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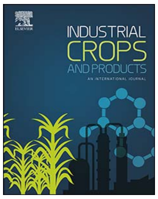

Characterization of *Jatropha curcas* accessions based in plant growth traits and oil quality

Nuria Alburquerque[a,*], Roque Carlos García-Almodóvar[a], Juan Miguel Valverde[b], Lorenzo Burgos[a], Domingo Martínez-Romero[b]

[a] Departamento de Mejora Vegetal, Grupo de Biotecnología de Frutales, CEBAS-CSIC, Apdo. 164, 30100 Murcia, Spain
[b] Departamento de Tecnología de Alimentos, EPSO, Universidad Miguel Hernández, Ctra. Beniel, km 3,2, 03312 Orihuela, Alicante, Spain

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ABSTRACT

Variability in growth and reproductive characteristics, seed traits, quality and oil content of six accessions of *Jatropha curcas* growing in Murcia (Spain) under greenhouse conditions were analysed. Differences were found among accessions in branch length, ratio female to male flowers (F/M), productions, seed weight, endosperm weight, percentage of endosperm and percentage of aborted seeds oil contents, fatty acids composition and quality indexes of the biodiesel. Two genotypes (8-8 and 6-3) have shown a good vigor plant, the best F/M ratio, and appropriate average productions. In addition, these accessions showed the highest average single seed weights, and the best average endosperm weights. The highest total seed oil percentages were observed in the accessions 4-5 (42.42 ± 0.26), 6-3 (41.51 ± 0.20), and 8-8 (40.41 ± 0.73), whereas percentages lower than 30% were recorded in the others. The percentage of endosperm oil ranged from 42.72 ± 0.98% to 65.71 ± 0.36%. In relation to the fatty acids composition, the highest monounsaturated fatty acids percentages with the highest concentration of oleic acid and the lowest concentration of linolenic acid were observed in three genotypes (4-5, 8-8 and 6-3). The biodiesel obtained from these accessions presented high cetane number and low iodine value, which indicates the good ignition quality and stability of the oil.

The knowledge of plant growth parameters and oil quality of the studied genotypes could be useful to choose the appropriate ones to be grown under subtropical climatic conditions or to be used as parents in a breeding program.

1. Introduction

Nowadays there is a growing interest in seeking renewable energy due to the reduction of fossil fuel reserves along with the recognition that increased carbon dioxide emissions are leading to climate change. The use of biofuel feedstocks that do not conflict with the food market and whose production is carried out in a sustainable way has been promoted (Wassner et al., 2016).

Jatropha curcas L. is a perennial oilseed crop belonging to the family of Euphorbiaceae. It is suitable for cultivation in tropical and sub-tropical regions and has a considerable potential for the use of its seed oil as a sustainable alternative source of biofuels (Yue et al., 2013). In addition, this crop shows easy propagation, rapid growth, short development period, adaptation to a wide range of agro-climatic conditions, and it is able to improve soil quality (Anand et al., 2015; Kumar and Sharma, 2008).

The *Jatropha* biodiesel has been proven to be one of the best biodiesels made from vegetable oils due to its good qualities for engine performance and low emissions of exhaust particles (Fairless, 2007; Ghosh et al., 2007). Biodiesel quality depends on several factors that reflect its chemical and physical characteristics. It is desirable for a good biodiesel that it does not solidify at low temperatures forming wax crystals, which can obstruct the circuits and filters of the vehicle's engines. In this sense, there is a close relationship between the number of carbon and unsaturations and the degree of solidification (the lower the number of carbons and unsaturations the greater the crystallization capacity). On the other hand, the oxidative stability of biodiesel measures the stability and durability of the fuels during the storage. However, a fuel has a worse oxidative stability when the number of carbons and unsaturations is too high. The crystallization capacity and the oxidative stability of a biodiesel can be evaluated by knowing the percentages of saturations, the number of carbons of the fatty acid methyl esters (FAMEs), the molecular weight of the fatty acids and also, in the case of oxidative stability, using the iodine value (IV) (Gopinath

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[*] Corresponding author.
E-mail address: nalbur@cebas.csic.es (N. Alburquerque).

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et al., 2014). The saponification value (SV) and IV of the oil are indicative of the structure of fatty acid such as chain length of fatty acid and the degree of unsaturation, respectively (Meher et al., 2013). The cetane number (CN) of a biodiesel influences the combustion process and engine performance. It measures the availability of fuel to switch on automatically when injected into the engine. Many performance characteristics, such as density, heating value are related to the CN, which is used to determine the ignition biodiesel quality (Gopinath et al., 2009). Higher Heating Value (HHV) is an important property that characterizes the energy content of solid, liquid and gaseous fuels. HHV is also called calorific value or heat of combustion and it is related to the length of the carbon chain. The longer the carbons chain of the ethyl ester the higher the calorific value (Demirbaş, 1998). There are different regulations adopted in different regions of the world that standardize commercial biodiesel quality indexes such as SI, IV, CN and HHV (Barabás and Todoruţ, 2011).

Despite the high potential of *Jatropha curcas*, there are public concerns over the accessibility of enough feedstock to provide to the huge fuel needs (Singh et al., 2016). Since *Jatropha curcas* is a semi-domesticated species, plant breeding and domestication of *Jatropha* is paramount in order to reduce the risk of future unsustainable practices, and thus improve future crop routine or further selection.

At the moment, there is not available elite germplasm with combination of several desirable traits needed to establish successful large-scale plantation (Fairless, 2007; Singh et al., 2016). It is necessary to characterize different germplasm adapted to specific agro-climatic conditions to ensure that they have desirable growth, reproductive, productive and biochemical characteristics. Therefore, existing variability is the pre-requisite for any breeding program aimed at improving the oil production and other characters (Sunil et al., 2013). Many works have been focused on evaluation of phenotypic and genotypic variability in *J. curcas* for selective breeding during the past few years (Pecina-Quintero et al., 2014; Rao et al., 2008; Singh et al., 2016; Sunil et al., 2013). Osorio et al. (2014) found a higher genetic variability in Central American accessions compared to Asian, African and South American accessions.

Several conventional breeding programs of *J. curcas* are ongoing in India, China, Thailand, Philippines Mexico, Guatemala, and Brazil (Carels, 2009). The more desirable traits are female to male flower ratio, synchronistic of flowering and fruiting, seed yield, oil quality and overall increasing the oil yield (Divakara et al., 2010).

For selecting breeding material, a good practice is to use the best-performing trees in the location of interest.

The objective of the present work is to study the growth and reproductive characteristics, variation in seed traits, quality and oil content of six accessions of *Jatropha curcas* growing in Murcia (Spain) under greenhouse conditions.

2. Material and methods

2.1. Plant material and culture conditions

Seedlings from Colombia were grown in Canary Islands (Gáldar, Gran Canaria 28° 8′ 38″ N, 15° 39′ 1″ W). As a previous step to establish a breeding program a mass selection was carried out and six accessions were studied in detail.

Cuttings were taken from the six selected *J. curcas* plants. The cuttings were immersed in a solution with 100 mg L^{-1} 3-indolacetic acid during 24 h before rooting and planting under greenhouse conditions in Murcia to be used as parents in a further breeding program.

The experiment design was laid out in a randomized complete block design with one plant per genotype in each of the three replication blocks. The planting density was 33 plants in a total area of 400 m^2 (3 m × 3 m planting distance).

Greenhouse *Jatropha* culture was carried out in pots of 90 L and the mix of peat (50%), perlite (30%) and coconut coir (20%) was used as soil substrate. Nutrients were supplied through a complex fertilizer at the rate of 13:5:25:10 (1 g L^{-1}) N:P_2O_5:K_2O:CaO, respectively. Additionally, phosphoric acid at 0.2 10^{-3}% and Dropomix (Haifa Iberica, S.L.) at 35 mg L^{-1} were added to the soil substrate. Plants were watered two times a day and irrigation time was variable according to season, fitting it to obtain drainage close to 15%.

Greenhouse temperature was controlled by cooling system to prevent an excessive temperature.

2.2. Plant growth characters and analyses of seeds

Growth data were recorded from three replications of each accession. The parameters studied were branch length, number of female and male flowers and ratio female to male flowers (F/M).

Fruits were collected at the black maturity stage (ripe capsule ~45–60 days after pollination), and seeds were removed from the fruits and wrapped in paper. Production was calculated as g of harvested seeds $plant^{-1}$.

Jatropha seeds from all accessions were dried in a vacuum oven (Selecta S.A. model Vacioterm T. Barcelona Spain) at 45 °C for 24 h in the laboratory. Average of single seed weight, single endosperm weight, percentage of endosperm (weight of endosperm x weight of whole $seed^{-1}$ × 100) and percentage of aborted seeds (number of seeds with endosperm failed to develop x total number of $seeds^{-1}$ × 100) were calculated in 3 lots of 50 seeds from each accession.

The endosperm was extracted from each seed and then lots of endosperms were dried in the vacuum oven at 45 °C for 24 h. Each batch of endosperms was milled with an analytical mill (Mortar Grinder Mill, Retsch GmbH model RM 200 Haan Germany). Each *Jatropha* milled batches (20 g) was packed with N_2 in hermetically sealed glass containers and stored at −70 °C for further use.

2.3. Extraction of oil

Two 22 × 50 mm cellulose extraction cartridges (Filter-Lab, Barcelona Spain) were prepared with 6 g of each batches of ground *Jatropha* endosperm. The oil was extracted from the endosperm by adding 100 mL of diethyl-ether (Yoon, 2015). The extraction was carried out by the Soxhlet method in six parallel extraction tubes for 8 h at 55 °C.

The mixture of oil and extraction solvent was separated via vacuum evaporation in a Rotavapor (Selecta S.A. model RS 3000-V Barcelona-Spain). Finally the vegetable oil was filtered and analysed. The total seed oil percentage was calculated by dividing the weight of extracted oil by the weight of seeds and expressed as percentage ± SE. The endosperm oil content of the seeds was calculated by dividing the weight of extracted oil by the weight of endosperm and expressed as percentage ± SE.

2.4. Fatty acid composition

Jatropha oil (25 mg) was submitted to methylation of fatty acids by adding 1 mL boron trifluoride/methanol at boiling temperature during 10 min. Methylated fatty acids were extracted with hexane (600 μL), taken to dryness and dissolved in 200 μL chloroform before injection. Fatty acids were separated and quantified by gas chromatography (GC, Hewlett-Packard model 6890) equipped with flame ionization detector (FID). Five microliters in split mode was injected into a capillary column (HP-Innowax Polyethylene glycol, 30 m × 250 μm × 25 μm). A gradient of temperature was used for fatty acid separation: initial temperature 120 °C for 2 min and then a rate at 4 °C min^{-1} to 190 °C which was held 5 min, and final rate at 4 °C/min to 242 °C. Identification of fatty acids was performed by comparing retention times with authentic standards (purchase from Sigma, Sigma–Aldrich, Madrid, Spain).

2.5. Biodiesel properties

Taking into account the FAMEs oil composition, different equations were used to calculate quality indexes of the *Jatropha* oil (Demirbaş, 1998; Kalayasiri et al., 1996; Gopinath et al., 2009). The SV, the IV, the CN and the HHVs of oil in the studied genotypes:

$$SN = \sum (560 \times A_i)/MW_i \quad (1)$$

$$IV = \sum (254 \times D \times A_i)/MW_i \quad (2)$$

$$CN = 46.3 + 5458/SN - 0.225 \times IV \quad (3)$$

$$HHVs = 49.43 - [0.041 \times SN + 0.015 \times IV] \quad (4)$$

Where, for each FAME, A_i is the percentage, D is the total number of double bonds, and MW_i is the molecular weight.

2.6. Statistical analysis

Data of plant growth characters, seed traits, oil seed contents, oil composition and the values of SV, IV, CN and HHVs were statistically analysed by the general lineal model (GLM) using SAS GLM version 9.4 (SAS Institute Inc., Cary, NC, USA). Percentages were transformed by arcsine root square and ANOVA analysis was carried out. When necessary, a LSD test was used to determine differences between means.

3. Results and discussion

3.1. Plant growth and seed characteristics

The branch length was recorded in all accessions as a measure of the vigor tree (Fig. 1). There were differences among genotypes ($P \le 0.05$), and the most vigorous plants were found in 8-8, 6-3 and 7-6 lines with branches more than 75 cm in length. Pruning that allows the mechanization of the fruit cropping is performed by eliminating the branches below 50 cm and over 80 cm (Carels, 2009). According to this, most of the accessions of our study have a good branch length, since only 8-8 exceeds 80 cm.

The number of male, female flowers and the ratio F/M can be observed in Fig. 2. Some authors have observed variation in the number of male and female flowers per inflorescence depending on the study, but the male-to-female flower ratio was similar in the two studies reporting it (Achten et al., 2010). However, Rao et al. (2008) indicated that the female to male ratio is a highly inheritable character. According with this, we have found differences in the number of male and females flowers ($P \le 0.05$) as in the F/M ratio among the accessions studied ($P \le 0.001$). The best ratio was recorded in 5-5, 6-3 and 8-8 lines. Since the low number of female flowers is one of the major factors limiting *Jatropha* seed production and consequently oil yield (Carels, 2009; Rao et al., 2008), the genotypes with the highest female/male ratios can be considered as good candidate for parental in a breeding program.

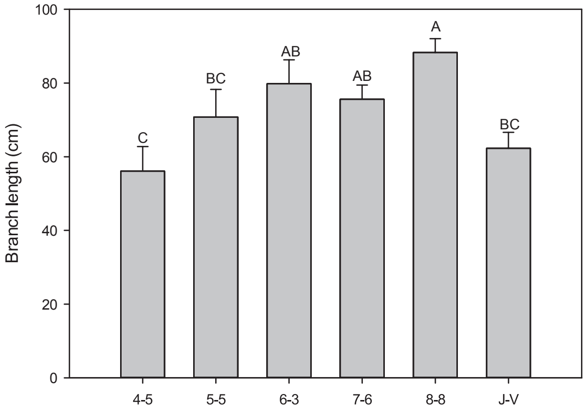

Fig. 1. Branch length observed in six *Jatropha curcas* accessions. Data are average ± SE. Different capital letters represent differences among accessions at 0.05 probability level (LSD test).

Non-synchronized fruiting was observed in all accessions tested and seeds were harvested several times, when they showed the black maturity stage. The harvest was carried out manually before the fruits dropped and capsule dehiscence was not observed in any accession.

The best productions (weight of harvested seeds plant^{-1}) were recorded in the 6-3 and 8-8 accessions with averages of 1943 and 1967 g, respectively. The genotypes 5-5 and 4-5 produced a smaller amount of seeds (from 1260 to 1648 g) and the less productive accessions were 7-6 and J-V with less than 400 g. Plants producing approximately 2 kg of seeds have been considered as plus trees (Singh et al., 2016).

The single seed weight, endosperm weight and the percentage of endosperm were different among the accessions ($P \le 0.001$). The genotypes with highest values of these parameters were 6-3 and 8-8 (Figs. 3 and 4). The seed weight and percentage of endosperm were correlated with a regression coefficient $r^2 = 0.6$ (Fig. 3). The best average single seed weights were observed in 6-3 and 8-8 (0.67 ± 0.01 g), following by J-V (0.65 ± 0.01 g), and the best average endosperm weights were also recorded in 6-3 (0.44 ± 0.01 g) and 8-8 (0.42 ± 0.01 g). Differences of seed weight were indicated by Saikia et al. (2015) that recorded 783.53 g as the highest 1000 seed weight in one accession. Similarly, Singh et al. (2016) found a high variability in the single seed weight of several *Jatropha* accessions with a maximum value of 0.73 g and a minimum value of 0.47 g.

The highest percentages of endosperm were observed in lines 6-3 (64.95 ± 0.28), 4-5 (64.55 ± 0.35) and 8-8 (63.05 ± 0.37). The percentage of endosperm in the others accessions (J-V, 5-5 and 7-6) were less than 60%, similar to the 59% of endosperm in the total seed weight found by Liu et al. (2009).

Regarding percentages of aborted seeds, the lowest values (less than 10%) were obtained in the accessions 8-8, 4-5, 6-3 and J-V, while in 5-5 and 7-6 the aborted seeds were 36.7 ± 0.3 and 22.7 ± 0.2, respectively.

3.2. Oil analysis

The total seed oil percentage was different among accessions ($P \le 0.001$). The highest oil percentages were observed in the lines 4-5 (42.42 ± 0.26), 6-3 (41.51 ± 0.20), and 8-8 (40.41 ± 0.73), whereas the others recorded percentages lower than 30% (7-6: 24.98 ± 0.38; J-V: 23.49 ± 0.69; 5-5: 21.46 ± 0.36). Different authors have described variation in the seed oil content among accessions using a solvent (hexane) extraction. Percentages varied from 27.68% to 37.49% with a mean of 33.79% when different genotypes of *Jatropha* were studied (Singh et al., 2016). The oil content of 162 accessions collected in India ranged from 22% to 42% (Sunil et al., 2008) and the average content of different Mexican populations varied from 42.35 to 55.39% (Martínez-Díaz et al., 2017). Trees with a seed oil content higher than 35% can be considered good accessions (Carels, 2009). In our study three accessions showed oil seed content higher than 40%.

The percentage of endosperm oil was also different among the accessions ($P \le 0.001$). It ranged from 42.72 ± 0.98% in J-V to 65.71 ± 0.36% in 4-5 (Fig. 4). High oil contents of 50–60% are presented in the kernel (Liu et al., 2011). The correlation between endosperm weight and percentage of endosperm oil were low ($r^2 = 0.52$, Fig. 4), which means that the higher seeds do not have the higher oil content. Adriano-Anaya et al. (2014) found a correlation between the ratio oleosomes tissue^{-1} (volume volume^{-1}) and total oil content of the seed in Mexican genotypes of *J. curcas*. Furthermore, the variables oleosomes·μL of tissue^{-1} and oleosomes cell^{-1} had high heritability.

The analysis of the *Jatropha* oil composition is shown in Fig. 5. The fatty acids identified were: C14:0 (mystiric acid ranged 0.36–0.56%), C16:0 (palmitic acid ranged 11.64–15.45%), C16:1 (palmitoleic acid

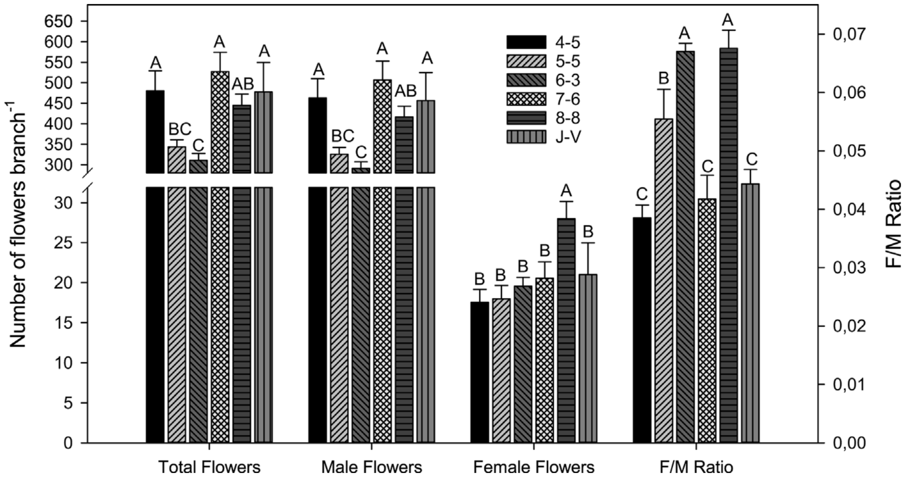

Fig. 2. Flower production and female-to-male ratio (F/M) recorded in six *Jatropha curcas* accessions. Data are average ± SE. Different capital letters represent differences between accessions at 0.05 probability level (LSD test).

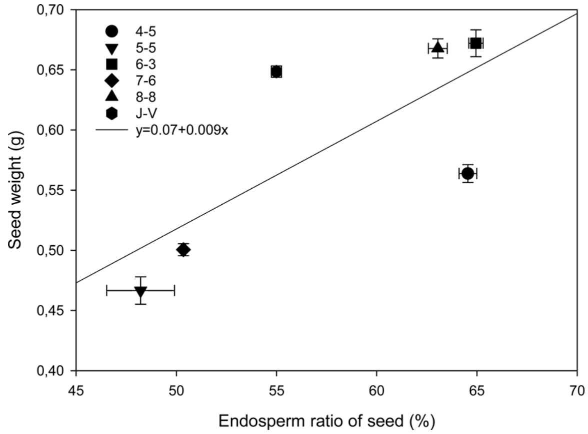

Fig. 3. Relation between seed weight and percentage of endosperm observed in six *Jatropha curcas* accessions. Data are average ± SE.

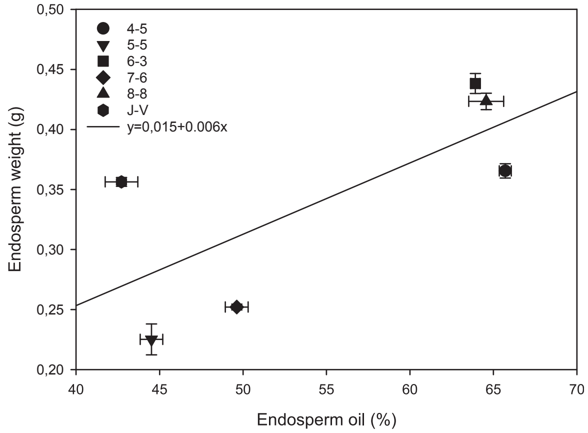

Fig. 4. Relation between endosperm weight and percentage of endosperm oil observed in six *Jatropha curcas* accessions. Data are average ± SE.

ranged 0.97–1.46%), C18:0 (stearic acid ranged 3.52–8.18%), C18:1 (oleic acid ranged 25.92–44.08%), C18:2 (linoleic acid ranged 33.79–52.20%), C18:3 (linolenic acid ranged 0.49–0.69%) and C20:0 (arachidic acid ranged 0.42–0.48%). All accessions presented the same profile of fatty acids; however the amount was different among accessions for some of the fatty acids. Thus, differences were observed among the accessions for oleic acid, linoleic acid, palmitic acid and stearic acid ($P \leq 0.001$). The main fatty acids were oleic acid (C18:1) and linoleic acid (C18:2) with percentages over 30% in most of the genotypes. The highest concentration of oleic acid was found in 6-3, 4-5 and 8-8 genotypes, however, the highest concentration of linolenic acid concentration was recorded in J-V, 7-6, and 5-5 genotypes. Sinha et al. (2015), Parawira (2010), Martínez-Díaz et al. (2017) described similar fatty acid profiles in oil of *Jatropha curcas* accessions from different geographic origin. The oil composition seems to have a genetic component. Different key genes of oil and lipid biosynthesis have been identified and their expression has been correlated with *J. curcas* seed developmental stages (Gu et al., 2012; Xu et al., 2011). Recently, Sood and Chauhan (2015) studied the expression profile of 18 genes encoding enzymes catalyzing the fatty acid and triacylglycerol biosynthetic pathway in various developmental stages of *J. curcas* seeds with high and low oil content, trying to identify regulatory elements and transcription factors specific to oil or lipid accumulation. They found several genes and transcription factors which are expected to play a major role in engineering Jatropha varieties with enhanced and improved oil production.

The saturated, unsaturated, monosaturated and polysaturated fatty acids of the different *Jatropha* seeds were analysed (Fig. 6). Differences were found among the saturated acids percentages ($P \leq 0.005$), with values between 22.5 ± 1.2% in 8-8 and 18.3 ± 0.5% for 5-5. Total unsaturated fatty acid percentages were the highest values observed with differences among genotypes ($P \leq 0.005$). All the accession showed percentages over 75%. The monounsaturated and polyunsaturated fatty acids presented significant differences ($P \leq 0.001$). The highest monounsaturated percentages were recorded in the lines 4-5, 6-3 and 8-8. In these genotypes the polyunsaturated acids were the lowest. On the contrary, the lowest values of monounsaturated and the highest values of polyunsaturated acids percentages were found in the rest of the accessions. Jatropha oil has been widely described as rich in unsaturated fatty acids (Gopinath et al., 2009; Kumar and Sharma, 2008; Martínez-Díaz et al., 2017; Parawira, 2010), but with enough quantity of saturated acids, which are less susceptible at oxidation (Cordeiro et al., 2016).

A seed oil is a good candidate for biodiesel if fits to the current standards for regulating the quality of biodiesel (Barabás and Todoruţ, 2011). In this work we have calculated different quality indexes of the *Jatropha* oil to be used as biodiesel. The values of SV, IV, CN and HHVs of oils are different among genotypes (Table 1). The differences in SV among accessions are slight and the values are similar to those found by other authors (Meher et al., 2013). The CN values in the accessions with the higher oleic acid and lower linoleic acid content are more than 51 (4-5, 6-3 and 8-8; Table 1), which satisfy EN 14214, IS 15607 and ASTM D 6751-09 specifications (Meher et al., 2013). The IV is less than 120 in all accessions indicating a low oxidation power. Despite all accessions had a high HHV, the genotypes 4-5, 8-8 and 6-3 recorded the best oil properties because the presence of a high CN and low IV indicates the

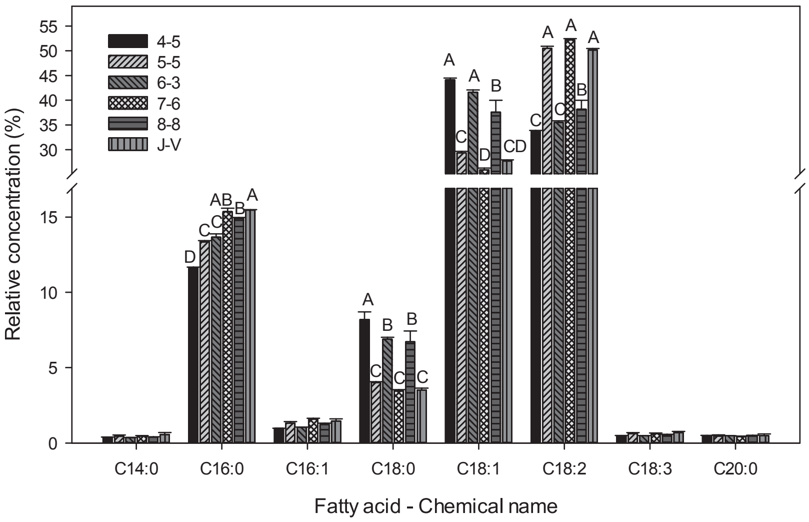

Fig. 5. Fatty acids content (%) observed in the oil of six *Jatropha curcas* accessions: mystiric acid (C14:0); palmitic acid (C16:0); palmitoleic acid (C16:1); stearic acid (C18:0); oleic acid (C18:1); linoleic acid (C18:2); linolenic acid (C18:3); arachidic acid (C20:0). Data are average ± SE. Different capital letters represent differences among accessions for each fatty acid at 0.05 probability level (LSD test).

superior ignition quality and the higher stability of the oil. High IV and low CN have been observed in fuels with greatly unsaturated components like soybean, sunflower and grape seed oils, which generally show poorer oxidation stability than biodiesel of almond, olive, corn, rapeseed and high oleic sunflower oils with a greater monounsaturated content (Ramos et al., 2009). Overall, cetane number, heat of combustion, melting point, and viscosity of fatty compounds increase with increasing chain length and decrease with increasing unsaturation (Knothe, 2005).

On the other hand the oxidation stability of *Jatropha* based biodiesel can be enhanced by the use of natural and synthetic antioxidants or free radical scavengers (Meher et al., 2013), but a crude oil with high quality is desirable.

Biodiesels emit more nitrogen oxides (NOx) exhaust emissions than conventional diesels. Reduced NOx emissions have been correlated with increasing CNs, saturation and length of the fatty ester chain, and decreasing IV values (Knothe, 2008; McCormick et al., 2001). In this sense, biodiesels obtained from the genotypes 4-5, 8-8 and 6-3, with high CN, low IV and high percentage of saturated fatty acids are the best candidates to be the most environmentally-friendly oils with the lowest NOx emissions.

4. Conclusion

In this work information about promising *Jatropha* accessions is provided. The knowledge of plant growth parameters and oil quality of the studied genotypes could be useful to choose the appropriate ones to be grown under subtropical climatic conditions (Canary Island) or to be used as parents in a breeding program. Thereby, the genotypes 8-8 and 6-3 have shown a good vigor plant, the best F/M ratio, the highest single seed and endosperm weight, in addition to high oil content. Regarding the quality of the seed oil, the genotypes 4-5, 8-8 and 6-3 presented the highest monounsaturated fatty acids percentages with the highest concentration of oleic acid and the lowest concentration of linolenic acid. The biodiesel obtained from these genotypes had worthy properties due to the presence of high CN and low IV, which indicates the good ignition quality and stability of the oil.

Combination of agronomical data with biochemical data shows that the accessions showing the best plant growth characteristics are also the best in oil yield and quality.

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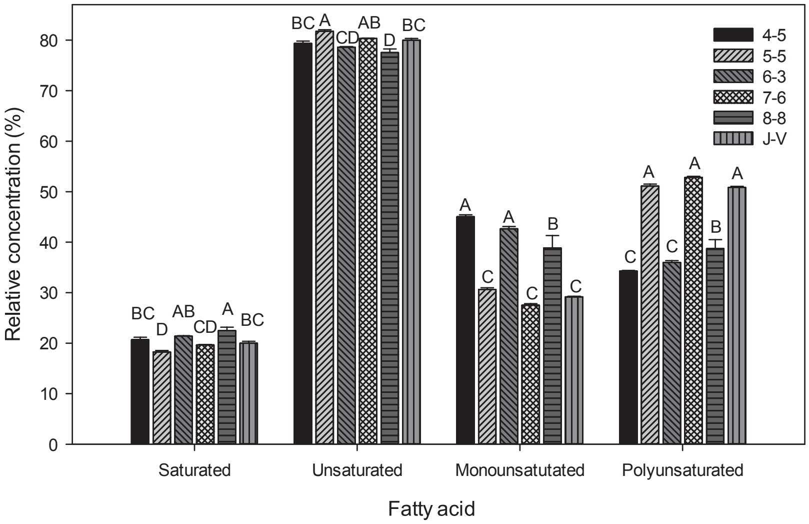

Fig. 6. Saturated, unsaturated, monosaturated and polysaturated fatty acids content (%) recorded in the oil of six *Jatropha curcas* accessions. Data are average ± SE. Different capital letters represent differences among accessions for each type of fatty acid at 0.05 probability level (LSD test).

Table 1
Fuel properties of *Jatropha curcas* oil seed. Data are average ± SE. Different letters represent differences among accessions for each index at 0.05 probability level (LSD test).

Accession	Saponification Number (SN)	Iodine Value (IV)	Cetane Number (CN)	Higher Heating Values (HHVs)
4-5	191.59 ± 0.01^{a}	98.21 ± 0.54^{a}	52.69 ± 0.12^{c}	43.05 ± 0.01^{a}
5-5	192.27 ± 0.05^{c}	115.06 ± 0.50^{e}	48.80 ± 0.11^{a}	43.27 ± 0.01^{b}
6-3	192.00 ± 0.04^{b}	99.10 ± 0.17^{b}	52.43 ± 0.04^{c}	43.04 ± 0.00^{a}
7-6	192.73 ± 0.03^{d}	115.37 ± 0.08^{e}	48.66 ± 0.02^{a}	43.26 ± 0.01^{b}
8-8	192.32 ± 0.00^{c}	100.60 ± 0.62^{c}	52.05 ± 0.14^{b}	43.05 ± 0.01^{a}
J-V	192.73 ± 0.06^{d}	113.40 ± 0.42^{d}	49.11 ± 0.09^{a}	43.23 ± 0.01^{b}

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