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SCIENTIFIC ARTICLE

Agronomic and morphological screening of American *Jatropha curcas* L. accessions for crop improvement in biofuels production

Evaluación agronómica y morfológica de accesiones americanas de *Jatropha curcas* L. para la mejora del cultivo en la producción de biocombustibles

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ABSTRACT

Jatropha curcas L. is a potential biomass source for biofuel production. This study evaluated the phenotypic variation of ten *J. curcas* accessions —seven from Mexico (Chiapas and Yucatan) and three from Honduras— based on four agronomic traits and 15 morphological characters of plants, fruits and seeds, in Yucatan, Mexico, for future crop improvement. The most significant variation among accessions was explained by morphological plant characters (37%), followed by yield and seed proportions (22%). Two Yucatan accessions had the lowest plant proportions and the highest agronomic yield, while two Chiapas accessions exhibited both high plant proportions and agronomic yield. In contrast, all Honduras accessions had both low plant proportions and agronomic yield. Dissimilarity analysis grouped the accessions based on their geographical origin, agronomic yield, and proportions of plants, fruits, and seeds. The agromorphological characterization of *J. curcas* enabled the identification of accessions with desirable traits for parent selection in breeding programs.

KEYWORDS

Aerial biomass, geographical origin, oil yield, phenotypic diversity, plant proportions, seed yield.

RESUMEN

Jatropha curcas L. es una fuente potencial de biomasa para la producción de biocombustibles. Este estudio evaluó la variación fenotípica de diez accesiones de *J. curcas* —siete de México (Chiapas y Yucatán) y tres de Honduras— basándose en cuatro características agronómicas y 15 características morfológicas de las plantas, frutos y semillas en Yucatán, México, con el objetivo de mejorar el cultivo en el futuro. La mayor variación entre accesiones fue explicada por las características morfológicas de la planta (37%), seguida por el rendimiento y las proporciones de semillas (22%). Dos accesiones de Yucatán presentaron las menores proporciones de planta y el mayor rendimiento agronómico, mientras que dos accesiones de Chiapas mostraron tanto altas proporciones de planta como alto rendimiento agronómico. En contraste, todas las accesiones de Honduras tuvieron tanto bajas proporciones de planta como bajo rendimiento agronómico. El análisis de disimilitud agrupó las accesiones según su origen geográfico, rendimiento agronómico y proporciones de plantas, frutos y semillas. La caracterización agromorfológica de *J. curcas* permitió identificar accesiones con rasgos deseables para la selección de progenitores en programas de mejoramiento genético.

PALABRAS CLAVE

Biomasa aérea, origen geográfico, rendimiento de aceite, diversidad fenotípica, proporciones de planta, rendimiento de semilla.

Introduction

Jatropha curcas L. is a fast-growing plant, with a high energy content, capable of adapting to poor-quality soils without competing with agricultural food production (Massoukou et al., 2024; Neupane et al., 2021; Qaseem and Wu, 2021). For this reason, it remains a potential source of biomass for biofuel production, either through hydrogenation and deoxygenation of the oil from its seeds or through thermo- and biochemical processing of the residual biomass, which can also produce biogas, bioalcohol, biopetroleum, liquid hydrocarbons, paraffin and olefins (Alherbawi et al., 2021a; Ruatpuia et al., 2023).

Despite the enormous potential of *J. curcas*, the commercially profitable production of biofuels and other products, is still far from viable (Makepa et al., 2024). To overcome this limitation, it has been proposed, among other measures, to increase seed production per hectare, which in turn enhances biofuel yield per unit of surface, as well as to maximize the use of the entire plant biomass (Alherbawi et al., 2021b). However, *J. curcas* is an incipiently domesticated species that has not undergone intensive genetic improvement; therefore, elite germplasm is not yet available for large-scale of commercial plantations (Adebusuyi et al., 2021).

To establish profitable *J. curcas* crop, it is necessary to have germplasm with desirable characteristics, such as high seed and oil yield, adequate plant morphology to facilitate seed harvesting, and tolerance to abiotic stress and diseases (Ewunie et al., 2021). Therefore, characterizing different *J. curcas* germplasm under various environmental conditions is essential to identify genotypes with desirable agronomic, morphological, and physiological traits for mass cultivation or for use as parent material in breeding programs before large-scale planting (Borah et al., 2018; García-Alonso et al., 2023).

The agromorphological characterization of *J. curcas* has been widely used to identify genotypes with improved traits that enhance crop profitability. Differences have been observed in traits associated with fruit, seed and of oil yield, as well as plant growth and proportions, among the germplasm collected from different geographic and climatic regions (Adebusuyi et al., 2021; García-Alonso et al., 2023; Salazar-Villa et al., 2020).

Mesoamerica is the center of origin of *J. curcas*, so it is expected that the greatest genetic variability will be found among germplasm collected from different localities in this region (Heller, 1996; Neupane et al., 2021). The Yucatan peninsula in Mexico, is a region with soils of limited agricultural importance due to their high alkalinity (pH 7.0-8.5), rocky and shallow nature, and susceptibility to erosion (Bautista-Zúñiga et al., 2003). However, these conditions make the region ideal for *J. curcas* cultivation due to the plant´s ability to thrive in such soils. Moreover, this region aligns with Mexico´s Law for the Promotion and Development of Bioenergy, which stipulates that bioenergy production should not compromise national food security and sovereignty (Law for the promotion and development of bioenergy –Presidencia de la República, 2008–). Therefore, the aim of this study was evaluated the variation in agronomic and morphological traits among ten preselected *J. curcas* accessions from Mexico and Honduras, under Yucatan peninsula conditions, as a preliminary screening for parent material in crop improvement programs in the region.

Materials and methods

Origin of the *J. curcas* accessions and establishment of the experimental plantation

The agromorphological characteristics of ten American accessions of *J. curcas*, from Mexico and Honduras (Table 1) were evaluated in the northwest of the state of Yucatan, Mexico (N 21° 3′ 22′′, W 89° 36′ 43′′).

The experimental plantation was established through vegetative reproduction. Cuttings of each accession were collected one day before planting from a single mature parental plant. These cutting were taken from lignified branches (from previous year´s growth cycle), measuring 50 cm in length and at least 1.5 cm in diameter. The branches were cut 10 cm above the stem intersection. The cuttings were wrapped in paper and stored in the shade until planting (Martínez-Sebastián et al., 2018).

The plant material was obtained from the germplasm bank of the company Agroindustria Alternativa del Sureste S.P.R. of R.L. de C.V. These ten accessions exhibited the best agronomic characteristics in the Yucatan peninsula region among 33 previously eva-

Table 1. Origin of the *Jatropha curcas* accessions evaluated in northeastern Yucatan, Mexico.

Accession	Country	State/ Department	Latitude (N)	Longitude (W)
B2F86P87	Honduras	Yoro	15° 11′ 50.9′′	87° 14′ 8.9′′
B2F91P6	Honduras	Yoro	15° 11′ 12.8′′	87° 19′ 1.0′′
B2F91P16	Honduras	Yoro	15° 09′ 32.6′′	87° 14′ 56.4′′
B5F83P1	Mexico	Chiapas	16° 45′ 11.0′′	93° 06′ 56.0′′
B3F119P1	Mexico	Chiapas	16° 45′ 11.0′′	93° 06′ 56.0′′
B5F59P19	Mexico	Chiapas	16° 10′ 55.02′′	91° 58′ 3.80′′
GAGI10	Mexico	Yucatan	21° 08′ 43.69′′	88° 08′ 58.59′′
GAGI28	Mexico	Yucatan	20° 43′ 21.0′′	89° 41′ 08.0′′
SUCILATEBEC	Mexico	Yucatan	20° 42′ 35.56′′	89° 05′ 39.62′′
CAM32	Mexico	Yucatan	21° 00′ 28.26′′	89° 38′ 28.31′′

luated in a characterization study conducted by the same company (unpublished data).

Twenty-four cuttings per accession were planted during the region´s dry season (March 2013) at a depth of 10-15 cm using a randomized block design. Each block consisted of six cuttings, with four replicates per accession, arranged in a 2 m × 1.5 m spatial pattern, as previously optimized for this species in the Yucatan peninsula (Góngora-Canul et al., 2018; Martínez-Sebastián et al., 2018). The plantation was irrigated via a drip system every four days until the onset of the rainy season in the region (May). Conventional agronomic practices, such as mechanical weed removal, were applied, and no agrochemicals (fertilizers, hormones, herbicides, or pesticides) were used.

Agronomic and morphological characterization

At the end of the first reproductive cycle, nine months after planting, 15 traits related to plant, fruit and seed proportions, along with four characters associated with the agronomic yield were measured on four plants of each accession, one for each block, selected at random.

The measured morphological traits have been widely used for the phenotypic characterization of this species, as they exhibit high variability. Some of these traits are correlated with agronomic and economic variables, such as seed and oil yield, and can help identify low-proportion accessions with high agronomic yield, faciliting seed harvesting (Gohil and Pandya, 2008; Montes et al., 2013; Rao et al., 2008; Shabanimofrad et al., 2013; Wani et al., 2012).

The 15 morphological traits analyzed were plant height (m), measured from the soil surface to the apex; aerial biomass (g); stem base diameter (cm); number of primary branches; length of primary branches (cm); number of leaves; wood volume (cm^3); wood density ($g\ cm^{-3}$); diameter (cm), length (cm) and thickness (cm) of ripe fruits, and width (cm), thickness (cm), length (cm) and volume (cm^3) of seeds.

Aerial biomass was determined by dehydrating the stem and branches of each plant in an electric oven at 80 °C until a constant weight was reached (Achten et al., 2010). Stem base diameter was calculated by dividing its circumference by the constant π.

Wood volume (V_W) was calculated using equation 1 (West, 2009), where the total of wood volume of a plant was obtained by summing of all its branches and its stem. Wood density was determined by dividing the aerial biomass by the fresh volume of the branches and stem, as calculated using equation 1 (Achten et al., 2010).

$$V_W = \left[\frac{\pi}{4}\right]\left[\frac{(D_m)^2+(D_M)^2}{2}\right][L] \quad (1)$$

Where, V_W is the volume of the wood (cm^3); L is the length (cm); D_m is the lower diameter (cm) and D_M is the upper diameter (cm) of the stem or branches, π=3.1416.

Seed volume (V_S) was calculated using equation 2 (Gohil and Pandya, 2008; Wani et al., 2012).

$$V_s = \frac{\left[\frac{4}{3}\pi(L \times W \times T)\right]}{2} \quad (2)$$

Where, V_s is the seed volume (cm^3); and L, W, and T are the length (cm), width (cm), and thickness (cm) of the seeds, respectively, π = 3.1416.

The agronomic yield traits measured are of economic interest and they exhibit high variability and heritability in this species (Gohil and Pandya, 2008; Rao et al., 2008; Shabanimofrad et al., 2013).

The four agronomic yield traits measured were: the number of seeds per plant, seed yield per plant (g), which is the total weight of seeds harvested from each plant over one year; the oil content of the seeds (%) and the weight of 100 seeds per accession (g).

For oil extraction, approximately 20 g of dry seeds, without testa, and 150 mL of hexane were used in a recirculation process for 8 h at 60 °C, in Soxhlet equipment. The solvent was later recovered with a rotary evaporator at 38 °C. Hexane residues in the oil were eliminated by heating at 80 °C for one hour while stirring (Srivastava et al., 2011). The oil content was calculated as the percentage by weight of oil relative to the weight of the seed sample used.

Statistical analysis

For the 19 characters recorded in each *J. Curcas* accession, basic descriptive statistics were calculated. A one-way analysis of variance (p = 0.05) and multiple comparisons of means were performed to identify significant differences among accessions (Fisher´s least significant difference, p = 0.05). A correlation analysis was conducted for the 19 agromorphological characters using Pearson´s coefficient (p = 0.05) to determine associated variables.

To identify the main traits contributing to the greatest variation among *J. curcas* accessions, a multivariate principal component analysis (PCA) was performed, excluding dependent variables that were highly correlated ($r \geq 0.7$) as identified in the previous correlation analysis, in order to avoid redundancies among variables by components (Shabanimofrad et al., 2013; Singh et al., 2016a, 2016b).

To represent the dissimilarity relationships among the accessions based on their agromorphological traits, a cluster analysis was conducted using the dissimilarity coefficient (Euclidean Distance) and the unweighted pair group method with arithmetic mean (UPGMA) (Pecina-Quintero et al., 2014). Only the variables included in the first two principal components were used in this analysis. All statistical analyses were performed in InfoStat *software*, version 2020 (InfoStat Group, FCA, National University of Cordoba, Argentina).

Results and discussion

Survival of cuttings

The percentage of survival of the cuttings in the field —i.e., those that rooted, developed shoots, and prospered as complete plants—, varied among the ten accessions of *J. curcas* (Table 2). The survival of the cuttings from accessions B2F86P87, B2F91P6, B2F91P16, B5F83P1, B3F119P1 and GAGI28 was 100%, while those from SUCILATEBEC, CAM32, and B5F59P19 had survival rates of 79.2, 75 and 66.7%, respectively. The accession GAGI10 had the lowest survival rate, at 58.3% (Table 2).

The total survival rate of the cuttings was 87.92%, with 29 cuttings dead out of the 240 planted (Table 2). The rooting percentage obtained in this study agrees with previous reports, as it has been observed that this propagation method typically achieves an average rooting success of 80%, provided the cuttings are taken from lignified branches. This is because new shoots dehydrate easily and are more susceptible to rot (Góngora-Canul et al., 2018). Additionally, the propagation of *J. curcas* by direct cutting sowing was done during the dry season to avoid rotting caused by rain and to promote a higher rooting percentage, as recommended for this species in the region (Martínez-Sebastián et al., 2018).

Table 2. Survival of cuttings of ten accessions of *J. curcas* planted in northwestern Yucatan, Mexico.

Accession	Planted cuttings	Rooted cuttings	Dead cuttings	Survival (%)
B2F86P87	24	24	0	100
B2F91P6	24	24	0	100
B2F91P16	24	24	0	100
B5F83P1	24	24	0	100
B3F119P1	24	24	0	100
B5F59P19	24	16	8	66.7
GAGI10	24	14	10	58.3
GAGI28	24	24	0	100
SUCILATEBEC	24	19	5	79.2
CAM32	24	18	6	75

The survival results suggest that the accessions B2F86P87, B2F91P6, B2F91P16, B5F83P1, B3F119P1, and GAGI28 are more competitive under the environmental conditions in which they were planted, likely due to their genotype, as all cuttings were exposed to the same environmental conditions in the experimental plot. Conversely, the accessions SUCILATEBEC, CAM32, B5F59P19, and GAGI10, in descending order, proved to be the least competitive.

The length of the planted cuttings was similar among the *J. curcas* accessions, while the diameter varied. Although a directly proportional relationship between cutting volume and energy reserves has been reported, it has also been shown that the length and the diameter of cuttings do not significantly affect the survival rate of *J. curcas* (Enciso and Castillo, 2010) or other plant species, such as *Trichanthera gigantea* (Bonpl.) Nees (Moreno and Guerrero, 2003). This reinforces the hypothesis that the genotype of the plant material is what makes it competitive under specific environmental conditions.

Asexual propagation of *J. curcas* by cuttings is a method that preserves the characteristics of the mother plant in the offspring, such as resistance or tolerance to diseases, productivity and/or morphological traits. The establishment of *J. curcas* plantations by direct sowing of cuttings, without prior rooting in a nursery, has certain advantages, such as reduced time and lower maintenance in the nursery. Moreover, it has been reported that with this propagation method leads to earlier flowering and fruiting compared to plants propagated with seeds (Góngora-Canul et al., 2018).

Agromorphological characterization of the accessions

The vegetative characters indicated that the accessions ranged in height from 1.26 to 2.32 m, with 2-6 branches ranging from 0.62-1.56 m in length, and 62-241 leaves, supported by 0.406 to 1.544 kg of biomass, and wood volumes of 788.75 at 5,385.32 cm^3, with B3F119P1 being the standout accession (Table 3).

Significant differences were observed in all the agromorphological characters among accessions (Table 3), indicating that the materials are morphologically and agronomical distinct. This suggests that different genotypes may be better suited to various environments. Understanding the behavior of the vegetative and agronomic characters in this industrially important shrub species is crucial from the early stages of cultivation, given its long growth period before yielding utilitarian benefits.

The highest seed yield per plant was observed in accessions B5F83P1, B2F91P16, CAM32 and GAGI10 with values ranging from 104.07 to 117.99 g per plant, along with oil contents exceeding 36.01% (Table 3). When considering seed yield and oil content as the most important of the crop variables, the accessions B5F83P1 and B2F91P16, followed by CAM32 and GAGI10, were identified as the most outstanding for cultivation in the state of Yucatan.

The oil content values found in this study are within the range reported for *J. curcas* (30-60%) cultivated in Mesoamerica (Aguilera-Cauich et al., 2015; Herrera et al., 2010; Ovando-Medina et al., 2011), Europe (Montes et al., 2013), Asia and Africa (Jonas et al., 2020; Kumar and Das, 2018; Rao et al., 2008; Senger et al., 2016). It has been proposed that the variation in oil content in *J. curcas* seeds is influenced by origin, genotype-environment interactions (Heller, 1996; Montes et al., 2013), and environmental factors such as precipitation and soil fertility (Escobar et al., 2009; Mishra, 2009). Since all accessions in this study were cultivated in the same locality under identical conditions, the differences in oil content are likely due to the genotypes of the individuals.

The coefficients of variation (CV) of the 19 agromorphological characters of *J. curcas* ranged from 5.51 to 59.93 %, with the highest values observed for two variables related to plant proportion, aerial biomass (59.93%) and wood volume (56.91%), and two related to agronomic performance, number of seeds per plant (57.18%) and seed yield per plant (55.49%). The variation range observed in this study was greater than that reported for germplasm from India and Cape Verde (1.49-10.53%) (Singh et al., 2016a) and similar to that of other American populations cultivated in Yucatan, Mexico (6.72-65.65%) (Aguilera-Cauich et al., 2015).

The variation among American accessions indicates a high degree of diversity compared to that in other regions, which is consistent with the fact that Mesoamerica is considered the center of origin and diversity for the species (Heller, 1996; Kamel et al., 2018; Montes et al., 2013; Neupane et al., 2021).

Table 3. The behavior of 19 characters of ten accession of *J. curcas* in the northwestern of Yucatan, Mexico.

Accession	SBD (cm)	PH (cm)	NPB	LPB (cm)	NL	ABM (g)	WV (cm^3)	WD (mg cm^{-3})	FL (cm)	FD (cm)
B2F86P87	6.39 ^{ab}	200.00 ^{ab}	3.25 ^{bc}	138.78 ^{ab}	138.25 ^{b}	1119.5 ^{ab}	3460.34 ^{ac}	202.89 ^{a}	3.04 ^{de}	2.80 ^{bc}
B2F91P6	5.65 ^{bd}	205.00 ^{ab}	3.75 ^{bc}	153.65 ^{a}	94.00 ^{bd}	951.00 ^{bc}	3656.61 ^{ac}	178.67 ^{a}	3.47 ^{a}	2.64 ^{de}
B2F91P16	6.20 ^{bc}	217.25 ^{ab}	3.75 ^{bc}	147.41 ^{ab}	113.75 ^{bd}	1106.00 ^{ab}	3341.67 ^{ac}	207.41 ^{a}	3.42 ^{ab}	2.71 ^{cd}
B5F83P1	5.79 ^{bd}	207.88 ^{ab}	4.25 ^{b}	156.33 ^{a}	137.25 ^{b}	822.50 ^{bd}	4327.09 ^{ab}	162.61 ^{a}	3.21 ^{c}	2.80 ^{bc}
B3F119P1	7.45 ^{a}	232.00 ^{a}	4.25 ^{b}	149.26 ^{ab}	241.25 ^{a}	1544.00 ^{a}	5385.32 ^{a}	169.60 ^{a}	3.23 ^{c}	2.91 ^{a}
B5F59P19	4.91 ^{de}	161.00 ^{bc}	2.25 ^{c}	114.17 ^{ac}	69.25 ^{cd}	415.50 ^{d}	1901.63 ^{cd}	167.48 ^{a}	3.26 ^{bc}	2.83 ^{ab}
GAGI10	4.29 ^{e}	160.75 ^{bc}	3.75 ^{bc}	100.00 ^{bc}	92.50 ^{bd}	464.50 ^{cd}	2597.63 ^{bd}	177.40 ^{a}	3.18 ^{cd}	2.56 ^{ef}
GAGI28	5.17 ^{be}	206.00 ^{ab}	3.50 ^{bc}	124.50 ^{ab}	128.00 ^{bc}	667.00 ^{bd}	3456.85 ^{ac}	199.59 ^{a}	3.26 ^{bc}	2.81 ^{ac}
SUCTEBEC	4.63 ^{de}	163.75 ^{bc}	3.00 ^{bc}	115.38 ^{ab}	83.75 ^{bd}	406.00 ^{d}	2744.79 ^{bd}	200.12 ^{a}	3.44 ^{ab}	2.66 ^{de}
CAM32	4.97 ^{ce}	125.75 ^{c}	6.75 ^{a}	62.19 ^{c}	62.00 ^{d}	471.50 ^{cd}	788.75 ^{d}	202.82 ^{a}	3.02 ^{e}	2.49 ^{f}
n	40	40	40	40	40	40	40	40	153	153
Mean	5.54	187.94	3.85	126.16	116.00	796.75	3166.07	186.86	3.25	2.72
Minimum	2.70	56.00	1.00	6.50	15.00	32.00	197.67	123.52	2.60	2.20
Maximum	7.79	280.00	8.00	176.00	323.00	1742.00	9023.47	354.13	3.90	3.10
SD	1.18	48.96	1.58	42.47	63.07	477.52	1801.77	42.16	0.23	0.18
CV (%)	21.29	26.05	40.98	33.66	54.37	59.93	56.91	22.56	6.98	6.66
Error (%)	3.37	4.12	6.48	5.32	8.60	9.48	9.00	3.57	0.56	0.54

Accession	FT (cm)	SL (cm)	SW (cm)	ST (cm)	SV (cm^3)	NS (plant^{-1})	SY (plant^{-1} g)	OC (%)	HSW (g)
B2F86P87	0.41 ^{bc}	1.82 ^{cd}	1.08 ^{cd}	0.95 ^{ab}	3.90 ^{b}	137.88 ^{a}	101.0 ^{ac}	41.12 ^{cd}	67.80
B2F91P6	0.46 ^{ab}	1.79 ^{d}	1.02 ^{f}	0.96 ^{ab}	3.65 ^{c}	110.13 ^{ab}	77.14 ^{ac}	38.58 ^{de}	79.60
B2F91P16	0.47 ^{a}	1.91 ^{b}	1.10 ^{bc}	0.86 ^{d}	3.76 ^{bc}	165.25 ^{a}	113.70 ^{a}	42.50 ^{bc}	76.20
B5F83P1	0.41 ^{b}	1.99 ^{a}	1.13 ^{a}	0.95 ^{ab}	4.44 ^{a}	146.63 ^{a}	117.99 ^{a}	42.18 ^{bc}	84.70
B3F119P1	0.45 ^{ab}	1.89 ^{b}	1.11 ^{ab}	0.97 ^{a}	4.26 ^{a}	127.88 ^{ab}	99.36 ^{ac}	43.96 ^{ab}	76.90
B5F59P19	0.45 ^{ab}	1.84 ^{c}	1.06 ^{de}	0.93 ^{bc}	3.81 ^{bc}	45.67 ^{b}	36.50 ^{c}	45.38 ^{a}	79.60
GAGI10	0.38 ^{cd}	2.00 ^{a}	1.09 ^{bc}	0.93 ^{bc}	4.25 ^{a}	170.67 ^{a}	104.07 ^{ac}	36.01 ^{e}	82.30
GAGI28	0.44 ^{ab}	1.90 ^{b}	1.06 ^{de}	0.94 ^{b}	3.96 ^{b}	88.75 ^{ab}	63.83 ^{bc}	40.77 ^{cd}	73.90
SUCILATEBEC	0.40 ^{bd}	1.89 ^{b}	1.02 ^{f}	0.91 ^{c}	3.66 ^{c}	58.50 ^{b}	39.45 ^{c}	36.74 ^{e}	71.40
CAM32	0.35 ^{d}	1.91 ^{b}	1.05 ^{e}	0.90 ^{c}	3.77 ^{bc}	142.60 ^{a}	106.26 ^{ab}	37.88 ^{e}	75.10
n	153	300	300	300	300	65	65	30	1000
Mean	0.42	1.89	1.07	0.93	3.95	119.39	85.93	40.51	76.75
Minimum	0.30	1.20	0.95	0.53	2.20	23.00	20.70	34.36	67.80
Maximum	0.50	2.10	1.50	1.33	6.10	363.00	200.20	46.48	84.70
SD	0.07	0.11	0.06	0.07	0.47	69.71	49.29	3.29	5.06
CV (%)	15.54	5.81	5.51	7.02	11.92	57.18	55.49	8.12	6.59
Error (%)	1.26	0.34	0.32	0.41	0.69	7.09	6.88	1.48	2.08

SBD: stem base diameter; PH: plant height; NPB: number of primary branches; LPB: length of primary branches; NL: number of leaves; ABM: aerial biomass; WV: Wood volume; WD: Wood density; FL: fruit length; FD: fruit diameter; FT: fruit thickness; SL: seed length; SW: seed width; ST: seed thickness; SV: seed volume; NS: number of seeds; SY: seed yield; OC: oil content; HSW: hundred-seed weight; SD: standard deviation; CV: coefficient of variation. Means with a common letter in the same column, are not significantly different (Fisher's least significant difference, $p > 0.05$)

Correlation among morphologic and agronomic traits

The correlation among 19 agromorphological traits of ten *J. curcas* accessions is shown in Table 4. A significant, positive and strong correlation ($r \geq 0.7$) was observed between the number of seeds per plant with the seed yield, between stem basal diameter and aerial biomass, between fruit thickness and seed volume, and between the number of leaves and wood volume. To reduce the number of variables and avoid redundancy, SY, ABM, SV and WV were excluded from the PCA analysis.

The strong correlation between SBD and ABM is consistent with previous findings for these ten accessions, as reported by Teco-Bravo et al. (2015), where SBD was used as an independent variable to estimate ABM in allometric models. Similar results have been reported for this species by other authors (Achten et al., 2010).

FT was strongly correlated with SL, likely because larger seeds require greater protection from the fruit, which must have a thicker pericarp to prevent breakage due to seed size. The correlation between NL and WV could be attributed to the fact that a greater number of leaves enhances photosynthetic activity, resulting in increased carbon availability for wood production (West, 2009).

Phenotypic behavior of the accessions

Multivariate principal component analysis (PCA) indicated that the first four principal components explained 85% of the total variation (Table 5 and Figure 1). The first principal component (PC1) accounted for 37% of the variation and was composed of vegetative traits (SBD, PH, LPB, NL, FD, FT) as well as oil content (Table 5 and Figure 1).

The second principal component (PC2) explained 22% of the variation and included two agronomic yield traits (NS per plant and HSW), one related to plant proportions (NPB) and two related to seed proportions (SL and SW) (Table 5 and Figure 1). Only the variables from PC1 and PC2 were included in the subsequent cluster analysis.

The accessions GAGI10 and CAM32 exhibited the highest values in PC2 and the lowest values in PC1. This indicates that they had the best agronomic traits (NS per plant, which was highly correlated with SY,

Table 4. Person´s correlation coefficients among 19 characters measured in ten accessions of *J. curcas* in northwestern of Yucatan, Mexico.

	SBD (cm)	PH (cm)	NPB	LPB (cm)	NL	ABM (g)	WV (cm^3)	WD (mg cm^{-3})	FL (cm)	FD (cm)
SBD (cm)	1.00	0.00	0.39	0.00	0.00	0.00	0.03	0.40	0.94	0.14
PH (cm)	0.49 ***	1.00	0.83	0.01	0.01	0.00	0.00	0.82	0.39	0.02
NPB	0.14	0.03	1.00	0.04	0.71	0.24	0.46	0.96	0.12	0.13
LPB (cm)	0.59***	0.41**	-0.33*	1.00	0.00	0.00	0.00	0.13	0.98	0.57
NL	0.61***	0.41**	-0.06	0.52***	1.00	0.00	0.00	0.15	0.85	0.02
ABM (g)	0.95***	0.53***	0.19	0.57***	0.62***	1.00	0.01	0.48	0.93	0.34
WV (cm^3)	0.34*	0.55***	-0.12	0.49**	0.77***	0.42**	1.00	0.18	0.82	0.10
WD (mg cm^{-3})	-0.14	0.04	-0.01	-0.24	-0.23	-0.12	-0.21	1.00	0.19	0.95
FL (cm)	-0.01	0.14	-0.25	0.00	-0.03	-0.02	0.04	0.21	1.00	0.00
FD (cm)	0.24	0.36*	-0.24	0.09	0.35*	0.16	0.26	-0.01	0.38***	1.00
FT (cm)	0.34*	0.33*	-0.08	0.27	0.23	0.32*	0.30	-0.08	0.52***	0.53***
SL (cm)	-0.17	-0.01	0.09	-0.20	0.00	-0.11	0.00	0.20	0.10	0.03
SW (cm)	0.14	-0.13	-0.03	0.06	0.33*	0.12	0.14	-0.08	-0.04	0.16*
ST (cm)	-0.17	0.06	0.08	-0.03	-0.06	-0.16	-0.01	-0.15	0.14	-0.02
SV (cm^3)	0.29	0.25	-0.05	0.19	0.33*	0.29	0.32*	-0.02	0.49***	0.53***
NS (plant^{-1})	0.03	0.25	0.38*	-0.19	0.16	0.10	0.28	0.01	-0.05	-0.03
SY (plant^{-1} g)	0.14	0.23	0.36*	-0.11	0.26	0.16	0.28	-0.01	-0.08	0.03
OC (%)	0.39*	0.21	-0.26	0.35	0.31	0.31	0.23	-0.14	0.11	0.49**
HSW (g)	-0.56	-0.05	0.11	-0.34	-0.03	-0.64*	0.33	-0.24	-0.13	0.15

Table 4. Continued.

	FT (cm)	SL (cm)	SW (cm)	ST (cm)	SV (cm^3)	NS ($plant^{-1}$)	SY ($plant^{-1}$ g)	OC (%)	HSW (g)
SBD (cm)	0.03	0.29	0.37	0.29	0.07	0.84	0.42	0.03	0.09
PH (cm)	0.04	0.93	0.42	0.70	0.12	0.13	0.17	0.26	0.90
NPB	0.61	0.57	0.87	0.64	0.78	0.02	0.02	0.16	0.77
LPB (cm)	0.09	0.21	0.73	0.86	0.25	0.26	0.51	0.06	0.34
NL	0.15	1.00	0.04	0.70	0.04	0.34	0.12	0.09	0.93
ABM (g)	0.05	0.51	0.45	0.32	0.07	0.56	0.34	0.10	0.05
WV (cm^3)	0.06	1.00	0.40	0.95	0.04	0.09	0.09	0.23	0.35
WD (mg cm^{-3})	0.63	0.21	0.61	0.35	0.89	0.97	0.95	0.46	0.51
FL (cm)	0.00	0.23	0.60	0.08	0.00	0.68	0.53	0.57	0.72
FD (cm)	0.00	0.72	0.05	0.85	0.00	0.83	0.83	0.01	0.68
FT (cm)	1.00	0.38	0.84	0.62	0.00	0.76	0.58	0.01	0.80
SL (cm)	-0.07	1.00	0.00	0.05	0.00	0.67	0.69	0.41	0.46
SW (cm)	-0.02	0.34***	1.00	0.83	0.00	0.97	0.61	0.17	0.62
ST (cm)	0.04	0.11*	-0.01	1.00	0.06	0.25	0.26	0.69	0.15
SV (cm^3)	0.87***	0.31***	0.38***	0.16	1.00	0.60	0.38	0.01	0.70
NS ($plant^{-1}$)	0.04	0.05	0.00	-0.15	0.07	1.00	0.00	0.86	0.15
SY ($plant^{-1}$ g)	0.07	0.05	0.06	-0.14	0.11	0.97***	1.00	0.53	0.12
OC (%)	0.48**	-0.16	0.25	-0.08	0.47**	0.03	0.12	1.00	0.65
HSW (g)	0.09	0.26	-0.18	0.49	0.14	0.49	0.52	-0.16	1.00

SBD: stem base diameter; PH: plant height; NPB: number of primary branches; LPB: length of primary branches; NL: number of leaves; ABM: aerial biomass; WV: Wood volume; WD: Wood density; FL: fruit length; FD: fruit diameter; FT: fruit thickness; SL: seed length; SW: seed width; ST: seed thickness; SV: seed volume; NS: number of seeds; SY: seed yield; OC: oil content; HSW: hundred-seed weight. *, ** and *** = significant correlation at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, respectively.

Table 5. Analysis of the phenotypic behavior of ten accessions of *J. curcas* with 15 characters in Yucatan, Mexico.

Characters	PC1	PC2	PC3	PC4	PC5
Stem base diameter (cm)	0.33	0.08	-0.29	0.08	0.34
Plant height (m)	0.39	0.03	-0.07	0.28	0.02
Number of primary branches	-0.19	0.31	-0.17	0.12	0.57
Length of primary branches (m)	0.37	-0.03	0.04	0.28	-0.11
Number of leaves	0.32	0.19	-0.16	-0.01	0.28
Wood density (mg cm^{-3})	-0.17	-0.21	-0.41	0.31	-0.08
Fruit length (cm)	0.12	-0.27	0.35	0.48	-0.07
Fruit diameter (cm)	0.37	-0.06	-0.05	-0.34	-0.05
Fruit thickness (cm)	0.34	-0.21	0.10	0.18	-0.04
Seed length (cm)	-0.08	0.44	0.12	0.01	-0.35
Seed width (cm)	0.21	0.41	-0.11	-0.12	-0.36
Seed thickness (cm)	0.05	0.14	0.52	-0.04	0.43
Number of seeds ($plant^{-1}$)	-0.01	0.42	-0.24	0.39	-0.11
Oil content (%)	0.32	-0.02	-0.05	-0.40	-0.03
Hundred-seed weight (g)	0.04	0.36	0.44	0.13	-0.05
Autovalue	5.61	3.24	2.49	1.36	0.98
Proportion	0.37	0.22	0.17	0.09	0.07
Accumulated proportion (%)	37	59	76	85	91

PC: principal component; SBD: stem base diameter; PH: plant height; NPB: number of primary branches; LPB: length of primary branches; NL: number of leaves; WD: wood density; FL: fruit length; FD: fruit diameter; FT: fruit thickness; SL: seed length; SW: seed width; ST: seed thickness; NS: number of seeds ($plant^{-1}$); OC: oil content; HSW: hundred-seed weight.

and HSW) but lower plant, fruit and seed proportions (Figure 1 and 2).

The accession B5F83P1 displayed larger plants and fruit proportions, high agronomic performance and the largest seeds (Figure 1 and 2), making it the most productive accession. In contrast, SUCILATEBEC and all Honduras accessions had low values in both components, indicating that this germplasm were characterized by small-sized plants, fruits, and seeds, as well as low agronomic yield (Figure 1 and 2).

The diversity explained by the first four principal components in this study (85%) was higher than that reported for *J. curcas* in India and Cape Verde (69.2%) and was similar to that found in other American accessions cultivated in Yucatan, Mexico (84.19%) (Aguilera-Cauich et al., 2015).

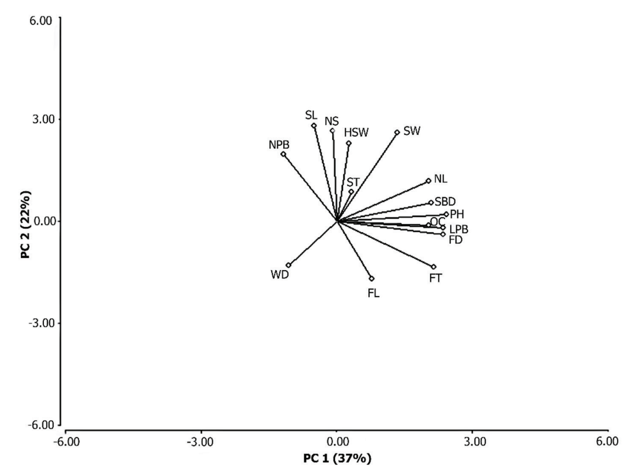

Figure 1. Analysis of the agromorphological variation of 15 characters of ten accessions of J. curcas in Yucatan, Mexico.

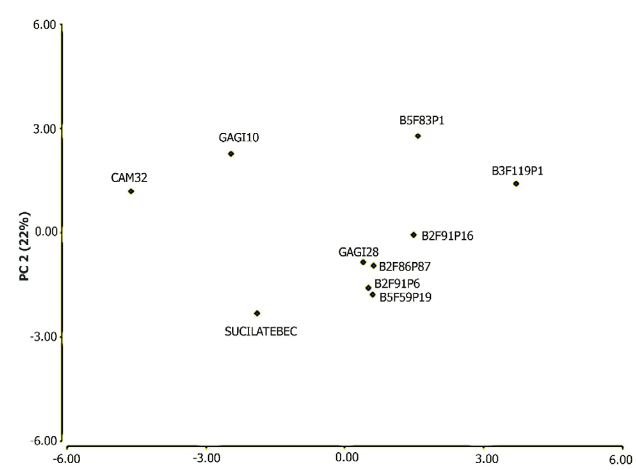

Figure 2. Response of ten *J. curcas* accessions based on 15 agromorphological characters. PC: principal component.

Dissimilarity analysis

The ten *J. curcas* accessions were grouped into four similarity clusters at a Euclidean distance of 4.36 (Figure 3), based on the variation of 12 agromorphological traits previously identified in the PCA analysis (Figure 2).

Group I included the GAGI10 and CAM32 accessions, both collected in Yucatan, Mexico. Group II consisted of B5F83P1 and B3F119P1, both from Chiapas, Mexico. In group III comprised SUCILATEBEC from Yucatan and B5F59P19 from Chiapas. Finally, group IV included all Honduran accessions (B2F91P6, B2F91P16, and B2F86P87) along with one from Yucatan (GAGI28) (Figure 3).

Regarding agromorphological characteristics, Group I and II had the highest values for agronomic yield traits (NS, SY, and HSW) and seed proportions (SL and SW), while exhibiting the lowest values for plant (SBD, PH and LPB) and fruit (FD and FT) proportions. In other words, these accessions had smaller plants but higher seed production (Figure 2 and 3, Table 3).

Group III accessions had the lowest values for plant, fruit, and seed proportions, as well as agronomic yield, indicating that these plants were small and with low seed production (Figure 2 and 3, Table 3). Meanwhile, group IV accessions exhibited intermediate values for plant proportions and agronomic yield, representing medium-sized plants with intermediate seed production (Figure 3 and Table 3).

The cluster analysis revealed phenotypic similarities among accessions based on their geographical origin. In most cases, accessions from the same country or region were gropued together, as seen in groups I and II, where the Mexican accessions were from the

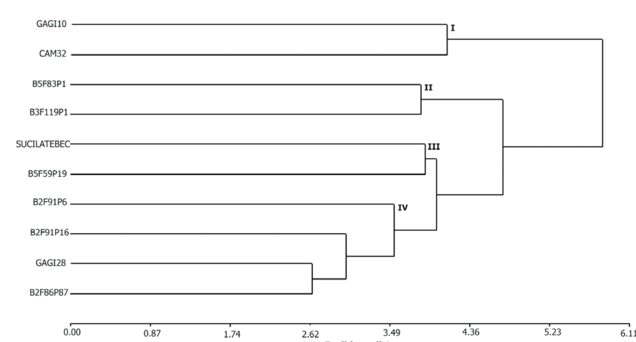

Figure 3. Cluster analysis of ten *J. curcas* accessions based on 13 agromorphological traits, according to the UPGMA method.

same state (Yucatan and Chiapas, respectively), and in group IV, where three of the four accessions originated from Honduras. This suggests that the phenotypic variation among accessions may be influenced by geographic barriers (Aguilera-Cauich et al., 2015; Montes et al., 2013).

This hypothesis is further supported by the fact that two of the four highest-yielding accessions (GAGI10 and CAM32) were collected in Yucatan, the same state where the experimental plantation was established (Table 1). This suggests that these germplasms were better adapted to the local environment than others. Similarly, previous studies have classified *J. curcas* based on geographic origin using agromorphological traits (Rao et al., 2008; Shabanimofrad et al., 2013).

The variation *J. curcas* accessions in this study was primarily explained by plant proportions (37%) and agronomic yield (22%), similar to findings in other regions. For example, a study of 48 accessions of *J. curcas* in Malaysia, based on 14 agromorphological traits, identified six groups (Euclidean distance of 4.8), where variation was mainly driven by agronomic yield traits such as NS per plant, SY per plant, and oil yield per hectare (Shabanimofrad et al., 2013).

The GAGI10 and CAM32 accessions from Group I showed potential as energy crops for biodiesel production due to their high NS and SY. Additionally, their small size and low volume could facilitate seed harvesting (Singh et al., 2016a). These accessions could serve as parental lines for hybridization in mass *J. curcas* plantation, given that SY and plant height are highly heritable traits (83.6% and 87.7%, respectively) (Rao et al., 2008; Shabanimofrad et al., 2013).

Group II plants, with both high proportions and agronomic yield, have potential for bioenergy production beyond biodiesel, as their aerial biomass and wood volume could be converted into biofuels through the thermochemical and biochemical process, such as biogas, bioalcohols, biopetroleum, liquid hydrocarbons, paraffin and olefins (Alherbawi et al., 2021a). Additionally, these accessions have high CO_2 capture capacity, as previously reported for them (Teco-Bravo et al., 2015).

For the genetic improvement of *J. curcas* for energy purposes, hybridizing accessions from group I (GAGI10 and CAM32) with those of group IV (B2F91P16, B2F91P6, GAGI28, and B2F86P87) could generate greater variation in progeny. To maximize heterosis and develop superior genotypes, it is recommended to cross the most divergent lines (Abdullah et al., 2011; Gohil and Pandya, 2009; Latif et al., 2011).

The analysis of morphological and agronomic traits facilitates reliable classification of accessions and helps identify subgroups for germplasm selection and breeding. The diversity and associations identified in this study using phenotypic markers could be validated through molecular markers in future research.

Conclusions

The variation among the ten *J. curcas* accessions was explained by traits related to plant proportions (height and number of branches), fruit (diameter and thickness), and seed (length, width, and volume), as well as agronomic yield (number of seeds and seed yield per plant). The GAGI10 and CAM32 accessions stand out as candidates for breeding programs, as they combine the highest agronomic yield with lower vegetative proportions, facilitating seed harvesting in commercial plantations.

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