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ORIGINAL PAPER

Physico-Chemical Analysis, NMR Spectroscopy and Gas Chromatographic Studies of *Jatropha curcas* L. Germplasm

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Abstract A systematic collection of *Jatropha curcas* germplasm was carried out from five distinct ecogeographic zones of peninsular India in 2008. This involved recording passport data, documentation of important plant traits in situ, ecogeographic parameters and assessment of variability in 82 accessions. Extraction of oil was done by the Soxhlet method which gave an average yield of 38%. Oil content of collected accessions was determined by nuclear magnetic resonance and Soxhlet extraction. Oil variability ranged from 45.5% in JC-8 to 11.5% in JC-30. The level of unsaturated fatty acid ranged from 85% in JC-57 to 75.5% in JC-10. Saturated fatty acids ranged from 24% in JC-43 to 15% in JC-54, the oxidative stability index was highest at 2.1 in JC-30 and lowest at 0.68 in JC-17. The seed weight was greatest in JC-8 and the lowest in JC-30. This paper provides information that will facilitate the selection of promising accessions for genetic enhancement of *Jatropha* germplasm through conventional breeding.

Keywords *Jatropha* · Physico-chemical · NMR · Fatty acid

Introduction

Utilization of *J. curcas* oil as a new source for diesel engines has the potential for meeting a growing need for energy resources in India. Several research institutions are working on the possibility of creating energy crops that could increase biomass yields. However, there is much to be learned about *Jatropha* production, commercialization, and genetic improvement. No work on genetic improvement aspects of this species has been undertaken in India. Systematic provenance trails at different locations have not been carried out with *J. curcas* in India either. Owing to its importance, the production of this species has increased substantially in different parts of India [1]. Nearly 40% of India is waste land. There is a good reason for developing *J. curcas* as a new energy crop, as it does not compete with conventional food crops for land, water and manpower resources and it also has the ability to make a significant contribution to the nation's growing needs for energy through large-scale cultivation with ease [2]. In this respect *Jatropha* is a multipurpose shrub fit for agroforestry and other afforestation programmes in wasteland development. It grows well under diverse climatic conditions because of its low moisture demands, fertility requirements and tolerance of high temperatures. There are readily available methodologies for characterizing the accessions reliably for germplasm collected systematically from wild and diverse locations of seed traits [3]. Systematic provenance trials and analysis of genetic resources of *J. curcas* is very sporadic. Forty-two accessions from Thailand studied did not show significant morphological, developmental and seed yield variation [4]. On the other hand, a five cultivar trial for both *J. curcas* and *J. gossypifolia* L. in India and Africa revealed variations in characteristics such as plant height, branches per plant, and seed yields [5]. Recently genetic variability in growth performance, oil yields, contents and seed traits of *J. curcas* accessions collected from Madhya Pradesh, Andhra Pradesh and Haryana have been analyzed from India [6–8]. Under the rain shadow area

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development (RSAD) project modest levels of genetic variation were found among the 42 accessions of *Jatropha* [9].

The objective of the present study was to investigate the variation in *Jatropha* seeds with respect to oil content, seed weight, the oxidative stability index (OSI) and fatty acid estimation of 82 accessions collected from different parts of India to be utilized for reforestation and future genetic improvement work to become a profitable crop for fuel production.

Materials and Methods

Seed Samples

Mature fruits of *Jatropha* were collected during the year 2008 from various parts of Maharashtra, Gujarat, Karnataka, Andhra Pradesh and Tamilnadu States. Fruits were chosen from a single plant to provide potentially useful genetic variation. Seeds from capsules were removed through manual threshing. The seeds were separated from the fruit which is moist, then cleaned and stored in plastic bottles under ambient conditions. All seed lots were dried under similar temperature and humidity conditions to reach a constant weight. For seed weight, three samples of seeds including 100 seeds each were taken from each seed lot, measured for weight and expressed in grams.

Oil Extraction and Nuclear Magnetic Resonance (NMR) Analysis

The seed oil was extracted with petroleum ether (60–80 °C) in a Soxhlet apparatus for 6 h. Solvent was removed under reduced temperature and pressure and the yield of oil was calculated. The oil content of seeds was estimated by the Soxhlet method. The level of oil in seed germplasm was analyzed by an NMR method at 5 MHz using a low resolution Oxford 4000 NMR analyzer calibrated by Soxhlet-extracted *Jatropha* oil. Seed samples dried for 3 h in an oven (50 °C) were used for analysis and the results expressed as percentages. All measurements were performed in triplicate.

Physico-Chemical Properties

The iodine value, saponification value, and acid value of the oil were estimated by standard procedures [10, 11]. The mean molecular mass (MMM) was estimated from the relationships (560/saponification value) × 100 [12–15]. The FFA was calculated from the relationship given in [11]. The specific gravity was determined by the method in [16]. Crude nitrogen was determined by the Micro Kjeldhals method and protein was calculated by N × 6.25. The ash content determination was carried out in a muffle furnace at 560 °C and the moisture content by a Sartorius MA 45 autoanalyser at 100 °C.

Fatty Acid Analysis

Powdered seeds of collected samples were used for the determination of fatty acids [17]. The fatty acid estimation was done on an Agilent 6890N gas chromatograph with an auto sampler and an auto injector. The samples were injected in a 30-mm long and 0.32-mm diameter HP-Innovax Capillary Column. Auto Injector, oven temperature and flame ionization Detector (FID) were adjusted to 225, 115, 275 °C, respectively. The initial oven temperature was 150 °C ramped at 15 °C/min up to 250 °C. An FID was used to detect the signals. Hydrogen and air with flow rates of 30 ml/min and 400 ml/min, respectively, were used to ignite the flame of the FID. Nitrogen gas (2 ml/min) was used as the carrier gas. Standard fatty acids from Sigma Chemicals Limited were used as standards to calibrate the method. The signals from the detector were integrated as normal percentages of the calibration curve by using HP Chemstation software. Furthermore, the OSI ratios were found out by the monounsaturated fatty acids/polyunsaturated fatty acids (MUFA/PUFA) method [18, 19].

Statistical Analysis

This was carried out using Agrobase/4TM (Agronomix Software Inc., MB, Canada) by one-way ANOVA and an analysis of different sources of variability was performed. The parameters include: the R^2 value, the coefficient of variation (CV) and heritability with respect to saturated fatty acids (SFA), unsaturated fatty acids (UFA), oil content and seed weight. R^2 values were 0.9973, 0.9817, 0.9921, 0.9998; CV 0.80, 0.52, 2.40, 0.38% and heritability was 0.995 0.963, 0.984, 1.000, respectively.

Results and Discussions

The utility of any vegetable oil largely depends upon its chemical properties and fatty acid composition. Physico-chemical parameters viz. acid value, iodine value, saponification value, free fatty acid content, MMM, specific gravity and cetane number of seed oil showed that all values fall within the range of other non-edible oil resources like *Aphanamixis, Bauhinia* and *Caesalpinia* [20, 21] (Table 1). The low acid value and high content of polyunsaturated fatty acids in *Jatropha* oil affects its acceptability for biofuel purpose. However, in the present investigations, 82 accessions collected from different ecotypes were studied

Table 1 Physico-chemical characteristics of *Jatropha* species

Parameters	Results
Oil content of seeds (%)	38.0
Saponification value (mg/g)	324.6
Iodine value (g/100 g)	139.0
Acid value (mg/g)	1.86
Free fatty acids (mg/g)	10.4
Mean molecular mass (MMM)	172.5
Specific gravity at RT (28 °C)	0.922
Cetane number (CN)	1.48
Nitrogen in fatted cake (%)	2.6
Nitrogen in defatted cake (%)	5.0
Protein (%) fatted	16.5
Protein (%) defatted	26.9
Ash (%)	9.3
Moisture (%)	4.86

for 100 seed weight, oil content, fatty acid and OSI by various biometrical traits. NMR analysis showed that 28 accessions recorded oil percent range between 35 and 40% (34%). While 18 accessions like JC-10, JC-14, JC-15, JC-16, JC-17, JC-39, JC-53, JC-59, JC-64, JC-65, JC-68, JC-69, JC-70, JC-73, JC-75, JC-77, JC-78, JC-83 recorded more than 40% (10%), 46 accessions (56%) showed less than 35% oil. The ratio of MUFA to PUFA is an indicator of oxidative stability of the oil, the OSI of 82 accessions was found to be in the range of 0.68–2.0 (Table 2). It is higher than the soybean and sunflower oil [22]. SFA and UFA play important role in degree of saturation and trans-esterification of oil. Present investigation showed a lower percentage of SFA and a higher percentage of UFA and is suitable for trans-esterification. Data studied were subjected to statistical analysis (ANOVA) indicated that the population investigated was heterogeneous. Identification of promising lines among the germplasm collections would require a concerted study over a period of time, usually 5–10 years in a under tree species like *Jatropha* [23]. However, a method of scoring of collected *Jatropha* accessions in situ for all traits has been used as an attempt to identify superior genotypes under uniform climatic conditions at Hol and Sonagaon villages, Baramati taluka of Agharkar Research Institutes agricultural farm. Based on the observations, superior accessions for each zone will be identified. The top ranked accessions within each group will be selected and analyzed for the eco-geographic conditions. Thus, it focuses on necessity of a study to be done in different agroclimatic zones to understand the consistency of characters in elite *Jatropha* germplasm. The present research is one step in evaluating the best germplasm from India to identify the promising *Jatropha* type for improvement and further breeding purposes.

Table 2 Variability for biometrical traits, fatty acid profile (%, w/w), oxidative stability index (OSI) and oil content (%, w/w)

Sr. no.	Acc. no.	Wt. of 100 Seeds (g)	SFA	UFA	OSI	Oil (%)
1.	JC-2	58.18	19.74	80.26	0.69	35.9
2.	JC-3	62.5	22.46	77.54	0.68	37.2
3.	JC-5	65.0	20.23	79.76	0.88	38.1
4.	JC-8	88.0	20.41	79.60	0.98	36.7
5.	JC-9	76.5	19.09	80.92	0.72	35.0
6.	JC-10	73.3	20.52	73.48	0.82	43.3
7.	JC-11	44.9	19.95	80.04	0.69	34.5
8.	JC-13	48.9	19.45	80.54	0.81	31.5
9.	JC-14	67.4	20.12	79.87	0.81	40.4
10.	JC-15	61.3	21.15	78.85	0.79	44.7
11.	JC-16	69.7	19.58	80.43	0.74	45.5
12.	JC-17	76.0	21	78.99	0.68	44.4
13.	JC-18	52.9	22.44	77.57	0.78	37.5
14.	JC-20	58.5	19.58	80.43	0.74	36.2
15.	JC-21	38.2	19.32	80.69	1.52	39.0
16.	JC-22-1	38.3	20.74	79.26	1.81	31.0
17.	JC-22-2	38.3	20.74	79.26	1.81	31.0
18.	JC-23	58.4	20.21	79.79	1.64	30.3
19.	JC-24	49.2	20.83	79.17	0.92	31.6
20.	JC-25	59.1	20.36	79.63	0.90	37.5
21.	JC-26	36.4	20.01	79.99	1.60	23.2
22.	JC-27	65.4	18.56	81.43	0.95	34.0
23.	JC-28	30.8	21.56	78.43	2.01	13.5
24.	JC-29	50.7	23.32	76.67	1.63	25.7
25.	JC-30	28	22.72	77.28	2.11	11.5
26.	JC-31	53.4	19.84	80.16	1.06	28.6
27.	JC-32	31.4	20.99	79.01	1.67	17.2
28.	JC-33	50.2	19.98	80.03	1.85	28.7
29.	JC-34	52.2	21.93	78.08	0.84	36.5
30.	JC-35	49.4	20.86	79.13	0.73	36.6
31.	JC-36	57	20.97	78.83	0.91	39.4
32.	JC-37	51.8	20.41	79.60	0.99	27.6
33.	JC-38	49.6	20.33	79.67	0.82	35.3
34.	JC-39	53.8	20.2	79.79	0.82	40.8
35.	JC-40	60.4	18.96	80.13	0.80	36.1
36.	JC-41	44.8	20.78	79.22	0.96	30.4
37.	JC-42	60.4	21.14	78.85	0.95	34.1
38.	JC-43	48.4	24.36	75.72	1.34	35.4
39.	JC-44	58	19.44	80.55	0.82	35.3
40.	JC-45	55.6	20.09	79.90	0.78	37.2
41.	JC-46	53.8	22.45	77.54	0.96	33.9
42.	JC-47	52.2	20.24	79.76	0.87	23.6
43.	JC-48	71.2	19.68	80.31	0.85	35.4
44.	JC-49	52.2	19.24	80.75	0.94	33.3
45.	JC-50	48.0	21.01	78.99	0.82	29.1
46.	JC-51	62.8	21.33	78.67	0.79	33.9
47.	JC-52	45.4	21.34	78.67	1.10	32.5

Table 2 continued

Sr. no.	Acc. no.	Wt. of 100 Seeds (g)	SFA	UFA	OSI	Oil (%)
48.	JCH	48.9	19.86	80.13	0.90	27.4
49.	JC-53	57.0	19.98	80.03	1.85	40.8
50.	JC-54	63.4	14.8	85.21	0.99	30.4
51.	JC-55	41.0	16.23	83.77	1.98	24.7
52.	JC-56	59.8	17.84	82.16	0.91	36.2
53.	JC-57	44.4	15.62	84.65	0.91	24.2
54.	JC-58	57.6	17.26	82.73	0.90	35.8
55.	JC-59	73.0	16.52	83.48	0.86	40.4
56.	JC-60	56.6	16.72	83.27	0.88	35.0
57.	JC-61	59.6	16.69	83.31	0.71	39.7
58.	JC-62	64.2	17.21	82.80	0.89	39.4
59.	JC-63	68.2	16.76	83.31	0.70	38.9
60.	JC-64	63.4	17.53	82.46	0.86	40.8
61.	JC-65	67.8	16.53	83.47	0.89	43.4
62.	JC-66	64.6	16.62	83.38	0.78	37.9
63.	JC-67	62.5	16.6	83.38	0.78	37.9
64.	JC-68	61.5	15.91	84.18	0.86	41.3
65.	JC-69	62.6	16.78	83.22	0.81	42.7
66.	JC-70	52.01	17.33	82.66	0.91	42.0
67.	JC-71	56.6	17.03	83.16	0.89	37.0
68.	JC-72	59.1	16.16	83.83	0.98	25.8
69.	JC-73	67.4	17.04	82.96	0.86	42.2
70.	JC-74	69.4	16.44	83.56	0.81	38.5
71.	JC-75	75.4	16.95	83.05	0.81	42.1
72.	JC-76	66.8	15.91	84.18	0.86	37.0
73.	JC-77	68.4	16.19	84.18	0.86	41.1
74.	JC-78	61.8	17.39	82.62	0.92	42.0
75.	JC-79	55.5	16.8	83.20	0.78	35.7
76.	JC-80	55.4	17.41	82.58	0.94	34.3
77.	JC-81	70.0	17.19	82.81	0.82	38.7
78.	JC-82	78.0	17.3	82.70	0.84	35.9
79.	JC-83	71.0	17.53	82.66	0.91	40.1
80.	JC-84	62.2	16.28	83.72	1.02	30.9
81.	JC-85	73.0	17.54	82.43	0.99	36.5
82.	JC-86	57.2	17.75	82.25	1.11	27.0

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