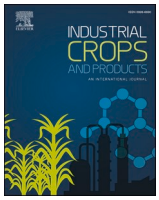
Industrial Crops & Products 198 (2023) 116717

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Parental selection and the diversity of the F_1 *Jatropha curcas* genotypes: Seed quality and phytochemistry

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ARTICLE INFO

Keywords:
Biodiesel
RAPD
X-ray
HPLC
Arboviruses

ABSTRACT

Jatropha curcas is a germplasm that produces seeds used for biodiesel production. Characterizing different germplasms is always necessary to select varieties with potential for industrial use. This study aimed to screen the parentals used in partial diallel crosses using DNA-RAPD and phenology. The most divergent parentals were evaluated for their seed traits and oil composition. The hybrids were tested for seed oil composition, optimal maturation stadium for physiological seed quality (germination and vigour), and physical seed quality using X-ray tests. The hybrids and parentals were compared to estimate heterosis and heterobeltiosis. The hybrid leaf extracts were tested against *Aedes aegypti*. The parentals had a mean genetic similarity of 52.6%. 003 × 013 and 004 × 005 had the highest heterosis and heterobeltiosis for seed traits. The 001 × 005 hybrid stood out for its seed biometry and physical seed quality, showing full seeds, although it had the lowest oil content. Phenological stadium I fruit maturation showed full seeds for 004 × 005, 004 × 005, 001 × 008, and 001 × 013, which differed in their phytochemical composition, and the leaf extracts did not show potential use as an insecticide against *A. aegypti*. In the future, new approaches could be used with the genotypes that were selected for seed and phytochemistry characterization under different edaphoclimatic conditions.

1. Introduction

Given the recognition of the impact of carbon dioxide emissions on climate change, there has been a growing interest in renewable energy sources to reduce dependence on fossil fuel reserves. The use of vegetable oils for biofuel production has increased to meet global demand (Lama, 2018; Albuquerque, 2017). In addition to traditional crops, there are many new oilseeds that are suitable for biofuel production. *Jatropha*

Abbreviations: GC-MS, Gas chromatography-mass spectrometry; HPLC, High-performance liquid chromatography; RAPD, Random Polymorphic DNA; UPGMA, Unweighted Pair-Group Method using Arithmetic Averages; 1stC, First count; %G, Total germination percentage; SGI, Speed of germination index; CG-MS/FID, Gas chromatography-mass spectrometry/flame ionization detector; MFS, Membranes; SPE, Solid-phase extraction; HPLC-DAD, High performance liquid chromatography-Diode Array Detector; PCA, Principal Component Analysis; JCUFS, *Jatropha curcas*/Universidade Federal de Sergipe; LC-grade, Liquid chromatography grade; σg^2, Genetic Variance; σe^2, Environment Variance; σph^2, Phenotypic Variance; h^2, Heritability; CVg, Coefficient of Variation Genetic; CVe, Coefficient of Variation Environmental; CV, Coefficient of Variation; NPK, Nitrogen: Phosphorous: Potassium; FA, Fatty acid.

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https://doi.org/10.1016/j.indcrop.2023.116717
Received 27 March 2022; Received in revised form 31 March 2023; Accepted 11 April 2023
Available online 19 April 2023

curcas L. is one such source of seeds that can be used to obtain renewable oil and produce sustainable and affordable biofuels (Wassner et al., 2016; Kumar et al., 2018; Singh et al., 2016). However, it should be noted that the viability, vigor, and oil richness of the seeds obtained from crosses may not always be guaranteed.

Developing cultivars with seeds that possess both physical and physiological quality is a significant challenge for oilseed production. In addition, genotypes must also demonstrate potential for producing co-products from other plant parts, such as leaves. In recent decades, *Jatropha* has emerged as an interesting species-genus, as it can produce oil as a primary source for biodiesel production (Purwati et al., 2022).

Jatropha curcas, commonly known as the physic nut, is a shrub belonging to the Euphorbiaceae family. Its inedible fruits contain oil-rich seeds, with reported oil yields of 35–40% of the seed mass, and a yield of 1–1.5 tonnes of oil per hectare in a single harvest (Sahoo, 2009; Shukla et al., 2022; Lee et al., 2013). The high-quality oil is suitable for biodiesel and even biofuel for airplanes (Nietsche et al., 2015; Escalante et al., 2022). Compared to other oilseed crops, *Jatropha* can tolerate moderate water deficits and can recover with available water. When faced with intense drought and herbivorous stress, it produces flavonoids that protect the leaf tissue from oxidative stress and photo-damage (Lama et al., 2016; Pemmaraju et al., 2022). As a versatile tropical plant with many attributes, it can be grown in low to high-rainfall areas and can be used as a hedge or commercial crop, providing employment opportunities and improving the environment, as well as enhancing the quality of rural life (Priyanka et al., 2021). The species can also be used to prepare fungicides (Cordova-Albores et al., 2014; Ribeiro et al., 2020), insecticides (Ahuchaogu et al., 2014), larvicides (Kovendan et al., 2011), and acaricides (Juliet et al., 2012). Additionally, it has been studied as a reinforcing element against slope erosion for its use in soil conservation, as it protects from the impact of raindrops with the plant shoots and root system anchorage (Islam et al., 2011; Ferreira et al., 2022).

The fruit residues of *Jatropha* can be converted into fertilizers or animal feed due to their NPK and protein content (Tomar et al., 2014). Additionally, the plant has shown potential for cytotoxic activity in preliminary studies, making it a potential candidate for medicinal applications (Ribeiro et al., 2012; Chijindu et al., 2022).

Characterizing diverse germplasms is necessary to identify and select genotypes with potential for production and biochemistry. In this regard, phenological investigations can significantly aid in the selection of genotypes that can acclimate to specific regional conditions (Domiciano et al., 2014).

Genetic markers can be used to access the characterization of genetic diversity, and studies have already been conducted on the physic nut using such markers (Gangapur et al., 2018; Ribeiro et al., 2019; Souza et al., 2019), as well as on oil production (Abdelra Abdelrahman et al., 2020; Francis et al., 2018), and phenology (Castillo-Ugalde, 2018; Fernandes, 2013; Kumar, 2014; Santos et al., 2005). Several studies have also been conducted worldwide on plant extracts and their compounds against larvae (Beserra et al., 2014; Alvarez et al., 2016). However, to select parentals for crossings to obtain productive hybrids, it is important to use genotypes that are suitable for the region of interest, with associated studies of production, variations in seed development, and genetic diversity, all of which are crucial.

Oilseed crops are grown specifically for their seeds or fruits that are rich in fat and can be extracted for plant oil, which can be used for food, energy, and other industrial purposes. Some of the by-products of oil extraction, such as cake, can be used for animal feed. Several oil plants, including sunflower, palm oil, and other oilseeds, can be targeted for biofuel production (Ndiaye et al., 2022). It is essential to select species that demonstrate rusticity and tolerance to semi-arid regions. Increasing the oil output from seeds is one of the factors that can be exploited in plant breeding programs (Jonas et al., 2020).

Jatropha can be evaluated for potential uses by using plant tissues in the form of extracts that can be tested for insect control. One of the most significant arboviruses impacting humans, dengue fever, is transmitted by *Aedes aegypti*. The disease has been controlled through measures that target the vector at different stadium of the insect's life cycle. However, such control is often conducted using synthetic insecticides, which have faced challenges related to insect resistance and environmental pollution. The search for alternative plant-derived insecticides has grown to reach diverse objectives, particularly those in line with the United Nations' sustainable development goals. *In vitro* studies have shown varying levels of insecticidal activity against *A. aegypti*, depending on the genotype (Njom, 2022).

In this context, this study aims to address the gaps in earlier studies on parental selection by using phenology and genetic diversity to aid in the crossings. The study also estimate progeny gains for physical and physiological seed quality, as well as heterosis and heterobeltiosis when compared to parental performance. Furthermore, the study will consider the insecticide potential of the hybrids against *A. aegypti*.

2. Material and methods

2.1. Plant material

Over a period of 36 months (three crop years), seventeen genotypes from Minas Gerais, Goiás, Espírito Santo, Sergipe, and Bahia (Fig. 1) were studied. The evaluation was conducted at latitude 10 ° 55' 27" S, longitude 37 ° 12' 01" W, and an altitude of 46 m in a region highlighted in orange on the map (Fig. 1). The region is characterized by an As-type climate (tropical rainy, with a dry summer, according to Köppen's classification) (Köppen, 1936) and an average annual temperature of 25.2 °C. The provenances and herbarium codes of the parental genotypes are presented in Fig. 1.

2.2. Parental genotypes DNA similarity

DNA was extracted from the leaves (Doyle et al., 1987; Ribeiro et al., 2017) using the Random Polymorphic DNA (RAPD) technique with 19 primers (Williams et al., 1990). The amplified products were separated in a 1% agarose gel and stained with ethidium bromide (25 μg/mL). The presence (1) and absence (0) of fragments were used to estimate similarity (Jaccard coefficient) (El-Sayed et al., 2020), and the data was expressed in the dendrogram (Rohlf, 2000) by the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) (Sneath, 1973). The consistency of each group was bootstrapped (Coelho, 2002).

2.3. Phenological phases for the parental genotypes

Phenological phases were evaluated every two weeks for 36 months (Fournier, 1974), and each phase was determined and associated with temperature and precipitation. The phases were scored from 0 to 4, with 0 representing the absence of the phenomenon, 1 representing 1–25%, 2 representing 26–50%, 3 representing 51%– 75%, and 4 representing 76–100% of the phenological event (Table 1).

2.4. Biometry, mass, and the oil content of the seeds' parental genotypes

The fruits were harvested, and the seeds were dried and stored at 8 °C with 12% humidity. Seed biometry and mass were determined using four replicates of 25 seeds for each genotype (Brasil, 2009).

Oil extraction was performed using 20 g of macerated seeds in a Soxhlet apparatus (80 mL) with hexane (150 mL) for 6 h, and the data was estimated using a completely randomized design. The samples were placed in cylindrical cellulose cartridges, and triplicate extractions were performed on a Quimis® plate at 70 °C under continuous reflux for 4 h. The samples were then kept under reflux without hexane for 1 h.

Municipality	State	Herbarium
Lavras	Minas Gerais	15256
Lavras	Minas Gerais	15259
Lavras	Minas Gerais	15249
Lavras	Minas Gerais	15258
Lavras	Minas Gerais	15257
Lavras	Minas Gerais	15243
Lavras	Minas Gerais	15250
Lavras	Minas Gerais	15246
Lavras	Minas Gerais	15254
Rio Verde	Goiás	15253
Rio Verde	Goiás	15252
Lavras	Minas Gerais	15251
Rio Verde	Goiás	15262
Alegre	Espírito Santo	15255
Rio Verde	Goiás	15248
Lagarto	Sergipe	15261
Valente	Bahia	15260

Fig. 1. *Jatropha curcas* parentals origin and herbarium number in Federal University of Sergipe (the area of evaluations is orange-coloured on the map).

Table 1
Phenophases by Fournier (1974) when applied on the parentals of *Jatropha curcas* L.

Phenological Stadium	Code	Phenophases
Sprouting season	1	Leafless
	2	Sprouting
	3	Adult leaves
Flowering season	1	Presence of floral buttons
	2	Full blossom
	3	End of flowering
Fruiting season	1	Early fruiting stage
	2	Green fruits
	3	Brown fruits

2.5. F_1 plants

Six parentals (JCUFS005, JCUFS008, JCUFS013, JCUFS001, JCUFS003, and JCUFS004) were selected based on phenology and DNA-RAPD diversity for the partial diallel crossings (Geraldi and Miranda Filho, 1988) as conducted by Santana et al. (2013). Each experimental plot consisted of six plants that received the same fertilization and crop management as the parental plants.

2.6. Biometry of the F_1 seeds

The fruits were separated according to their maturation stage using colour attributes, and the seeds' morphometry was measured using a digital caliper (Model ZAAS) with 4 replicates of 50 seeds. The seeds were then subjected to X-ray and germination tests (Brasil, 2009) to evaluate their physical and physiological quality.

2.7. Derivatisations of the oil and the fatty acid profiles of the hybrids

The derivatizations and determinations of the fatty acids were adapted from ISO (5508, 1999) and ISO (5509, 2000) (Antolín et al., 2008; Petrovic et al., 2010). The oil was analyzed using the GC-MS/FID GCMS-QP2010 Ultra Model (Shimadzu Corporation, Kyoto, Japan), with an AOC-20i (Shimadzu). Separation of the extracts was performed on a Restek Rtx®− 5MS fused silica capillary column (5% diphenyl-95% dimethylpolysiloxane) with a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm. The analysis was performed under a constant flow of helium (99.999%) at a rate of 1.2 mL/min. An aliquot of 1 mL (10 mg/mL) was used with a split ratio of 1:10. The temperature was programmed to 50 °C (1.5 min isotherm), then increased by 4 °C/min to 200 °C, followed by an increase of 10 °C/min to 250 °C, with a 5 min isotherm at 250 °C.

2.8. Reagents

For HPLC, the methanol LC-grade (Tedia, Fairfield, OH, USA) and formic acid (JT Baker, Philipsburg, PA, USA) were used. The deionised water was purified by a Milli-Q system (Millipore, São Paulo, SP, Brazil). All of the solvents were filtered through 0.45 nylon membranes (MFS), and degassed by an ultrasonic bath before use.

2.9. Leaf hybrid extracts HPLC analysis

For the extraction of compounds, 2.0 g of dried leaves were mixed with 10 mL of ethyl alcohol solvent, and the samples were homogenized using an ultra-turrax® (UT) for 10 min. Then, they were centrifuged at 4000 rpm for 10 min. The solvent was removed by a rotary evaporator after three extractions, and the ethanolic extracts were stored at − 21 °C until chromatographic analysis. Aliquots of 30 mg were dissolved in 1 mL of methanol: water solution (1:1). Solid-phase extraction (SPE) using C-18 cartridges (Phenomenex) was performed to purify the solutions prior to high-performance liquid chromatography with Diode Array Detector (HPLC-DAD) analysis (Vega-Ruiz et al., 2021).

The samples were analyzed using a Shimadzu Prominence® high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan), consisting of two LC-20AT pumps, an SPD-M20A photodiode array detector, a CTO-20A column oven, a DGU-20A3 degasser, a SIL-20AHT autoinjector, and a CBM-system controller 20A. Data collection was performed using Shimadzu LC Solution software. The chromatographic profile was obtained using a Phenomenex Kinetex® phenyl-hexyl column (150 ×4.6 mm i.d., 5 μm particle diameter) under the following conditions: flow rate of 1 mL/min, injection volume of 20 μL, with the column at 25 °C, and a mobile phase consisting of 0.1% aqueous formic acid and methanol. The elution gradient for the samples was 5–100% for 60 min, then remaining at 100% for 5 min, and returning to 5% in 1 min. The photodiode array detector was set at 254 and 350 nm for acquiring the chromatograms, and the ultraviolet spectra were recorded at 190–800 nm.

2.10. Hybrid extracts essay for the Aedes aegypti

The assay was performed using adult mosquitoes that were provided with a 10% ad libitum sucrose solution. To stimulate oogenesis, the insects were deprived of sugar for 48 h and then fed with defibrillated

and pure sheep blood (EBE-FARMA Biológica e Agropecuária LTDA., Rio de Janeiro, Brazil) until they were fully engorged. The mosquitoes were kept in a room at 26 ± 1 °C, with a relative humidity of 80 ± 2%, and a 12:12 (light: dark) photoperiod.

The larvicidal tests were performed following the standard procedures of the World Health Organization, with some modifications. The extract concentrations were set at 50 and 200 ppm. Fifty milliliters (50 mL) of the extracts were added to the cups, and ten third-instar *A. aegypti* larvae were introduced, with triplicate bioassays. The mortality rate was determined after 24 and 48 h for each concentration.

2.11. Data analyses

The data were evaluated using the Shapiro-Wilk test for normality and for homoscedasticity of variances. ANOVA was performed with a 5% significance level, and means were grouped by the Scott-Knott method at 5%, using SISVAR® statistical software (Ferreira, 2008). Heterosis and heterobeltiosis were estimated using Genes® software (Cruz, 2013).

3. Results and discussion

3.1. Parental plants

The DNA diversity of the RAPD markers varied among the genotypes. Previous studies have shown that RAPD markers efficiently estimate genetic variability in physic nut genotypes (Kumar et al., 2009). Similarly, RAPD markers have been effective in detecting polymorphism in *Jatropha* (El-Sayed et al., 2020; Gopale et al., 2013; Rosado, 2010). In this study, 110 fragments were obtained using RAPD, and 64 of them were polymorphic (58%). The mean similarity was 52.6%. Subramanyam et al. (2010) reported 60.8% polymorphism in studies using RAPD for physic nut from different provenances, while Ram et al. (2008) found 80.2% polymorphism using 18 primers. Other studies have revealed low to moderate levels of genetic diversity (Gangapur et al., 2018). The data was evaluated for normality and homoscedasticity using the Shapiro-Wilk test, and the F-test was conducted using ANOVA at 5%. The means were clustered using Scott-Knott at 5%, and the heterosis and heterobeltiosis were estimated using Genes® software (Cruz, 2013).

JCUFS-007 and JCUFS-008 showed higher divergence (76% ± 0.05), whereas JCUFS-015 and JCUFS-017 showed the lowest divergence (25% ± 0.05). JCUFS-007 and JCUFS-008 were the most divergent genotypes from JCUFS-015 and JCUFS-017 (25% ± 0.05). However, the level of divergence observed in the present study was lower than that reported by Rafii et al. (2012), who found 72.88% of total variation among the genotypes. The current study classified the genotypes into five groups (Fig. 2) based on their level of divergence.

The phenology and RAPD analysis were subjected to Principal Component Analysis to better discriminate the genotypes (Fig. 3). A total of 112 variables were evaluated in this process.

The budding phase occurred from July to December, shortly after the end of the rainy season. Genotypes JCUFS-008, JCUFS-009, JCUFS-014, and JCUFS-015 showed flowering in October; JCUFS-012 and JCUFS-013 in November, while the remaining genotypes flowered in December.

Flowering occurred during the dry and warm seasons, with the first flowering stadium taking place in the months with the highest rainfall, from January to April (444.80 mm). Flowering was also observed from October through December. Fruiting was initiated from January to June (28.37 °C) during low rainfall (36.50 mm) and reached its peak during the rainy season (Santos et al., 2013). All genotypes produced fruits from April to June, during the region's highest rainfall volume.

The second fruiting occurred in December (8.70 mm and 28.42 °C), with fruits at different stadium of maturity on the same branch.

The photoperiod, humidity, temperature, pollinators, and agents of dispersal can interfere with the flowering and fruiting stadium.

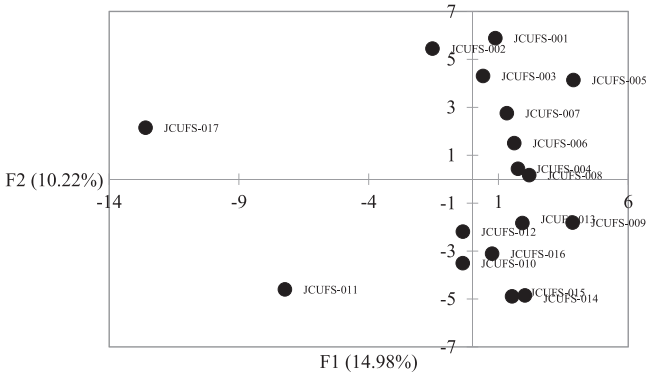

Fig. 3. Principal Component Analysis (PCA) that was based on the Mahalanobis distance, estimated from 112 variables (48 phenological and 64 RAPD markers).

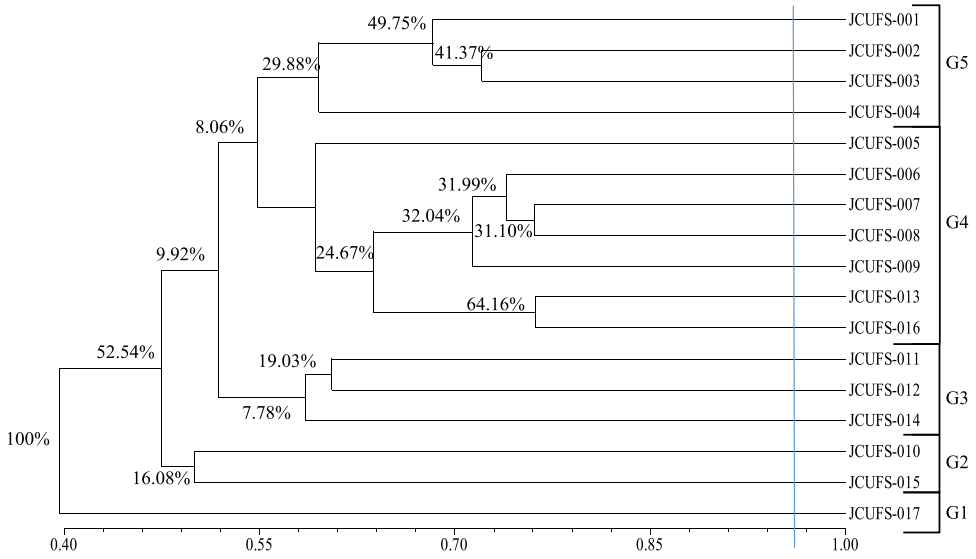

Fig. 2. The genetic similarity by RAPD when using the UPGMA method, which was based on the Jaccard coefficient when using the RAPD markers in *Jatropha curcas* L.

Understanding the phenology of the species is crucial for crop management and for studying genetic and environmental interactions (Gurgel et al., 2011; Bai et al., 2022). The mean product of production for *J. curcas* is the seeds. There were differences in the seeds' biometry, mass, and oil content for the parentals, with the best yield in JCUFS-001, JCUFS-011, and JCUFS-006. The highest widths were measured in JCUFS-015, JCUFS-003, JCUFS-001, JCUFS-006, JCUFS-011, and JCUFS-013 (Table 2).

The phenotypic variance indicated the predominance of environmental influence, which highlights the importance of phenophases. The heritability of the seed measurement variables was superior at 92%. Kaushik et al. (2015) reported high heritability and genetic gains for oil content and seed weight, implying additive gene action. Seed mass could indicate oil content, as already noted for hybrids by Santana et al. (2013), with moderate heritability for other seed indices (Joshi et al., 2021).

The seed mass had the highest heritability (98.43%), indicating potential for genotype selection. The magnitude of variability present in a crop provides the potential for effective selection. Heritability refers to the portion of phenotypic variation transmitted from the parentals to the hybrids (Sharma and Bora, 2013). JCUFS-001, JCUFS-006, and JCUFS-011 presented the highest seed measurements.

An informative genetic parameter is heterosis, which evaluates the performance of a hybrid by comparing its increase or decrease in performance with the mean of the two parentals (heterosis). Moreover, hybrid vigor can be evaluated from the parentals, and the best performance can be estimated by heterobeltiosis.

Heterobeltiosis is considered to be more effective than heterosis in identifying superior hybrids. The selection of promising parentals to obtain superior hybrids is dependent on the predominance of genes with additive effects (Gowda et al., 2010; Beche et al., 2013).

Heterosis is a genetic parameter that highlights the importance of non-additive genetic effects such as dominance and epistasis, which are generally associated with the heterozygous condition. However, a hybrid will be advantageous if it is superior to both parentals. In this study, the authors evaluated heterosis and heterobeltiosis (Godoy et al., 2008).

The parentals were used in the partial diallel crosses to evaluate the general and specific combining capacity, and Santana et al. (2013) previously observed positive genotypic correlations.

3.2. Hybrids oil content and seed traits

A significant difference ($p < 0.05$) was observed in the oil content and seed biometry of all hybrids. The oil content ranged from 33.27% to 52.20%, and the weight of 100 seeds ranged from 50.25 to 55.97 g, with higher values particularly noted for 004 × 005 (Table 3).

Furthermore, 004 × 013 presented small seeds and high oil content, with no significant difference ($p < 0.05$) in the mass of 100 seeds. In studies of genetic diversity using morphological traits, the mean oil content of 31% was observed in 75 progenies in Brazil and three regions of Cambodia, with no correlations found between the morpho-agronomic traits and the oil content (Freitas et al., 2011).

The heritability, in the broadest sense, of the hybrids was favorable (above 80%) for most of the traits (Table 4). However, they were all lower than the values reported by Rao et al. (2008), who found an average heritability of 99.61% for oil content and 93.16% for 100-seed weight.

The highest σg^2 was estimated for the oil content and the weight of 100 seeds. High h^2 and σg^2 indicate high genetic variation for the hybrids (Diédhiou et al., 2016; Galapia et al., 2012). The unsaturated fatty acids, such as oleic acid and linoleic acid, were found in higher ratios (Table 5). Methyl laureate, methyl myristate, methyl palmitoleate, methyl elaidate, methyl linoleic acid, methyl arachidonate, and methyl behenate were not detected.

For all hybrids, oleic and linoleic acids were predominant, ranging from 35.7 (004 × 013) to 41.8 (003 × 005) and 30.6 (004 × 005) to 37.8 (001 × 013), composing 73.2% of the oils.

The composition of fatty acids (FAs) primarily determines the properties of biodiesel and triglycerides. Biodiesel derived from linolenic and oleic acids yields low cetane numbers. Saturated FA alkyl esters affect thermal stability, cetane number, and slow deterioration rate, while unsaturated FAs affect oxidative stability. A smaller amount of polyunsaturated and saturated FAs and a higher amount of monounsaturated fatty acids such as palmitoleic acids can result in better-quality biodiesel (Singh et al., 2016).

The values for oleic and linoleic acid in the seeds were similar in India and Brazil (Jain et al., 2010; Silva et al., 2014; Barros et al., 2015)

Table 2
Jatropha curcas L. parental genotypes for seed size, the mass of 100 seeds, and oil content (%).

Access	Length (mm)	Width (mm)	Thickness (mm)	Mass (g/plant)	Oil yield (%)
JCUFS-001	18.75 a	12.00 a	9.00 a	15.00 d	34.00 a
JCUFS-002	18.00 b	11.00c	9.00 a	15.25 d	26.50 b
JCUFS-003	18.00 b	12.00 a	9.00 a	17.00 b	25.50 b
JCUFS-004	18.00 b	11.50 b	9.00 a	13.50 e	27.00 b
JCUFS-005	18.00 b	11.00c	9.00 a	14.75 d	23.00 b
JCUFS-006	18.50 a	12.00 a	9.00 a	16.25c	23.50 b
JCUFS-007	16.50c	10.50 d	8.00 b	13.00 e	22.00 b
JCUFS-008	18.00 b	11.50 b	9.00 a	15.00 d	25.50 b
JCUFS-009	17.00c	11.00c	8.00 b	12.50 e	29.50 a
JCUFS-010	18.00 b	11.25c	9.00 a	16.00c	34.00 a
JCUFS-011	18.50 a	12.00 a	9.00 a	15.00 d	24.00 b
JCUFS-013	18.25 b	12.00 a	8.75 a	18.25 a	32.00 a
JCUFS-015	18.00 b	12.00 a	9.00 a	15.00 d	18.50 b
JCUFS-016	18.25 b	11.25c	8.75 a	13.25 e	32.00 a
JCUFS-017	17.75 b	11.00c	9.00 a	14.75 d	30.50 a
Mean	17.92	11.46	8.77	14.93	31.31
Phenotypic variance (σ^2ph)	0.30	0.01	0.11	2.10	8.59
Environmental Variance (σ^2e)	0.01	0.01	0.01	0.03	0.63
Genotypic variance (σ^2g)	0.29	0.13	0.11	2.07	7.96
Heritability (h^2) - %	96.21	93.01	94.06	98.42	92.69
CV (%)	1.1964	1.7120	1.8679	2.43831	5.06

The means with a different letter in the columns are different at 5% by the Scott-Knott test.

Table 3
Seed biometry and the oil content of the hybrids of *Jatropha curcas* L.

Hybrid	Length (mm)	Width (mm)	Thickness (mm)	Mass 100 Seeds (g)	Oil Content (%)
001 × 005	19.12 a	11.80 a	8.80 a	55.97 a	46.30 a
001 × 008	18.47 b	11.32 b	8.52 a	53.31 a	43.87 a
001 × 013	17.98c	11.17 b	8.13 b	52.44 a	46.07 a
003 × 005	18.30 b	11.25 b	8.45 a	54.21 a	42.80 a
003 × 008	18.33 b	11.27 b	8.57 a	54.75 a	42.40 a
003 × 013	17.51 d	10.97c	8.59 a	50.28 a	52.20 a
004 × 005	17.78c	11.20 b	8.44 a	52.05 a	46.47 a
004 × 008	17.87c	11.23 b	8.42 a	55.58 a	33.27 b
004 × 013	17.17 d	10.90c	8.23 b	50.25 a	43.03 a
004 × 015	18.01c	11.18 b	8.39 a	52.85 a	43.00 a
CV (%)	1.44	0.98	1.99	5.64	7.48

The mean values followed by the same letter in the column belong to the same group, according to the Scott-Knott Test, at 5% of probability.
001 × 005 showed distinguishable characteristics for seed length and width, while the highest oil content was found in 003 × 013, reaching 52.20%. Notably, several hybrids exhibited higher values compared to those reported in the literature (Pessoa et al., 2012; Christro et al., 2012; Pimenta et al., 2014; Fernandes et al., 2015; Evangelhista et al., 2015).

Table 4
Genetic (σg^2), Environmental (σe^2), Phenotype (σph^2) variance, and heritability, in the broadest sense (h^2), in the hybrids of *Jatropha curcas* L.

Parameter	Length (mm)	Width (mm)	Thickness (mm)	Mass-100 Seeds (g)	Oil (%)
Mean	18.06	11.23	8.45	53.17	20.70
Genetic variance (σ_g^2)	0.27	0.05	0.03	1.91	19.33
Environment variance (σ_e^2)	0.02	0.01	0.01	2.09	3.33
Phenotypic variance (σph^2)	0.29	0.06	0.04	4.01	22.67
Heritability (h^2)	92.50	93.24	80.20	47.75	85.29
Genetic coefficient of variation ($CV_g\%$)	2.88	2.12	1.98	2.60	29.95
Environment coefficient of variation ($CV_e\%$)	5.07	5.12	1.99	1.24	33.87
$CV_g\%/CV_e\%$	1.76	0.75	1.01	0.48	7.20
CV (%)	1.64	1.11	1.97	5.44	7.19
Mass of Seeds (g)	1.17**	0.23**	0.14**	16.02	60.00*

**, * = Significant at 1% and 5% of probability.

Table 5
Fatty acid profiles of the hybrids of *Jatropha curcas* L. (% peak area on GC-FID).

Hybrid	Palmitic (%)	Stearic (%)	Oleic (%)	Linoleic (%)
001 × 005	17.50	8.10	38.90	35.50
001 × 008	21.90	8.20	37.70	32.30
001 × 013	16.30	7.30	38.70	37.80
003 × 005	16.50	8.00	41.80	33.30
003 × 008	20.50	7.90	38.30	33.30
003 × 013	15.10	7.00	40.50	37.40
004 × 005	22.30	8.30	38.80	30.60
004 × 008	20.20	8.80	38.80	32.20
004 × 013	21.80	6.70	35.70	35.80
004 × 015	17.80	7.90	38.80	35.40

and were higher than those obtained in the Republic of Congo (Nizkou et al., 2009) and China (Senou et al., 2016) for palmitic acid (4%). A study in Cuba reported higher values for stearic and oleic acid (Rodríguez et al., 2011), while values obtained in Nigeria and Mexico were in the range of 9.76 – 13% (Mohammed-Dabo et al., 2012; Zavala--Hernández et al., 2015). The accessions grown in different regions of Zimbabwe and India showed differences in fatty acid composition (Parthiban et al., 2011; Sunil et al., 2009). The seed oil of *J. curcas* is characterized by oleic-linoleic traits (Akbar et al., 2009), and the fuel quality is affected by its composition. Ideally, the oil should have low saturation and high amounts of monounsaturated fatty acids. The richness in polyunsaturated acids, such as linoleic and linolenic, tends to generate methyl ester fuels with poor oxidation stability (Waghmare et al., 2015). Oleic acid is a promising alternative for fuels due to its conflicting requirements of one-sided cold flow properties, oxidative stability, and NOx (nitrogen oxide) emissions (Mazumddar et al., 2013), which are essential for maintaining biodiesel qualities during product storage.

The quality of oil in *J. curcas* is influenced by various factors such as climatic conditions, soil characteristics (physical and chemical), genetic variability, and seed development (Turynaio et al., 2015). The physiology of the seed can also affect the pathways of triacylglycerols, fatty acids, and sterols, resulting in variations in the lipid content. In this study, a low correlation was found between the oil content and fatty acid composition of ripe *Jatropha* nuts, which is consistent with previous reports (Booranasrisak et al., 2013). The authors also observed significant variations in fatty acid composition. A comprehensive investigation of the correlation between morphological traits, characterizations, and seed yield is essential for assessing genetic variability and selecting the most favorable genotypes.

Diallel crosses are commonly used to estimate genotype combinations in breeding and pre-breeding programs. In a study of *J. curcas* diallel crosses, general and specific combining ability, as well as reciprocal effects, were significant for yield and 1000 - seed weight. This allowed for the evaluation of genetic, additive, non-additive, and cytoplasmic effects (Laviola, 2018). The better interaction and complementation of genotypes, which is reflected in yield, can be observed through significant estimations of dominant effects, and the parentals were divergent for most loci. Heterotic manifestations can result in genetic loci complementation. Therefore, methods that prioritize heterosis should be used to improve genetic dominance and divergence (Teodoro et al., 2017). The mean heterosis for the parentals, when considering seed biometry and oil yield, ranged from − 0.95 – 0.75 for length, − 1.03 – 0.30 for width, − 0.74 to − 0.20 for thickness, and 7.03 – 23.42 for oil content. Hybrids 001 × 005 (length and width of the seed) and 003 × 0013 (mean value of 23.42) were superior.

The lowest oil yield was observed in 004 × 008 (33.27%), which was 18.93% lower than the oil content of 003 × 013. The hybrid combinations showed strong heterosis, resulting in divergence, and the parental and F_1 stability was expressed in the yield and stable inheritance of the agronomic traits (Fu et al., 2014). However, the superiority in seed volume did not always result in germinative benefits for propagation because there could be abnormal and damaged embryos, or even internal empty seeds. Therefore, tools such as X-rays and germination tests are essential for assessing the physical and physiological quality of the seeds.

3.3. X-ray tests of the seeds

The hybrids with better performance were evaluated by using X-ray tests to identify the seed quality in the two fruit maturation stadiums. The analysis of the images allowed for the visualisation of the internal seed structures (Bianchini et al., 2021). The low water content in the seeds and the higher optical density allowed for adequate differentiation and the clear visualisation of the internal structures in the X-ray tests (Simak, 1991). The better visualisation of the internal structure permitted the study to classify the seeds into five categories: a) full and well-formed seeds, with all the essential tissues for germination; B) seeds with space and well-formed; C) seeds with space and malformed; D) stained or damaged seeds; and E) empty seeds, with the absence of any internal content (Fig. 4).

There were variations in the seeds, with a class of full seeds observed in stadium I. The hybrid 004 × 005 showed superiority in this evaluation (Table 6).

Differences in normal seedling (NS) among the hybrids were observed in stadium I, with the highest value observed for 004 × 005. However, in stadium II, the opposite trend was observed. The quality of the seeds depends on the genotype and could be associated with the oil content and composition. Therefore, 004 × 005 presented the highest NS values in stadium I (Table 7).

The previous category of seeds allowed the study to observe the hybrids' viability for germination and the normal seedlings, independently of the fruit stadium maturation. The hybrids were qualified to produce seeds with physiological quality, and they were essential for the breeding programmes and the viability of the specific crosses.

3.4. HPLC-DAD of the hybrid extracts

Initial investigations were carried out on the ethanolic leaf extracts of *J. curcas* hybrids using HPLC-DAD. This allowed for the evaluation of the chromatographic profiles at two selected wavelengths (254 nm and 350 nm). These UV spectrum profiles are typical of flavonoids, with bands at 258 – 271 nm (band II) and 335 – 348 nm (band I). Differences

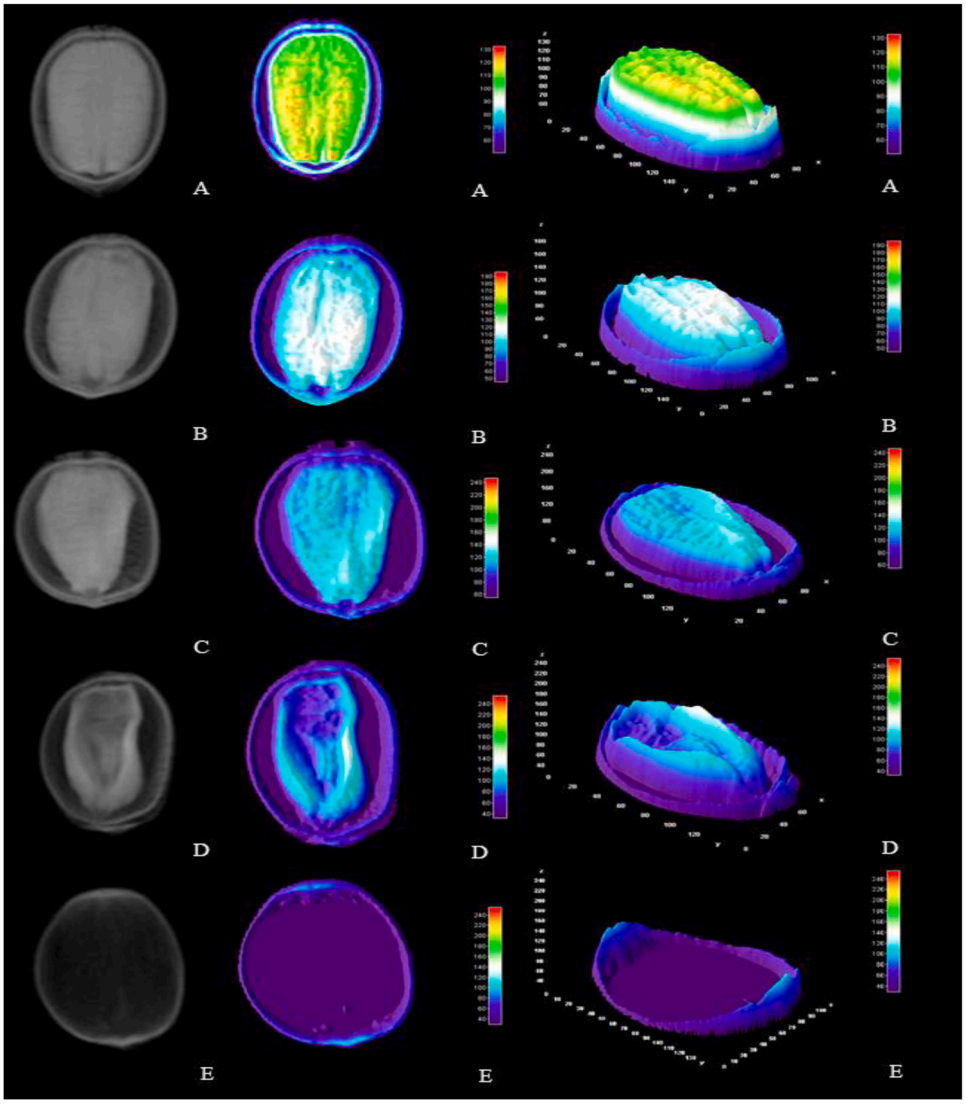

Fig. 4. The internal morphology of *Jatropha curcas* L. when using the X-ray tests. (A) Full and well formed; (B) with space and well formed; (C) with space and poorly formed; (D) stained or damaged; and (E) empty; and with the core representations (2D and 3D) of density, respectively.

were observed, especially for the 001 × 008, 001 × 013, and 004 × 005 hybrids (Fig. 5).

The HPLC-DAD analysis of the ethanolic leaf extracts of *J. curcas* hybrids revealed differences in chromatographic profiles at two selected wavelengths (254 nm and 350 nm). The 001 × 008 and 001 × 013 hybrids were similar but showed divergent peak intensities compared to 001 × 005, indicating differences in phytochemical composition. The chromatograms of 001 × 008 and 001 × 013 were also different from those of 003 × 005, 003 × 008, 003 × 013, 004 × 008, and 004 × 015 hybrids.

Previous studies have reported that *J. curcas* leaves contain compounds such as apigenin (vitexin and isovitexin), which are influenced by jasmonic acid application (Thomas et al., 2008, Lucho-Constantino et al., 2017). Jasmonic acid was found to be a precursor of flavonoid biosynthesis in *J. curcas*, with apigenin (vitexin and isovitexin) and kaempferol being identified in the leaf extracts. Phenylpropanoids, including lignins, coumarins, and flavonoids, are metabolic constituents that accumulate through the action of phenylalanine ammonia-lyase, which biotransforms L-phenylalanine into trans-cinnamic acid and ammonia. The first key enzyme of the phenylpropanoid sequence and the operative pathway of the secondary metabolites are related to the defence mechanisms in most plants (Macdonald, 2007).

Chalcones are precursors to various flavonoid-derived groups found in plants. Many contain a six-sided heterocyclic ring, forming a flavanone, with flavonoid compounds such as luteolin at the end product and apigenin, including vitexin, isovitexin, orientin, and rhoifolin. The biosynthesis of compounds such as luteolin mono-glycosidic, glycosidic apigenin, and mono-glycosidic apigenin is directly influenced by biotic and abiotic stress, water deficit, and UV radiation levels (Verdan et al., 2011; Van der Staaij et al., 2002; Dewick 2002).

The dissimilarities observed in the hybrids were investigated in the work of Ribeiro et al. (2017) using molecular markers with the same hybrids. The phytochemical composition of *J. curcas* extract can be considered a potential alternative for pest control, as it has been shown to possess toxic properties against several species (Ribeiro et al., 2012; Bassem et al., 2014). The larvicidal activity of *J. curcas* leaf extracts on *A. aegypti* is mainly due to the presence of alkaloids, steroids, and flavonoids (Gutierrez et al., 2014; Vinchurkar et al., 2017).

3.5. Larvicide bio essay

J. curcas has shown potential as a pesticide (Rahmam et al., 2014; Ratnadass et al., 2012), herbicide (Lemos et al., 2009; Reichel et al., 2013), and fungicide (Rahmam et al., 2011; Alonso et al., 2013).

Table 6
The percentage of the seeds that were categorised as full and well formed (A), with space and well formed (B), with space and malformed (C), with spots and damages (D), and empty (E), which were obtained in the hybrids of *Jatropha curcas* L. (JC), at the different stages of maturity (I = yellow fruit and II= brown fruit).

Hybrid	A		B		C		D		E	
	I	II	I	II	I	II	I	II	I	II
001 × 013	1.00dA	0.75bA	8.00 aA	8.20 aA	11.00 aA	10.75 aA	3.75bA	3.00 aA	1.25 aA	2.25 aA
003 × 005	3.25cA	2.75 aA	7.50 aA	9.00 aA	11.00 aA	9.25 aA	1.75bA	3.00 aA	0.50 aA	1.00 aA
004 × 005	9.50 aA	3.00aB	6.75 aA	10.25 aA	5.00bB	8.50 aA	1.50bA	1.50 aA	1.00 aA	1.75 aA
004 × 013	3.50cA	1.00bB	6.00 aA	7.50 aA	10.50 aA	12.25 aA	4.50 aA	2.75 aA	0.50 aA	1.50 aA
003 × 013	5.25bA	2.75 aA	6.75 aA	9.00 aA	9.25 aA	10.75 aA	1.75bA	1.50 aA	2.00 aA	1.00 aA
001 × 005	1.25dA	1.50bA	5.25 aA	7.00 aA	9.00 aA	10.25 aA	7.25 aA	4.75 aA	2.25 aA	1.50 aA
CV %	27.13		15.60		14.62		25.60		40.64	

The means followed by the same lowercase letter in the column, and uppercase in the line (when comparing I and II for each hybrid) did not differ statistically from one another by the Scott-Knott test at 5% probability.
The hybrids 001 × 005 and 001 × 013 had few well-formed and full seeds. However, there were no significant differences among the hybrids 001 × 005, 001 × 013, 003 × 005, and 003 × 013 for both maturation stages (I and II).
The frequency of well-formed seeds (A) did not differ among the hybrids in the different maturation stadiums. The seeds with space and malformation (B) and those that were stained or damaged (C) showed differences in stadium I, with 004 × 005 having a higher percentage of defective seeds compared to stadium II. There was no significant difference among the hybrids in the maturation for the empty seeds (E).
There were significant differences of the hybrids for the three variables and the two stadiums of maturity. In the first, 004 × 005 showed better germination compared to other hybrids. In the second stadium, 004 × 005 showed similar germination to 003 × 005 and 004 × 013. 001 × 005, 003 × 005, and 004 × 013 had similar germination rates in both stadiums.
There were differences in viability and vigor of the hybrids in the two maturation stadiums. In stadium I, 004 × 005 showed superior results, while in stadium II, it was similar to 003 × 005 and 004 × 013. Interestingly, Silveira et al. (2015) reported different results with *Jatropha* seeds, showing higher germination rates in black fruits (50% dry) compared to green and yellow fruits. Ducca et al. (2015) also reported an increase in seed quality with the advancement of fruit maturation. In stadium II, 001 × 013, 004 × 005, and 003 × 013 showed a significant reduction in vigor (speed of germination index), which could be related to the initiation of physiological maturity in yellow fruits, with maximum seed viability (Pessoa et al., 2012). The differences observed might be associated with environmental variations and the high genetic variability of jatropha. Another factor could be the asynchronous variation in fruit maturation.

Table 7
Seedlings and the seeds attributes of the *Jatropha curcas* L. from fruits at the two maturation stadiums.

Hybrid	Normal seedlings (NS)		Abnormal seedlings (AS)		Deteriorated seeds (DS)	
	I	II	I	II	I	II
001 × 013	11.00 b A	7.00 a A	2.27 b A	2.34 a A	8.25 b B	13.00 a A
003 × 005	11.50 b A	9.25 a A	2.52 a A	2.90 a A	7.25 b A	7.75 b A
004 × 005	18.50 a A	10.25 a B	1.93 b B	3.15 a A	3.25c A	5.00 b A
004 × 013	7.75c A	7.75 a A	2.52 a A	2.74 a A	10.75 a A	9.75 a A
003 × 013	12.25 b A	10.50 a A	2.79 a A	2.64 a A	5.25c A	8.00 b A
001 × 005	5.00c A	7.75 a A	2.80 a A	3.07 a A	12.25 a A	7.75 b B
CV %	15.12		14.25		16.22	

The means followed by the same lowercase letter in the column and the uppercase in the row did not differ statistically from one another by the Scott Knott test at 5% probability.

However, in this study, no larvicidal effect was observed at concentrations of 50 and 200 ppm.

Other studies have reported low larval mortality, ranging from 9% to 28%, with alcoholic extracts of *J. curcas* at concentrations between 500 and 1300 ppm (Mahyoub et al., 2014). LC90 was observed at a concentration of 400.68 ppm of petroleum ether extract in *A. aegypti* larvae (Sakthivadivel et al., 2008). These bioactivities are likely related to flavonoid compounds such as apigenin, vitexin, and isovitexin (Lans et al., 2001; Tomass, 2012). Similar results were also observed with the ethanolic extract of *J. curcas* leaves at a concentration of 800 ppm on third instar larvae, with 84–88% mortality at 24 and 48 h, respectively (Alvarez et al., 2015). The toxicological, cytotoxic, or other effects may be related to physiological changes in hormonal metabolism (Schloms, 2014), which influence the secondary metabolites, such as flavonoids (López-Lázaro, 2009), and warrant further study.

4. Conclusion

This study aimed to address gaps in previous research on parental selection using phenology and DNA genetic diversity. The study evaluated the most divergent parentals for use in crossings and estimated their progenies' potential for physical and physiological seed quality. The hybrids were evaluated for heterosis and heterobeltiosis to determine gains in biometry, mass of 100 seeds, and oil content. In addition, their insecticidal action against *A. aegypti* was tested. The hybrids 003 × 013 and 004 × 005 showed the highest heteroses and heterobeltiosis. When considering the influence of maturation stadiums on seed quality, stadium II must be considered to select genotypes for seed viability and vigor. However, results may vary depending on genotype, oil composition, and content.

The initial analysis of ethanolic leaf extracts for the hybrids using HPLC-DAD allowed for the differentiation of genotypes for future studies. It is crucial to consider the potential use of different plant tissues and organs for industrial purposes. This study evaluated seed formation, oil content, and potential insecticide use using leaves.

Some hybrids presented many malformed and empty seeds, indicating that not all plant varieties in the crosses are viable for generating vigorous and viable seeds. This highlights the importance of evaluating seed traits to define new approaches while considering the genotypes selected for characterization under different edaphoclimatic conditions. Seeds are crucial for establishing production fields.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Olavo José Marques Ferreira: Conceptualisation, methodology, research data. **Daniel Ornelas Ribeiro:** Data curation, writing, research data. **Angela Maria dos Santos Pereira:** research data. **Allívia Rouse Carregosa Rabbani and Valdinete Vieira Nunes:** Software, validation. **Crislaine Costa Calazans:** Visualisation, Investigation. **Paulo Cesar de Lima Nogueira:** chromatographic analysis, contributed with materials and analysis tools. **Renata Silva-Mann:** Conceptualisation, methodology, research data. Supervision, guided the research, revised the text, and contributed to the writing of the manuscript.

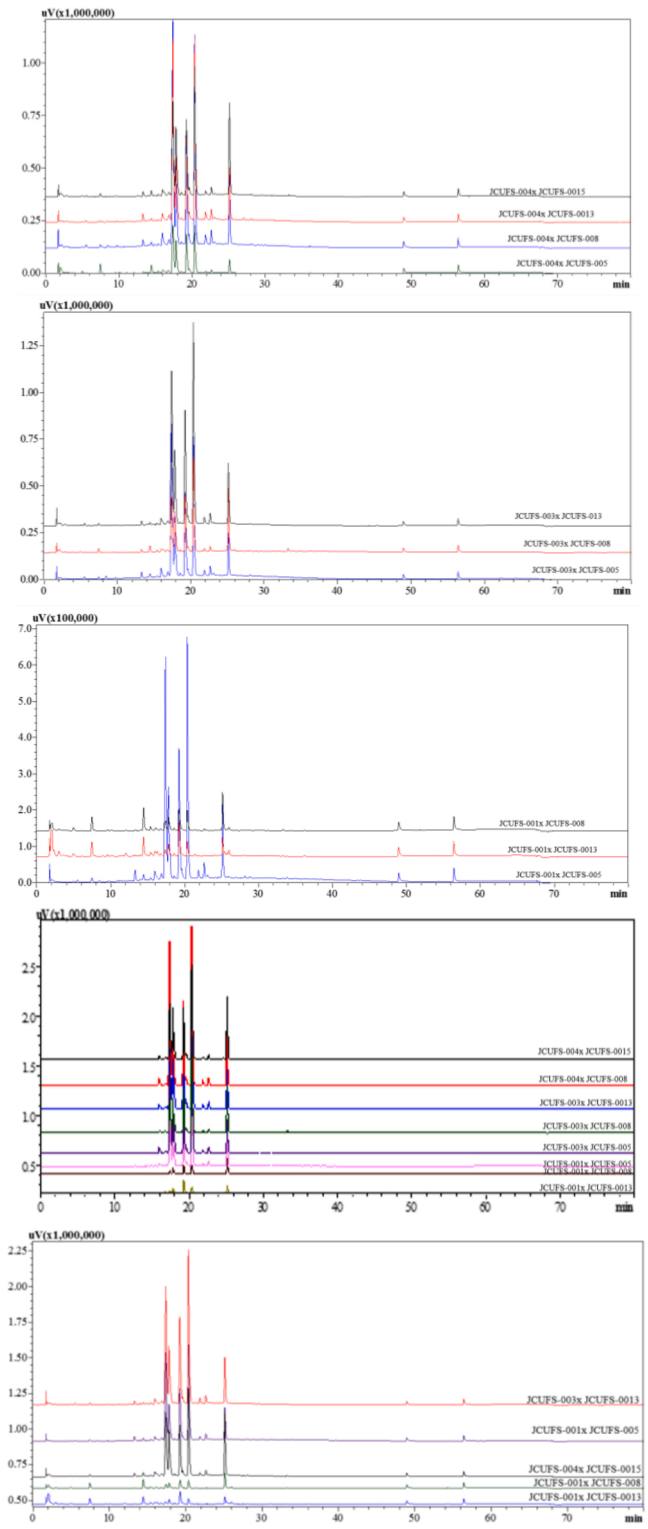

Fig. 5. The chromatograms that were obtained by HPLC-DAD (254 nm) when using the extracts of *Jatropha curcas* L., and obtained by ultra-turrax.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data sets produced and/or analysed during the current study are available from the corresponding author upon request.

Acknowledgements

The authors would like to express their gratitude to Graciele de Andrade Costa for the technical support, as well as to the National Council for Scientific and Technological Development Brazil (CNPq), the Coordination for the Improvement of Higher Education Personnel Brazil (CAPES-001), Research and Projects Financing Institution (FINEP), and the Research Group on Conservation, Breeding, and Management of Genetic Resources (GENAPLANT) for their support.

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