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Phenotypic and Chemical Diversity of Seed Oils Across *Jatropha curcas* L. Germplasm Collections

K. T. Parthiban*, P. Selvan, M. Paramathma, S. Umesh Kanna, P. Kumar,
V. Subbulakshmi and S. Vennila

Forest College and Research Institute, Tamil Nadu Agricultural University, Mettupalayam – 641 301, Tamil Nadu, India.

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Studies were carried out to assess seed physico-chemical properties of *Jatropha curcas* genetic resources. Twenty-five seed sources of *J. curcas* investigated in the current study exhibited wide variation for seed physio-chemical properties. The chemical properties of seed sources indicated superiority of more number of seed sources for iodine value, cetane number and saponification value. Hence the *J. curcas* genetic resources tested in this current study could be the potential sources of raw material for biodiesel production.

Key words: *Jatropha curcas*, iodine number, saponification value.

INTRODUCTION

Jatropha curcas is a perennial shrub to small evergreen tree of upto 6 m height, adapted to all kinds of soil and does not demand any special nutritive regime (Patil and Singh, 1991). It has been introduced in Africa and Asia and is now cultivated worldwide. In India, it was believed to be introduced by the Portuguese settlers during 16th century. It is a multipurpose, deciduous, small tree, reported to be cultivated in drier regions of central and western parts of India. Recently, it has also been introduced in the northern and southern states of India. The plant is widely distributed and fits easily into agricultural system in the form of hedges, windbreak, and erosion barrier or as a source of firewood (Paramathma et al., 2004). In recent years there are growing concerns about the utility of *J. curcas* as a source of biofuel plantations in order to generate alternate source of energy. This is mainly due to the suitability of oil derived from *Jatropha* seeds for biofuel purposes (Paramathma et al., 2004). The oil contains 21.0% saturated fatty acids and 79.0% unsaturated fatty acids (Bhasabutra and Sutiponpeibun, 1982). The quality of oil in terms of its fatty acid composition is very important. The current studies on the fatty acids distribution of *Jatropha* oil and physico-chemical properties of oil viz., specific gravity, refractive

index, acid value, free fatty acid, iodine value, saponification value and cetane number indirectly influence the quality of oil for biodiesel production. However, such studies are limited in *J. curcas* which thus warrants systematic investigation. Hence studies were carried out on physico-chemical characterization of various *Jatropha* genetic resources.

MATERIALS AND METHODS

The experimental material for the present study consisted of 25 seed sources of *J. curcas* collected from different parts of Tamil Nadu, Andhra Pradesh, Rajasthan and Zimbabwe (Table 1). The regulations for seed source sampling concerning minimum number of trees and distance between parents trees were followed (Lauridsen and Olesen, 1990). Seeds from individual trees were mixed and used as seed source in the present investigation. These experiments were carried out at Forest College and Research Institute, Mettupalayam (11°19'N; 76°56'E; 300 MSL). The actual locations of the seed sources are presented in Table 1.

Oil was extracted by means of solvent extraction using hexane (40 – 60°C) as solvent (Soxhlet extraction) AOAC. (1975). The physico-chemical properties of seed oils of 25 seed sources were assessed as mentioned below.

Specific gravity / density

The specific gravity of *J. curcas* seed sources' oils was determined by specific gravity bottles method using IS: 1460-2000. The

*Corresponding author. E-mail: ktparthi2001@yahoo.com.

Table 1. Details of seed sources.

S/N	Seed source	Location	State	Country	Latitude	Longitude
1	TNMC 01	Uppupallam	Tamil Nadu	India	11°11'N	76°56'E
2	TNMC 02	Palapatti	Tamil Nadu	India	11°19'N	76°57'E
3	TNMC 03	Vedar Colony	Tamil Nadu	India	11°20'N	76°58'E
4	TNMC 04	Thayanoor	Tamil Nadu	India	11°15'N	76°54'E
5	TNMC 05	Vachinampalayam	Tamil Nadu	India	11°20'N	76°59'E
6	TNMC 06	Kurunthamalai	Tamil Nadu	India	11°16'N	76°55'E
7	TNMC 07	Thondampudhur	Tamil Nadu	India	11°00'N	76°51'E
8	TNMC 08	Palayampudhur	Tamil Nadu	India	12°00'N	78°05'E
9	TNMC 09	Nagercoil	Tamil Nadu	India	8°10'N	77°25'E
10	TNMC 16	Pethikuttai	Tamil Nadu	India	11°15'N	77°03'E
11	TNMC 19	Satyamangalam	Tamil Nadu	India	11°29'N	77°09'E
12	TNMC 20	Anthiyur	Tamil Nadu	India	11°34'N	77°35'E
13	TNMC 21	Anaikatti	Tamil Nadu	India	11°05'N	76°50'E
14	TNMC 22	Siruvani	Tamil Nadu	India	11°56'N	76°43'E
15	TNMC 23	Churu	Rajasthan	India	25°30'N	71°36'E
16	TNMC 24	Manjolo	Victoria	Zimbabwe	18°00'N	31°00'E
17	TNMC 26	Himmat Nagar	Andhra Pradesh	India	16°40'N	78°46'E
18	TNMC 28	Azhagiapandipram	Tamil Nadu	India	8°16'N	77°26'E
19	TNMC 29	Madavilagam	Tamil Nadu	India	8°17'N	77°22'E
20	TNMC 30	Kadukarivilagam	Tamil Nadu	India	8°15'N	77°24'E
21	TNMC 31	Barailiyaru	Tamil Nadu	India	8°16'N	77°28'E
22	TNMC 32	Thadikarandonam	Tamil Nadu	India	8°14'N	77°25'E
23	TNMC 33	Vadhabachery	Tamil Nadu	India	10°39'N	76°59'E
24	TNMC 34	Mulli	Tamil Nadu	India	11°7'N	76°56'E
25	TNMC 35	Varasanadu	Tamil Nadu	India	11°01'N	77°28'E

experiments were conducted at 25°C.

Specific gravity of test sample = $(W_3 - W_1) / (W_2 - W_1)$

Where, W_1 – Weight of empty specific gravity bottle, W_2 – Weight of water + specific gravity bottle, W_3 – Weight of test sample + specific gravity bottle.

Refractive index

The refractive index of seed sources of *J. curcas* oils was determined by a standard instrument employing the principle of the critical angle using diffused daylight as suggested in IS: 326-1968. The experiments were conducted at 20°C and reported as a number corrected to four decimal places.

Determination of acid number and free fatty acid

The acid number and free fatty acid of oil was determined by the procedures of AOAC. (1975).

Acid value = $(V \times N \times 56.1) / W$

Where, V = volume of potassium hydroxide used, N = normality of

Potassium hydroxide, W = weight in g of the sample

Free fatty acid as oleic acid, per cent by weight = $(28.2 \times V \times N) / W$

% FFA $\times 1.99$ = Acid value

Determination of iodine number

The iodine number was determined by Hanus iodine method (AOAC, 1975).

Iodine number = $\{(B - S) \times N \times 12.69\} / \text{Weight of the sample}$

Where, B = ml of 0.1 N sodium thiosulfate required by blank, S = ml of 0.1 N sodium thiosulfate required by sample, N = Normality of sodium thiosulfate solution.

Estimation of saponification number

The saponification number was estimated as per the method of AOAC. (1975).

Acid value = $\{(b - a) \times 0.02805 \times 1000\} / \text{Weight of the sample}$

Where, b = ml of 0.5 N HCl required by the blank, a = ml of 0.5 N HCl required by the sample.

Determination of cetane number

Cetane number of the *Jatropha* methyl esters was calculated empirically as per Krisnangkura (1986).

Cetane number = $46.3 + (5458 / \text{SN} - 0.225 \times \text{IV})$

Table 2. Physical properties of different seed sources of *Jatropha*.

Seed source	Refractive index at 20°C	Specific gravity at 25°C
TNMC01	1.4647	0.9073
TNMC02	1.4619	0.9094
TNMC03	1.4650	0.9045
TNMC04	1.4678	0.9103
TNMC05	1.4645	0.9083
TNMC06	1.4640	0.9113
TNMC07	1.4718	0.9103
TNMC08	1.4633	0.9123
TNMC09	1.4611	0.9114
TNMC16	1.4653	0.9134
TNMC19	1.4708	0.9135
TNMC20	1.4640	0.9053
TNMC21	1.4598	0.9132
TNMC22	1.4635	0.9123
TNMC23	1.4702	0.9122
TNMC24	1.4645	0.9100
TNMC26	1.4638	0.9122
TNMC28	1.4695	0.9130
TNMC29	1.4633	0.9125
TNMC30	1.4623	0.9062
TNMC31	1.4693	0.9066
TNMC32	1.4600	0.9106
TNMC33	1.4665	0.9129
TNMC34	1.4705	0.9122
TNMC35	1.4610	0.9130
Mean	1.4651	0.9105
SEd	0.0064	0.0020

Where, SN is the saponification number, IV is the iodine value

Statistical analysis

The data collected were subjected to the analysis of variance after Panse and Sukhatme (1978).

RESULTS

Twenty Five seed sources were deployed for physico chemical analysis in order to assess the quality of oil suitable for biodiesel production. The analysis of physical properties indicated that all the seed sources of *J. curcas* did not show significant difference for physical properties of oil viz., refractive index and specific gravity. All the seed sources exhibited an average refractive index of 1.4 and an average specific gravity of 0.9 (Table 2).

The analysis for chemical properties indicated that significant differences were recorded for acid value and free fatty acid (%) of the oil of *Jatropha* seed sources. Among 25 seed sources, twenty seed sources recorded significantly lower values for acid value and free fatty acid (Table 3). In the present investigation, seed sources differed significantly due to saponification value (Table 3).

The saponification value was significantly higher in three seed sources viz., TNMC 24 (202.73), TNMC 06 (202.63) and TNMC 20 (202.63).

The seed sources registered significant variation for iodine value, which measures the degree of unsaturation in *Jatropha* methyl esters as it restricts the fatty acid methyl esters from solidification. However, with higher degree of unsaturation, *Jatropha* methyl esters are not suitable for biodiesel (Mohibbe Azam et al., 2005). In the current study, only three seed sources viz., TNMC 22(121.64), TNMC 23 (116.59) and TNMC 21 (116.46) recorded significantly higher value for this property (Table 3). The investigated seed sources differed significantly due to Cetane number. Two seed sources viz., TNMC 35 (52.76) and TNMC 30 (50.80) recorded significantly higher Cetane number. Twenty two seeds expressed parity with general mean (48.79) (Table 3).

DISCUSSION

Biofuels and biodiesel are the possible environmental friendly alternative sources of energy, for the predominantly used fossil fuels. These biofuels are

Table 3. Chemical properties of different seed sources of *Jatropha*.

Seed source	Acid value	Free fatty acid (%)	Saponification value	Iodine value	Cetane number
TNMC01	4.83	2.42	202.40	114.02	48.25
TNMC02	5.18	2.60	202.04	114.96	47.25
TNMC03	4.26	2.14	202.07	112.71	48.83
TNMC04	4.60	2.31	201.54	114.26	46.54
TNMC05	5.05	2.54	202.19	112.28	49.02
TNMC06	4.20	2.11	202.63*	109.59	47.78
TNMC07	5.27	2.65	202.50	114.81	48.70
TNMC08	3.90	1.96	202.30	107.56	51.37
TNMC09	4.23	2.12	202.24	111.50	49.17
TNMC16	4.83	2.42	202.30	111.65	48.46
TNMC19	5.65*	2.84*	201.97	111.90	48.35
TNMC20	4.40	2.21	202.63*	111.74	48.74
TNMC21	5.60*	2.81*	202.45	116.46*	47.96
TNMC22	7.05*	3.54*	202.47	121.64*	45.87
TNMC23	5.78*	2.90*	202.45	116.59*	46.20
TNMC24	4.65	2.34	202.73*	111.98	47.84
TNMC26	4.55	2.29	201.90	113.76	48.49
TNMC28	5.10	2.56	201.96	111.40	48.72
TNMC29	6.25*	3.14*	201.73	114.80	48.07
TNMC30	3.77	1.89	202.27	98.51	52.80*
TNMC31	4.35	2.19	201.61	110.34	49.55
TNMC32	4.35	2.19	202.09	109.38	49.30
TNMC33	4.15	2.09	201.83	112.71	48.83
TNMC34	4.65	2.34	201.81	108.79	50.11
TNMC35	4.20	2.11	201.67	101.11	52.76*
Mean	4.83	2.43	202.15	111.82	48.79
SEd	0.34	0.17	0.20	1.84	1.28
CD(0.5)	0.69	0.35	0.42	3.78	2.64

* Significant at 5% level.

considered as offering many priorities including sustainability, reduction of green house gases, regional development, social structure and improvement in agriculture coupled with security of supply (Ayhan, 2006). Against this backdrop *Jatropha* and other tree borne oil seeds have been promoted as a source of renewable and alternate fuels due to their adoptability and non edible nature (Paramathma et al., 2004). Under such circumstances, the current study has been carried out to assess and identify various *Jatropha* seed sources for their suitability towards biodiesel utility. The analysis of physical properties particularly the refractive index and specific gravity parameters in the current study indicated the average of 1.4 and 0.9 respectively which are highly useful to check for contamination / adulteration of oil (Anon, 2003) and also as reported in *J. curcas*, *Jatropha glandulifera*, *Jatropha gossypifolia* and *Jatropha multifida* (Banerji et al., 1985), in *Chukrasia tabularis* (Jain et al., 1997) and in *Taxus baccata* (Goel, 1998).

Higher amounts of NaOH catalyst were required for

higher free fatty acid oil. Otherwise higher amount of NaOH resulted in reduced recovery. These values increase during storage and are specified to ensure proper ageing of the biodiesel. The high acid value can cause damage to engine parts. Higher acid value and free fatty acids (%) were reported in *J. curcas* (Bhasabutra and Sutiponpeibun, 1982), in *Simarouba glauca* (Armour, 1959; Joshi and Hiremath, 2000), *T. baccata* (Goel, 1998), *Pongamia glabra* (Raheman and Phadatare, 2004), in *Madhuca indica* (Ghadge and Raheman, 2005) and also in *Tectona grandis* (Radhakrishna, 2003). However, in the present investigation 20 seed sources exhibited lower acid value and free fatty acid content which are recommended for commercial biofuel programme. The free fatty acid content of the biodiesel should be less than one per cent. It was observed that lesser the free fatty acid in oil, better is the bio diesel recovery. Higher free fatty acid oil can also be used but the bio diesel recovery will depend upon oil type and the amount of NaOH used (Anon, 2003).

Saponification value is an index of the average size of fatty acid present, which depends upon the molecular weight and percentage concentration of fatty acids components in the oil. Saponification value will be higher if the oil contains more of saturated fatty acids (C14:0, C16:0, C18:0) as it determines the length of carbon chain and increase cloud point, cetane number and improve stability of bio-diesel (Anon, 2003). Similar results were earlier documented in *J. curcas* (Bringi, 1987; Bhasabutra and Sutiponpeibun, 1982), *J. glandulifera*, *J. gossypifolia* and *J. multifida* (Banerji et al., 1985) and in other tree borne oilseeds viz., *Azadirachta indica*, *Pongamia pinnata*, *Calophyllum inophyllum*, *Salvadora oleoides* and *Salvadora persica* (Bringi, 1987) which thus extended support to the current study. In contrary, low saponification value (102.9) was reported in the *Jatropha* species (Banerji et al., 1985) which was not reported in the current investigation.

The iodine value should be less than 115 as per bio-diesel standards (Anon, 2003). Poor oxidation stability can cause fuel thickening, formation of gums and sediments, which in turn, can cause filter clogging and injector fouling. Iodine number indicates the tendency of a fuel to be unstable as it measures the presence of C = C bonds that are prone to oxidation. Generally instability increase by a factor of one for every C = C bond on the fatty acid chain. Thus, C18:3 are three times more unstable than C18:0 fatty acids (Anon, 2003). Higher iodine values were also reported in *C. tabularis* (Jain et al., 1997), *T. baccata* (Goel, 1998) and also in *T. grandis* (Hilditch and Williams, 1964). Therefore, seed sources with higher iodine value may not be suitable for the production of bio-diesel. In the current investigation, twenty two seed sources of *Jatropha* recorded less than 115 for iodine value and these seed sources may be qualified for the production of bio-diesel. *Jatropha* fatty acid methyl ester, with higher cetane number is preferred for use as biodiesel. The specifications of bio-diesel are such that it can be mixed with any diesel. Cetane number of the bio-diesel is in the range of 48-60 (Anon, 2003). Hence the seed source with the higher cetane number identified in the current study may be fit for commercial biofuel programme.

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