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Research Article

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Genetic Diversity of *Jatropha curcas* L. accessions and screening for high Biodiesel production

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ABSTRACT

Morphological, physico-chemical and molecular characterization of Jatropha curcas L. populations were carried out to unravel the causes of conspicuous intra-specific morphological variation. Morphological differences between accessions were clearly evident and the quality of seed-oil was evaluated for suitability to produce Biodiesel. Although highest seed-weight and seed-oil content were recorded in KM accession followed by RSAD accession, the latter was more suited for production of Biodiesel due to its superior physico-chemical characteristics. Molecular study confirmed the genetic variability between the accessions in relation to the variation in morphological and physico-chemical traits and superior accessions could be distinguished by RAPD markers.

Keywords: *Jatropha accessions, RAPD markers, physico-chemical analysis, biodiesel.*

INTRODUCTION

Fuel consumption has increased with increasing energy demands for industrialization and transport. Wildly fluctuating oil prices in the global market is a destabilizing element for the country's economy. The use of biofuels also affects the CO2 balance by limiting its accumulation in the biosphere, which may be considered as one of the principal causes of the greenhouse effect[1]. Biodiesel, an alternative diesel fuel is made from vegetable oils and animal fats through a process of transesterification. It is biodegradable, non-toxic, has low emission profiles and so is environmentally beneficial. Biodiesel from edible oils such as soybean oil in USA, rapeseed oil in Europe and palm oil in countries with tropical climate such as Malaysia are underway[2]. However, in the Indian context, the use of edible oils for engine fuel is not feasible. There are several non-edible oil-yielding plants such as *Jatropha curcas* L. (Physic nut), *Pongamia pinnata* (Karanj), *Simarouba glauca* (Paradise tree) etc. which could be utilized for the production of biodiesel. Among the non-edible oilseed resources, *J. curcas* has gained tremendous importance as it is easily adaptable to the semiarid conditions, drought tolerant, pest resistant and produces seeds early[3,4]. *Jatropha curcas* L. member of the Euphorbiaceae family is a shrub or a small tree, commonly known as purging nut or physic nut. It is found in a semi-wild condition in many tropical countries of Asia and Africa[5]. It is thought to have originated either in Peru or Mexico, but naturalized in Asia and Africa. Seeds contain 40-60 % (w/ w) non-edible oil[6,7]. The seed-oil was reported to possess insecticidal[8], molluscicidal[9] and fungicidal[10] properties. The crude oil can easily be converted to biodiesel by a process of transesterification and glycerine (by-product) is used in soap industries[11]. In India, *Jatropha* has been traditionally planted as a fence since it is not browsed by cattle, besides being used for control of soil erosion[12]. However, despite its abundance and use as an oil and reclamation plant, none of the *Jatropha* species have been properly domesticated.

Considerable genetic diversity exists among the 12 *Jatropha* species[13]. Further, the Indian germplasm of *J. curcas* exhibit clear differences between the races in terms of seed size, shape and fatty acid composition[14]. The goal of germplasm conservation in gene banks is to maximize genetic variation. Collecting explorations would be more efficient if factors that predict areas and habitats associated with greater genetic differences and diversity could be identified.

DNA marker based fingerprinting can distinguish species rapidly using small amounts of DNA and therefore can assist to deduce reliable information on their phylogenetic relationships. DNA markers are not typically influenced by environmental conditions and therefore can be used to help describe patterns of genetic variation among plant populations and to identify duplicated accessions within germplasm collections. RAPD is an inexpensive and rapid method not requiring any information regarding the genome of the plant, and has been widely used to ascertain the genetic diversity in several plants[15,16]. RAPD analysis requires only a small amount of genomic DNA and can produce high levels of polymorphism and may facilitate more effective diversity analysis in plants[17]. RAPD analysis provides information that can help define the distinctiveness of species and phylogenetic relationships at molecular level. Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique genotypes or sources of genetically diverse genotypes. The present study was undertaken to assess the genetic relationships between different Jatropha species using RAPD markers.

Therefore, the objective of this research on *Jatropha curcas*, the Biodiesel plant was to investigate whether ecogeographical variables have significant associations with patterns of genetic variation in populations of *Jatropha curcas* comprising elite accessions growing at Osmania University in terms of suitability of the seed-oil for conversion to Biodiesel.

MATERIAL AND METHODS

Plant material

Seeds of 14 accessions were collected from different locations in India, including the wild locations in and around the state of Andhra Pradesh (AP) (Table-1). Seeds and seedlings of the above 14 accessions of *Jatropha curcas*, were raised as a plantation in the Experimental Garden of Department of Botany, Osmania University, Hyderabad, Andhra Pradesh, India.

Study of quantitative and qualitative traits

Plants belonging to all the 14 accessions of *Jatropha curcas* started producing seeds from the third year. The morphological data were recorded from these 3-year-old plants in the plantation. Data pertaining to quantitative traits recorded from 30 plants per accession comprised of plant height (m), number of primary branches, number of secondary branches, Leaf area index (with C1-203 laser digital leaf area index meter CID Instruments), number of pods per plant, seed-yield per plant (gm) and 1000-seed weight (gm). The recorded data was statistically analyzed and presented as mean ± S.E. Data on qualitative characters was also collected along with details of diseases and pests.

Seed characteristics

Seeds of the 4 elite accessions were harvested from the 4-year-old plants after 60 days of flowering. They were carefully dried and seed-coats (shells) removed mechanically and the weight of kernels (gm) recorded in gm.

Extraction of seed-oil: The kernels were ground in a grinder to a fine powder and transferred to the thimble of the Soxhlet extraction apparatus. Enough hexane was added to wet the powder completely and the contents heated to 100-110^{o}C for about 12 hours allowing at least 10 siphonings. Oil collected at the bottom was taken out and the hexane was evaporated to leave the pure oil. The weight (gm) of the oil was recorded after vacuum drying at 110^{o}C for four hours. Percentage of oil was calculated as below:

$$\text{Percentage of oil} = \frac{\text{Wo (gm)}}{\text{Ws (gm)}} \text{ X } 100$$

Where Wo = Weight of oil extracted (gm)
Ws = Weight of seeds taken (gm).

Physico-chemical characterization of seed-oil

Physico-chemical characterization of seed-oil of the 4 elite accessions was carried out according to Horowitz method of 1984: Acid value, Saponifiable number, Unsaponifiable number, Refractive index, Iodine number, Moisture and volatile matter % by the specific AOCS Official Methods (Revised 2003). Color (units) and Density (g/c3) (recorded using DMA 48 Density meter), Viscosity (cSt) (with Viscometer Model 83545-2 SETAVIS 'Double 6'), Phosphorus content (by IUPAC method), Hydrocarbon content (HC), Free fatty acids (FFA), Diglycerides (DG) and Triglycerides (TG) (by column chromatographic method), Conversion of oil into Methyl esters (biodiesel) and estimation of Fatty acid composition by Gas Chromatography (by AOCS Official Method, Ce 1-62 Revised 2003) and Gas Chromatogram (GC6890 model, supplied by Agilent Technologies, USA) recorded by capillary column of DB 225 MS J&W Scientifics, USA.

Molecular characterization of the accessions

The 4 elite accessions of *Jatropha curcas* were subjected to molecular analysis which included the isolation of DNA from the 4 accessions of *Jatropha*, random amplification of DNA samples by Polymerase Chain Reaction (PCR) using random primers and analysis of amplified products through gel electrophoresis and NtSYSpc software (RAPD analysis).

Isolation of plant genomic DNA

DNA was isolated from the leaves of the 4 elite accessions of *Jatropha* by CTAB method. The quality of the DNA was assessed on a 0.8 % agarose gel containing 0.5 µg/ml ethidium bromide. The quantitation of DNA was done using a UV-vis spectrophotometer (Elico S L 150) and used for PCR analysis.

Amplification of DNA samples by Polymerase Chain Reaction (PCR) with random primers (RAPD analysis)

One µg of RNase treated DNA was used as template for PCR amplification. A selection of ten 10-mer primers from the Operon Technologies RAPD primer set (OPM primer) was screened for amplification on four DNA samples. Each PCR reaction was performed in a total volume of 25 µl. The PCR reaction mixture contained 2.5 µl PCR reaction buffer (10X) (Sigma), 2.0 µl Template DNA (0.2 µg), 0.5 µl dNTPs (10mM) (Sigma), 0.75 µl $MgCl_2$ (50mM) (Sigma), 0.5 µl RAPD Primers (0.1µM) (Operon Technologies), 18.5µl Sterile distilled water and 0.25µl Platinum Taq DNA Polymerase (1 unit) (Sigma).

The amplification of DNA was performed by using the Thermal Cycler with hot lid (MJ Research INC, PTC 100) according to the following program: One cycle of 94° C for 1 min, 45 cycles of 94° C for 1 min., 37° C for 1 min., 72° C for 2 min, and finally one more cycle for 72° C for 5 min.

The PCR products were visualized by electrophoresis using 15-20 µl aliquots on 1.5 % agarose gels with 120 V in a 1xTAE buffer system. One liter 50 x TAE buffer was prepared by using 242 g Tris base, 57.1 ml Acetic acid, 100 ml 0.5 M EDTA and required volume of distilled water and pH of 8.5. After completion of electrophoresis, the agarose gels were visualized and photographed under Ultraviolet light on a Transilluminator. The PCR reaction was conducted twice to confirm the reproducibility of the RAPD products.

Analysis of amplified products through gel electrophoresis and NtSYSpc software.

The RAPD products between 100 and 2000 bp were scored for presence (1) and absence (0) irrespective of band intensity, since each PCR product of identical molecular weight was supposed to represent a single locus. The data were analyzed based on the Dice Coefficient for binary data via SIMQUAL of the NTSYSpc ver. 2.1 Package[18]. Analysis utilized the SAHN clustering program of the NTSYSpc Package to construct an UPGMA (Unweighted Pair Group Method of Arithmetic Mean) dendrogram. Similarity coefficients from the squared data matrix were used for Principal Coordinates Analysis (PCA) via PCOORDA of NTSYSpc Package.

RESULTS AND DISCUSSION

Quantitative and qualitative traits

Fourteen accessions of *Jatropha curcas* growing in the plantation were screened for superior quantitative traits (Fig. 1 and Table 2) and 4 elite accessions with superior traits were identified viz. RSAD, KM, MB and FWB based on physico-chemical and analysis. Whereas, the MB, KM and RSAD accessions produced higher values of quantitative traits like plant height, number of branches, dense flowers, number of pods per plant and seed-yield compared to the other accessions, FWB produced a high 1000 seed-weight. Further, KM was also the tallest with highest leaf area index, higher number of secondary branches, highest kernel weight and highest percentage of oil in seeds and kernels. It was followed closely by RSAD FWB and MB accessions with respect to 1000-seed weight, kernel weight, percentage of oil in seeds and percentage of oil in kernels. Among the elite accessions, the RSAD and MB accessions had darker leaves and flowers. Some fungal diseases like damping off (*Phytophthora* sp) and root rot (*Pythium* sp) were spotted in some accessions and insect pests like *Pinnapsis strachani* (cushion scale), *Ferrisia vergata* (woody aphid) and *Spodoptera litura.* However, the KM and RSAD accessions were highly resistant to diseases and pests.

Similar to the present results, morphological variations and variations in seed-traits were also reported earlier in different parts of the world[3,4,14]. Highly significant correlations between 1000 seed-weight and percentage of crude fat content was reported earlier[14] and these results are in agreement with our observations in RSAD accession with 1000 seed-weight (620 gm) and hydrocarbon content (0.477). Highest 1000 seed-weight (640 gm) and seed-oil content (50.16 %) was recorded in the KM accession. Therefore, the study of quantitative and qualitative traits point to the fact that KM is the most desirable followed by the RSAD accession.

Seed characteristics

Data of seed characteristics show that the KM and RSAD accessions yielded the highest kernel weight, percentage of oil in whole seeds, percentage of oil in kernels with lower shell weight (Table 3). Although the MB accession produced highest number of seeds, the seeds yielded the least percentage of oil with highest shell weight. Further, the MB accession produced the bulkiest seeds and the FWB accession produced the smallest seeds as evidenced by the data of seed-length and seed-width. Seeds of KM accession have the biggest kernel and smallest shell content followed by the RSAD accession as evidenced by the kernel to shell percentage ratios. Maximum percentage of oil from kernels was recorded in KM accession followed by RSAD, FWB and MB accessions and the highest weight of seed-cake was obtained from KM accession. Oil extraction was definitely more efficient when shells were removed since the percentage of oil from kernels was higher than from seeds with shells intact (Table 2). Variation in seed-oil content was reported earlier in *Jatropha* growing in different climatic conditions and altitudes[19]. In the present study, highest oil content (50.16 %) was recorded in KM accession followed by RSAD (45.66%), FWB (44.26%) and MB (40.73%) accessions. Therefore, the study of quantitative and qualitative traits point to the fact that KM is the most desirable followed by the RSAD accession with optimum height, higher number of primary and secondary branches, leaf area index, optimum number of pods per plant, bulk of seed, seed-yield and weight of seed-cake, highest 1000-seed weight, kernel weight, percentage of oil content in seeds with and without shells and resistance to diseases and pests.

Physico-chemical characterization of seed-oil

Physico-chemical characteristics and fatty acid profiles of seed-oil of the 4 elite accessions of *Jatropha* are presented in Table 4. The seed-oil of the MB accession contained the highest acid value and free fatty acids and that of RSAD accession contained least acid value and free fatty acids. Seed-oil of RSAD accession contained least values of phosphorus content, viscosity and iodine number but, that of MB accession contained the highest values of phosphorus content, viscosity and iodine number (Table 4). High values of phosphorus content, viscosity and iodine number interfere with conversion to biodiesel. Seed-oil of RSAD accession contained highest hydrocarbon content, and the MB accession contained the least and the amounts of triglycerides and diglycerides ranged from 1.2 to 1.3% in all the accessions as revealed by Column chromatographic studies (Table 4). The Fatty acid profiles of the 14 oil samples estimated by Gas chromatography (Table 4) help in assessing the feasibility of blending the crude oil with diesel.

Parameters like high free fatty acid content (recorded in MB and FWB accessions) and moisture content of seed-oil affect the transesterification process and also interfere with the separation of fatty esters and glycerol. Further, high phosphorus content and iodine number interfere with conversion to biodiesel. In this context, the seed-oil of RSAD accession was found to be most suitable for Biodiesel production followed by KM, FWB and MB accessions (although its yield was lower than KM), since it has lower free fatty acids, acid value, viscosity, triglycerides, iodine number and unsaturated fatty acids like linolenic acid. These results and those of quantitative and qualitative analyses confirm that KM and RSAD are the most desirable accessions for biodiesel production. Apart from the above properties, those of the individual fatty esters that comprise biodiesel determine the overall fuel properties of the biodiesel fuel[20,21]. In turn, the properties of the various fatty esters are determined by the structural features of the fatty acid and the alcohol moieties that comprise a fatty ester. Structural features that influence the physical and fuel properties of a fatty ester molecule are chain length, degree of unsaturation, and branching of the chain. Important fuel properties of biodiesel that are influenced by the fatty acid profile among others are viscosity, and lubricity. Iodine number and unsaturated fatty acids like linolenic acid[22]. Presently, the seed-oil of RSAD accession was found to be most suitable for production of Biodiesel, followed by the KM, FWB and MB accessions (although its yield was lower than KM), since it contains lower free fatty acids, acid value, viscosity and diglycerides,

Molecular analysis for estimation of genetic diversity using RAPD

Molecular analysis was carried out by RAPD method employing 10 random primers for assessing the genetic diversity. Polymerase chain reaction (PCR), an in vitro molecular technique that amplifies microgram quantities of DNA copies of the desired specific sequences of DNA present even as a single copy in the sample within few hours. The PCR analysis and the SAHN clustering program of the NTSYSpc package was used to construct an UPGMA dendrogram and the similarity coefficients were studied from the squared data matrix of the Principal Coordinate Analysis (PCA).

PCR analysis revealed that a total of 10 primers could elicit reliable banding patterns with high reproducibility and clear band resolution (Fig. 2). Hence, these were used for further analyses. These 10 primers (OPM-3, OPM-4, OPM-9, OPM-10, OPM-11, OPM-12, OPM-13, OPM-14, OPM-17, and OPM-19) produced a total of 63 distinct amplification products ranging from 100 to 2000 bp and some representative agarose gels are presented (Fig. 2). The number of scored bands per primer ranged from 3 (OPM-14) to 11 (OPM-10) with mean of 6.3 bands per primer (Table 5). Whereas, OPM-13 and OPM-14 primers produced zero polymorphic products, the highest polymorphic bands (9) were produced by OPM-10 primer. Among these amplified products, and the total of 63 bands, a total of 30 were polymorphic with an average mean of 3 and the total polymorphism was 402.98 with an average mean of 40.298, and percentage polymorphism ranging from 0 % (produced by primers OPM-13 and OPM-14) to 83.33 % (obtained by OPM-11 primer).

Jacquard's pair-wise similarity coefficient (Genetic similarity matrix) values for the four accessions of *J. curcas* were also calculated by using NTSYS pc ver. 2.0 (Table 5). Analysis of the relationship was based the on the 63 RAPD bands, which revealed that the genetic similarity of the four accessions ranged from 0.5079365 to 0.9682540. Among the four accessions, the least similarity (0.5079365) was observed between RSAD and MB accessions and the highest similarity (0.9682540) was observed between KM and FWB accessions. The Principal Coordinate Analysis (PCA) based on similarity matrix of the four accessions showed that the RSAD, FWB and KM accessions were grouped together and MB accession was grouped separately since it was distinct from others.

Results of UPGMA dendrogram (Fig. 3), with the four accessions were similar to those of PCA. Genetic similarity (Cluster analysis) matrices using the UPGMA ranging from 52% (48% variability) to 95 % (5 % variability) was obtained using RAPD primers (OPM-3, OPM-4, OPM-9, OPM-10, OPM-11, OPM-12, OPM-13, OPM-14, OPM-17, and OPM-19). From the dendrogram generated by UPGMA cluster analysis, the four accessions could be split into two groups of 3 and 1 at 52 % similarity. First group of three accessions could be divided into cluster "A" consisting of 1 and cluster "B" consisting of 2 accessions.

These two clusters reflected the genetic variability of 12% with a similarity of 88 %. The B cluster was sub-divided into two groups with a similarity of 95%. These results and conclusions are based on Knothe[2].

The principal coordinate analysis (PCA) indicated that RSAD, FWB and KM accessions were distinct in the coordinate plot whereas the MB accession was reasonably distinct from the others. The PCA showed similar clustering method compared to UPGMA dendrogram. The genetic similarity matrix revealed least similarity (0.5079365) between RSAD and MB accessions and highest similarity (0.9682540) between KM and FWB accession. Further, the UPGMA dendrogram analysis indicated that the first group of three accessions could be divided into a cluster 'A' consisting of 1 and cluster 'B' consisting of 2 accessions. These two clusters reflected the genetic variability of 12 % with 88 % similarity. The 'B' cluster was subdivided into two groups with a similarity of 95 %.

DNA markers have been used to quantify genetic diversity and to determine the phenetic relationships in several plant species[13,23,24,25,26]. The present results and conclusions are based on an earlier report [23] where, a 96.3% similarity between accessions by using with OPJ and OPM sets of RAPD primers was reported. In the present investigation also, OPM set of RAPD primers were used to amplify the DNA of the 4 accessions. Similarity and cluster analysis can therefore be used as a tool to identify desirable characteristics of accessions in biodiesel production programs.

In the present study, although the KM accession yielded the highest seed-weight and seed-oil content, the RSAD accession was more suited for production of Biodiesel as revealed by the physico-chemical characteristics. The study reveals considerable variability in the *J. curcas* accessions in accordance with morphological traits and provides genetic markers for their morphological identification. The narrow genetic base revealed in the present study emphasizes the need to exploit large germplasm collections having diverse morpho-agronomic traits in accession improvement programs[27].

Table-1: Information pertaining to the fourteen collected accessions of *Jatropha curcas* at Osmania University

S. No.	Accession	Name of location	GPS Coordinates:	
			Latitude	Longitude
1	A.P. Government's Rain Shadow Area Department ("RSAD")	Collection from Rajasthan State	19° 37'N	78° 30'E
2	Farm Wealth Biotech Pvt Ltd (FWB)	Hyderabad, A.P.	20° 59'N	78° 30'E
3	Karimnagar (KM)	Wild plants from Venkatapur Karimnagar, A.P.	19° 37'N	78° 96'E
4	Mahabubnagar ("MB")	Wild plants from Bapanpalli, Mahabubnagar, A.P.	16° 74'N	77° 98'E
5	Mahabubnagar – 2 (MB2)	Wild plants 2 Km away from "MB" ("MB2")	16° 49'N	77° 30'E
6	Ranga Reddy ("RR")	Wild plants from Chandanagar Ranga Reddy, A.P.	17° 49'N	78° 34'E
7	Narsipatnam ("NP")	Wild plants from Narsipatnam Vishakapatnam A.P.	17° 66'N	82° 61'E
8	Jodhpur ("JP")	Wild plants from Jodhpur, Rajasthan.	26° 17'N	73° 11'E
9	Adilabad ("AB")	Wild plants from Kanagutta village, Adilabad, A.P.	19° 40'N	78° 32'E
10	Sumerpur - 11 ("S-11")	Sumerpur, Rajasthan State Collection Centre.	25° 09'N	73° 10'E
11	Sumerpur - 12 ("S-12")	Sumerpur, Rajasthan State Collection Centre.	25° 09'N	73° 10'E
12	Sumerpur - 13 ("S-13")	Sumerpur, Rajasthan State Collection Centre.	25° 09'N	73° 10'E
13	Sumerpur – 14 ("S-14")	Sumerpur, Rajasthan State Collection Centre.	25° 09'N	73° 10'E
14	Sumerpur - 15 ("S-15")	Sumerpur, Rajasthan State Collection Centre.	25° 09'N	73° 10'E

Table-2: [a] Quantitative characteristics of 14 accessions of *Jatropha curcas* recorded from 3-year-old plants at Osmania University

S.No	Accession	Plant height (m) Mean± S.E.	No. of primary branches Mean± S.E.	No. of secondary branches Mean± S.E.	Leaf area index (c^2) Mean± S.E.	No. of pods per plant Mean± S.E.	Seed-yield per plant (gm)	1000 Seed-weight (gm)
1	KM	3.0 ± 0.24	3.16 ± 0.47	17.6 ± 0.09	152.1 ± 1.28	68.56 ± 0.50	385	640
2	MB	3.0 ± 0.18	3.57 ± 0.20	15.4 ± 1.35	151.1 ± 1.04	72.66 ± 0.05	394	589
3	S-15	2.8 ± 0.18	2.85 ± 0.16	12.2 ± 1.03	139.1 ± 0.28	60.71 ± 0.49	258	554
4	S-12	2.8 ± 0.17	2.67 ± 0.10	11.5 ± 1.25	147.1 ± 0.75	57.31 ± 0.28	285	520
5	RSAD	2.6 ± 0.08	2.84 ± 0.21	14.0 ± 1.44	140.3 ± 1.47	65.28 ± 0.47	379	620
6	AB	2.6 ± 0.48	2.54 ± 0.18	11.4 ± 1.08	115.4 ± 0.09	57.18 ± 0.21	250	565
7	S-13	2.6 ± 0.16	2.71 ± 0.47	12.4 ± 1.38	119.1 ± 0.63	57.26 ± 0.21	258	549
8	S-11	2.5 ± 0.26	2.48 ± 0.07	12.5 ± 1.06	135.2 ± 0.85	59.65 ± 0.34	252	567
9	MB2	2.5 ± 0.15	2.35 ± 0.53	12.8 ± 1.78	125.1 ± 0.95	54.20 ± 0.25	225	515
10	S-14	2.5 ± 0.08	2.62 ± 0.05	11.4 ± 1.47	130.1 ± 0.14	58.04 ± 0.06	290	440
11	NP	2.4 ± 0.19	2.38 ± 0.54	11.9 ± 1.54	129.1 ± 0.28	58.67 ± 0.08	235	520
12	JP	2.3 ± 0.33	2.34 ± 0.22	11.8 ± 1.17	118.2 ± 0.31	51.24 ± 0.17	268	553
13	RR	2.2 ± 0.21	2.35 ± 0.28	12.9 ± 1.24	132.2 ± 0.10	54.40 ± 0.38	229	528
14	FWB	2.0 ± 0.13	2.33 ± 0.58	11.8 ± 1.77	128.1 ± 0.95	56.40 ± 0.31	240	520

[a] Data was recorded from 30 plants (3-year old plants) in the plantation and presented as mean ± S.E

Table-3:[a] Seed-characteristics of 4 accessions of *Jatropha curcas* recorded from 3-year-old plants at Osmania University

Accession	Seed-length (cm) Mean ± S.E	Seed-width (cm) Mean ± S.E	% weight of kernels (gm)	% weight of shells (gm)	% oil in seeds with shell	% oil in kernels	Weight of seed cake (gm)
RSAD	2.68 ± 0.07	3.72 ± 0.06	60.0	40.0	27.4	45.66	323
KM	2.43 ± 0.02	3.62 ± 0.05	61.0	39.0	30.6	50.16	299
MB	2.86 ± 0.06	3.83 ± 0.04	46.4	53.6	18.9	40.73	272
FWB	2.36 ± 0.04	3.25 ± 0.05	56.7	43.3	25.10	44.26	297

[a] Data was recorded from 30 plants (3-year old plants) in the plantation and presented as mean ± S.E

Table-4: Physico-chemical properties of the seed-oil of 4 elite accessions of *Jatropha curcas* recorded from 3-year-old plants at Osmania University

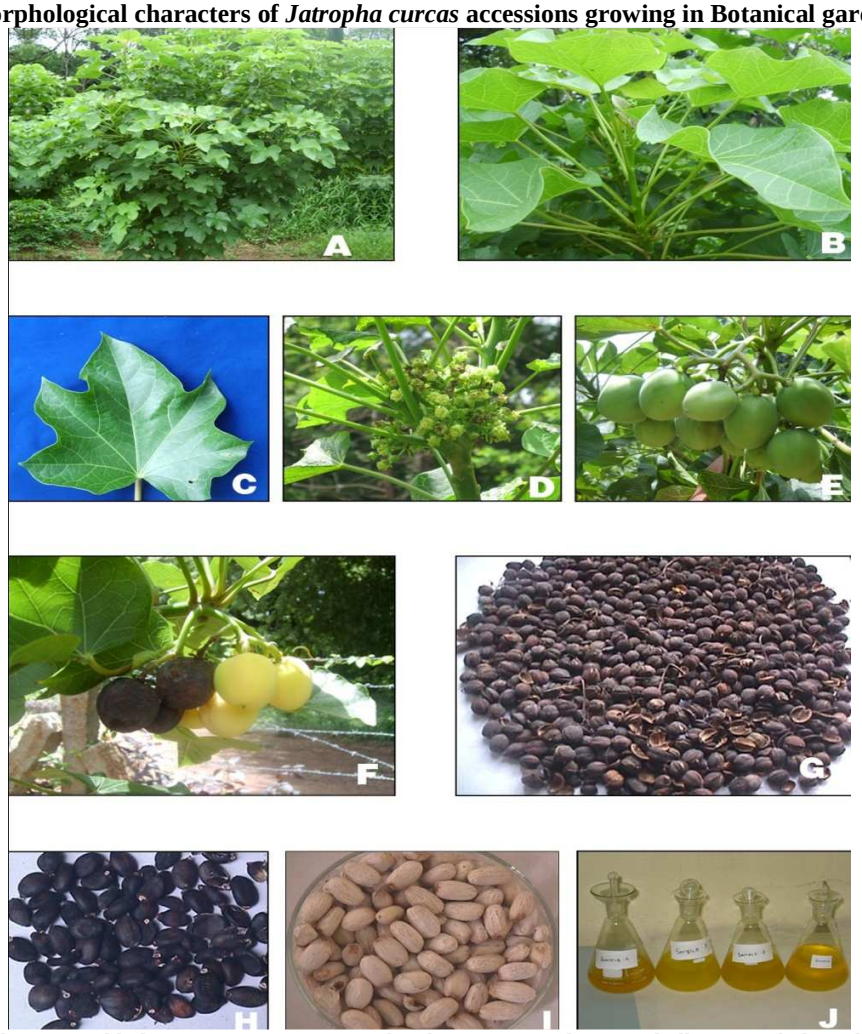
S.No	Parameter	RSAD	KM	MB	FWB
1	Acid value mg KOH/g	3.6895	5.4306	5.8858	4.9838
2	Saponification number (mg/g)	197.4753	195.322	198.1402	184.4394
3	Unsaponifiable number (%)	0.9755	0.5268	0.7226	0.6218
4	Refractive index 40^0C	1.4768	1.46642	1.46642	1.46728
5	Iodine values (% absorbed)	98.2635	98.5656	102.8190	96.0641
6	Moisture & volatile matter (%)	0.0402	0.0457	0.0184	0.0269
7	Color units	1.2	1.1	0.95	1.1
8	Density at 40^Ocg/cm^3	0.9078	0.9077	0.9071	0.9079
9	Viscosity (Cst) at 40^0C	34.6	34.65	37.20	34.72
10	Phosphorus ppm	114.8386	128.7386	153.4651	139.7449
11	Column chromatographic analysis:				
	% Hydrocarbons	0.477	0.452	0.314	0..381
	% Triglycerides	95.740	95.631	95.620	95.804
	% Free Fatty Acids	2.189	2.632	2.851	2.390
	% Diglycerides	1.383	1.274	1.201	1.391
12	Gas chromatographic analysis:				
	Myristic Acid %	0.06	0.06	-----	0.07
	Palmitic Acid %	15.16	13.92	13.51	15.31
	Palmitolic Acid %	0.83	0.77	0.72	0.87
	Stearic Acid %	6.49	6.70	7.33	6.63

	Oleic Acid %	39.65	41.99	52.97	38.77
	Linoleic Acid %	37.38	36.11	24.87	37.90
	Linolenic Acid %	0.19	0.20	0.28	0.21
	Arachidic Acid %	0.20	0.21	0.28	0.20
	Total saturated fatty acids %	21.91	22.21	20.89	21.12
	Total unsaturated fatty acids %	78.05	77.75	79.07	78.84

Table-5: Estimation of genetic variation in terms of the number of amplified products, polymorphic bands and percentage of polymorphism scored with RAPD Primers

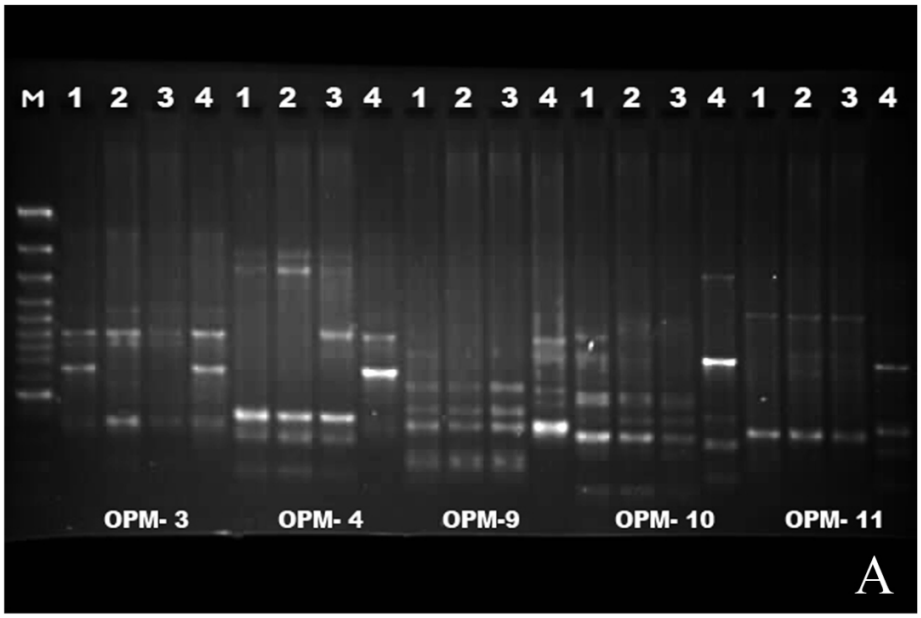
Primer	Sequence	G-C%	No of Bands	Polymorphic Bands	Polymorphism
OPM-3	3'-GGG GGA TGA G - 5'	70	6	2	33.33
OPM-4	3'-GGC GGT TGT C -5'	70	7	3	42.85
OPM-9	3'- GTC TTG CGG A - 5'	60	8	6	75.00
OPM-10	3'-TCT GGC GCA C - 5’	70	11	9	81.81
OPM-11	3'- GTC CAC TGT G- 5'	60	6	5	83.33
OPM-12	3'- GGG A CG TTG G- 5'	70	6	1	16.66
OPM-13	3'- GGT GGT CAA G- 5'	60	5	0	0
OPM-14	3'- AGG GTC GTT C- 5'	60	3	0	0
OPM-17	3'- TCA GTC CGG G- 5'	70	6	3	50.00
OPM-19	3'- CCT TCA GGC A- 5'	60	5	1	20.00
Total			63	30	402.98
Mean			6.3	3.0	40.298

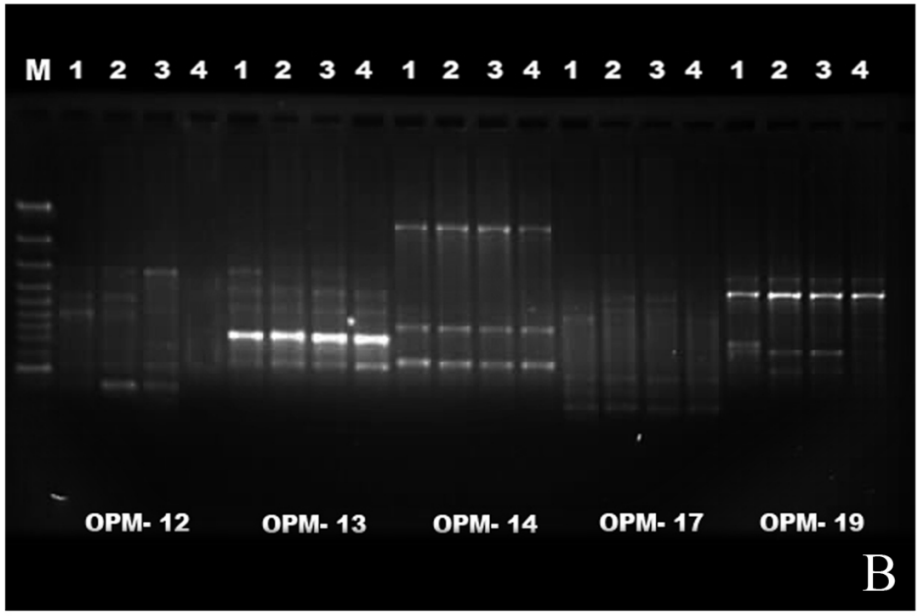
Fig. 1. Morphological characters of *Jatropha curcas* accessions growing in Botanical garden, O.U.



A.Three year old plant (RSAD) approximate height: 4.0 m, **B.** Alternate phyllotaxy with dense leaves (KM), **C.** Dark-green leaf (MB), **D.**Inflorescence (Panicle) (FWB), **E.** Immature Capsules (RSAD), **F.** Yellow mature Capsules (RSAD), **G.** Dry Capsules (KM), **H.** Seeds (KM), **I.** Kernels (seed coat removed) (MB), **J.** Seed-oil extracted from 4 accessions: Sample 1 = RSAD, Sample 2 = KM, Sample 3 = MB and Sample 4 = FWB

Fig. 2: Agarose gel (1.5%) showing amplified DNA patterns of 4 accessions of *Jatropha curcas* obtained with a RAPD–PCR reaction using OPM set of primers

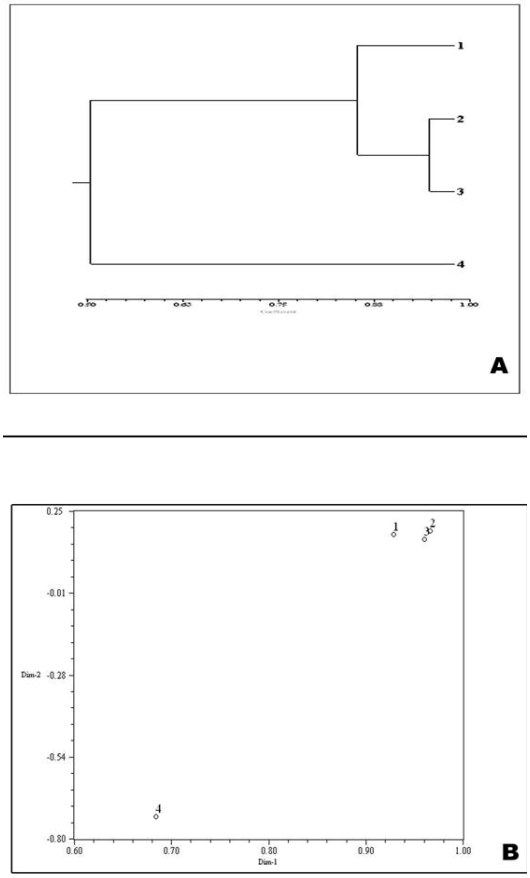




A) RAPD profile of the 4 accessions of *Jatropha curcas* obtained with OPM-3, OPM-4, OPM- 9, OPM-10 and OPM11- primers. Lane M is 1 kb DNA ladder marker and 1, 2, 3 and 4 represent RSAD, FWB, KM and MB accessions respectively.

B) RAPD profile of 4 accessions of *Jatropha curcas* obtained with OPM-12, OPM-13, OPM-14, OPM-17 and OPM-19 primers. Lane M is 1 kb DNA ladder marker and 1, 2, 3 and 4 represent RSAD, FWB, KM and MB accessions respectively.

Fig. 3: Dendrogram and Principal Coordinate Analysis (PCA) using RAPD data of 4 accessions of *Jatropha curcas* performed by NTSYSpc software



A. Dendrogram illustrating genetic relatedness among four accessions of *Jatropha curcas* generated by UPGMA Cluster Analysis based on 63 RAPD-bands. 1, 2, 3 and 4 represent RSAD, FWB, KM and MB accessions respectively.
B. PCA using RAPD data of four accessions of *Jatropha curcas* 1, 2, 3 and 4 represent RSAD, FWB, KM and MB accessions respectively.

CONCLUSION

In the study conducted to select and characterize the most desirable Jatropha accession for production of biodiesel, KM accession recorded the highest seed-weight and seed-oil content followed by the RSAD accession. However, the latter was more suited for production of Biodiesel due to its superior physico-chemical characteristics. Molecular analysis confirmed the genetic variability between the accessions in relation to the variation in morphological and physico-chemical traits and superior accessions could be distinguished by RAPD markers.

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