International Journal of Multidisciplinary Research and Development

Volume: 2, Issue: 9, 197-200
Sep 2015
www.allsubjectjournal.com
e-ISSN: 2349-4182
p-ISSN: 2349-5979
Impact Factor: 4.342

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Intra-specific hybridization using selected accessions of *Jatropha curcas* L.

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Abstract
Jatropha curcas L. is a shrub belonging to Euphorbiaceae family. There is a worldwide interest to cultivate this plant in large scale for the production of biodiesel from its seed oil. Hence, improvement to get commercially profitable quantities of seed yield with high oil content for biodiesel production is the prime objective. Intra-specific hybridization by xenogamy (cross-pollination) was carried out to meet this objective. A total of 50 accessions were identified from the Jatropha germplasm centre of Labland Biotech Private Limited, Mysore for the purpose of hybridization studies. Out of many highly successful crosses made in this study, a maximum of 80% effective fruit formation and 61% quality seed formation were achieved. The efficiency of hybrid seed production is discussed.

Keywords: *Jatropha curcas*, Intra-specific hybridization, Hybrids, Cross-pollination

1. Introduction
Jatropha curcas L. has been identified as an alternative source of energy for the production of biodiesel all over the world. However, availability of quality planting material of *J. curcas* has been one of the shortfalls resulting in slow progress in establishing quality plantations. As of now, there is lack of identified germplasm for development of high yielding hybrids. The need of the hour is to improve the genus Jatropha and domesticate it to suit the current required varieties which will fulfil the gap of feedstock required to produce biodiesel. One important approach is to opt for conventional breeding techniques. Development of pure lines and hybrids is one of the several different studies that are being carried out at Labland Biotech Private Limited (Labland), Mysore for catering to the needs of quality planting material. With a strategic intention on the commercial production of quality hybrid seeds, intra-specific hybridization was carried out to develop improved varieties of *J. curcas* with higher seed yield and oil content from established selections maintained at Labland's germplasm centre. Jatropha is a potential candidate for inter and intra-specific hybridization and production of varieties having all the desirable qualities [1]. Intra-specific variation and interrelationships between morphology, nutritional content and enzymatic activity of *J. curcas* have been reported [2]. Inter-specific hybridization has been attempted about 20 years ago in Jatropha, between different species with limited success [3, 4]. Parthiban *et al.* [5] have attempted both intra- and inter-specific hybridization in Jatropha with some success. Molecular characterization of intra-specific variation in *J. curcas* has also been studied before [6, 7] but there are no reports to describe the standard procedure to develop hybrids and the efficiency of hybrid seed formation in Jatropha. Hence, the present study was carried out to develop F1 hybrids through hand pollination in *J. curcas* by crossing several identified parental accessions for the production of hybrid seeds.

2. Materials and Methods
2.1 Study site
The present work was carried out in the study site of Labland's Research Field located at Marati Kyathana Halli, Bogadi-Gaddige Road, Mysore, Karnataka, India (12°18′ 04.10"N,76°34′17.74" E) near Mysore city, with an elevation of 600 m and annual rainfall of 800 mm. The highest temperatures are from May to June (23 °C to 35 °C), and even at its coolest, the temperature rarely drops below 14 °C.

2.2 Parental lines and crossing scheme for hybridization
A total of 50 accessions from two blocks in the Germplasm Center of Labland were identified for the purpose of hybridization studies. The mother plants in these blocks had been collected from different places of India. Different morphological/yield traits are being recorded to

generate the passport data of these plants over a period of 10 years which are documented in the confidential records of Labland. The current investigation of 30 different cross pollinations were carried out in well established and well studied 5-year-old plants of two identified blocks.

The crossing scheme adopted in the present investigation involved parental accessions with varied degrees of seed yield and seed oil traits. In the trait specific crosses, high seed yielding accessions were crossed with low and medium yielding accessions. Similarly, accessions with high seed oil content were crossed with low and medium oil content accessions. In addition, based on the results of Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram made based on the Euclidean distance for 15 morphological traits in 50 different accessions [8], some accessions falling in specific groups were also crossed. The details of 30 various crosses made are presented in Table 1.

Table 1: Details of various intra-specific crosses from the selected accessions of *Jatropha curcas*

Sl. No.	Parental Accessions (♀ x ♂)	Sl. No.	Parental Accessions (♀ x ♂)	Sl. No.	Parental Accessions (♀ x ♂)
1	LBL1 x LBL27	11	LBL49 x LBL9	21	LBL136 x LBL146
2	LBL9 x LBL5	12	LBL70 x LBL8	22	LBL138 x LBL143
3	LBL9 x LBL8	13	LBL75 x LBL64	23	LBL146 x LBL140
4	LBL9 x LBL47	14	LBL85 x LBL112	24	LBL147 x LBL155
5	LBL10 x LBL12	15	LBL86 x LBL95	25	LBL154 x LBL180
6	LBL11 x LBL34	16	LBL94 x LBL101	26	LBL163 x LBL156
7	LBL12 x LBL44	17	LBL127 x LBL135	27	LBL174 x LBL193
8	LBL15 x LBL11	18	LBL128 x LBL140	28	LBL174 x LBL167
9	LBL30 x LBL41	19	LBL130 x LBL150	29	LBL104 x LBL76
10	LBL31 x LBL2	20	LBL131 x LBL143	30	LBL105 x LBL118

2.3 Method of crossing

Inflorescences were washed gently with distilled water by spraying/pouring on inflorescence to remove pollen from the surface of inflorescence. Male and female flowers were identified in inflorescence of the same plant before blooming. The male flowers were clipped off and only female flowers were retained in the mother plant and bagged carefully to avoid pollination. Insecticide was used on branch and flowers to avoid pollination by small insects, specially ants. After the flowers bloomed, the pollen were collected from different selected accessions and pollinated on the female flowers of mother plants (Figure 1A to D). After a week, the inflorescences were un-bagged and the fruit development was tracked. However, the number of female flowers used for crosses were limited to 30 in each specific crosses. Thus, 900 female flowers were crossed with specific identified male accessions.

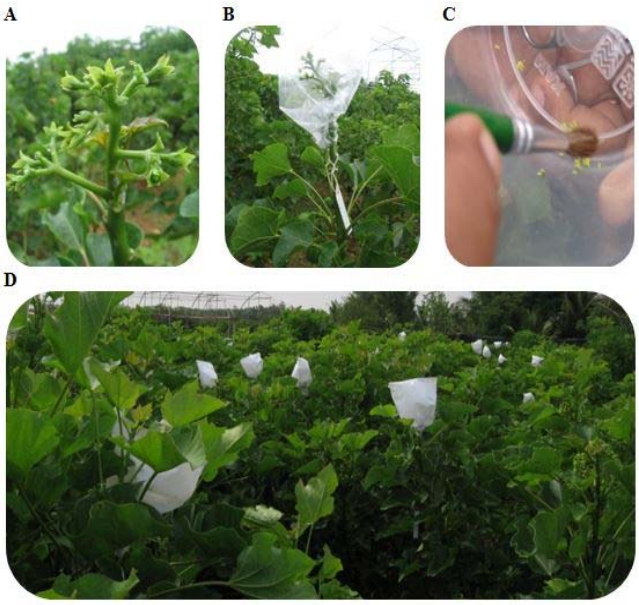

Fig 1: Method of intra-specific hybridization in 50 different accessions of *Jatropha curcas* by hand xenogamy. **A.** Emasculated inflorescence, **B.** Emasculated inflorescence bagged and labeled, **C.** Collection of pollen in to Petri dish, **D.** Experimental plot one week after cross pollination

2.4 Data collection and Statistical analysis

Efficiency rate of fruit formation, efficiency rate of hybrid seed production and average days to maturity after pollination (DAP) were analyzed. Data on number of fruit set, quantity of seeds, number of seeds/fruit collected, average of single seed weight, total weight of seeds harvested and 100 seeds weight were recorded. All the data collected here are from 30 flowers in each of the identified accessions. Description and methodology of data are presented in Table 2.

Table 2: Description and methodology of data collection from *Jatropha curcas* after intra-specific hybridization

Sl. No.	Data collected on yield traits	Descriptions and methodology
1	Total fruits harvested (no.)	Total number of fruits set harvested out of 30 female flowers pollinated by hand was visually counted
2	Total seeds harvested (no.)	Total number of seeds obtained out of 30 female flowers pollinated by hand was visually counted
3	Average of seeds/fruit (no.)	Total number of seeds obtained ÷ Total number of fruits set harvested
4	Total seed weight harvested (g)	Total number of seeds obtained was measured with an electronic weighing balance (Teraoka Seiko Co. Ltd, Japan)
5	Average of single Seed weight (g)	Total seed weight harvested ÷ Total number of seeds obtained
6	100 seed weight (g)	Average of single seed weight x100
7	Efficiency rate of hybrid fruits formation (%)	(Total number of set fruits harvested ÷ 30) x100
8	Efficiency rate of hybrid seeds formation (%)	(Total number of seeds obtained ÷ 90) x100
9	Average days to maturity after pollination (Days)	Average of total days from the date of pollination to final harvest recorded

3. Results and Discussion

3.1 Evaluation of the crosses of the parental lines

In the present investigation, 30 crosses were made. The intra-specific crosses of 50 identified accessions showed wide range of variations in the seed size (Figure 2) and number of seeds obtained. The data on the total fruits harvested, total seeds harvested, average number of seeds/fruit, average of single seed weight (g), total seed weight harvested, 100 seed weight, efficiency rate of hybrid fruits formation, efficiency rate of hybrid seeds formation and average days to maturity after pollination are presented in Table 3.

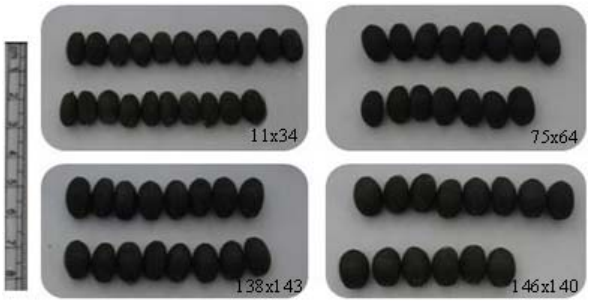

Fig 2: Variations in the seed size obtained in selected intra-specific hybrids

Table 3: Results of 30 different intra-specific hybridization made using 50 different accessions of *Jatropha curcas*

♀Parental accessions	♂ Parental accessions	TFH (no.)	TSH (no.)	AV. S/F (no.)	AV.SSW (g)	TSWH (g)	100SW (g)	EFF (%)	ESF (%)	AV.DAP (Days)
LBL1	LBL27	9	24	2.67	0.65	15.60	65.00	30.00	26.67	54
LBL9	LBL5	15	42	2.80	0.76	31.80	75.71	50.00	46.67	55
LBL9	LBL8	10	8	0.80	0.79	6.33	76.00	33.33	8.89	56
LBL9	LBL47	21	32	1.52	0.66	21.00	65.88	70.00	35.56	58
LBL10	LBL12	12	24	2.00	0.74	17.70	73.75	40.00	26.67	60
LBL11	LBL34	9	21	2.33	0.64	13.50	64.29	30.00	23.33	54
LBL12	LBL44	15	42	2.80	0.66	27.60	65.71	50.00	46.67	56
LBL15	LBL11	24	38	1.58	0.72	27.20	71.58	80.00	42.22	62
LBL30	LBL41	6	13	2.17	0.70	9.14	71.11	20.00	14.44	63
LBL31	LBL2	14	32	2.29	0.73	23.20	72.50	46.67	35.56	57
LBL49	LBL9	9	26	2.89	0.76	19.69	75.00	30.00	28.89	60
LBL70	LBL8	9	24	2.67	0.76	18.30	76.25	30.00	26.67	56
LBL75	LBL64	18	48	2.67	0.71	34.20	71.25	60.00	53.33	58
LBL85	LBL112	14	36	2.57	0.88	31.74	87.39	46.67	40.00	58
LBL86	LBL95	15	42	2.80	0.86	36.30	86.43	50.00	46.67	58
LBL94	LBL101	15	34	2.27	0.72	24.38	72.22	50.00	37.78	58
LBL127	LBL135	22	35	1.59	0.76	26.73	75.38	73.33	38.89	63
LBL128	LBL140	24	54	2.25	0.84	45.60	84.44	80.00	60.00	58
LBL130	LBL150	14	32	2.29	0.74	23.65	74.44	46.67	35.56	63
LBL131	LBL143	23	53	2.30	0.79	42.00	80.00	76.67	58.89	60
LBL136	LBL146	17	37	2.18	0.86	32.00	87.27	56.67	41.11	63
LBL138	LBL143	9	25	2.78	0.98	24.53	99.29	30.00	27.78	59
LBL146	LBL140	11	11	1.00	0.89	9.75	86.67	36.67	12.22	55
LBL147	LBL155	19	38	2.00	0.77	29.25	78.00	63.33	42.22	64
LBL154	LBL180	21	48	2.29	0.71	34.20	71.25	70.00	53.33	57
LBL163	LBL156	23	55	2.39	0.76	41.54	75.00	76.67	61.11	63
LBL174	LBL193	21	7	0.33	0.69	4.85	70.00	70.00	7.78	62
LBL174	LBL167	12	21	1.75	0.76	15.92	76.67	40.00	23.33	55
LBL104	LBL76	15	27	1.80	0.78	21.00	77.78	50.00	30.00	56
LBL105	LBL118	11	28	2.55	0.76	21.38	76.00	36.67	31.11	62
Min		6	7	0.33	0.64	4.85	64.29	20	7.78	54
Max		24	55	2.89	0.98	45.60	99.29	80.00	61.11	64
Mean		15	32	2.14	0.76	24.34	76.08	50.78	35.44	59

TFH: Total fruits harvested (no.), **TSH:** Total seeds harvested (no.), **AV. S/F:** Average of seeds/fruit (no.), **AV. SSW:** Average of single seed weight (g), **TSWH:** Total seed weight harvested (g), **100SW:**100 Seed weight (g), **EFF**: Efficiency rate of hybrid fruits formation (%). **ESF**: Efficiency rate of hybrid seeds formation (%), **AV. DAP**: Average days to maturity after pollination (Days)

In the conventional breeding approach of *J. curcas*, the most important phenotypic traits considered in the selection of accessions are seed yield, seed size and oil yield [9]. The present investigation was set up with a seed-based breeding approach wherein selections from Indian origin with high, medium and low yield traits were hybridized to achieve the set breeding objectives. Out of many highly successful crosses made in this study, a maximum of 80% effective fruit formation (Figure 3) with 61% seed formation was achieved. A total of 957 quality hybrid seeds were produced in one flowering season and were used for further evaluation. Apart from this, *J. curcas* accessions crossed among themselves in the specific manner falling in specific groups, exhibited diversity in days to maturity after pollination, percentage of fruit and seed formation in addition to seed weight characters. From this study, better parents and hybrids could be selected for future breeding program after evaluation of hybrids in the field. From literature survey, it is given to understand that this is the first report wherein such massive numbers of F1 hybrids have been produced for commercial purpose.

Fig 3: 50-day-old fruits of intra-specific hybridization seen in some of the hybrid plants

Biabani *et al.* [10] crossed 10 superior parents in a diallel manner to produce 45 hybrids. The hybrids and their parents were evaluated for their combining abilities and heterosis in greenhouse. Islam *et al.* [11, 12] crossed six *J. curcas* parental lines in half diallel fashion, and the F1 hybrids were evaluated to determine the combining ability for nine germination parameters and proposed that the crosses with desirable specific combining ability could be used for exploitation of heterosis in *J. curcas* for germination and seedling growth.

4. Conclusions

From the results of these studies, it can be concluded that it is possible to produce elite hybrids through cross-pollination in *J. curcas*. The procedure of emasculation is relatively simple and inexpensive except that it was laborious and time-consuming. Intra-specific crossing results in a maximum of 80% effective fruit formation with 61% seed formation.

A total of 957 quality hybrid seeds are produced in one flowering season from the 50 identified accessions. *J. curcas* accessions cross among themselves in specific manner, exhibiting diversity in days to maturity after pollination, percentage of fruit or seed formation and seed weight characters.

5. Acknowledgement

The first author acknowledges the University of Mysore, Mysore.

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