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Seed oil of *Jatropha curcas* L. germplasm: Analysis of oil quality and fatty acid composition

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ABSTRACT

Jatropha curcas L. is recognized as one of the important non-edible tree borne oilseeds (TBOs) in India and many other tropical countries. *J. curcas* is a promising bioenergy crop as its seed oil is a suitable feedstock for biodiesel production. This study focused on a total of 19 *J. curcas* accessions for seed oil extraction, oil quality analysis and fatty acid composition. Most of the accessions showed more than 30% oil content with free fatty acid content ranging from 0.21 to 1.82%. The oil samples were transesterified efficiently to fatty acid methyl esters as evident from proton nuclear magnetic resonance (^{1}H NMR) spectra. As revealed by gas chromatography, the contents of the four major fatty acids were found to significantly vary in the seed oils viz Palmitic acid (8.64–17.05%), stearic acid (4.34–7.94%), oleic acid (26.26–46.36%) and linoleic acid (28.72–53.78%). A number of seed oils showed high level of oleic acid (40.02–46.36%), and some other oil samples were rich in linoleic acid (~45%). These *J. curcas* accessions appeared to be promising with regard to clonal propagation, field trials, large-scale plantations for biodiesel feedstocks, other industrial applications, and also as prebreeding materials in crop improvement programs.

1. Introduction

Currently, many countries in the world focus on alternative energy sources to overcome the major challenges due to fast-depleting petroleum reserves and adverse effects of the greenhouse gas emissions (Balat and Balat, 2010; Huerga et al., 2014). Biological sources particularly fat- and oil-derived fuels are gaining considerable importance. Raw vegetable oil (RVO) is not applied as a fuel in CI engines because of very poor atomization, high viscosity, low volatility, and partial combustion causing increased soot and smoke formation (Nayak et al., 2017). The term 'biodiesel' refers to mono-alkyl esters of fatty acids having the following desirable attributes: renewability, biodegradability, low sulphur content, good lubricity, higher flash point and ease of oil extraction and processing. It can be blended in varying proportions with non-renewable petro-fuels. In addition, combustion of biodiesel produces reduced level of particulate matters, carbon monoxide (CO), hydrocarbons, soot and sulphur oxides (SOx). Relatively a new term i.e., 'renewable diesel' is coined which resembles petrodiesel produced by cracking or pyrolysis; hydrodeoxygenation is also gaining importance in the recent years (Berchmans and Hirata, 2008; Fukuda et al., 2001; Knothe, 2009, 2010).

USA and many countries in Europe and South-East Asia use various plant edible oils such as soybean, rapeseed, peanut, sunflower and palm seeds for biodiesel production. There is a short-supply of edible oils in Indian subcontinent; therefore, the focus has been shifted to various tree-borne oilseeds (TBOs) such as Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*), Mahua (*Madhuca indica*), Jatropha (*Jatropha curcas*) because a) they grow under different agro-climatic and edaphic conditions, b) ease of large-scale production of raw materials i.e., oilseeds, oil extraction, and process of transesterification, c) supportive to sustainable development, energy conservation, environment preservation, rural development, poverty alleviation and waste land reclamation (Achten et al., 2008; Demirbus, 2008; Dhyani et al., 2015). *J. curcas* also known as physic nut, a member of the *Euphorbiaceae* family, is a fast-growing, deciduous non-edible oilseed plant and shows considerable tolerance to drought and pest. It is a perennial multipurpose tree and gaining importance increasingly as a promising bioenergy crop because its seed oil, in particular, serves as a suitable feedstock in the biodiesel industries. As evident in the earlier reports, seed yield and oil content vary significantly between the *J. curcas* accessions. These traits are influenced by genotype, eco-geographic conditions, agronomic practices and various stresses (Divakara et al., 2010; Srivastava et al., 2011; Quinn et al., 2015).

The production of biodiesel from *J. curcas* seed oil through transesterification has become an area of intense research in the recent years. During this process, triacylglycerol (TAG) molecules react with an alcohol namely methanol or ethanol in the presence of a catalyst,

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usually potassium hydroxide to form fatty acid methyl/ethyl esters and glycerol. Various parameters are known to influence the transesterification reaction involving vegetable oils as evident from several reports. For example, oil resources and its fatty acid composition, oil quality, nature of the contaminants and their contents, methanol-to-oil ratio, temperature, nature of catalysts, reactor/separator design, hydrodynamic conditions such as volumetric flow rates, phase ratios and catalyst concentrations and other process conditions influence the transesterfication process. Some lipases are being used as substitute of base catalysts in the transesterification process (Ghesti et al., 2007; Klofutar et al., 2010; Wang et al., 2011; Likozar and Levec, 2014; Likozar et al., 2016; Rodrigues et al., 2016).

Quantitatively, seed oil content is a desirable attribute of the individual *J. curcas* accessions; but the parameters like free fatty acid (FFA) content and fatty acid composition (FAC) are major influencing factors in determining the quality of a biodiesel (Tiwari et al., 2007; Wang et al., 2011). High FFA content has adverse effects on the transesterification process because of soap formation causing low yield of biodiesel product. FAC analysis helps to assess the fuel properties of a biodiesel such as cetane number (CN), oxidative stability (OS), viscosity, lubricity and cold flow properties (Demirbus, 2008; Knothe, 2009; Ramos et al., 2009). The composition of a quality biodiesel should comply with the following parameters: presence of high amounts of monounsaturated fatty acids (MUFA) such as C16:1 and C18:1, reduced level of the polyunsaturated fatty acids (PUFA) and optimal presence of the saturated fatty acids (Knothe, 2009; Pinzi et al., 2009). Apart from searching the superior *J. curcas* genotypes with high seed oil content and desired FAC, some other strategies were also adopted successfully in developing the designer crops. For example, Qu et al. (2012) reported marker-free RNA interference transgenic *J. curcas* plants with significantly increased level of oleic acid in seed oil. A few *J. curcas* lines with very high oleic acid content were generated through genetic crossing (Sinha et al., 2016).

A thorough survey and analysis of the seed and oil traits helped us to identify a number of superior *J. curcas* genotypes in terms of both seed yield and oil content from different locations in Punjab, a North-Western state of India (Kumar and Das, 2018). This report presents extraction of seed oil from the *J. curcas* accessions, analysis of oil and FFA content, transesterification, quality checking of the fatty acid methyl esters by ^{1}H NMR followed by FAC analysis by gas chromatography (GC). The objective was to identify the promising *J. curcas* accessions suitable for multi-location trials, performance evaluation, large-scale cultivation, biodiesel production and crop improvement.

2. Materials and methods

2.1. Plant materials and reagents

A number of morphologically superior *J. curcas* candidate plus trees (CPTs) were identified from different agro-climatic zones covering some districts of Punjab, a North-Western state of India. Ecogeographical characteristics of the survey and collection sites are provided in Table 1. This study focused on a total of 19 *J. curcas* accessions. The mature seeds collected from these accessions were submitted to National Bureau of Plant Genetic Resources (NBPGR), New Delhi for assignment of the Indigenous Collection (IC) numbers. Methanol, potassium hydroxide, hexane and other chemicals were of analytical grade, and purchased from Himedia. For GC analysis, a standard fatty acid methyl ester (FAME) C_8–C_{24} was purchased from Sigma-Aldrich.

2.2. Extraction of seed oil and analysis of FFA content

J. curcas seed oil % on kernel basis was determined using Soxhlet apparatus (Kumar and Singh, 2014). Briefly, the healthy *Jatropha* seeds were dried under sun, followed by drying in hot air oven at 40 °C till they attain constant weight. For each seed sample, 50 g of kernel was broken into small pieces, and oil extraction was carried out at 70–80 °C for 8–10 h using hexane as solvent. To remove hexane completely from seed oil, the flask was kept in vacuum rotary evaporator. The oil % was calculated using the following equation

$$\% \text{ Oil} = [(W_b - W_a) \times 100]/\text{kernel weight} \quad (1)$$

where W_a was the weight of empty flask and W_b refers to weight of flask containing the extracted oil. The free fatty acid (FFA) content of each seed oil sample was determined by the titrimetric method (Rukunudin et al., 1998). Briefly, 10 g of oil was dissolved in a mixture of ethanol and diethyl ether (1:1 volume ratio), and titrated with 0.1 M KOH solution using phenolphthalein as indicator. FFA concentration in seed oil was calculated as percentage oleic acid on the basis of the following equation

$$\% \text{ FFA as Oleic acid} = [\text{alkali volume (mL)} \times \text{alkali normality} \times 28.2]/\text{Sample weight (g)} \quad (2)$$

Table 1
Ecogeographical characteristics of the survey areas of the North-Western part of India for collection of the *J. curcas* accessions.

S. No.	TI Acc. No.	District	Location			Agro-climatic zones of Punjab
			Altitude	Latitude	Longitude	
1	TJS-01# 04	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
2	TJS-02#01	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
3	TJS-04#42	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
4	TJS-05#10	Mansa	211 m	30°–58' N	74°–18' E	Western zone
5	TJS-06#24	Sangrur	259 m	30°–20' N	76°–28' E	Central plain zone
6	TJS-07#05	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
7	TJS-15#11	Hoshiarpur	260 m	31°–32' N	75°–55' E	Sub-mountain undulating zone
8	TJS-17#01	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
9	TJS-19#01	Ludhiana	259 m	30°–20' N	76°–28' E	Central plain zone
10	TJS-21#01	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
11	TJS-23#17	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
12	TJS-25#01	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
13	TJS-27#108	Ludhiana	259 m	30°–20' N	76°-28' E	Central plain zone
14	TJS-28#01	Patiala	259 m	30°–20' N	76°–28' E	Central plain zone
15	TJS-29#07	Barnala	259 m	30°–20' N	76°–28' E	Central plain zone
16	TJS-30#06	Barnala	259 m	30°–20' N	76°–28' E	Central plain zone
17	TJS-31#13	Barnala	259 m	30°–20' N	76°–28' E	Central plain zone
18	TJS-32#16	Barnala	259 m	30°–20' N	76°–28' E	Central plain zone
19	TJS-33#14	Barnala	259 m	30°–20' N	76°–28' E	Central plain zone