ORIGINAL RESEARCH

Fatty acid profile variability in *Jatropha curcas* oil and their use as varietal descriptors

Variabilidad del perfil de ácidos grasos en aceite de *Jatropha curcas* y su uso como descriptores varietales

Juan Ubaldo Sánchez-Velázquez, Jesús Alfonso Patrón-Vázquez, Neith Pacheco, Guadalupe Lopez-Puc, Ana Ramos-Díaz*

Subsede Sureste. Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco A. C. Tablaje Catastral 31264 Km 5.5 Carretera Sierra Papacal-Chuburna Puerto Parque Científico Tecnológico de Yucatán CP: 97302 Mérida, Yucatán, México

 *Corresponding author
E-mail address: aramos@ciatej.mx (A. Ramos-Díaz)

ABSTRACT

Because of its fatty acid profile (FAP) *Jatropha curcas* seed oil is considered suitable to produce biodiesel. FAP indicates the presence and proportion of different fatty acids such as oleic acid, linoleic acid, palmitic acid, and stearic acid. However, the FAP is variable between populations of the same species, therefore the quality of the biodiesel produced varies from one accession to another. Different genetic improvement programs select and cross plants to improve seed yield, nonetheless, it is necessary to obtain new varieties with the appropriate FAP for high-quality biodiesel production. In the present work, the variability of the FAP of 24 accessions (6 parents and 3 controls and 15 F1 hybrids) was evaluated, as well as the use of fatty acids as chemical descriptors. The FAP was determined by GC-MS chromatography, their variability and interdependence were evaluated to establish the fatty acids (or the relationship among them) that could be used as trait. Finally, a method is proposed to assign trait states considering the variability of the character value, for use as a varietal chemical descriptor.

Keywords: Fatty acids, *Jatropha curcas*, varietal descriptors.

RESUMEN

Debido a su perfil de ácidos grasos (FAP) el aceite de la semilla de *Jatropha curcas* se considera adecuado para la producción de biodiesel. FAP se refiere al presencia y proporción de diferentes ácidos grasos como ácido oleico, acido linoleico, ácido palmítico y ácido esteárico. Sin embargo, el FAP es variable entre poblaciones de una misma especie, por lo que la calidad del biodiesel producida varia de una accesión a otra. Diferentes programas de mejoramiento genético seleccionan y cruzan plantas para aumentar el rendimiento de la semilla, no obstante, es necesario obtener nuevas variedades con el FAP adecuado para producción de biodiesel de alta calidad. En el presente trabajo, la variabilidad de los FAP de 24 accesiones (6 parentales y 3 testigos y 15 híbridos F1) fue evaluada, así como la utilización de los ácidos grasos como descriptores químicos. Los FAP fueron determinados por cromatografía GC-MS, su variabilidad y su interdependencia fueron evaluadas para establecer los ácidos grasos (o la relación entre ellos) que podrían usarse como caracteres. Por último, se propone un método para asignar estados de caracter considerando la variabilidad del valor del carácter, para su uso como descriptor químico varietal.

Palabras clave: Acidos grasos, descriptores varietales, *Jatropha curcas*.

1. INTRODUCTION

Jatropha curcas is an oleaginous plant species that are used to produce oil as a raw material for biodiesel manufacture (Teo *et al.*, 2019). Its oil has physicochemical characteristics that allow it to produce high-quality biodiesel, such as its cetane number (CN >47) and oxidative stability (>6 h) (Ong *et al.*, 2011; Sivaramakrishnan & Ravikumar, 2012). These characteristics are directly associated with the fatty acid profile (FAP) of the oil since saturated fatty acids increase the values of CN and reduce oxidative stability, while unsaturated fatty acids reduce CN and increase oxidative stability (Ramos *et al.*, 2009; Rashed *et al.*, 2015). Few studies have proposed the relationship between FAP and produce high quality of biodiesel, FAP should have a predominance of monosaturated oil and a low proportion of polyunsaturated oils to ensure the high quality of biodiesel (Ramos *et al.*, 2009; Nakkash & Al-karkhi, 2012).

The characterization of *J. curcas* oil from different parts of the world has shown that the species exhibits a wide variation in the FAP. The predominant fatty acids are oleic acid (28.3% to 63%) and linoleic acid (19% to 43%); other acids that are present in proportions above 5% are palmitic acid (7% to 13.6%) and stearic acid (5.2% to 9.7%) (Rodrigues *et al.*, 2013; Montes *et al.*, 2014; Cruz Rubio *et al.*, 2015; Shalaby, 2015). The characterization of FAP of vegetable oil, and the use of the profile as a differentiating trait (descriptor) between accessions, is thus an important element in the development of the most suitable plant varieties for the production of biodiesel (King *et al.*, 2009).

This type of descriptor has been used in oleaginous plants to select varieties and improve the properties of the vegetable oil produced. Previous works have characterized two peanuts (*Arachis hypogaea*) varieties to select the one with the highest content of oleic acid and the lowest content of linoleic acid, to improve the oxidative stability of the oil produced (O'Keefe *et al.* 1993). Different authors have developed strategies to increase the proportion of oleic and linoleic acid in canola (*Brassica napus*) to produce an edible vegetable oil of better nutritional quality (Broglie *et al.*, 2006; Beyzi *et al.*, 2019). Furthermore, there are reports of sunflower varieties (*Helianthus annuus*) whose oil has a proportion of oleic acid that ranges from 24.6% to 89.3%, while the proportion of linoleic acid is higher in varieties with lower amounts of oleic acid (64.7%), and lower in those that contain a greater amount of oleic acid (4%) (de Carvalho *et al.*, 2019).

In *J. curcas*, unlike peanut, canola, and sunflower plants, the variability in the composition of fatty acids has not been considered as a descriptor, even though the biodiesel produced from different varieties and accessions of *J. curcas* has different physicochemical properties depending on that FAP. Although the FAP description of *J. curcas* has been described by different authors, the methodology for using this composition as a descriptor has not been evaluated for genetic improvement programs. The aim of the present work was to determine the variability of the FAP in different *J. curcas* accessions and to evaluate the use of these profiles as descriptors for distinguishing among accessions in genetic improvement programs focused on developing *J. curcas* varieties specialized in the production of oil with high concentrations of specific oleic acid.

2. MATERIALS AND METHODS

2.1. Plant material

An experimental plantation of 24 accessions of Jatropha curcas was established in Yucatan, Mexico 21 ° 08 '00.7 "N 89 ° 46' 49.6" W), the crop was started with 15 stakes per accession from a jatropha germplasm bank (Agroindustria Alternativa del Sureste SPR de RL de CV). Nine accessions were collected by the company from different regions, of which six were used as parental accessions JatroP1, JatroP2, JatroP3, JatroP4, JatroP5, JatroP6; three accessions were used as a control to define a better separation of crosses from parents: JatroT1, JatroT2, and JatroT3. And 15 accessions are crosses: JatroC1, JatroC2, JatroC3, JatroC4, JatroC5, JatroC6, JatroC8, JatroC8, JatroC8, JatroC12, JatroC13, JatroC14, and JatroC15 (Table 1). After one year, fully ripe fruits were harvested, and the nuts were extracted from the seeds by breaking the testa manually. They were then stored at 4 ° C until use.

Table 1. Description of the J. curcas accessions used.

Accession	Type of accession	Origin	Female parental	Male parental
JatroP1	Parental	Nicaragua	N.A.	N.A.
JatroP2	Parental	Nicaragua	N.A.	N.A.
JatroP3	Parental	Chiapas	N.A.	N.A.
JatroP4	Parental	Chiapas	N.A.	N.A.
JatroP5	Parental	Veracruz	N.A.	N.A.
JatroP6	Parental	Chiapas	N.A.	N.A.
JatroT1	Control	Veracruz	N.A.	N.A.
JatroT2	Control	Chiapas	N.A.	N.A.
JatroT3	Control	Yucatan	N.A.	N.A.
JatroC1	Cross	N.A.	JatroP1	JatroP2
JatroC2	Cross	N.A.	JatroP5	JatroP2
JatroC3	Cross	N.A.	JatroP2	JatroP4
JatroC4	Cross	N.A.	JatroP1	JatroP4
JatroC5	Cross	N.A.	JatroP1	JatroP5
JatroC6	Cross	N.A.	JatroP2	JatroP6
JatroC7	Cross	N.A.	JatroP1	JatroP6
JatroC8	Cross	N.A.	JatroP2	JatroP3
JatroC9	Cross	N.A.	JatroP1	JatroP3
JatroC10	Cross	N.A.	JatroP4	JatroP5
JatroC11	Cross	N.A.	JatroP4	JatroP6
JatroC12	Cross	N.A.	JatroP4	JatroP3
JatroC13	Cross	N.A.	JatroP5	JatroP6
JatroC14	Cross	N.A.	JatroP5	JatroP3
JatroC15	Cross	N.A.	JatroP6	JatroP3

N.A.: Not applicable

2.2 Oil extraction and analysis of FAP

The fractionated kernels (120 g) were dried for 1 hr. at 60 °C; subsequently, samples were placed in a hydraulic press and subjected to a force of 2 Ton/m for 5 min. The oil was collected and centrifuged at 6000 g for 10 min to remove solid residues. The oil obtained was stored in the dark at -20 °C until further analysis.

Acidic esterification was carried out according to Ichihara & Fukubayashi protocol (2010) with the following modifications, oil samples were diluted at 5000 ppm in 1 ml of hexane, 7.5 ml of methanol and 1.5 ml of HCl (8% in methanol) was added. The samples were then vigorously shaken for 10 sec. Subsequently, transesterification was carried out by heating the samples at 100 °C for 1.5 h. The fatty acid methyl esters (FAME) obtained were purified by adding 5 ml of hexane and 5 ml distilled water to the samples at room temperature. After the shaking, the

samples were sediment to promote phase separation, 200 μl of the organic phase were recovered for analysis by GC-MS.

The samples were analyzed in a chromatograph (Agilent 7890B-GC) coupled to a mass spectrometer (Xevo TQ-S micro) with a Waters Atmospheric Pressure Gas Chromatography (APGC) unit. FAME samples in hexane were injected in splitless mode (0.5 μl) at an injector temperature of 250 °C, using a 10 μl syringe. An HP-Innowax column (60 mx 0.25 mm x 0.25 μm) column was used as stationary phase. Nitrogen (99.999% purity) was used as carrier gas at a pressure of 10 psi, with a flow of 1 ml/min and an average linear velocity of 30 cm/s. A mixture of 37 components of FAME (SIGMA-Supelco CRM47885) was used as standard, diluted in septated chromatography vials at a concentration of 1000 ppm with n-hexane (HPLC) and analyzed directly. The oven program was as follows: 60 °C for 3.5 min, followed by increments of 10 °C/min to 200 °C, then increments of 1 °C/min to 211 °C, which was maintained for 5 min and then again increments of 2 °C/min to 240 °C, which was maintained for 7 min. The hexane solvent was detected between minutes 3.5 and 4.5. The ionization of FAME was carried out with argon gas (99.999%) at 1 eV. The auxiliary temperature was 300 °C, while the temperature of the source was 110 °C. The resolution of the MS was calibrated to a range of 50 to 600 Da. The data obtained were processed with the MassLynxTM software (v.4.1). The retention times of each peak were compared with a standard of 37 saturated and unsaturated fatty acids. The ionic fragmentation patterns were determined by MS to verify the identity of the unsaturated fatty acids.

2.3 Theoretical determination of cetane number in oil from *J. curcas* accessions and F1 hybrids

The CN of the oil of each accession was calculated based on the percentage composition of fatty acids in the oil, using the equation suggested by Bamgboye and Hansen (2008).

CN = 61.1 + (0.133 * %C16:0) + (0.152 + %C18:0) − (0.101 * C16:1Δ^9) − (0.039 *%C18:1Δ^9) − (0.243*C18:2$\Delta^{9,12}$) − (0.395 * C18:3)

2.4 Similarity analysis of accessions concerning their fatty acid composition

The similarity distance indices among accessions concerning the proportions of fatty acids were calculated using the PAST software (v.3.2), a Euclidean matrix was generated, the dendrogram was constructed with the neighbor-joining method, with the boot of 1000 and final Branch. On the other hand, Pearson's linear correlations were used to analyze the ratio between *J. curcas* accessions, made using the Pearson index, using the same statistical software (Hammer et al., 2001).

2.5 Characterization and assignment of traits states for fatty acids and classification of accessions and F1 hybrids

The assignment of trait states was based on the ranges of variation of fatty acid pairs with a linear correlation greater than 0.5 (Table 3). The fatty acid pairs that were not detected in at least 30% of the accessions were discarded. The relationship between fatty acid pairs was considered as a trait, while the range of total variation was the state of the trait. Three traits states were established based on the definition of three sub-ranges, each of which was assigned as a trait state using the following equations:

$$\lim sR\ II = (R/3) \rightarrow ((R/3 + R/3)) = \text{Trait state II (medium)}$$

$$\lim sR\ I = Vmin \rightarrow (R/3) = \text{Trait state I (minimum)}$$

$$\lim sR\ III = ((R/3 + R/3)) \rightarrow Vmax = \text{Trait state III (maximum)}$$

Where R is the total range among all accessions, Vmax is the ratio with the highest value of a pair of fatty acids in all accessions, and Vmin is the ratio with the lowest value of the same fatty acid pair in all accessions.

3. RESULTS

3.1 Analysis of FAP and CN in oil from *J. curcas* accessions and F1 hybrids

The fatty acids were esterified in preparation for their identification by GC-MS, using the acid esterification method of Ichihara & Fukubayashi (2010). This method allowed to detect the presence of fatty acids in the oil from *J. curcas* accessions, from C4 to C22. In the 24 accessions, the order of occurrence of fatty acids coincided with the 37 FAME mix standard. The presence and the percentage of fatty acids in the oil were used to establish FAP by accession. The FAP was used to calculate the CN, characterizing in this way the oil from each of the 24 accessions. The Table 2 shows the proportion of oleic (C18:1) and linoleic acid (C18:2) is the majority in the FAT of all the accessions, by contrast, C18N6 fatty acids were detected in a minority in two accessions and C18N3 in three accessions. The cetane number of all accessions was higher than that required by the biodiesel quality standards (Ong et al., 2011).

Table 2. FAP of 9 accessions and 15 F1 hybrids of *J. curcas* (% of total fatty acids).

Accession	C16	C16:1	C18	C18:1	C18:2	C18N6	C18N3	C20	CN
JatroP1	17.84	0.51	9.78	33.36	38.50	N.D.	N.D.	N.D.	54.25
JatroP2	15.75	0.36	12.75	39.25	31.88	N.D.	N.D.	N.D.	55.82
JatroP3	15.52	0.37	9.17	38.40	36.54	N.D.	N.D.	N.D.	54.14
JatroP4	17.00	0.44	8.83	38.30	35.44	N.D.	N.D.	N.D.	54.55
JatroP5	15.49	0.41	9.86	39.05	35.19	N.D.	N.D.	N.D.	54.54
JatroP6	16.37	0.55	8.22	34.84	40.02	N.D.	N.D.	N.D.	53.39
JatroT1	15.35	0.46	7.82	38.52	37.84	N.D.	0.03	N.D.	53.58
JatroT2	15.61	0.43	7.80	40.76	35.41	N.D.	N.D.	N.D.	54.13
JatroT3	16.30	0.47	9.41	42.39	31.41	N.D.	N.D.	0.01	55.37
JatroC1	16.32	0.51	8.12	30.39	44.67	N.D.	N.D.	N.D.	52.41
JatroC2	13.64	0.43	7.89	39.94	38.03	0.03	0.05	N.D.	53.24
JatroC3	14.60	0.47	8.29	40.47	36.17	N.D.	N.D.	N.D.	53.89
JatroC4	15.02	0.49	7.86	39.89	36.66	0.00	0.00	0.08	53.78
JatroC5	15.06	0.44	8.45	35.60	40.45	N.D.	N.D.	N.D.	53.13
JatroC6	13.48	0.38	8.25	41.40	36.46	N.D.	N.D.	0.03	53.63
JatroC7	15.28	0.46	8.47	38.90	36.89	N.D.	N.D.	N.D.	53.89
JatroC8	14.21	0.53	7.28	40.92	37.06	N.D.	N.D.	N.D.	53.44
JatroC9	15.13	0.43	8.03	37.47	38.93	N.D.	0.01	N.D.	53.36
JatroC10	14.21	0.38	6.58	39.95	38.88	N.D.	N.D.	N.D.	52.95
JatroC11	16.37	0.46	7.14	38.49	37.55	N.D.	N.D.	0.01	53.69
JatroC12	13.64	0.48	6.48	39.81	39.56	0.03	N.D.	N.D.	52.68
JatroC13	14.00	0.43	7.28	36.14	42.16	N.D.	N.D.	N.D.	52.37
JatroC14	13.18	0.33	7.60	42.47	36.40	N.D.	N.D.	0.02	53.47
JatroC15	14.38	0.44	6.93	42.66	35.60	N.D.	N.D.	N.D.	53.71

C16: Palmitic acid; C16:1: Palmitoleic acid; C18: Stearic acid; C18:1: Oleic acid; C18:2: Linoleic acid; C18N6: γ-linolenic acid; C18N3: linolenic acid; C20: Arachi-dic acid; N.D: no detected

3.2 Similarity between *J. curcas* accessions according to the FAP of the oil.

To assess the potential of fatty acids to be used as a trait, we calculated the Euclidean distance between accessions was calculated concerning their percentage composition of fatty acids, to build a distance dendrogram (Fig. 1).
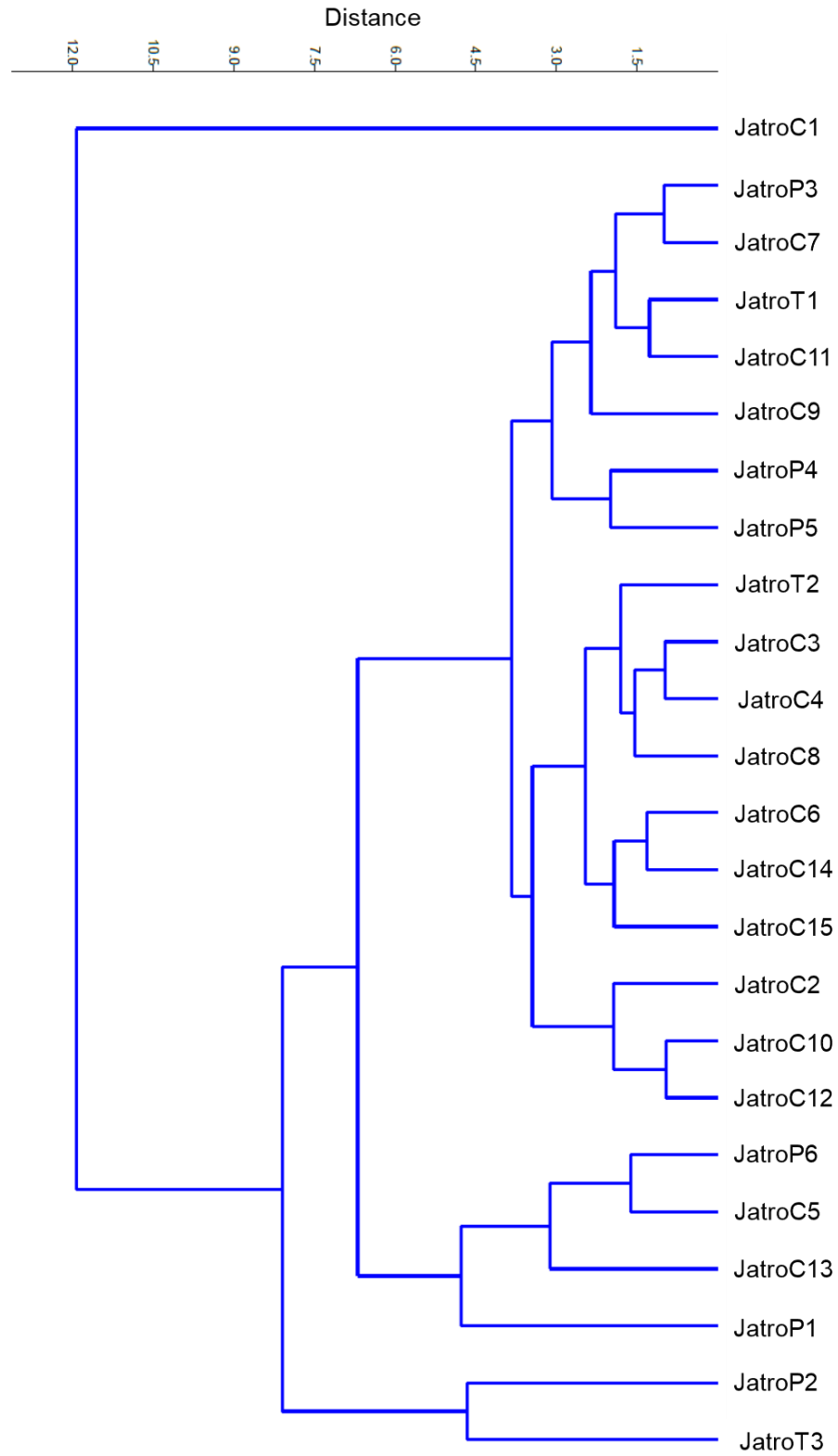
Fig. 1. Clusters of accessions and F1 hybrids by Euclidean distance, based on the composition of fatty acids in the oil of each accession.

The CN was not considered since CN is a chemical property derived from the total composition of fatty acids in the oil and would mask the differences in the amount of each fatty acid. The results showed that the accessions were divided into three well-defined clusters at a distance of 7.5, where the JatroC1 was completely separated from the rest of the accessions. This hybrid had the highest percentage of linoleic acid and the lowest percentage of oleic acid.

The cluster formed by the JatroP2 and JatroT3 accessions share three fatty acids above average (C18, C16, and C18:1), and fatty acid below average (18:2). In addition to the absence of fatty acids (C18N6 and C18N3). Although the CN was not considered as a differentiating trait between accessions and F1 hybrids, it is worth noting that these two accessions had the highest CN of all accessions, a further indication of the similarity in the chemical composition of the oil from these accessions.

The third cluster contains four subclusters, one formed by the JatroP6, JatroC5, JatroC13 and JatroP1, the latter of which being the most distant from the other accessions and F1 hybrids in the subcluster and the one that contains the highest percentage of palmitic acid. These subclusters are grouped based on similar average percentages of palmitic acid, oleic acid, and linoleic acid, with no particular fatty acid prevailing over the others (except in JatroP1) and none reaching such high levels as to become part of the other two clusters. Besides the CN was not considered as a differentiating trait, the third cluster excluded accessions and F1 hybrid with the highest or lowest values of CN, leaving only accessions with intermediate CN values.

3.3 Relationship between pairs of fatty acids.

Since it was proposed that fatty acids could be used as differentiating traits between accessions and F1 hybrids of *J. curcas*, it was necessary to determine the degree of interdependence between the fatty acids present in *J. curcas*, it has been demonstrated in other species that the accumulation of some fatty acids negatively influences the accumulation of other fatty acids (Shockey et al., 2017), considering the biochemical pathway of synthesis of fatty acids in plants. For example, varieties with high oleic acid content have low linoleic acid content. For this reason, a correlation analysis between fatty acids was performed using the Pearson index (r).

The correlation analysis between fatty acids (Table 3) showed the interdependence of the amount of a fatty acid concerning to the others. Given the biosynthetic relationship between fatty acids, it was considered that Pearson's correlation coefficient values lower than -0.45 and higher than +0.45 denoted enough independence and therefore the possibility of using those pairs of fatty acids as descriptors. Of the relationships between fatty acids that were considered independent, those of C18N6 and C18N3 were discarded because those fatty

91

Sánchez-Velázquez et al. / Mexican Journal of Biotechnology 2020, 5(3):83-98

acids were not detected in all accessions or their content was less than 0.05%. Thus, only the relationships between C16-C18N3 (-0.5481), C18N3-C18N6 (-0.72508), C18-C18N6 (-0.50035), C16-C18N6 (-0.81106) and C16-C18 (0.48) were used as a trait.

Table 3. Correlation between fatty acids by Pearson index.

Accession	C16	C16:1	C18	C18:1	C18:2	C18N6	C18N3
C16:1	0.417						
C18	0.486	-0.188					
C18:1	-0.546	-0.437	-0.149				
C18:2	-0.811	0.343	-0.500	-0.725			
C18N6	-0.359	0.036	-0.216	0.118	0.121		
C18N3	-0.224	-0.027	-0.096	0.053	0.079	0.558	
C20	-0.172	-0.014	-0.075	0.240	-0.145	-0.126	-0.117

3.4 Assignment of trait states by categories

To define the trait states among fatty acid pairs, the ratio between their proportions was first calculated (C18:1N9/C18:2N6, C16/C18, C18:1N9/C16, C18/C18:2N6 y C18:2N6/C16) to obtain ratio scales (Table 4). The ratio between oleic acid and linoleic acid (C18:1N9/C18:2N6), establish if the vegetal oil is suitable for biodiesel production, therefore, any particular accession of *J. curcas* should keep this ratio between oleic acid and linoleic acid in desirable proportions (Klopfenstein, 1985; Patil and Deng, 2009; ASTM D6751, 2011). Using the equations described above (lim sR II, lim sR I and lim sR III), three traits states were defined to classify accessions as low (state I), medium (state II), or high (state III) concerning each trait (Table 4). In crops used for oil production, this classification (high, medium, or low) of a certain fatty acid has been used as a descriptor for varietal differentiation (Rondanini *et al.*, 2011). However, the range of variability in the content of fatty acid is specific to each species (Purdy, 1985; O'Keefe *et al.*, 1993; Shockey *et al.*, 2017); thus, for the accessions of *J. curcas* evaluated in the present study, specific ranges of variability were established to be able to assign trait states to selected relationships between fatty acids.

Table 4. Relationship between fatty acids and the assignment of trait states between fatty acids of *J. curcas*.

Accession	C18:1/C18:2		C16/C18		C18:1/C16		C18/C18:2		C18:2/C16	
	R	S	R	S	R	S	R	S	R	S
JatroP1	0.866	I	1.824	II	1.869	I	0.254	II	2.157	I
JatroP2	1.231	III	1.236	I	2.491	II	0.399	III	2.023	I
JatroP3	1.051	II	1.692	II	2.474	II	0.250	II	2.354	II
JatroP4	1.081	II	1.925	II	2.253	I	0.249	II	2.085	I
JatroP5	1.11	II	1.572	I	2.520	II	0.280	II	2.271	I
JatroP6	0.871	I	1.992	III	2.128	I	0.205	I	2.444	II
JatroT1	1.018	II	1.964	III	2.509	II	0.206	I	2.465	II
JatroT2	1.151	III	2.003	III	2.610	II	0.220	I	2.267	I
JatroT3	1.349	III	1.732	II	2.599	II	0.299	II	1.926	I
JatroC1	0.68	I	2.011	III	1.862	I	0.181	I	2.737	III
JatroC2	1.05	II	1.728	II	2.929	III	0.207	I	2.789	III
JatroC3	1.119	II	1.76	II	2.772	III	0.229	I	2.477	II
JatroC4	1.088	II	1.912	II	2.655	II	0.214	I	2.440	II
JatroC5	0.88	I	1.782	II	2.363	II	0.209	I	2.684	III
JatroC6	1.135	III	1.633	II	3.071	III	0.226	I	2.705	III
JatroC7	1.055	II	1.804	II	2.545	II	0.229	I	2.413	II
JatroC8	1.104	II	1.954	III	2.878	III	0.196	I	2.607	II
JatroC9	0.962	II	1.885	II	2.476	II	0.206	I	2.573	II
JatroC10	1.028	II	2.161	III	2.811	III	0.169	I	2.735	III
JatroC11	1.025	II	2.294	III	2.351	II	0.190	I	2.293	II
JatroC12	1.006	II	2.105	III	2.918	III	0.163	I	2.899	III
JatroC13	0.857	I	1.924	II	2.581	II	0.172	I	3.011	III
JatroC14	1.167	III	1.735	II	3.223	I	0.208	I	2.762	III
JatroC15	1.198	III	2.076	III	2.967	III	0.194	I	2.476	II

R: ratio; S: trait state

4. DISCUSSION

The ASTM D6751-11b (2011), specifies the properties of biodiesel, establishes a minimum CN value of 47 for biodiesel, for acceptable quality. Lower CN leads to higher NOx emissions and accumulation of soot in the combustion chamber, causing damage to the engine and greater ecological impact. In the present work, the CN estimation was performed according to the equations proposed by Bamgboye and Hansen (2008), based on the fatty acid composition of the accessions, with an accuracy of 88%. All accessions and F1 hybrids had a CN higher than the established minimum since they had a higher proportion of oleic acid, which is considered appropriate to produce high-quality biodiesel, followed by varieties with a high proportion of saturated fatty acids of long-chain (Klopfenstein, 1985; Sivaramakrishnan and Ravikumar, 2012).

These fatty acids predominate in *J. curcas* plants (Table 2), which is consistent with what has been reported by different authors (Campuzano-Duque & Cardeño-López, 2020; Ong *et al.*, 2011; Rodrigues *et al.*, 2013). The reduction of short-chain fatty acids is another characteristic that increases the quality of the biodiesel produced. In Table 2, it is observed that the accessions with the least amount of C16 are the product of 13 crosses (Canoira *et al.*, 2010), which suggests that the accessions obtained are suitable to produce biofuel.

To maintain the proportion of fatty acids in the progeny, the use of these as chemical descriptors has been proposed (Ovando-Medina et al., 2011), for this reason, we carried out a study of similarity between parental, control and hybrid accessions about the composition of fatty acids (Fig. 1), using geographically distant and cultivated accessions under the same conditions, however, we did not find a grouping between accessions by geographic region or by progeny. The most influential trait was the proportion of oleic acid and linoleic acid fatty acids.

The above may be due to the degree of variability of the proportions of fatty acids, in additions, the relationship between these fatty acids is based on the biosynthetic route that connects them, the accumulation of fatty acid can negatively influence the accumulation of another due to the presence of genetic and metabolic regulations (Harwood, 1988). This ratio is particularly visible in oleic acid and linoleic acid (Ye *et al.*, 2009). To identify the interdependence between fatty acids, the correlation analysis between fatty acids was performed (Table 3). Correlations that resulted with values less than -45 or greater than 0.45 were proposed as traits, the character states were designated according to the degree of variability (Table 4).

Table 4 shows the influence of the fatty acid ratio on the formation of clusters (Fig. 1). JatroC1 was independent of the rest of the accessions and F1 hybrids, and had also the lowest ratio between oleic acid and linoleic acid, with 0.68, and JatroP2 and JatroT3 formed an independent cluster, with the highest proportion of oleic acid ratio (1.22 and 1.34 respectively). A third cluster divided into three sub-clusters, one of which included JatroP6, JatroC5, JatroC13 and JatroP1, all classified within the low oleic/linoleic acid category, followed by a subcluster formed by JatroP3, JatroC7, JatroT1, JatroC11, JatroC9, JatroP4 and JatroP5, all classified within the medium category of oleic/linoleic acid, and by a third subcluster formed by JatroC3, JatroC4, JatroC8, JatroC6, JatroC14, JatroC15, JatroC2, JatroC10, JatroC12 and JatroT2, which fell in both the medium and high categories of relationship C18:1/C18:2 (Fig. 1, Table 4).

The use of fatty acids as descriptors in Jatropha is feasible considering the relationship between oleic/linoleic acid, the ratio of palmitic/stearic acid, the ratio of oleic/palmitic acid, the ratio of stearic/linoleic acid and the ratio between linoleic/palmitic acid. By assigning character states concerning the proportional content in pairs of fatty acids, it is possible to follow a genetic improvement route focused on improving the quality of oil in F1 with a better CN, in addition to being

able to monitor the modification of this parameter when performing selective crosses and retro crosses of the F1.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

ASTM D6751-11b. 2011. Standard Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels, American Society for Testing and Materials International, West Conshohocken, PA, www.astm.org. DOI: 10.1520/D6751-11B

Bamgboye A. I., Hansen A. C. & Hansen A. 2008. Prediction of cetane number of biodiesel fuel from the fatty acid methyl ester (FAME) composition. International Agrophysics. 22: 21–29.

Beyzi E., Gunes A., Buyukkilic Beyzi S. & Konca Y. 2019. Changes in fatty acid and mineral composition of rapeseed (*Brassica napus* ssp. oleifera L.) oil with seed sizes. Industrial Crops and Products. 129: 10–14. doi.org/10.1016/J.INDCROP.2018.11.064

Broglie R. M., DeBonte L. R., Hitz W. D., Miao G. H. & Stefan R. 2006. Methods for increasing oleic acid content in seeds from transgenic plants containing a mutant delta 12 desaturase. U.S. Patent No. 7,109,392B1

Campuzano-Duque, Luis Fernando, & Cardeño-López, Fernando. (2020). Composición de los ácidos grasos de genotipos de Jatropha curcas L., en Colombia. Agronomía Mesoamericana, 31(1), 95-104. https://dx.doi.org/10.15517/am.v31i1.37574

Canoira, L., García Galeán, J., Alcántara, R., Lapuerta, M., & García-Contreras, R. (2010). Fatty acid methyl esters (FAMEs) from castor oil: Production process assessment and synergistic effects in its properties. Renewable Energy, 35(1), 208–217. https://doi.org/10.1016/j.renene.2009.05.006

Cruz Rubio B. A., Pérez-Vázquez A., García Perez E., Gallardo Lopez F. & Soto Hernández R. M. 2015. Comparative chemical-morphological analysis of

accessions of *Jatropha curcas* L. from the state of Veracruz. Revista Mexicana de Ciencias Agrícolas. 6: 589–601.

de Carvalho C. G. P., Mazzola L. F., Caldeira A., Dalchiavon F. C. & Mandarino J. M. G. 2019. Quality of Sunflower Oil Obtained in the Main Producing Region of Brazil: Adherence to the Codex Alimentarius. Journal of the American Oil Chemists' Society. 96: 789-794. https://doi.org/10.1002/aocs.12206

Hammer Ø., Harper D. A. T. & Ryan P. D. 2001. Paleontological statistics software: package for education and data analysis. Paleontologia Electronica. 4(1): 1-9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm

Harwood, J. L. (1988). Fatty Acid Metabolism. Annual Review of Plant Physiology and Plant Molecular Biology, 39(1): 101–138. https://doi.org/10.1146/annurev.pp.39.060188.000533

Ichihara K. & Fukubayashi Y. 2010. Preparation of fatty acid methyl esters for gas-liquid chromatography. Journal of Lipid Research. 51: 635–40. https://doi.org/10.1194/jlr.D001065

King, A. J., He, W., Cuevas, J. A., Freudenberger, M., Ramiaramanana, D., & Graham, I. A. (2009). Potential of *Jatropha curcas* as a source of renewable oil and animal feed. Journal of experimental botany. 60(10): 2897-2905.

Klopfenstein W. 1985. Effect of molecular weights of fatty acid esters on cetane numbers as diesel fuels. Journal of the American Oil Chemists' Society. 62: 1029–1031. https://doi.org/10.1007/BF02935708

Montes O. L. R., Torres S. A. F., Jongschaap R. E. E., Azurdia P. C. A., Berduo, S. J. E., Trindade L. M., Visser R. G. F. & van Loo E. N. 2014. High level of molecular and phenotypic biodiversity in *Jatropha curcas* from Central America compared to Africa, Asia and South America. BMC Plant Biology. 14:77. https://doi.org/10.1186/1471-2229-14-77

Nakkash N. B. & Al-karkhi S. R., 2012. Production of biodiesel fuel from oleic acid and comparison of its properties with petroleum diesel. Iraqi Journal of Chemical and Petroleum Engineering. 13(4): 13–25.

O'Keefe S. F., Wiley V. A. & Knauft D. A. 1993. Comparison of oxidative stability of high- and normal-oleic peanut oils. Journal of the American Oil Chemists' Society. 70: 489–492. https://doi.org/10.1007/BF02542581

Ong H. C., Mahlia T. M. I., Masjuki H. H. & Norhasyima R. S. 2011. Comparison of palm oil, *Jatropha curcas* and *Calophyllum inophyllum* for biodiesel: A review. Renewable and Sustainable Energy Reviews. 15: 3501–3515. https://doi.org/10.1016/j.rser.2011.05.005

96

Ovando-Medina, I., Espinosa-García, F. J., Núñez-Farfán, J., & Salvador-Figueroa, M. (2011). Genetic variation in Mexican Jatropha curcas L. estimated with seed oil fatty acids. Journal of Oleo Science, 60(6), 301–311. https://doi.org/10.5650/jos.60.301

Patil P. D. & Deng S. 2009. Optimization of biodiesel production from edible and non-edible vegetable oils. Fuel 88(7): 1302–1306. https://doi.org/10.1016/j.fuel.2009.01.016

Purdy R. H. 1985. Oxidative stability of high oleic sunflower and safflower oils. Journal of the American Oil Chemists' Society. 62: 523–525. https://doi.org/10.1007/BF02542324

Ramos M. J., Fernández C. M., Casas A., Rodríguez L. & Pérez Á. 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource Technology. 100(1): 261–268. https://doi.org/10.1016/j.biortech.2008.06.039

Rashed M. M., Kalam M. A., Masjuki H. H., Rashedul H. K., Ashraful A. M., Shancita I. & Ruhul A. M. 2015. Stability of biodiesel, its improvement and the performance and emission. Royal Society of Chemistry Advances. 5: 36240–36261. https://doi.org/10.1039/C4RA14977G

Rodrigues J., Miranda I., Gominho J., Vasconcelos M., Barradas G., Pereira H., Bianchi-de-Aguiar F. & Ferreira-Dias S. 2013. Variability in oil content and composition and storage stability of seeds from *Jatropha curcas* L. grown in Mozambique. Industrial Crops and Products. 50: 828–837. https://doi.org/10.1016/J.INDCROP.2013.08.038

Rondanini B. D. P., Castro D. N., Searles P. S. & Rousseaux M. C. 2011. Fatty acid profiles of varietal virgin olive oils (*Olea europaea* L.) from mature orchards in warm arid valleys of Northwestern Argentina (La Rioja). Grasas y Aceites 62(4): 399–409.

Sánchez-Velázquez, J. U., Pacheco, N., López-Puc, G., & Ramos-Díaz, A. (2018). Behavior of genetic diversity in F1 crosses of selected accessions of *J. curcas*. Industrial Crops and Products. 122: 669–674. https://doi.org/10.1016/j.indcrop.2018.05.029

Shalaby E. A. 2015. A Review of Selected Non-Edible Biomass Sources as Feedstock for Biodiesel Production. Biofuels-Status and Perspective. 30: 3-20. DOI:10.5772/59178

Shockey J., Dowd, M., Mack B., Gilbert M., Scheffler B., Ballard L., Frelichowski J. & Mason C. 2017. Naturally occurring high oleic acid cottonseed oil: identification and functional analysis of a mutant allele of *Gossypium barbadense* fatty acid desaturase-2. Planta 245: 611–622. https://doi.org/10.1007/s00425-016-2633-0

Sivaramakrishnan K. & Ravikumar P. 2012. Determination of cetane number of biodiesel and it's influence on physical properties. Asian Research Publishing Network Journal of Engineering and Applied Sciences. 7(2): 205–211.

Teo S. H., Islam A., Chan E. S., Thomas Choong S. Y., Alharthi N. H., Taufiq-Yap, Y. H. & Awual M. R. 2019. Efficient biodiesel production from *Jatropha curcas* using $CaSO_4/Fe_2O_3$-SiO_2 core-shell magnetic nanoparticles. Journal of Cleaner Production. 208: 816–826. https://doi.org/10.1016/J.JCLEPRO.2018.10.107

Ye J., Qu J., Bui H. T. N. & Chua N. H. 2009. Rapid analysis of *Jatropha curcas* gene functions by virus-induced gene silencing. Plant Biotechnology Journal. 7(9): 964–976. https://doi.org/10.1111/j.1467-7652.2009.00457.x