest College and Research Institute (FCRI), Tamil Nadu Agricultural University, Mettupalayam, has been involved in inter- and intra-specific hybridization mainly to develop varieties with higher seed and oil yield. During the process of the intensive hybridization programme, FCRI came out with some promising, early and superior hybrid progenies with distinct morphological characters, which are presented in this communication.

The materials for the hybridization programme consisted of 15 potential *J. curcas* clones and their eight related species assembled at the species germplasm bank of FCRI. The desirable features of the various *Jatropha* species are furnished in Table 1.

The intensive hybridization programme was initiated through inter- and intra-specific hybridization using identified *J. curcas* clones and related *Jatropha* species. However, the current study reports only the interspecific crosses followed by backcross breeding. For the current breeding programme, the crossing was attempted in the morning hours preferably between 7.30 and 9.30 am. The pollen grains of the identified species were collected at the time of anthesis and dusted on the identified *J. curcas* clones. The breeding method followed in successful crosses is depicted in Figure 1.

The cultivated species *J. curcas* was used as the female parent and the wild species, viz. *J. integerrima*, *J. podagrica*, *J. villosa*, *J. tanjorensis*, *J. gossypifolia*, *J. glandulifera*, *J. multifida*, *J. maheshwarii* and *J. villosa* were used as pollen donors. Self- and cross-pollination was made and the growth of pollen tube at different stages was recorded using a fluorescent microscope after staining with aniline blue. The F1 seeds were raised and analysed for yield characteristics. The F1 progenies were selected based on their morphological differences in terms of plant type, stem, leaf, flower, fruit and seed characteristics. The selected F1 plants were then backcrossed with *J. curcas* clone (MTP JC1) to increase the seed size.

The BC1F1 progenies were raised in the field and assessed for flowering and fruiting characters. From the segregating populations, 27 backcross derivatives were identified for their distinctiveness, uniqueness and variability of fruit size and colour over the *J. curcas* seed sources coupled with seed yield. From these identified hybrid progenies cuttings were collected and the rooted cuttings were established, which were referred to as hybrid clones. These hybrid clones were established in the form of clonal multiplication area (CMA) at the *Jatropha* clonal complex of the FCRI. From the assembled hybrid clones, cuttings were collected from individual clone-wise (ramets) and sufficient ramets for each clone were produced. The multiplied clones were established in the form of hybrid clonal testing trials (CTAs) at an espacement of 3 × 3 m for further testing and evaluation. Further back-crossing of superior hybrid clones with *J. curcas* (MTP JC1) clone resulted in successful BC2F1 hybrids. The seeds of BC2F1 were raised in the nursery and are under further evaluation.

The hybrid clonal plantation trial was established in a marginal degraded land and given assisted irrigation once in every 15 days during non-rainy months. During the planting operation, all the ramets were treated with chlorpyriphos (Trishul) at 0.5 ml/l, as a preventive measure against termite attack. In addition, 10 g of neem cake per plant was provided after weeding at 4 months after planting. Disc ploughing between rows was done at 7 months after planting as a part of the tending operation.

The interspecific crosses between various *Jatropha* species indicated a wide range of success. Among the interspecific crosses, the cross between *J. curcas* and *J. integerrima* was successful with seed production, while other crosses were either partly successful or failed due to the existence of pre- and post-zygotic barriers.

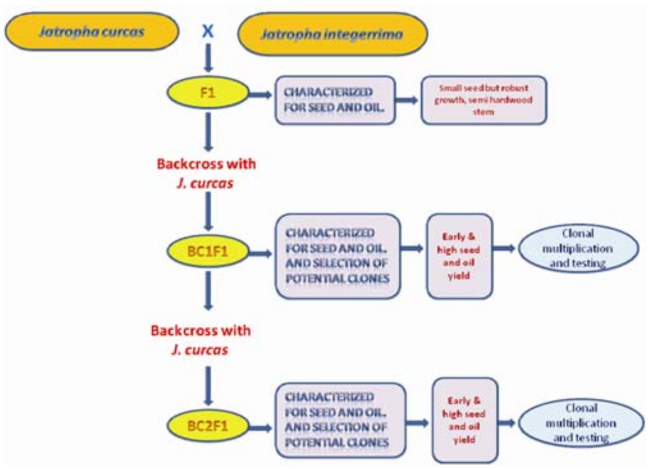

Figure 1. Diagrammatic representation of the breeding programme.

Table 1. Morphological description of *Jatropha* species

Species	Chromosome no.	Fertility status	Distinct morphological features	Desirable attributes
Jatropha curcas	22	Fertile	Tree/shrub, highly branching, cordate–palmately lobed leaves, greenish-yellow flowers, distinct coflorescence, tardily dehiscent fruits with black, large-sized ecarunculate seeds	High seed yield and oil content
J. integerrima	22	Fertile	Shrub, sparsely branched, ovate fiddle-shaped leaves, crimson-red flowers, dehiscent capsules, seeds small carunclate and brown with spots	Semi-hard wood stem and disease-resistant
J. podagrica	22	Fertile	Caudiciform shrubs, cordate leaves with peltate base, flat-topped corymbose cyme, bright scarlet flowers, violently dehiscent capsules with brown ecarunculate seeds	Bigger fruit and resistant to fusarial wilt
J. tanjorensis	Not conformed	Completely sterile	Shrub, profuse branching, cordate–palmately lobed leaves, margins distinctly serrate, greenish-yellow flowers with crimson-red tinge, no fruit-set	Robust and drought-hardy
J. gossypifolia	22	Fertile	Shrub, profuse branching, cordate leaves, glandular plant parts, dark crimson–purple flowers, violently dehiscent capsules with small brown caruncluate seeds	Drought-tolerant and profuse fruiting
J. maheshwarii	22	Fertile	Evergreen, drought-hardy and rhizomatous plant, leaves long, elliptical and resemble mango leaves, occur naturally in southern Tamil Nadu	Drought-hardy and rhizomatous plant
J. multifida	22	Fertile	Shrub/tree, uniform branching, leaves divided into 11 lobes, long petiole, long pedunculate flat-topped cyme, coral-red flowers and non-dehiscent capsules	Bigger fruit size and resistant to diseases
J. villosa	22	Fertile	Shrub, profuse branching, drought-tolerant, evergreen, rhizomatous plant	Evergreen and rhizomatous plant
J. glandulifera	22	Fertile	Smaller plant spread, and dichotomously branched, narrow leaves with serrated margin. Profused fruiting, but dehise before maturity	Profuse fruiting and drought-tolerant

The cross between *J. curcas* and *J. podagrica* exhibited significant reduction in pollen germination. The pollen tubes reached only up to midstylar region and no further growth was observed. Symptoms of crossability barriers, viz. bulging (Figure 2 *c*) and upward growth of pollen tubes were observed.

In the cross between *J. curcas* and *J. gossypifolia*, the pollen tube passed through the stigma 1 hour after pollination (HAP) the midstylar region at 2 HAP and finally reached the ovary at 3 HAP. Even though pollen tubes successfully reached the ovary, these crosses could not produce seeds which indicated the existence of post-zygotic barriers.

In the cross between *J. curcas* and *J. tanjorensis*, the pollen tube formation was completely arrested in the stigma region of *J. curcas*, which indicated the incompatibility barriers in the stigma region. The pollen grains of *J. tanjorensis* remained as such without germination even at 4 HAP (Figure 2 *d*), and there was no sign of pollen tube formation and fertilization.

The cross *J. curcas* and *J. maheshwarii* recorded significant reduction of pollen germination in the stigma region with germination percentage of 31.54 (Table 2). The pollen tubes passed through the stigma region at 1 HAP and reached the midstylar region at 3 HAP. Behind the midstylar region, no pollen tube growth was noticed (Table 3). The symptoms of crossability barriers, viz. partial germination of pollen grains and upward germination of pollen tubes towards the apex of the stigma were frequently observed (Figure 2 *e*).

The cross between *J. curcas* and *J. multifida* exhibited pollen germination percentage of 15.60, and the pollen tubes passed the midstylar region at 3 HAP. After that delayed pollen tube growth was noted and could not fertilize even at 4 HAP. However, the following symptoms of pre-zygotic barriers, viz. twisted growth pattern and partial germination of pollen grains were frequently observed.

The *J. curcas* and *J. villosa* cross exhibited significant reduction in pollen germination with a mean germination percentage of 34.32 and no fertilization occurred even after 4 HAP. The pollen tubes passed the stigma region at 1 HAP and reached midstylar region in 3 HAP. Behind the midstylar region, delayed rate of pollen tube growth was recorded. Symptoms of crossability barriers, viz. bulging of pollen tubes and crinkled growth pattern were observed (Figure 2 *f*).

In the cross *J. curcas* and *J. glandulifera*, the germination percentage of pollen grains recorded was 48.59. The

Table 2. Pollen tube growth in various interspecific crosses

Time after pollination (h)	No. of pistils observed	No. of pollen tubes observed in the stigma region	No. of pollen tubes reaching the midstylar region	No. of pollen tubes entering into the ovule
J. curcas × *J. curcas* (82.46% pollen germination)				
1	15	57	43	5.00
2	15	59	47	7.00
3	15	53	41	6.00
4	15	55	43	6.00
J. curcas × *J. integerrima* (50.02% pollen germination)				
1	15	61	30	0.00
2	15	59	23	2.00
3	15	63	29	5.00
4	15	62	29	5.00
J. curcas × *J. podagrica* (32.96% pollen germination)				
1	15	52	0.00	0.00
2	15	54	0.00	0.00
3	15	61	14	0.00
4	15	53	15	0.00
J. curcas × *J. tanjorensis* (0% pollen germination)				
1	15	23	0.00	0.00
2	15	32	0.00	0.00
3	15	27	0.00	0.00
4	15	35	0.00	0.00
J. curcas × *J. gossypifolia* (48.59% pollen germination)				
1	15	57	0.00	0.00
2	15	62	39	0.00
3	15	59	45	3.00
4	15	65	48	4.00
J. curcas × *J. maheshwarii* (31.54% pollen germination)				
1	15	25	0.00	0.00
2	15	31	0.00	0.00
3	15	27	9.00	0.00
4	15	21	7.00	0.00
J. curcas × *J. multifida* (15.60% pollen germination)				
1	15	25	0.00	0.00
2	15	27	0.00	0.00
3	15	19	6.00	0.00
4	15	22	8.00	0.00
J. curcas × *J. villosa* (34.32% pollen germination)				
1	15	25	0.00	0.00
2	15	27	0.00	0.00
3	15	19	5.00	0.00
4	15	22	8.00	0.00
J. curcas × *J. glandulifera* (48.59% pollen germination)				
1	15	25	0.00	0.00
2	15	27	0.00	0.00
3	15	19	7.00	0.00
4	15	22	9.00	0.00

pollen tube passed through the stigma, midstylar region and further movement was arrested at 3 HAP. Symptoms of crossability barriers, viz. crinkled growth pattern and reduction in the rate of pollen growth were noticed in the midstyler region.

The failure of inter-specific hybridization in the above-mentioned crosses was mostly due to pre-zygotic barriers barring the cross between *J. curcas* and *J. gossipifolia*, which might be due to the post-zygotic barrier. In the present investigation, it was observed that the pollen tubes had swollen tips, twisted growth pattern and reverse orientation in the styles of *J. curcas*. Similar pattern was also reported in other interspecific crosses involving populus[7], wheat and rye[8], sorghum and pearl millet[9] and *Sesamum* spp.[10], which thus lend support to the present investigation. We thus conclude that failure of interspecific hybridization in *Jatropha* species is due to the absence of pollen germination, and inhibition of pollen tube growth. A maximum degree of incompatibility barrier existed at the stigma and stylar level in the above crosses. Further studies to overcome pre- and post-zygotic barriers are under investigation.

The self-pollinated (*J. curcas* and *J. curcas*) pollen grains of *J. curcas* recorded pollen germination of 82.46% (Table 2). The pollen grains produced pollen tubes which rapidly entered into the stigma, midstylar region (Figure 2 *a*) and finally into the ovary within 1 HAP. Totally an average of 56 of pollen grains were observed in the stigma region, out of which 43.5 produced pollen tubes which were observed in the midstylar region. Among these, six pollen tubes entered into the ovary. The whole process was completed within 1 HAP. The entire process was observed till 4 HAP and no crossability barriers were noticed during the process.

In the cross between *J. curcas* and *J. integerrima*, the germination percentage of pollen grains recorded was 50.02. The pollen tube passed through the stigma and midstylar region (Figure 2 *b*) within 1 HAP and finally reached the ovary within 2 HAP. The cross-pollination exhibited reduction in pollen tube growth and germination in the stigma region recorded was almost 50%. A total of 61.25 pollen grains were observed in the stigma region in the first 1 HAP, and only 27.75 of them produced pollen tubes in the midstylar region. Of these, only three entered into the ovary. During the entire observation, minimal incompatibility barriers were found, viz. crooked pollen tube growth along the path and reduction of pollen tube growth in the midstylar region.

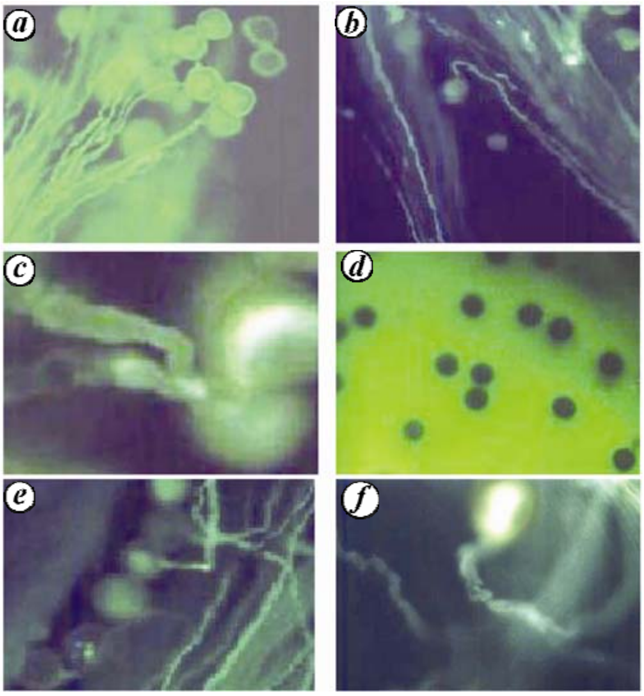

Figure 2. Success and failure of pollen tube growth to ovary. ***a***, *J. curcas* × *J. curcas* (selfing): No cross compatibility barriers were noticed. ***b***, *J. curcas* × *J. integerrima*: Fertilized at 2 HAP but interpollen tube competition observed at the midstylar region. ***c***, *J. curcas* × *J. podagrica*: Failed to fertilize due to bulging of pollen tubes. ***d***, *J. curcas* × *J. tanjorensis*: Pollen germination completely arrested even at 4 HAP. ***e***, *J. curcas* × *J. maheshwari*: Upward-growing pollen tubes were noticed. ***f***, *J. curcas* × *J. villosa*: Crinkling in the stylar region.

Among the various crosses, successful results were obtained between the self and the cross between *J. curcas* and *J. integerrima*. The F1 plants exhibited wider variations in terms of stem character (semi-hard wood), flower colour (pink, white and yellow) and fruit size (small and round). The seed size of the F1 plants was small and the yield was low, similar to *J. integerrima*, but exhibited robust growth particularly in terms of stem characters. The stem of F1 plants was robust and exhibited the character of semi-hard wood.

The BC1F1 plants raised in the field exhibited significant variation in terms of morphological features, fruit characteristics coupled with seed and oil content. Among the backcross derivatives, 27 distinct hybrid progeny clones were identified for their superiority in terms of growth, distinctness, seed and oil yield (Tables 4 and 5). At BC1F1, few new hybrid plants (FCRI HC 32 and 33) yielded significantly different and colourful *Jatropha* fruits (Figure 3), which are under investigation for seed and oil quality.

Among the 27 hybrid clones, three hybrid clonal progenies, viz. FC RI HC 3 (55.26%), FCRI HC 15 (48.50%), FCRI HC 13 (37.01%) exhibited superiority in terms oil content (Table 5). Similarly, the hybrid clonal

Figure 3. Distinct *Jatropha* hybrid progeny clones.

Table 3. Status of pollen tube growth at different time intervals

	Status			
Cross	1 h after pollination	2 h after pollination	3 h after pollination	4 h after pollination
J. curcas × *J. curcas*	Fertilization had occurred	Fertilization had occurred	Fertilization had occurred	Fertilization had occurred
J. curcas × *J. integerrima*	Pollen starts germinating	Fertilization	Fertilization	Fertilization
J. curcas × *J. gossypifolia*	Pollen starts germinating	Pollen tubes reached the midstylar region	Fertilization	Fertilization
J. curcas × *J. glandulifera*	Pollen starts germinating	Crinkling and twisting of pollen tubes in the upper stylar region	Crinkling of pollen tubes at the midstylar region	No fertilization
J. curcas × *J. mutlifida*	No pollen germination	Pollen starts germination	Delayed rate of pollen tubes coupled with twisted growth pattern	Twisting in the stylar region
J. curcas × *J. villosa*	Pollen starts germinating	Crinkling in the stylar region	Pollen tube gets arrested in the mid style	No fertilization
J. curcas × *J. maheshwarii*	Partial germination of pollen grains	Pollen tube in the midstylar region	Upward movement of the pollen tubes	No fertilization
J. curcas × *J. tanjorensis*	No pollen germination	No pollen germination	No pollen germination	No pollen germination
J. curcas × *J. podagrica*	Pollen starts germinating	Crinkling in the stylar region and upward germination towards the apex of the stigma	Pollen tube gets arrested in the mid style due to deposition of callose in pollen tubes	No fertilization

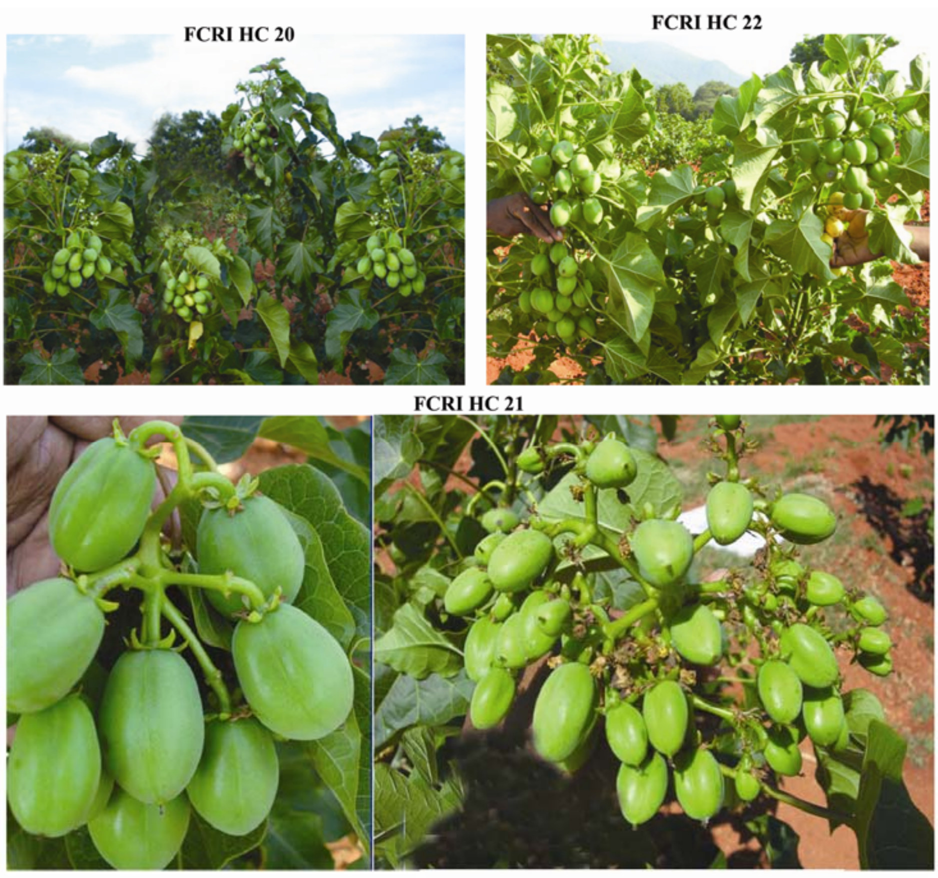

Figure 4. High-yielding hybrid progeny clones of *Jatropha*.

Table 4. Morphological descriptors of hybrid progeny clones

Hybrid clone no.	Plant type	Leaf: Axil colour	Leaf: Colour, Tender	Leaf: Colour, Full grown	Leaf: Length	Leaf: Width	Leaf: Texture	Bark: Colour, Stem	Bark: Colour, Tender
FC&RI HC 1	Tall erect	Green with light brown at joints	Light green	Green	10.8	10.2	Smooth	Light green	Green
FC&RI HC 2	Moderate bushy	Green with light brown at joints	Pale green	Light green	11.5	11.2	Smooth	Light green	Green
FC&RI HC 3	Tall erect	Light brown	Light green	Dark green	10.5	10.8	Smooth	Light green	Green
FC&RI HC 4	Dwarf spreading	Green with light brown at joints	Light green	Dark green	10.7	10.8	Slightly coarse	Light green	Green
FC&RI HC 5	Moderately tall erect	Green and light pinkish at joints	Light green	Pale green	12.2	13.2	Leathery	Light green	Green
FC&RI HC 6	Moderately tall	Light pink at ends	Light green	Dark green	11.0	10.5	Leathery	Light grey	Green
FC&RI HC 7	Moderately tall	Light green, light pink at ends	Pale green	Green	8.9	8.9	Leathery	Greenish-grey	Green
FC&RI HC 8	Dwarf	Light green, light pink at ends	Light green	Green	9.7	9.5	Rough	Green	Green
FC&RI HC 9	Semi spreading	Light green, light pink at ends	Light green	Dark green	9.4	8.5	Smooth	Grey	Green
FC&RI HC 10	Moderately tall bushy	Light green, light pink at ends	Pale green	Light green	8.8	8.3	Smooth	Greyish-green	Light green
FC&RI HC 11	Tall	Light green	Pale green	Dark green	10.3	10.6	Smooth	Light green	Green
FC&RI HC 12	Moderately bushy	Brown, pink at end	Pale green	Green	9.2	9.0	Smooth	Greenish-grey	Green
FC&RI HC 13	Moderately bushy	Light green	Pale green	Green	10.7	11.3	Slightly coarse	Greyish-green	Green
FC&RI HC 14	Medium tall	Light green, pink at tip ends	Pale green	Dark green	10.3	10.3	Leathery	Greyish-green	Light-greyish green
FC&RI HC 15	Moderately tall lean	Light green and yellow at tips	Pale green	Green	11.2	10.5	Leathery	Greyish-green	Light-greyish brown
FC&RI HC 16	Bushy spreading	Light green	Pale green	Dark green	9.5	9.5	Velvety	Greyish-green	Light green
FC&RI HC 17	Moderately tall	Light green	Pale green	Green	9.8	10.0	Smooth	Greyish-green	Green
FC&RI HC 18	Tall moderately bushy	Light pink at ends	Light green	Green	11.0	10.5	Smooth	Greyish-green	Green
FC&RI HC 19	Medium spreading	Tender brown, old light green	Light green	Green	7.6	7.0	Smooth	Greyish-green	Green
FC&RI HC 20	Medium tall	Green middle, pink at ends	Pale green	Dark green	11.0	11.0	Smooth	Greyish-green	Green
FC&RI HC 21	Moderately tall bushy	Pink at tip ends	Light green	Dark green	11.5	10.6	Rough	Greyish-green	Green brown
FC&RI HC 22	Bushy moderate	Green, pink at ends	Pale green	Dark green	11.5	12.1	Rough	Grayish green	Green
FC&RI HC 23	Tall	Pink at tip ends	Pale green	Dark green	11.0	10.7	Smooth	Greenish-grey	Light Greyish-green
FC&RI HC 24	Tall	Light green, pink at ends	Pale green	Dark green	11.0	10.8	Slightly coarse	Grayish-green	Green
FC&RI HC 25	Dwarf bushy	Green, pink at ends	Pale green	Slightly pale green	10.0	8.5	Smooth	Grey	Green
FC&RI HC 26	Tall erect	Light green, light brown at ends	Pale green	Green	10.3	10.0	Coarse	Greyish-green	Green
FC&RI HC 27	Medium bushy	Light green, light pink at ends	Pale green	Green	12.5	12	Leathery	Grayish-green	Light green

Table 5. Fruit characters and oil content of different *Jatropha* hybrid progenies

Hybrid clone no.	Fruit: Young fruit colour	Fruit: Mature fruit colour	Fruit: Shape of the fruit	Fruit length (mm)	Fruit breadth (mm)	Seed length (mm)	Seed breadth (mm)	100 seed weight (fresh; g)	100 seed weight after 30 days (g)	Yield (g/plant)	Oil content (%)
FC&RI HC 1	Light green	Yellow	Spherical	30.09	25.38	17.80*	9.60*	58.00*	90.00*	124.96	32.90*
FC&RI HC 2	Green	Yellow	Spherical	34.36*	28.62*	16.80	9.00*	50.00	64.00	92.41	23.30
FC&RI HC 3	Light green	Yellow	Oval, elongate	27.00	24.47	18.80*	9.80*	62.00*	96.00*	127.65	55.26*
FC&RI HC 4	Light green	Yellow	Spherical	30.18	27.29*	16.60	10.00*	58.00*	80.00	74.44	20.57
FC&RI HC 5	Light green	Yellow	Moderately spherical	29.54	27.51*	18.20*	10.20*	52.00	80.00	58.32	31.30*
FC&RI HC 6	Light green	Yellow	Broad spherical	26.65	23.94	18.20*	10.20*	56.00*	90.00*	83.60	34.36*
FC&RI HC 7	Light green	Yellow	Elongate	25.23	24.58	17.00	7.00	50.00	80.00	138.05	18.29
FC&RI HC 8	Green	Yellow	Small semi-spherical	25.53	22.04	16.60	6.60	56.00*	60.00	52.94	26.13
FC&RI HC 9	Green	Yellow	Semi-spherical	25.34	21.99	16.60	8.20	46.00	72.00	228.91	17.95
FC&RI HC 10	Light green	Yellow	Semi-oblong	28.92	26.38*	17.20	8.40	52.00	80.00	325.01*	22.10
FC&RI HC 11	Green	Yellow	Broad spherical	25.50	22.70	15.40	6.80	52.00	90.00*	160.16	26.84
FC&RI HC 12	Green	Yellow	Semi-elongate	36.67*	29.60*	15.40	5.60	46.00	90.00*	255.00*	24.67
FC&RI HC 13	Green	Yellow	Broad spherical	26.21	25.37	20.20*	12.00*	62.00*	96.00*	78.80	37.01*
FC&RI HC 14	Green	Yellow	Spherical	34.04*	25.18	19.20*	9.80*	58.00*	88.00*	174.84	32.06*
FC&RI HC 15	Light green	Yellow	Oval	29.71	27.77*	18.00*	8.40	70.00*	90.00*	97.48	48.50*
FC&RI HC 16	Green	Yellow	Medium cylindrical	32.23*	23.66	15.20	6.60	50.00	72.00	207.76	29.84*
FC&RI HC 17	Light green	Yellow	Broad spherical	27.20	25.55	18.60*	10.80*	55.00*	80.00	191.31	23.52
FC&RI HC 18	Green	Yellow	Small slightly oblong/cylindrical	27.09	25.08	16.00	9.00	46.00	68.00	305.43*	19.73
FC&RI HC 19	Green	Yellowish-orange	Small semi-spherical with six marginal lines	22.86	20.69	16.80	6.40	38.00	56.00	154.99	23.98
FC&RI HC 20	Green	Yellow	Broad semi-oblong with three distinct segments	35.04*	27.89*	18.00*	9.40	64.00	88.00*	252.26*	25.51
FC&RI HC 21	Light green	Yellow	Oblong	36.01*	24.52	15.20	7.00	50.00	60.00	328.07*	30.65*
FC&RI HC 22	Light green	Yellow	Broad moderately spherical	27.85	25.82	16.20	8.80	62.00*	96.00*	357.48*	23.89
FC&RI HC 23	Green	Yellow	Broad spherical	30.03	27.74*	18.20*	8.40	52.00	90.00*	156.28	20.42
FC&RI HC 24	Green	Yellow	Oval	27.94	24.23	17.80*	7.20	28.00	60.00	69.20	20.73
FC&RI HC 25	Greyish-green	Brownish-orange	Semi-oblong with three distinct segments	27.13	22.55	18.40	7.60	26.00	60.00	90.20	29.65*
FC&RI HC 26	Light green	Yellow	Spherical	27.00	22.23	15.20	7.00	44.00	72.00	150.00	20.82
FC&RI HC 27	Green	Yellow	Oblong	36.00*	25.83	20.20*	10.20*	32.00	80.00	250.00*	29.36*
Seed source	Green	Yellow	Spherical				Flowering not yet started				
Mean				29.31	25.13	17.33	8.52	78.81	50.93	169.84	27.75
SEd				0.64	0.37	0.17	0.23	1.33	0.97	35.49	0.22
CD (0.05)				1.28	0.73	0.34	0.47	2.67	1.94	70.37	0.43

*Significant at 5% level.

progenies FCRI 22 (357.48 g), FCRI HC 21 (328.07 g), FCRI HC 10 (325.01 g), FCRI HC 18 (305.43 g), FCRI HC 12 (255 g), FCRI HC 20 (252.26 g) and FCRI HC 27 (250 g) recorded maximum seed yield at 9 months after planting (Figure 4 and Table 5). These hybrid clones also expressed superiority in terms of early flowering and fruiting coupled with early yield, which thus lends scope for further promotion and utilization of *Jatropha* as a successful biofuel crop. The existing local seed sources of *Jatropha* are beset with problems of variation in seed yield, poor seed and oil yield and susceptibility to pest and diseases. The variable hybrid progenies developed so far and the hybrid progeny in the pipeline will help to solve the issue of seed yield and oil content.

All the identified hybrid mother plants exhibited distinct morphological features coupled with higher seed yield (700 g to 1.4 kg/plant) at the third year after establishment and oil content (17.95 to 55.26%). Except few hybrid clones, the others exhibited oil content more than 25%. The fruiting behaviour of some clones was unique, which produced fruits of different size, shape and colour (Figure 3). Five hybrid clones, viz. FCRI HC 2, 11, 21, 32 and 33, exhibited distinct variations in terms of oblong and coloured fruit coats. The hybrid clone 21 expressed oblong character coupled with continuous fruiting type from the base to top of the plant. In each branch, two to three bunches of fruits were seen from the base to top of the plants. In each bunch, a minimum of 15 fruits were observed. Three hybrid clones (FCRI HC 20, 21 and 22) recorded an average yield of 1.4 kg/plant (mother plant) on single plant basis at the third year after establishment. This yield was more than 300% than the local *Jatropha* seed sources yield, which was 200 to 300 g/plant at the same age and hence the hybrid clone proved to be promising. The superiority of the individual hybrid clone is now raised on a plantation scale which also expressed early superiority in terms of yield at 9 months after planting, thus lending scope for the promotion of a *Jatropha*-based biofuel plantation programme.

Systematic testing trials are already established and all the hybrid clones expressed early flowering and fruiting within 3 months after planting. Within 5 months, three hybrid clones, viz. FCRI HC20, 21 and 22 recorded excellent growth, including fruiting characteristics and seed yield. Such yield improvement in *Jatropha* through hybrid development is currently not available for utilization. Hence the present study is an attempt which will provide a scope to all user agencies. Further studies on testing of *Jatropha* hybrid genetic resources at multilocations are underway to screen and promote potential *Jatropha* high-yielders.

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Development of efficient techniques for clonal multiplication of *Jatropha curcas* L., a potential biodiesel plant

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Effect of auxins (IAA, IBA and NAA) and vitamin B_1 (thiamine) on rooting response of branch cuttings and air-layers of *Jatropha curcas* during spring and monsoon seasons was studied. Spring season was found best for clonal multiplication of genetically superior material in jatropha. Cuttings treated with 600 and 800 mg l^{-1} thiamine showed 100% sprouting during both seasons. The average sprout growth was also found maximum in thiamine treated cuttings. Auxins enhanced rooting of cuttings during spring season, but showed poor performance or even failed to root during monsoon. Interestingly, thiamine triggered highest

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