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Full Length Research Article

GENETIC DIVERSITY OF *JATROPHA TANJORENSIS* J.L ELLIS & SAROJA ACCESSIONS FROM FOUR STATES IN NIGERIA

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ABSTRACT
Sixteen accessions comprising sixty-four cultivars of *Jatropha tanjorensis* were collected from four states (Lagos, Edo, Ogun, and Oyo) in Nigeria. The *J. tanjorensis* accessions were transplanted on the field at a depth of 8 cm in perforated polythene bags filled with 10 kg of dried sandy-loam soil. The experiment was arranged in a complete randomized design with four replicates. The young leaves and roots from two-week-old plants were collected for DNA extraction. Five primer combinations were used, generating a total of 180 polymorphic amplifications across the *J. tanjorensis* accessions, achieving 100% polymorphism. Accessions JTshOg, JTaoOg, and JTowOg exhibited superior growth characteristics and performed best in the study. These accessions can be further improved for optimal productivity. The number of amplified polymorphic SSR bands per primer pair ranged from 18 to 80, with an average polymorphic percentage of 36.4%. The SSR20 primer produced the highest number of polymorphic bands (80). The Polymorphic Information Content (PIC) values ranged from 0.35 to 0.84, with the highest level of polymorphism observed for two primer combinations, SSR20 (0.84) and JCT7C (0.78). Factorial coordinate analysis based on molecular traits grouped the accessions into four clusters (I, II, III, and IV), highlighting genetic similarities and differences. This indicates the presence of substantial genetic variation among the accessions..

Keywords: Genetics diversity, *Jatropha tanjorensis*, Polymorphism, SSR Primers.

INTRODUCTION
Jatropha tanjorensis (family *Euphorbiaceae*) is a morphologically diverse genus comprising 160–175 species of trees, shrubs, rhizomatous subshrubs, and suffrutescent herbs (Dehgan, 1984; Noelly *et al*., 2020). Originally from tropical America, the *Jatropha* genus is now found throughout the tropics and subtropics of Asia and Africa. Dehgan and Webster (1978) categorized the genus into "Old World *Jatropha*s" and "New World *Jatropha*s," noting that most cataloged species originated from tropical, subtropical, and semiarid regions of the Americas, with only six species found in Africa and Asia. In West Africa, *Jatropha tanjorensis* is a common weed in field crops, bush regrowth, roadsides, and disturbed areas in high-rainfall forest zones. It is locally referred to as "Hospital-too-far," "Catholic vegetable," "Iyana-Ipaja," and "Lapalapa" (Iwalewa *et al*., 2005; Nwachukwu, 2018). Establishing correct descriptions and characterizations of accessions is essential for identifying individuals at the species or subspecies level, as well as for distinguishing varieties or inbred lines in phylogenetic studies and breeding programs (Alverson *et al*., 2011; Arnaud *et al*., 2020).

The name *Jatropha* was derived from Greek words "iatros" (doctor) and "trophe" (nutrition), reflecting its medicinal applications (Elbenri *et al*., 2013). The plant is a drought-resistant, perennial and multipurpose species similar to cassava. It sheds leaves during the dry season, typical of deciduous trees, and grows to a height of 3–5 m, remaining productive for 30–50 years (Elbenri *et al*., 2008). *Jatropha* species have multiple uses, including ornamentals, medicinal plants and energy crops (Heller, 1996). They also serve as germplasm resource for castor breeding due to their rare and beneficial traits (Sujatha, 1996). Some *Jatropha* species have been collected for castor improvement programs (Sujatha and Prabakaran, 1997).

Additionally, *Jatropha tanjorensis* is used for soil stabilization against landslides and erosion, fencing and as a vegetable in southern Nigeria. The plant has gained popularity as a natural remedy for various ailments, such as diabetes (Olayiwola *et al*., 2004), malaria and hypertension (Orhue *et al*., 2008). Its medicinal properties are attributed to bioactive compounds like alkaloids, flavonoids, tannins, cardiac glycosides, anthraquinones and saponins (Ehimwenma and Osagie, 2007). The increasing interest in medicinal plants has emphasized the need for scientific scrutiny of their bioactive compounds to provide reliable data for informed medical use (Ozolua *et al*., 2006; Oyewole *et al*., 2007). Recently, there has been greater emphasis on recovering or extracting high-value products using sustainable technologies.

Genetic variation is critical for the success of any genetic improvement or conservation program (Heller, 1996). The availability of a diverse gene pool allows for global exploration and characterization of germplasm samples, enabling efficient species management, conservation, and utilization (Hu *et al*., 2009). This is particularly relevant for *Jatropha* populations, which exhibit limited genetic variation due to their history as an introduced exotic species in many countries (Achten *et al*., 2010). Molecular markers, such as Simple Sequence Repeats (SSRs), have become indispensable tools for evaluating genetic diversity, mapping genes, and facilitating marker-assisted breeding (Phumichai *et al*., 2008). The SSRs are particularly useful due to their high reproducibility, polymorphism and co-dominant inheritance (Gupta and Varshney, 2000).

Several studies have assessed the genetic diversity of *Jatropha curcas* and other plant species using molecular markers like Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR), and SSR markers (Olawuyi *et al*., 2018; Azeez and

Olawuyi, 2020; Olawuyi *et al*., 2024). For instance, Sun *et al*. (2008) reported low genetic diversity in Chinese *J. curcas* landraces using SSR and AFLP markers. Similarly, Tatikonda *et al*. (2009) analyzed 48 elite germplasm collections in India using AFLP markers, clustering the accessions into four groups. Basha *et al*. (2009) demonstrated the rich allelic diversity of Mexican germplasm in a study involving 72 *J. curcas* accessions from 13 countries.

In contrast, other studies have reported low and moderate levels of interspecific and intraspecific genetic variation in *Jatropha* populations (Sudheer *et al*., 2009b; Shen *et al*., 2010; Guo *et al*., 2016). These findings underscore the importance of further studies on *Jatropha tanjorensis* to elucidate its genetic relationships and diversity. Genetic studies can aid conservation efforts and guide breeding strategies by characterizing germplasm based on agro-morphological traits, biochemical markers, and molecular tools like SSRs and nrDNA ITS (Prabakaran and Sujatha, 1999; Pamidiamarri *et al*., 2009).

Despite recent studies on *Jatropha curcas* using molecular markers (Wen *et al*., 2010; Osorio *et al*., 2014; Maghuly *et al*., 2015), detailed analyses of the genetic diversity, relationships, and differentiation of *Jatropha tanjorensis* from various geographical regions in Nigeria remain limited. Additional research is needed to provide taxonomic data for accurate classification and identification. Characterizing *Jatropha tanjorensis* germplasm from Nigeria is essential to understanding its genetic variation and evolutionary relationships.

This study examines the morphological traits and molecular characteristics of *Jatropha tanjorensis* accessions from four Nigerian states, using SSR markers. The findings will provide critical data for the conservation, characterization, and breeding of this important plant species.

MATERIALS AND METHODS

Source of Planting Material

Sixteen accessions shown in Table 1 comprising a total of sixty-four cultivars of *Jatropha tanjorensis* were collected from four states (Lagos, Edo, Ogun and Oyo State) in Nigeria. Identification of the accessions was carried out at the Department of Botany University of Ibadan prior to the study.

Table 1: The sources of *Jatropha tanjorensis* accessions and their locations

GENOTYPE	SOURCE	STATES	GEO LOCATION / GPS
		EDO	
JTegEd	Egor LGA.		6⁰ 21' 30. 0''N 5⁰ 3700.8'' E
JTorEd	Oredo LGA.		6⁰ 20' 43.8'' N 5⁰ 38' 02.1'E
JTioEd	Ikpoba Oka LGA		6⁰ 21' 04.9'' N 5⁰ 38' 51.1'E
JTeiEd	Ehor Ihunmwonde LGA		6⁰ 36' 26.6 N 5⁰ 58' 46.1'E
		LAGOS	
JTaliLa	Alimosho LGA		6⁰ 34'17N 3⁰ 16' 14'' E
JTagLa	Agege LGA		6⁰ 40' 3'' N 3⁰ 16' 51'' E
JTiiLa	Ifako Ijaiye		6⁰ 37' 34'' N 3⁰ 18' 22''E
JTikLa	Ikorodu LGA.		6⁰ 35' 55''N 3⁰ 23'37''E
		OGUN	
JTshaOg	Shagamu LGA		6⁰ 45' 50''N 30 30''0''E
JTaoOg	Adodo Ota LGA		6⁰ 33'13'N 3⁰ 3'57⁰ E
JTowOg	Owode LGA		
JTifOg	Ifo LGA.		6⁰ 42 42''N 3⁰ 16'14E
		OYO	
JTinOy	Ibadan North LGA.		7⁰ 23'4''N 3⁰ 53' 58 E
JTisOy	Ibadan South LGA.		
JTakOy	Akinyele LGA.		
JToaOy	Ona-Ara LGA.		7⁰ 24' 2'' N 3⁰ 56' 31'' E

Experimental Design and Planting

Jatropha tanjorensis was planted in polyethylene bags filled with 10 kg of topsoil and arranged in a complete randomized design with four replicates. Each *Jatropha tanjorensis* landrace was planted per bag and watered with 75mL of distilled water every three days. Planting was conducted during the rainy season (May to September 2022) following the procedure described by Verheiji (2004). The seedlings were transplanted into the field at a depth of 8 cm using perforated polyethylene bags filled with 10 kg of dried sandy-loam soil. Transplanting was carried out early in the morning, and the plants were watered every three days throughout the experiment. Weeding was conducted within the first ten days and subsequently as needed.

Study Site

The experiment was conducted at the University of Ibadan, Department of Botany experimental field in Oyo State, Nigeria. The site lies in the tropical savanna zone, characterized by sandy loam

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soil, light to darkish in color. It is located at Longitude 7.417°N and Latitude 3.900°E, with an elevation of 212 m above mean sea level. The mean annual rainfall is 1230 mm, distributed over approximately 123 days, with a mean daily temperature of 26.46°C and relative humidity of 74.55% (University of Ibadan Meteorological Station, 2022).

Field Procedure

The land was cleared manually, measured, and demarcated with pegs into a 15 m² plot. Polyethylene bags filled with topsoil were arranged at 30 × 30 × 30 cm spacing (Gubits *et al*., 1999). Sixty-four *J. tanjorensis* landraces were transplanted on-site, with 16 used for cytological and molecular studies. Forty-eight plants were randomly selected for data collection (Fig 1a & b).

Fig. 1: Experimental design and field layout (a) before (b) after planting

Growth performance of *Jatropha tanjorensis* accessions.

Growth parameters were measured weekly starting from the second week after transplanting. Quantitative traits included the number of leaves, number of stems, and number of lobes, as well as petiole length, leaf width, plant height, and leaf length, measured using a meter rule. Qualitative traits such as leaf pilosity, type of plant ramification, stem waxiness, pigmentation of young and adult leaves, color of foliar veins, petiole position and color, leaf texture, and leaf phyllotaxy were also recorded (Table 2).

Table 2: Quantitative and qualitative growth characters

Character	Character States
Leaf pilosity	Present, Absent
Type of plant ramification	Trifurcate, Bifurcate, Cup-like, Universal
Stem waxiness	Present, Absent
Color of young leaves	Green, Red, Purple
Color of adult leaves	Dark Green, Light Green
Color of foliar veins	Green, Pale Green, Purple
Petiole leaf position	Opposed, Alternate, Mixed
Petiole color	Dark Green, Light Green
Phyllotaxy	Whorled, Opposed, Alternate
Leaf texture	Rough, Smooth
Petiole Length (cm)	Quantitative
Numbers of Leaf Lobes	Quantitative
Leaf Length (cm)	Quantitative
Leaf Width (cm)	Quantitative
Number of Stem	Quantitative
Number of leaves	Quantitative
Leaf Axil Angle	Quantitative

Molecular diversity studies on *Jatropha tanjorensis* using SSR markers

Molecular studies were conducted at the Bioscience Center of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Fresh, young apical leaves were collected two weeks after planting, placed in ice bags, and transported to the laboratory. Upon arrival, the leaves were stored at -80°C until molecular analyses were performed. The molecular experiment was carried out at the Bioscience unit of Genetic Resources Centre, International Institute for Tropical Agriculture (IITA). The Nine SSR primers used are presented in Table 3.

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Table 3. SSR primers used for diversity studies in *Jatropha tanjorensis*.

Primers	Forward sequence	Reverse sequence
SSR 20	CGCTCTGTGAGAATCAAATGGT	GGACTCTTATTAGCCAATGGGATG
SSR 16	GCTTTATCCACATCAATATCC	TCCTACAATAATAACTTGCC
EF592203	TCTGACCCAACAAAGAACCA	TCCTCCTCGTCCTCATCATC
JCT 17	TCTCTCATTGTTGCGCTGTC	TAACAAGTCCTCCCCCTCCT
JCT 158	CCTCTCTCAATTGCCTCTCC	CAAAAGGCGCCTCTAATGAT
mJCENA41	CTTTCTTACCCCTCATCCTT	AAAGCCAGGACATACTTGAA
mJCENA87	ATCTGGAGTGAAACCAAAGA	CACATGGTAAGCATTCAAGC
JCT7[c]	CGAAGTGAATGCACAACACA	TGCTATTCAAATGGAACAAGTGA
JCT5[pc]	CATGCTAACGATAGAGGA	TTTTCAGCCACTACCTCA

DNA Extraction, Quantification and PCR Amplifications

Young leaves of *Jatropha tanjorensis* landraces were surface sterilized using 70% ethanol and ground in liquid nitrogen using The reaction was prepared as a 10 µl reaction mixture consisting of the following components: 1 µl of 10× PCR buffer, 0.8 mM dNTPs, 0.4 mM $MgCl_2$, 0.06 µl of Taq polymerase, 0.8µl of DMSO, 1.94 µl of PCR-grade H_2O, 1 µl of each primer, and 3 µl of DNA. Amplification was performed in a thermocycler programmed with a touchdown (TDSSR) protocol. The initial denaturation was set at 94°C for 2 minutes, followed by nine cycles of 93°C for 20 seconds, annealing at 65°C for 35 seconds, and extension at 72°C for 45 seconds. This was followed by 24 cycles at 93°C for 20 seconds (denaturation), 55°C for 35 seconds (annealing), and 72°C for 45 seconds (extension). A final extension step was performed at 72°C for 5 minutes, and the reaction was held at 4°C indefinitely.

Agarose Gel Electrophoresis and Scoring of Bands of SSR Primers

Agarose gel electrophoresis was conducted to verify the presence of DNA in the extracted plant samples. One gram (1 g) of agarose was dissolved in 100 mL of 0.5× Tris-borate-EDTA (TBE) buffer using a microwave oven for 5 minutes. The solution was cooled under running tap water for 1 minute. Subsequently, 2 µl of ethidium bromide (EtBr) stain was added to the gel solution as a dye. The agarose gel solution was then poured into a gel casting tray, and a comb was set in place.

The solidified gel was transferred to an electrophoresis tank containing the 0.5× TBE buffer. DNA samples, including 3 µl of the dye-extracted DNA and a 3 kb plus mid-range DNA ladder from Jena Bioscience, were loaded into the wells. Electrophoresis was conducted for 30 minutes at 100 volts. A negative control lacking a DNA template was included.

Visualization of the separated amplified fragments was performed under a UV transilluminator, where the formation of DNA bands was observed. Nine SSR primer pairs (forward and reverse oligonucleotides) were initially optimized, and the best five primers were selected for the genetic diversity study based on previously published data (Yaowalak *et al*., 2011; Ajayi *et al*., 2019). The bands produced were scored as discrete variables into an Excel sheet, with "1" indicating the presence of a band and "0" indicating its absence.

Molecular Statistical Analysis

The presence or absence of scorable bands was transformed into a binary matrix (1 for the presence and 0 for the absence of a band at a specific position). Phylogenetic relationships among the samples were determined through cluster analysis using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) implemented in the DARwin software package (version 6.0.021; Perrier and Jacquemoud-Collet, 2021). Multivariate grouping was analyzed using Factorial Coordinate Analysis (FCO) in the same DARwin software. Polymorphic Information Content (PIC) values for each primer combination were calculated following the method described by Ojuederie *et al*. (2014).

Thus: PIC = 1- ∑pi2; where: pi is the frequency of the ith allele.

Morphometric Statistical Analysis

Morphometric analysis was conducted on *Jatropha tanjorensis* species grown in the field. The quantitative traits (the number of leaves, number of stems, number of lobes, petiole length, leaf width, leaf angle, leaf length, and plant height) were measured using a meter rule. Qualitative traits such as leaf color, stem color, hairiness of the stem and leaf, leaf type, leaf shape, leaf arrangement, and leaf apex were also observed and recorded. The morphological data were analyzed using Analysis of Variance (ANOVA) in the Statistical Analysis System (SAS) software version 9.4 (SAS, 2012). After testing the assumptions of ANOVA, differences in means were separated using Duncan's Multiple Range Test (DMRT) at a 95% probability level ($p < 0.05$).

Subsequently, the data were subjected to Principal Component Analysis (PCA) and Cluster Analysis, which were computed based on the selected quantitative traits measured. These analyses were performed to identify patterns of variation and grouping among the *J. tanjorensis* accessions.

RESULTS

Mean Square Variance and Interaction Effects on Growth Characters of *Jatropha tanjorensis*

The analysis of variance (ANOVA) of the performance of the sixteen genotypes of *J. tanjorensis* reflected in the mean square values of the eight quantitative traits studied shown across accessions, weeks, and their interaction over a period of 10 weeks are presented in Table 2. Significant differences were observed among accessions and weeks for most traits, while the interaction effects were variable. Significant variation ($P<0.001$) was observed

among accessions for plant length (PL), leaf length (LL), leaf width (LW), number of leaves (NOLEA), and plant height (PH). However, the number of stems (NOS) showed a moderate significance ($P<0.05$), while leaf axial length (LAL) was non-significant ($P>0.05$).

Weeks had highly significant impact ($P<0.001$) on all traits except NOS, indicating that plant growth parameters varied considerably over time. The traits PL, LL, LW, NOLEA, LAL, and PH showed strong temporal variations, while NOS remained non-significant ($P>0.05$). The interaction between accessions and weeks showed varying levels of significance. Leaf length (LL) and leaf axial length (LAL) exhibited significant effects ($P<0.05$ and $P<0.001$, respectively), while plant height (PH) showed moderate significance ($P<0.05$). Other traits, including PL, LW, NOS, and NOLEA, did not show significant interactions, indicating that these traits were more influenced by either accession or time rather than their combined effects. Replication effects were mostly non-significant, except for LAL, which showed slight significance ($P<0.05$). The error variance remained low across traits, indicating a reliable experimental setup.

Mean Performance on Growth Characters of *Jatropha tanjorensis*

The mean performance of *J. tanjorensis* accessions presented in Table 3 revealed significant variability in growth traits. Among the accessions, JTshOg exhibited the highest plant length (7.55 cm), while JTagLa recorded the shortest (2.94 cm). Similarly, JTaoOg, JTowOg, and JToaOy showed superior plant height, whereas JTagLa had the lowest PH (1.84 cm). Leaf length (LL) and leaf width (LW) also varied, with JTowOg (2.81 cm LL) and JTifOg (3.01 cm LW) having the largest leaves, while JTagLa had the smallest. The number of leaves (NOL) was highest in JTshOg (3.08) and lowest in JTikLa and JTagLa (2.16 each). Leaf axial length (LAL) was highest in JTegEd (1.01 cm) and lowest in JTikLa (0.72 cm), further highlighting morphological differences among the accessions. The number of stems (NOS) varied, with JTalLa (1.75) and JTegEd (1.70) having the highest values, while JTioED recorded the lowest (1.31).

Principal Component Analysis of morphological traits of *Jatropha tanjorensis*

Principal Component Analysis (PCA) identified the first five principal component (PC) axes, which explained 91.1% of the total variation shown in Table 4. The first principal component (PC1), with an eigenvalue of 3.318, accounted for 42.8% of the total variability. PC2, with an eigenvalue of 0.258, explained 17.3%, while PC3, PC4, and PC5 contributed 15.3%, 13.7%, and 9.2%, respectively. Quantitative traits such as leaf width (0.408), leaf length (0.405), petiole length (0.397), and plant height (0.384) had the highest loadings on PC1, alongside qualitative traits like number of leaves (0.331) and petiole leaf position (0.342). On PC2, traits such as the number of stems (0.556), leaf axil length (0.574), and adult leaf color (0.216) showed significant contributions. PC3 was dominated by the number of leaf lobes (0.610), plant ramification (0.437), and leaf phyllotaxy (0.428).

The biplot (Fig.2) visualizes the relationships between variables and genotypes along PC1 and PC2. Notably, genotypes JTagLa, JTiKLa, and JTioEd showed the highest negative correlations with plant ramification. JTalLa correlated strongly with adult leaf color, while JTshOg exhibited a high positive correlation with foliar vein color.

Pearson correlation coefficients of agro-morphological variables of *Jatropha tanjorensis*

Pearson's correlation coefficients among the 13 agro-morphological traits studied in Table 5 revealed strong positive correlations between several traits. Petiole length was significantly correlated with leave length ($r = 0.82$), leaf width ($r = 0.84$), number of leaves ($r = 0.65$), plant height ($r = 0.77$), and adult leaf color ($r = 0.39$).

The number of leaf lobes also correlated positively and significantly with leaf axial length ($r = 0.47$). The leaf width exhibited a strong significant correlation with leaf length ($r = 0.97$), the number of leaves showed strong positive correlation with leaf width ($r= 0.57$), while leaf axial length had a weak positive correlation with plant length ($r = 0.32$). The number of leaves was significantly correlated with plant height ($r = 0.60$). Plant height showed strong correlations with plant width ($r = 0.80$) and adult leaf color ($r = 0.40$). Additionally, leaf phyllotaxy was correlated with the number of stems ($r = 0.67$), plant height ($r = 0.43$), plant ramification ($r=0.60$), and adult leaf color (0.81).

Amplification of *Jatropha tanjorensis* genotypes with Five SSR Primers

Amplification using five SSR primers shown in Fig. 3 generated a total of 182 polymorphic bands across the fourteen *J. tanjorensis* genotypes. The number of bands per primer ranged from 18 (JCT5BC) to 80 (SSR20), with an average of 36.4 bands (Table 3). The SSR20 produced the highest number of polymorphic bands (100%) and had the highest Polymorphic Information Content (PIC) value of 0.837, making it the most informative primer for genetic diversity assessment. This was followed by JCT7C, with a PIC value of 0.734, further highlighting its utility for genetic diversity studies in *J. tanjorensis*.

Cluster analysis of the *Jatropha tanjorensis* genotypes

Cluster analysis using Ward's minimum variance method grouped the sixteen genotypes into two clusters based on Gower distances (R-squared distance = 0.15). The dendrogram, presented in Fig. 4a, illustrates the clustering. The first cluster contained nine genotypes (56.25%), with JTaoOg and JTakOy being the most closely related (distance = 0.0071). The second cluster consisted of seven genotypes (43.75%). The distances between accessions ranged from 0.0071 to 0.3561.

Using Jaccard's dissimilarity coefficient and unweighted paired group method with arithmetic averages (UPGMA), a dendrogram grouped the fourteen genotypes into four clusters at a distance of 0.05 (Fig. 4b). Cluster I included a single genotype, JToeOy, which was genetically distinct. Cluster II contained six genotypes (42.86%), including JTaoOg and JTshOg, while Cluster III grouped three genotypes (21.43%). Cluster IV included four genotypes, with JTioEd, JTiiLa, and JTinOy closely related, and JTowOg being more isolated.

Factorial coordinate analysis

Factorial Coordinate Analysis (FCA) using DARwin software version 6.0 confirmed the grouping from the UPGMA analysis as shown in Fig. 5. Genotype JToeOy, identified as distinct in Cluster I, was also placed alone in Group I. Genotypes JTisOy, JTikLa, JTalLa, and JTowOg were grouped into Group II. Five genotypes (JTegEd, JTeiEd, JTifOg, JTshOg, and JTaoOg) formed Group III, while four (JTakOy, JTioEd, JTinOy, and JTiiLa) constituted Group IV.

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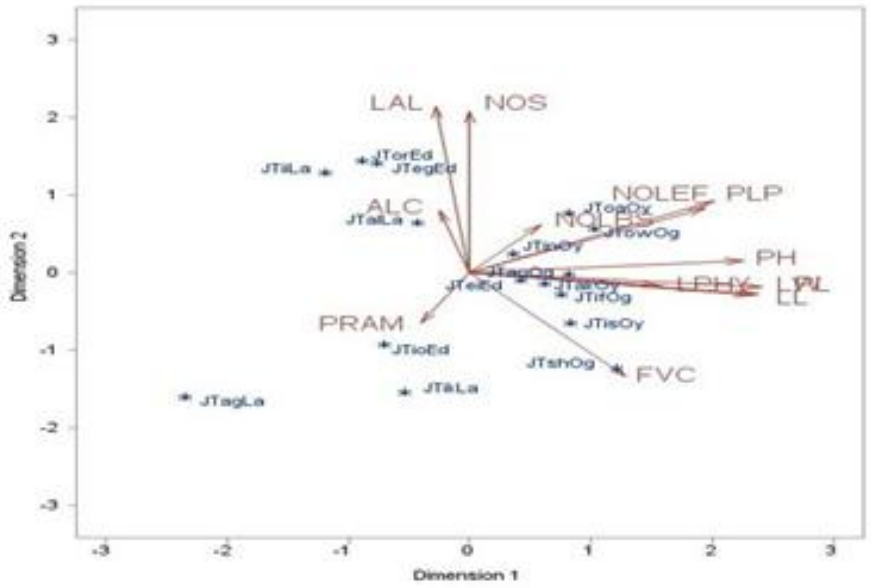

Fig. 2. Vector view of genotype by trait biplot of the first and second principal component axes showing the relationship of 13 morphological traits among 14 *Jatropha* genotypes. NOLEF-number of leaves, NOS- number of stems, NOLBS-number of leaves lobes, PL- petiole length, LW- leaf width, PH-plant height, LAL-leaves axil length, LL-leaf length PLP-plant leaf pilosity, PRAM- plant ramification, YLC-young leaves color, ALC-adult leaves color, FVC-foliar veins color, PLP- petiole leaf position, and LPHY- leaves phyllotaxy.

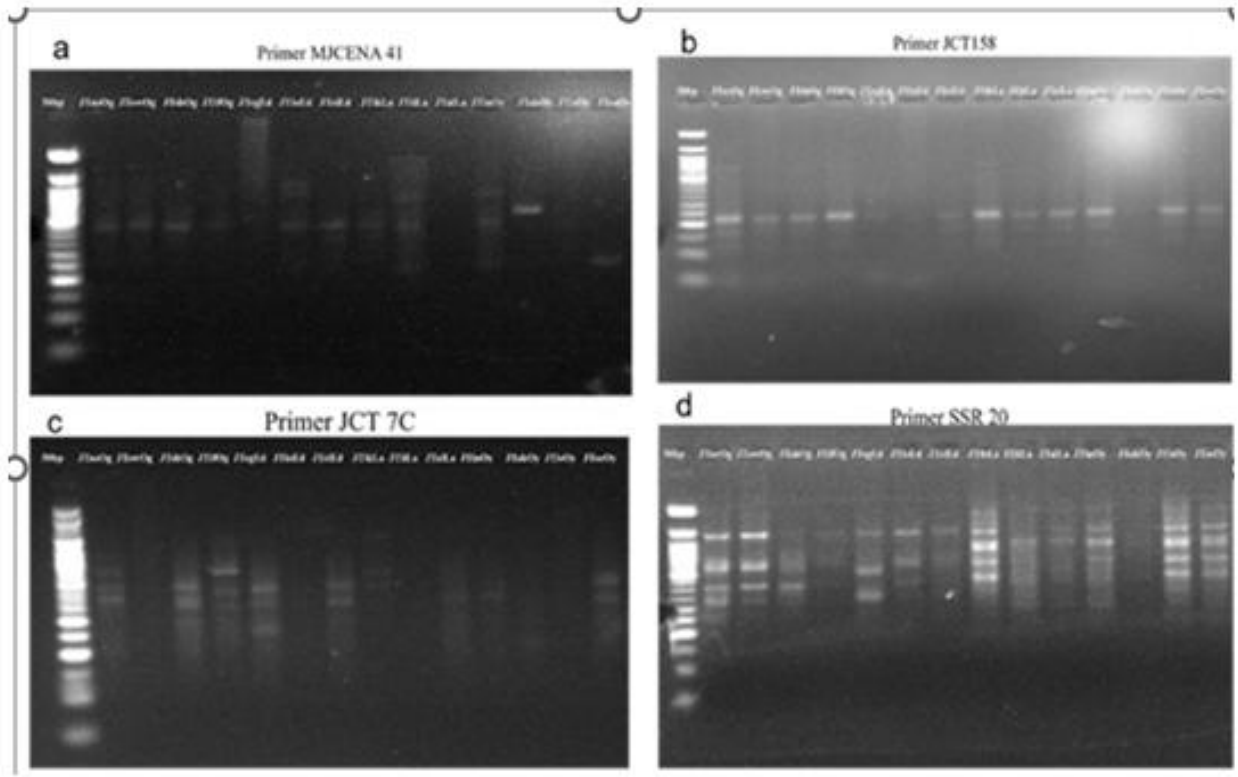

Fig 3. Gel picture showing PCR amplification of the four SSR markers (a) MJCENA 41 (b) JCT 158 (c) JCT 7C (d) SSR 20

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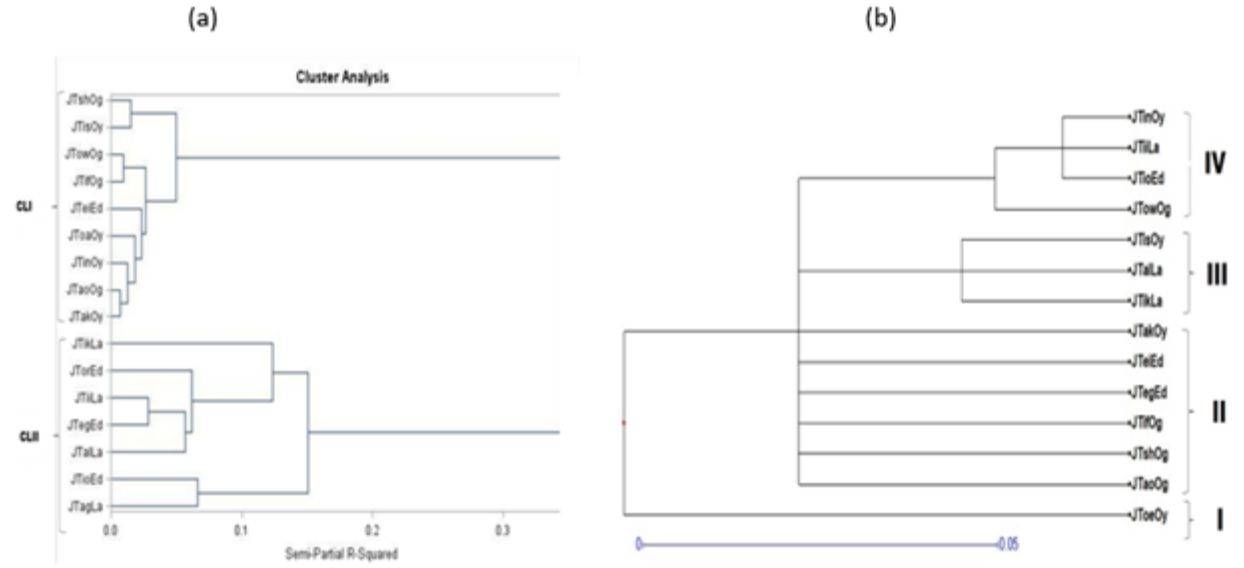

Fig 4: Genetic relationship (dendogram) among the 14 *Jatropha* genotypes revealed by the four SSR markers (a) using Ward's minimum variance (b) UPGMA method

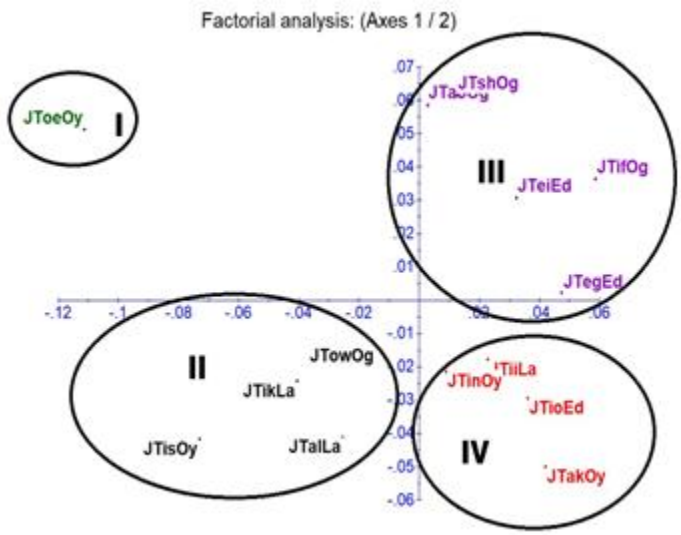

Fig 5. Factorial coordinate analysis of 14 *Jatropha* genotypes using dissimilarity matrix

Table 2. Mean square variance on growth characters of *Jatropha tanjorensis* accessions

SOURCE OF VARIATION	DF	PL	LL	LW	NOS	NOLEA	LAL	PH
Accessions	15	22.02***	0.76***	0.94***	0.22*	1.30***	0.09	2.36***
Weeks	9	161.8***	9.85***	13.96***	0.05^{ns}	8.85***	0.38***	19.34***
Accession x Weeks	130	3.46^{ns}	0.14*	0.15^{ns}	0.05^{ns}	0.21ns	0.16***	0.31*
Rep	2	8.16^{ns}	1.21^{ns}	1.72^{ns}	0.48^{ns}	0.72ns	0.35*	1.36ns
Model	156	16.35	0.86	1.17	0.08	0.98	0.17	1.76
Error	179	2.09	0.1	0.12	0.12	0.42	0.08	0.22
Corrected Total	335							

*** P<0.001 (highly significant), * P<0.05 (significant), ns- non-significant, DF-degree of freedom, PL- plant length, LL-leave length, LW-leave width, NOS-number of stems, NOL-Number of Leaves, LAL-Leave Axial Leave, PH-Plant Height

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Table 3. Mean Performance on Growth Characters of *Jatropha tanjorensis* accessions

Accessions	PL (cm)	NOLB	LL	LW	NOS	NOL	LAL	PH
JTshOg	7.55a	3.09^{abc}	2.73^{abc}	2.91^{a}	1.49^{bcd}	3.08^{a}	0.79^{bc}	3.06^{a}
JTaoOg	7.15^{ab}	3.00^{bcd}	2.71^{abc}	2.83^{a}	1.53^{abcd}	2.96^{ab}	0.98ab	3.12^{a}
JTisOy	6.79^{ab}	3.13^{abc}	2.68^{abc}	2.87^{a}	1.42^{cd}	300^{a}	0.80^{abc}	2.93^{b}
JTinOy	6.67^{ab}	3.14^{abc}	2.55^{cde}	2.78^{a}	1.48^{bcd}	2.93^{ab}	0.88^{abc}	2.65^{b}
JToaOy	6.49^{b}	3.00^{bcd}	2.70^{abc}	2.89^{a}	1.54^{abcd}	2.90^{ab}	0.97^{ab}	3.11^{a}
JTeiEd	6.30^{bc}	3.45^{ab}	2.56^{bcde}	2.78^{a}	1.51^{abcd}	2.92^{ab}	0.89^{abc}	2.53^{c}
JTakOy	6.27^{bc}	3.00^{bcd}	2.62^{abcd}	2.80^{a}	1.43^{cd}	2.90^{ab}	0.93^{abc}	2.99^{a}
JTowOg	6.15^{bc}	3.00^{bcd}	2.81^{a}	2.96^{a}	1.60^{abc}	2.99^{ab}	0.95^{ab}	3.08^{a}
JTifOg	5.38^{cd}	3.00^{bcd}	2.78^{ab}	3.01^{a}	1.51^{abcd}	2.94^{ab}	0.87^{abc}	2.98^{a}
JTikLa	5.04^{d}	1.94^{e}	2.35^{efd}	2.45^{b}	1.40^{cd}	2.16^{c}	0.72^{c}	2.41^{cd}
JTegEd	4.79^{e}	3.21^{abc}	2.32^{fg}	2.48^{b}	1.70^{ab}	2.87^{ab}	1.01^{a}	2.35^{cd}
JTioED	4.71^{ed}	3.52^{a}	2.42^{defg}	2.49^{b}	1.31^{d}	2.32^{c}	0.91^{abc}	2.16^{d}
JTalLa	4.64^{cd}	2.57^{d}	2.43^{def}	2.47^{b}	1.75^{a}	3.00^{ab}	0.86^{abc}	2.61^{c}
JTiiLa	3.99^{e}	3.00^{bcd}	2.05^{hi}	2.19^{c}	1.70^{ab}	2.82^{ab}	0.98^{ab}	2.17^{d}
JTorEd	3.96^{e}	3.00^{bcd}	2.20^{gh}	2.28^{c}	1.57^{abcd}	2.53^{bc}	1.00^{a}	2.26^{c}
JTagLa	2.94^{f}	2.75^{cd}	1.85^{i}	1.88^{d}	1.35^{cd}	2.16^{c}	0.83^{abc}	1.84e

PL-plant length, NOLB-number of lobes, LL-Leave Length, Key: LW-Leaves width, NOL-number of stems, NOL-number of leave, LAL-Leave Axial, PH-plant height. Means with the same letter in the same column are not significantly different at $p<0.05$ using Duncan's Multiple Range Test (DMRT)

Table 4. Principal component analysis of the morphological characters of *Jatropha* accessions

Traits	PC1	PC2	PC3	PC4
PL	0.397	-	-	-
NOLBS	-	-	0.61	-
LL	0.405	-	-	-
LW	0.408	-	-	-
NOS	-	0.556	-	0.407
NOLEF	0.331	0.224	-	0.379
LAL	-	0.574	0.274	-
PH	0.384	-	-	-
PRAM	-	-	0.437	0.521
ALC	-	0.216	-	-0.562
FVC	0.218	-0.357	0.189	-
PLP	0.342	0.249	-0.225	-
LPHY	0.268	-	0.428	-0.246
Eigen value	3.318	0.258	0.849	0.228
Proportion (%)	42.8	17.3	15.3	87.4
Cumulative (%)	0.428	0.6	0.753	0.841

Key: **PC**- Principal Component. Only Eigen vectors with values ≥ 0.20 which largely controlled each **PC** axes are shown. **NOLEF**-number of leaves, **NOS**- number of stems, **NOLBS**-number of leaves lobes, **PL**- petiole length, **LW**- leaf width, **PH**-plant height, **LAL**-leaves axil length, **LL**-leaf length **PLP-**plant leaf pilosity, **PRAM**- plant ramification, **YLC**-young leaves color, **ALC**-adult leaves color, **FVC**-foliar veins color, **PLP**- petiole leaf position, **LPHY**- leaves phyllotaxy

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Table 5. Pearson Correlation Coefficient matrix of 13 *Jatropha* traits evaluated in this study

	PL	NOLBS	LL	LW	NOS	NOLEF	LAL	PH	PRAM	ALC	FVC	PLP
NOLBS	0.23											
LL	0.82***	0.24										
LW	0.84***	0.21	0.97***									
NOS	0.02	0.13	-0.05	-0.05								
NOLEF	0.65***	0.12	0.56	0.57***	0.48***							
LAL	0.24	0.47***	0.32***	0.30	0.25	0.2						
PH	0.77***	0.10	0.79***	0.80***	0.05	0.60***	0.34					
PRAM	-0.22	-0.03	-0.26	-0.25	0.1	-0.14	-0.06	-0.24				
ALC	0.39***	0.08	0.40***	0.40***	0.01	0.34	0.13	0.37	-0.11			
FVC	0.05	-0.07	0.05	0.05	-0.14	-0.01	-0.21	0.06	0.05	0.03		
PLP	0.08	-0.09	0.07	0.07	0.02	0.09	-0.07	0.04	0.03	-0.01	0.14	
LPHY	0.12	0.11	0.23	0.23	0.67***	0.1	0.18	0.43***	0.60***	0.81***	0.01	

Key: PL- Petiole length, **NOLBS**-Number of lobes, **LL**-Leaf length, **LW**-Leaf width, **NOS-**Number of stems, **NOLEF**-Number of leaves, LAL-Leaf axial length, **PH**-Plant height, **PRAM**-Plant ramification, **ALC**-Adult leaf colour, **FVC**-Foliar vein colour, **PLP**-Plant phyllotaxy. Significant at $p < 0.05$, ** = Highly significant at $p < 0.01$, ns = Non –significant, df = degree of freedom.

Table 6. Polymorphism obtained from five SSR Markers on 14 *Jatropha* genotypes

SSR Primers	Number of bands	Number of Monomorphic bands	Number of Polymorphic bands	Percentage polymorphism	PIC
JCT5BC	18	0	18	100	0
JCT7C	36	0	36	100	0.784
JCT158	22	0	22	100	0
MJCENA41	26	0	26	100	0.142
SSR20	80	0	80	100	0.837
Average			36.4		0.353

PIC-Polymorphic information content

DISCUSSION

These findings suggest that genetic variability among accessions plays a critical role in influencing plant growth traits, while environmental factors significantly affect developmental changes over the study period. The lack of strong interaction effects for most traits suggests that the trends observed across weeks were relatively consistent among different accessions. The variation in these traits suggests strong influence of genetic and environmental factors on growth performance. Accessions JTshOg, JTaoOg, and JTowOg demonstrated superior growth characteristics, making them potentially suitable for further cultivation and breeding. On the other hand, accessions JTagLa, JTiiLa and JTorEd showed lower values for most traits, indicating their comparatively weaker growth potential. This variability emphasizes the importance of selecting high-performing accessions for improved productivity and adaptability in different environmental conditions as similarly reported in *Garcinia kola* by Azeez *et al*. (2020) and pearl millet by Olawuyi *et al*. (2020).

Temporal analysis over ten weeks highlighted dynamic growth patterns, with traits such as petiole length, leaf length, and plant height exhibiting substantial variability over time. These temporal dynamics could reflect a combination of genetic potential and environmental influences, such as soil fertility, water availability, and light intensity. Similar environmental modulation of phenotypic traits has been observed in *Jatropha curcas* under variable agro-climatic conditions (Ovando *et al*., 2011). These findings also align with earlier reports indicating the sensitivity of petiole and leaf dimensions to changes in nutrient and moisture regimes (Dominguez *et al*., 2015). Correlation analysis revealed strong positive associations among several traits. For example, the significant correlation between petiole length and leaf dimensions (length and width) suggests co-regulation and utility in indirect selection similar to the report by Dalla Vecchia and Bolpagni (2022). Similarly, the correlation between plant height and adult leaf color may serve as a practical marker for assessing plant maturity or vigor in breeding populations. These relationships are consistent with findings in other studies on *Jatropha* and related species, where morphological markers have been used to predict performance traits (Shabanimofrad *et al*., 2013; Debnath *et al*., 2018).

The agro-morphological and molecular analyses of *J. tanjorensis* genotypes revealed substantial genetic diversity, underscoring its potential for genetic improvement and conservation efforts. The significant variability ($p < 0.001$) observed in traits such as petiole length, leaf dimensions, number of leaves, and plant height demonstrates robust phenotypic diversity, which can be harnessed in breeding programs. These findings are consistent with studies in *Jatropha curcas*, which have reported similarly high variability in economically important traits such as seed yield, oil content, and growth characteristics (Nwachukwu and Mbagwu, 2006; Guan *et*

al., 2013; Saadaoui *et al*., 2015). The observed variability emphasizes the genetic plasticity of *J. tanjorensis*, similar to the adaptability seen in other *Jatropha* species under diverse agroecological conditions (Shabanimofrad *et al*., 2013; Laviola *et al*., 2018). In contrast, traits like the number of stems and leaf axil length showed minimal variability, suggesting these may be more genetically conserved or less influenced by environmental factors.

Cluster analysis using Ward's method revealed two major groups among the genotypes, reflecting clear genetic divergence. Notably, the close genetic relationship between JTaoOg and JTakOy highlights potential redundancy, which could inform germplasm management by identifying and eliminating duplicates in breeding populations. PCA identified traits like leaf width, leaf length, and plant height as major contributors to phenotypic variability, with their high loadings on the first principal component axis emphasizing their agronomic importance. These results align with findings *in J. curcas*, where morphological traits, such as plant height and leaf size, were similarly identified as significant contributors to variability (Shabanimofrad *et al*., 2013; Saadaoui *et al*., 2015). Furthermore, the role of plant ramification and phyllotaxy as structural determinants, as shown by their association with PC3, reinforces the importance of these traits in optimizing canopy architecture for higher productivity (One *et al*. 2014).

Molecular characterization using SSR markers further reinforced the genetic diversity within the genotypes. The SSR markers generated 182 polymorphic bands, with SSR20 producing the highest number of polymorphic bands (100%) and achieving the highest Polymorphic Information Content (PIC) value (0.837). This result underscores the utility of SSR20 as the most informative primer, consistent with findings in *Jatropha curcas* by Ajayi *et al*. (2019) and Ravishankar *et al*. (2011). High polymorphism levels observed in this study are indicative of extensive genetic diversity, critical for genetic improvement and conservation efforts, as reported in previous research on *Jatropha* species (Pamidimarri *et al*., 2010; Wen *et al*., 2010; Tanya *et al*., 2010). However, the lower average number of alleles per SSR locus (1.4) compared to related studies (Basha *et al*., 2009; Wen *et al*., 2010) may reflect narrower allelic richness in the sampled populations. This finding highlights the need for broader sampling across diverse regions to capture the full extent of genetic variation (Suwarno *et al*., 2021).
The clustering patterns observed in UPGMA analysis, corroborated by Factorial Coordinate Analysis (FCA), provided a robust framework for understanding genetic relationships and structuring breeding programs. Notably, JToeOy consistently emerged as a unique genotype, potentially harboring unique alleles or adaptations. Such genotypes are invaluable for future studies aimed at understanding gene-environment interactions or developing superior cultivars (Amad and Ming, 2024).

Conclusion

The comprehensive phenotypic and molecular characterization of *J. tanjorensis* genotypes underscores their genetic diversity and potential for genetic improvement. The integration of agro-morphological and molecular data provides a strong basis for selecting genotypes with desirable traits and optimizing conservation strategies. Accessions JTshOg, JTaoOg, and JTowOg that demonstrated superior growth characteristics can be improved for optimal productivity. The SSR markers, particularly SSR20, proved highly effective in genetic discrimination and diversity assessment, reinforcing their utilities in germplasm management. Future studies should explore the functional significance of identified genetic variations and assess the adaptability of *J. tanjorensis* under diverse environmental conditions to maximize its agricultural and industrial potential. These findings align with earlier research emphasizing the importance of genetic diversity in sustainable agriculture and bioenergy applications.

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