International Journal of Agricultural Technology 2026 Vol. 22(4):1703-1726
DOI: https://doi.org/10.63369/ijat.2026.22.4.1703-1726
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Genetic characterization of *Jatropha* landraces in Nigeria: unlocking potential through *rbcl* marker analysis

Edu, N. E.[1], Uzoma, L. G.[1], Edem, U. L.[1]*, Phillip, J. O.[1], Obua, P. A.[1], Idu, J. I.[1], Ogbaji, H. O.[1], Osim, P. B.[1], Aboh, A. P.[1], Abu, G. I.[1], Uno, U. U.[1, 2] and Ekerette, E. E.[1].

[1]Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar P.M.B 1115, Calabar, Nigeria; [2]Department of Biology, University of Education and Entrepreneurship, P.M.B. 1171, Akamkpa, Cross River State, Nigeria.

Edu, N. E., Uzoma, L. G., Edem, U. L., Phillip, J. O., Obua, P. A., Idu, J. I., Ogbaji, H. O., Osim, P. B., Aboh, A. P., Abu, G. I, Uno, U. U. U. and Ekerette, E. E. (2026). Genetic characterisation of *Jatropha* landraces in Nigeria: Unlocking potential through *rbcL* marker analysis. International Journal of Agricultural Technology 22(4):1703-1726.

Abstract *Jatropha* is a medicinal plant commonly found in many parts of Nigeria, but despite its usefulness, its genetic resources have not been fully explored. While different Jatropha species have been studied in several countries, there is still very little information available on the genetic diversity of Nigerian landraces. In this study, forty *Jatropha* landraces were collected from different locations and identified using their local names as well as visible morphological features recognised by local farmers. Fresh leaf samples from the collected landraces were analysed using the rbcL gene for genotyping and sequencing. The resulting data were further examined using Analysis of Molecular Variance (AMOVA) in GenALEx 6.5, phylogenetic analysis based on the Neighbour-Joining method, and SNP detection with DnaSP software. The results of the phylogenetic analysis separated the landraces into three major clusters, although the clustering pattern did not correspond to their geographical locations. This suggests the existence of substantial genetic variation among the landraces. A total of 685 mutations and 39 haplotypes were detected, with a very high haplotype diversity value of 0.999. This high level of diversity may be linked to mutation and recombination events within the population. In addition, nucleotide diversity was relatively high (0.70410), further indicating considerable genetic differences among the sampled landraces. AMOVA results showed that only 1% of the total variation occurred among populations, whereas 99% existed within populations, revealing a high level of diversity at the local population level. These findings provide useful baseline information for future conservation efforts and for the selection of promising parent materials in Jatropha breeding programmes.

Keywords: Jatropha, Genetic diversity, RbcL gene, Phylogenetic analysis, Haplotype diversity, Nigerian landraces

* **Corresponding Author:** Edem, U. L. **Email:** edemlinus@unical.edu.ng

Introduction

Food and nutritional insecurity remain major concerns across Africa, particularly in Nigeria, where the situation is increasingly being intensified by climate change and environmental instability (Edem *et al*., 2025a; Akinkuolie *et al*., 2025). Despite the availability of several indigenous and multipurpose crops with significant economic and nutritional value, many of these species are still neglected and underutilised (Edem *et al*., 2025b). *Jatropha* spp*.,* popularly known as physic nut, is one of such crops. It belongs to the *Euphorbiaceae* family and has attracted attention because of its diverse industrial, medicinal, and agricultural applications (Openshaw, 2000; Islam *et al*., 2011; Sangale *et al*., 2022; Edu *et al*., 2025). In recent years, there has been growing interest in exploring underutilised plant species such as *Jatropha curcas* as part of broader efforts to improve food security and reduce malnutrition, especially in developing countries (Murthy *et al*., 2020; Mudau *et al*., 2022). However, the effective improvement and conservation of such crops depend largely on a proper understanding of their genetic makeup and the level of variation that exists within and among populations. This includes both cultivated accessions and local landraces that may possess unique adaptive traits. For this reason, genetic diversity studies have become increasingly important in *Jatropha* research. Although, several approaches involving morphological, biochemical, cytogenetic, and molecular analyses have all been used to provide base-line data into the genetic structure of plant populations (Pecina-Quintero *et al*., 2014; Salgotra and Chauhan, 2023; Guo *et al*., 2024), little or no information has been reported with the use of rbcL. It is important to note that understanding genetic diversity forms the basis for sustainable utilisation of plant genetic resources and remains a critical requirement for successful crop breeding programmes (Salgotra and Chauhan, 2023).

Jatropha species have reportedly been used in different parts of the world for a wide range of medicinal purposes. Traditionally, the plant has been used in the treatment of several health conditions, including alopecia, burns, cough, diarrhoea, fever, ulcers, tumours, convulsions, dermatitis, dyspepsia, dropsy, syphilis, and yaws (Ciappina *et al*., 2017; Babil, 2021). Among the different parts of the plant, the latex is very valued in traditional medicine and has commonly been applied in the management of burns, haemorrhoids, ringworm, and skin ulcers. Several studies have also shown that *J. curcas* contains a number of biologically active compounds that may explain many of its medicinal properties. This has been proven in phytochemical investigations of the latex which revealed the presence of peptides such as curcucyclins A and B, which are reported to possess cytotoxic activities (Sharma *et al*., 2016; Tinpun *et al*., 2020; Komolafe

et al., 2024). Additionally, curcain, a protease isolated from the latex, has demonstrated wound-healing efficacy in murine models (Salim *et al.,* 2018; Tinpun *et al.,* 2020).

Plant germplasm is characterised by highly heritable traits, including seed proteins, molecular markers, and morphological or agronomic characteristics (Pipan *et al.,* 2023). Molecular markers are increasingly replacing morphological descriptors for applications such as evolutionary studies, genetic diversity estimation, and identification of duplicates (Bidyananda *et al.,* 2024). Understanding genetic diversity within and among germplasm samples enables effective characterisation and supports crop improvement efforts (Govindaraj *et al.,* 2015; Osuagwu and Edem, 2020; Edem *et al.,* 2024).

The theoretical benefits of genetic markers and linkage maps in plant breeding were first proposed eight decades ago (Lateef *et al.,* 2015; Alemu *et al.,* 2024). However, the development of DNA marker technology in the 1980s provided the tools necessary to trace agronomically significant traits accurately (Amiteye, 2021) DNA-based molecular markers have since become essential in fields such as genetic engineering, plant breeding, and taxonomy (Nadeem *et al.,* 2017). Biochemical markers, including secondary metabolites and macromolecules such as proteins and DNA, have been widely used, though environmental influences limit the reliability of some markers. Unlike other marker types, DNA markers, especially those derived from the chloroplast genome tend to be more stable and reliable, making them particularly useful for studying evolutionary patterns and resolving phylogenetic relationships (Pezoa *et al.,* 2021).

The rbcL gene is responsible for producing the large subunit of the enzyme ribulose-bisphosphate carboxylase/oxygenase (RuBisCo), which plays a major role in photosynthesis. RuBisCo is directly involved in carbon fixation and helps plants adjust to changing levels of atmospheric CO_2 (Ichikawa *et al*., 2008; Waheeda *et al*., 2023; Zhao *et al*., 2024; Shi *et al*., 2024). The gene exists as a single-copy sequence and is approximately 1,430 base pairs in length. The slow rate of evolution in the rbcL region has made it one of the most widely used genetic markers in phylogenetic and molecular systematic studies (Waheeda *et al*., 2023; Zhao *et al*., 2024). Researchers often rely on this gene when studying evolutionary relationships among plant species because its sequence is relatively stable and easy to compare across taxa. It has also been useful in investigating variation within species and in tracing the evolutionary and geographic origins of different plant groups (Jiang *et al*., 2016; Yong *et al*., 2024).

Despite the considerable research attention given to Jatropha species worldwide, much of the existing work has centred on their cultivation practices and geographical distribution. In contrast, studies focusing on genetic variation

and crop improvement remain relatively limited (Pecina-Quintero *et al*., 2014; Montes Osorio *et al*., 2014; Vandepitte *et al*., 2019). The lack of well-developed cultivars that combine resistance to major pests and diseases with high productivity under large-scale cultivation highlights the need for more focused breeding and genetic improvement efforts (Van Esse *et al*., 2020). Although *Jatropha curcas* is widely recognised in Nigeria for its economic value and environmental benefits, its potential has yet to be fully exploited. This is largely due to limited information on its nutritional and antinutritional characteristics (Francis *et al.,* 2005). In this context, DNA-based markers, particularly the *rbcL* gene, provide a dependable approach for exploring genetic variation and supporting crop improvement efforts (Bidyananda *et al.,* 2024). Even though *Jatropha* has been studied in different parts of the world, there is still a shortage of detailed studies focusing on the genetic diversity of Nigerian landraces. Therefore, this study set out to characterise *Jatropha* landraces collected from various locations across Nigeria using genetic data, with emphasis on *rbcL*-based markers. The goal was to better understand their genetic diversity and contribute useful information for the development of improved varieties.

Materials and methods

Study location

This study was conducted at Inqaba Biotech, Ibadan, Oyo State, Nigeria.

Sample collection

Leaf samples were collected from forty (40) *Jatropha* landraces obtained from diverse agroecological regions of Nigeria. Fresh leaves from each landrace were carefully harvested and placed in appropriately labelled sample bags to ensure accurate identification and prevent mix-ups during subsequent analyses (Table 1).

Table 1. Forty landraces of *Jatropha spp* used in the study and their locations

Sample No.	Location	Latitude	Longitude
1	KaruKarama, Jos, Plateau	9.744537	8.837208
2	Ndegwu, Owerri, Imo	5.543245	6.986405
3	Rumodome, Rivers	4.888140	7.007098
4	Kanshio, Asuir, Benue	7.697007	8.537146
5	Rimi, kokona, Nassarawa	8.915968	7.889378
6	Amassoama, Bayelsa	4.971469	6.124349
7	Aba, Abia	5.135215	7.354580
8	Kaba, Kubwa, F.C. T	9.126189	7.312542

Sample No.	Location	Latitude	Longitude
9	Kudy-kogin, Dutse, Jigawa	11.725733	9.367135
10	Opi, Nsukka, Enugu	6.769912	7.434066
11	Obubra, Cross River	5.991089	8.258368
12	Ashaka, Gombe	10.915930	11.480775
13	Faggae, Kano	12.024042	8.528676
14	Aguata, Anambra	6.018366	7.075524
15	Uyo, Akwa-ibom	5.008406	7.898525
16	Yola, Adamawa	9.203496	12.495390
17	Ikorodu, Lagos	6.629484	3.518274
18	Bauchi	11.398440	6.793149
19	Ozoro, Delta	5.538727	6.228469
20	Ekete, Orhuwhorun, Delta	5.500385	5.820495
21	Biu Emirates, Borno	10.617181	12.152730
22	Abakaliki, Ebonyi	6.336659	8.087300
23	Jalingo, Taraba	8.903073	11.314469
24	Umuonaje, Asaba, Delta	6.196002	6.718493
25	Alafia, Ibadan, Oyo	7.362198	3.819935
26	Ota, Ogun	6.592366	3.201195
27	Ojota, Lagos	6.584031	3.376412
28	Safana, Katsina	12.402568	7.405300
29	Ushafa, F.C. T	9.228900	7.390215
30	Newkaru, Karu, Nassarawa	9.013259	7.632999
31	Ado, Abuja, F.C. T	9.040931	7.584636
32	VandeIkya, Benue	6.811492	9.050438
33	Calabar South, Calabar, Cross River	4.982873	8.334503
34	Calabar Municipal, Calabar, Cross River	5.026875	8.373303
35	Nung-ukana, Ibesikpo, AkwaIbom	4.921256	7.966280
36	Obudu, Cross River	6.672476	9.163086
37	Ita, Uyo, Akwa-Ibom	4.989842	7.888318
38	Ikot–Ekpene, Akwa-Ibom	5.200653	7.700433
39	Itu, Akwa-Ibom	5.050375	7.889212
40	Idomi, Cross-river	5.758464	8.084197

Genomic DNA extraction

DNA extraction was carried out on the collected samples using the ZR Quick-DNA Plant/Seed Miniprep™ Kit (Zymo Research, California, USA) according to the manufacturer's guidelines. The concentration and purity of the extracted genomic DNA were then evaluated using a NanoDrop Lite ND-2000 spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA) before further analysis.

PCR amplification

Amplification of the ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene region was carried out using OneTaq® Quick-Load®

2X Master Mix (NEB, Catalogue No. M0486). The reaction mixture was prepared according to the conditions presented in Table 2. PCR amplification employed the primer pair rbcL-F535 (5′-ATGTCACCACAAACAGAGACTAAAGC-3′) and rbcL-R705 (5′-GTAAAATCAAGTCCACCRCG-3′). Reactions were performed in an Eppendorf Mastercycler Nexus Gradient 230 thermocycler using the cycling parameters outlined in Table 3. Following amplification, the PCR products were stained with GelRed nucleic acid stain (Thermo Fisher Scientific) and separated on a 2% agarose gel to confirm successful amplification. The gels were visualised under UV light using the Gel Doc EZ Gel Documentation System (Bio-Rad). Purification of the amplified products was conducted using the Zymo DNA Clean & Concentrator Kit (Zymo Research).

Table 2. PCR amplification

Component	Volumes for a 12.5µL reaction
Template DNA	2µL
10µM Forward Primer	0.5µL (10nM)
10µM Reverse Primer	0.5µL (10nM)
One Taq Quick Load 2X Master Mix with Standard Buffer	12.5µL
Nuclease free water	9.5µL

Table 3. Thermal cycling conditions

Step	Stage of PCR	Temperature (°C)	Time (min: secs)
1	Initial Denaturation	94	5:00
2	Denaturation	94	0:30
3	Annealing	50	1:00
4	Extension	68	1:30
5	Final Extension	68	10:00
6	Hold	4	Hold

DNA sequencing

Sequencing of the purified fragments was performed using the Nimagen Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1 (BRD3-100/1000) following the manufacturer's guidelines. The labelled products were subsequently cleaned using the ZR-96 DNA Sequencing Clean-up Kit (Catalogue No. D4053) and analysed on an Applied Biosystems ABI 3500XL Genetic Analyser equipped with a 50 cm array and POP7 polymer.

Data analysis

The gene sequences were visualised and edited as chromatograms using Chromas software version 2.6.4. Genetic diversity parameters, such as the

number of segregating sites, nucleotide diversity (Pi), number of haplotypes (h), and haplotype diversity (Hd), were calculated using GenAlEx 6.5 (Peakall and Smouse, 2012). Sequence identities and similarities were determined using the BLASTn search tool available through the NCBI platform. To investigate the evolutionary relationships among the sampled accessions, a phylogenetic tree was constructed using the Neighbour-Joining approach described by Saitou and Nei (1987). The robustness of the inferred relationships was evaluated through bootstrap analysis based on 1,000 replicates, and the resulting bootstrap consensus tree was used to represent the evolutionary history of the sequences (Soltis and Soltis, 2003). Genetic distances among sequences were estimated using the Maximum Composite Likelihood model (Tamura *et al*., 2004), with values expressed as the number of nucleotide substitutions per site. Variation in substitution rates across sites was accounted for by applying a gamma distribution with a shape parameter of 1. In addition, Analysis of Molecular Variance (AMOVA) was performed in GenAlEx 6.5 to further assess the distribution of genetic variation within and among the studied populations.

Results

Evaluation of genetic variability

*DNA polymorphism and phylogenetic analysis of the rbc*L *gene in Jatropha*

The DNA polymorphism generated from the analysis of 40 *rbcL* gene sequences of *Jatropha* accessions are presented in Table 4. A total of 229 segregating sites and 685 mutational events were detected across the dataset, indicating the presence of considerable sequence variation among the sampled accessions. The analysis also identified 39 distinct haplotypes from the 40 sequences examined, reflecting a remarkably high level of haplotypic variation. This observation was further supported by a haplotype diversity value of 0.999, suggesting that nearly every accession possessed a unique genetic profile within the *rbcL* region. The low variance (0.00004) and standard deviation (0.006) associated with this estimate indicate that the observed diversity was consistent across the dataset. Nucleotide diversity (Pi) was relatively high at 0.70410, while the average number of nucleotide differences (k) was estimated at 161.238. The overall variance for nucleotide differences was calculated to be 56.502.

Table 4. DNA polymorphism parameters analyzed for *rbc*L gene from the 40 different *Jatropha* landraces

Parameters	Scores
Number of sequences:	40
Selected region	1-603
Number of sites:	603
Total number of sites (excluding sites with gaps / missing data)	229
Number of polymorphic (segregating) sites, S	229
Total number of mutations, Eta	685
Number of Haplotypes, h	39
Haplotype (gene) diversity, Hd	0.999
Variance of Haplotype diversity	0.00004
Standard Deviation of Haplotype Diversity	0.006
Nucleotide diversity, Pi:	0.70410
Total variance of k (free recombination), V(k):	56.502

BLAST results

A total of 43 nucleotide sequences were included in the analysis, taking into account codon positions at the 1st, 2nd, and 3rd sites, along with noncoding regions. Sites with gaps or missing data were removed to ensure accuracy, leaving a final dataset of 194 positions. Phylogenetic analysis was then carried out using MEGA5 software. The results of the BLAST similarity search for the sequences obtained from the 40 *Jatropha* samples are summarised in Table 5, where both sequence similarity and inferred phylogenetic relationships are presented.

Analysis of molecular variance, shannon information, diversity, and allele frequency of selected rbcL sequences

To assess the genetic structure of the selected *rbcL* sequences, an Analysis of Molecular Variance (AMOVA) was performed by dividing the sequences into two populations. As shown in Table 5 and illustrated in Figure 1, the majority of genetic variation (99%) was found within populations, with only 1% of variation occurring between populations. The estimated variation among populations was 0.763, whereas variation within populations was much higher at 137.379. Additional diversity metrics for both populations are provided in Table 6. The total number of different alleles in each population was 2,000. The mean number of effective alleles was 1.909 for population 1 and 1.904 for population 2. Shannon's information index was 0.667 for population 1 and 0.665 for population 2, reflecting similar levels of genetic information across both groups. The observed diversity values were very similar between the two populations,

recorded as 0.474 for population 1 and 0.473 for population 2. A similar pattern was seen in the unbiased diversity estimates, which were slightly higher at 0.499 and 0.496, respectively.

Phylogenetic tree reconstruction of the sequences

The phylogenetic analysis of the rbcL sequences resolved the samples into two major groups. Group I consisted mainly of *Cnidoscolus aconitifolius*, while Group II was made up primarily of *Jatropha curcas* (Figure 2). A closer look at the tree further showed some variation within particular clades in both groups (Figure 3). The clade values, which ranged from 0 to 3, reflected differences in how closely related the sequences are, with some showing strong similarity while others were more genetically distinct. Sequences with a clade value of 2 exhibited greater similarity, whereas those with a value of 3 demonstrated a higher level of dissimilarity (Figure 2).

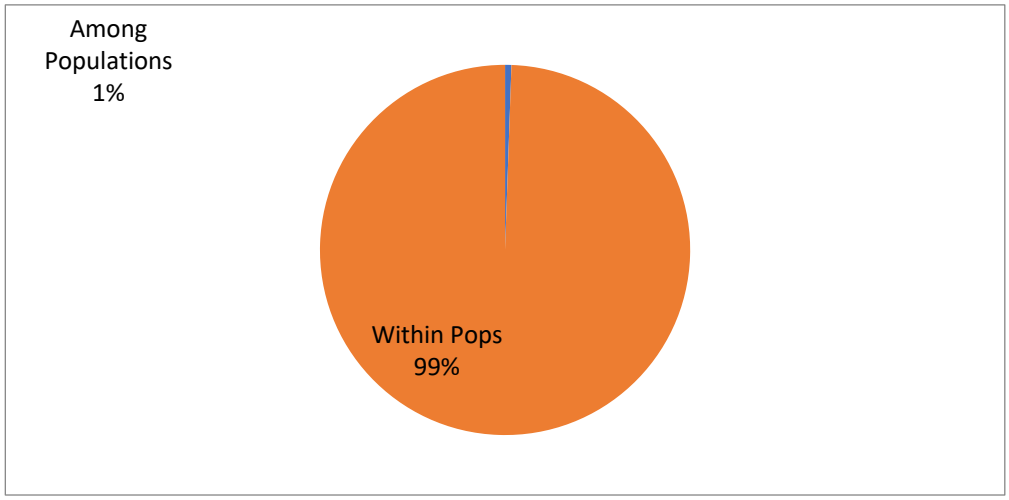

Figure 1. Analysis of molecular variance of the populations of *Jatropha* studied

Table 5. BLAST similarity search of the sequences obtained for the 40 *Jatropha* samples

S/N	Sequence Name	Hit in NCBI Database	Old Name	Total Score	Query Coverage	E-Value	% Identity	Accession No
1	10_RBCLA_R_G10_19	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
2	11_RBCLA_R_E11_14	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
3	12_RBCLA_R_G09_21	*Jatropha Curcas*	*Jatropha Curcas*	1014	100	0	99.82	MT385752
4	13_RBCLA_R_F11_17	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
5	14_RBCLA_R_G11_20	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
6	15_RBCLA_R_G12_21	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
7	16_RBCLA_R_H11_23	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
8	17_RBCLA_R_A11_02	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
9	19_RBCLA_R_A12_03	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
10	1_RBCLA_R_A11_02	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
11	25_RBCLA_R_B12_06	*Jatropha Curcas*	*Jatropha Curcas*	1018	100	0	100	KP827667
12	29_RBCLA_R_B10_04	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
13	2_RBCLA_R_B11_05	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	357	100	6.00E-94	99	PP063988
14	31_RBCLA_R_H12_24	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
15	34_RBCLA_R_A01_01	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1016	100	0	99.82	MZ045411
16	35_RBCLA_R_B01_04	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1018	100	0	100	MZ045411
17	36_RBCLA_R_C01_07	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
18	37_RBCLA_R_D01_10	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
19	38_RBCLA-R_A01_01	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1022	100	0	100	MZ045411
20	39_RBCLA_R_E01_13	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
21	40_RBCLA_R_F01_16	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1020	100%	0	100	MZ045411
22	5_RBCLA_R_C11_08	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1016	100	0.00E+00	99.82	MZ045411
23	7_RBCLA_R_F10_16	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	998	100	0	99.46	MZ045411
24	4_RBCLA_F_H07_22	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	998	100	0	99.46	MZ045411
25	18_RBCLA_F_C10_07	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	996	99	0	99.63	MZ045411

S/N	Sequence Name	Hit in NCBI Database	Old Name	Total Score	Query Coverage	E-Value	% Identity	Accession No
26	30_RBCLA_R_H07_22	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
27	33_RBCLA_R_B12_06	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
28	20_RBCLA_R_F07_16	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
29	27_RBCLA_R_H11_23	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
30	28_RBCLA_R_G07_19	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
31	32_RBCLA_R_A12_03	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
32	6_RBCLA_R_E07_13	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411
33	9_RBCLA_F_B08_05	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	999	99	0	99.45	MZ045411
34	21_RBCLA_F_C08_08	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	999	100	0	99.63	MZ045411
35	23_RBCLA_F_A09_03	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	999	100	0	99.63	MZ045411
36	3_RBCLA_F_G07_19	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	977	100	0	99.08	MZ045411
37	8_RBCLA_F_A08_02	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	994	99	0	99.63	MZ045411
38	22_RBCLA_F_H08_23	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	987	100	0	99.45	MZ045411
39	12_RBCLA_R_G09_21	*Jatropha Curcas*	*Jatropha Curcas*	1014	100	0	99.82	MT385752
40	24_RBCLA_H09_24	*Cnidoscolus aconitifolius*	*Jatropha tanjorensis*	1003	99	0	99.82	MZ045411

E-value = expected value

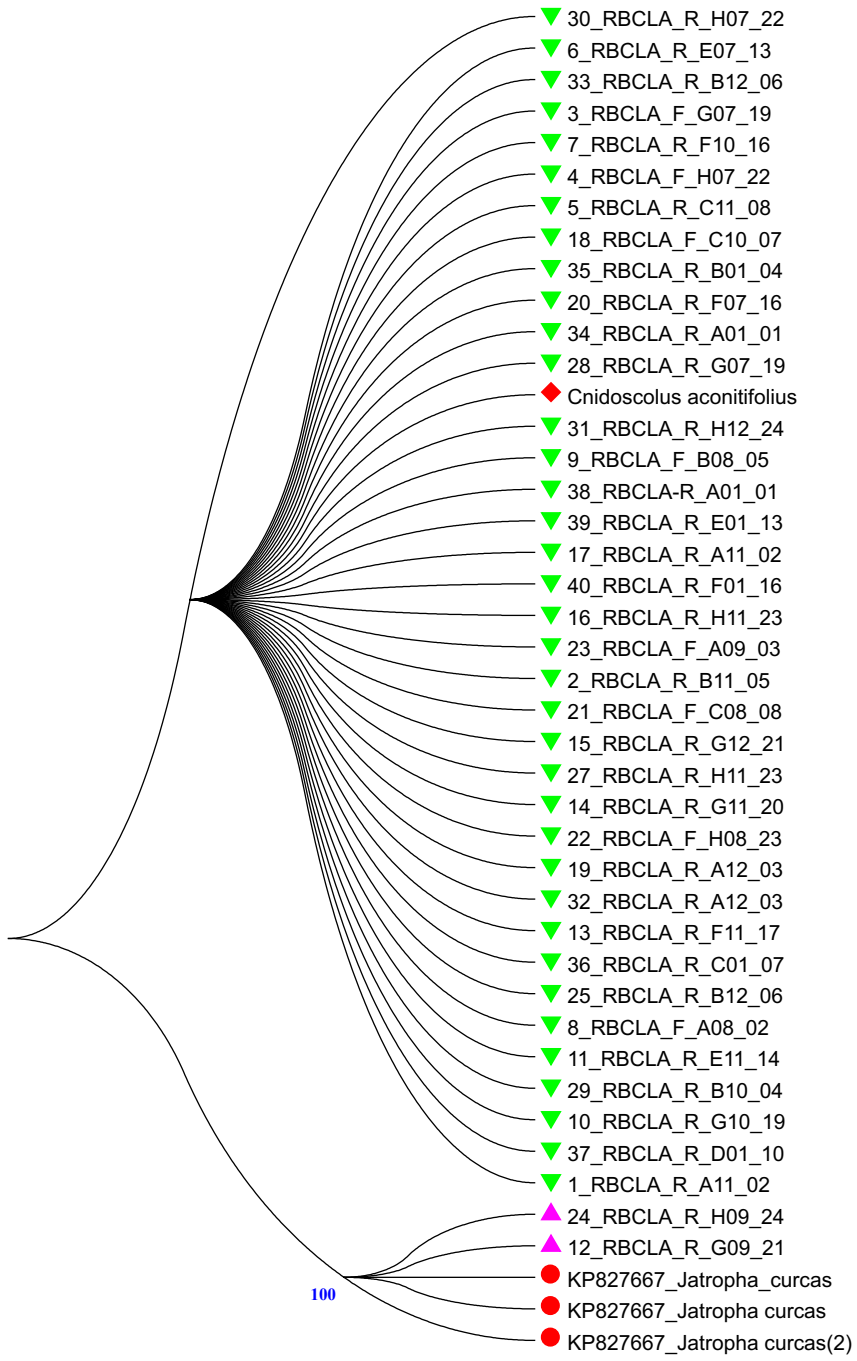

Figure 2. Consensus phylogenetic trees of generated sequences from NCBI

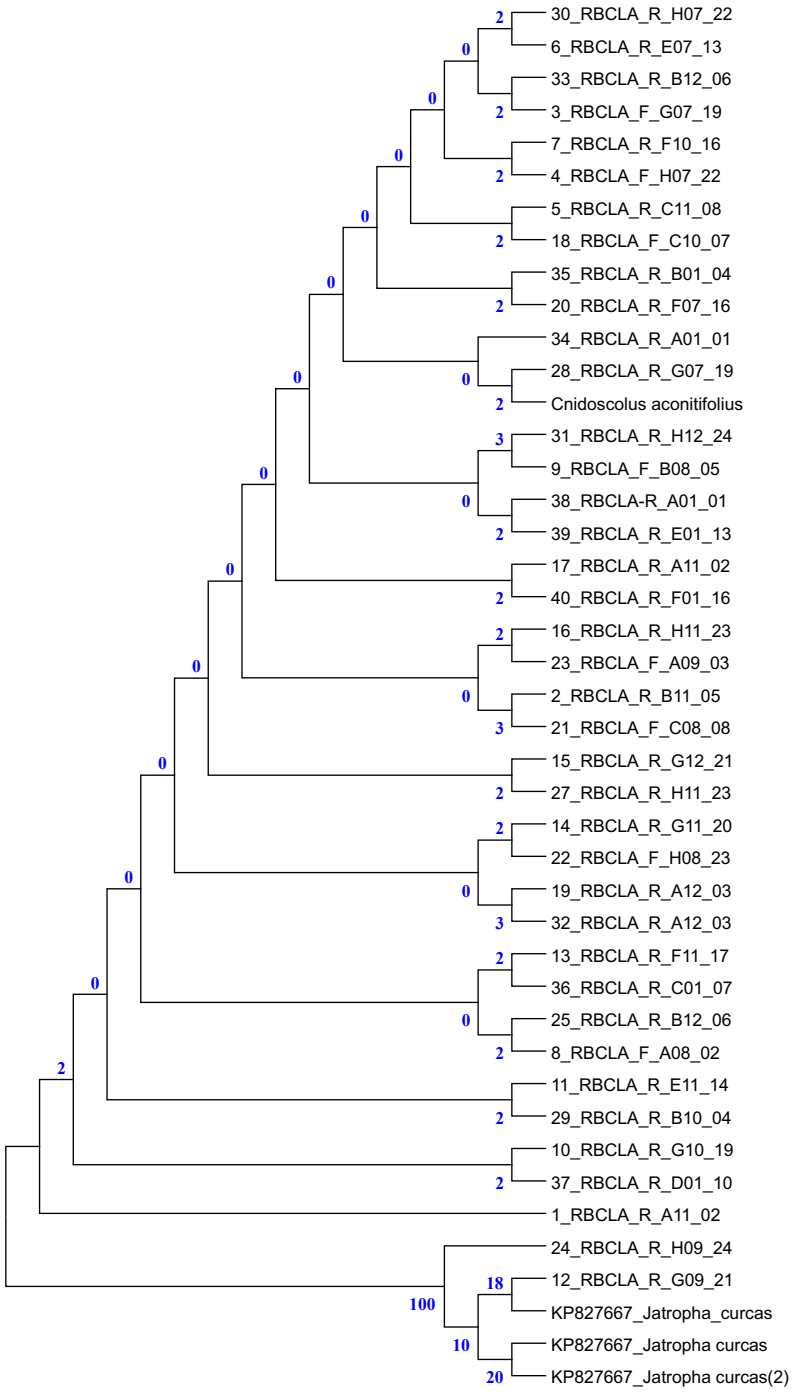

Figure 3. Original phylogenetic trees of generated sequences from NCBI

Table 6. Means and Standard Error (S.E.) over Loci for each Population

Pop		N	Na	Ne	I	H	Uh
Population 1	Mean	20.000	2.000	1.909	0.667	0.474	0.499
	SE	0.000	0.000	0.005	0.002	0.001	0.002
Population 2	Mean	21.000	2.000	1.904	0.665	0.473	0.496
	SE	0.000	0.000	0.005	0.002	0.002	0.002
Total	Mean	20.500	2.000	1.907	0.666	0.473	0.498
	SE	0.015	0.000	0.003	0.001	0.001	0.001

Key: Na = Number of Different Alleles, Ne = Number of Effective Alleles, I = Shannon's Information Index, h = Diversity, uh = Unbiased Diversity, Pop = Population.

Discussion

DNA polymorphism analysis involves two or more variant forms of a DNA sequence (Trent, 2012). Single-nucleotide polymorphisms are the most common type of genetic variation (Kaushik *et al.,* 2019). Various parameters have been developed to estimate DNA polymorphism, including haplotypes, nucleotide diversity, segregating sites, and total number of mutations. The results of the DNA polymorphism analysis provide valuable insights into the genetic diversity within *Jatropha* species based on the *rbcL* gene sequences. The large number of segregating sites (229) and mutations (685) observed among the analysed sequences points to a high degree of genetic variability within the studied accessions. This level of variation is often associated with the evolutionary diversification of populations through the gradual accumulation of genetic changes over time (Li, 2011).

A haplotype is a group of alleles in an organism that are inherited together from a single parent (Balasubramanian *et al.,* 2004). It is a measure of the uniqueness of a particular haplotype in a given population (Quan *et al.,* 2021). The detection of 39 haplotypes, coupled with a haplotype diversity rate of 0.999, underscores the substantial genetic richness within the dataset. This near-maximal haplotype diversity suggests that the *rbcL* gene exhibits significant variation among the sampled populations, which may be attributed to evolutionary pressures, ecological factors, or geographical influences affecting these populations (Qi *et al.,* 2023). The minimal variance (0.00004) and low standard deviation (0.006) of haplotype diversity highlight the robustness of this metric, suggesting that the genetic variation observed is consistent and reliable.

Nucleotide diversity (π) is widely used as an indicator of the level of genetic variation present within a population (Buckler *et al*., 2002). In the present study, the nucleotide diversity value of 0.70410 was relatively high, reflecting substantial sequence variation among the *Jatropha* accessions. Since π represents

the average number of nucleotide differences per site between pairs of sequences, the observed value suggests a considerable degree of genetic differentiation within the sampled population (Cork *et al*., 2005). A similar pattern was observed for the average number of nucleotide differences (k), which was estimated at 161.238. This relatively large value indicates that numerous nucleotide substitutions separated the sequences, providing further evidence of the high level of polymorphism detected among the accessions.

The BLAST analysis of the 43 nucleotide sequences, including the first, second, and third codon positions as well as non-coding regions, revealed useful patterns of genetic relationships among the Jatropha samples. During data preparation, sites containing gaps or missing information were excluded to improve the quality of the sequence alignment. After this filtering step, 194 reliable nucleotide positions were retained for further analysis. The use of a refined dataset provided a more accurate basis for assessing genetic variation and understanding the level of diversity present among the studied samples (de Boer *et al*., 2022).

The phylogenetic analysis provided a clearer view of the evolutionary relationships among the 40 Jatropha samples. The BLAST similarity results indicated that several samples had closely related nucleotide sequences and were grouped together within the same clusters, whereas some samples showed greater genetic differences. These patterns reveal the presence of genetic variation among the Jatropha samples and demonstrate the usefulness of sequence-based analysis for understanding diversity that can support future breeding programmes and genetic improvement strategies (Wang *et al*., 2009).

The sequence identity results presented in Table 5 reveal that some groups of Jatropha samples share a high level of genetic similarity. This finding agrees with the report by Guo *et al*. (2024), who observed considerable genetic relatedness among Chinese Jatropha populations, which was attributed to their common evolutionary background and possible recent divergence. Similarly, the pattern observed in this study suggests that samples within the same clusters may have originated from related ancestral sources or may have experienced some level of gene exchange over time. However, the samples showing greater sequence differences may indicate the presence of separate genetic lineages with distinct evolutionary histories.

The Analysis of Molecular Variance (AMOVA) results obtained from the rbcL gene sequences provide important information on the genetic variation and population structure of the samples examined. The partitioning of sequences into two populations yielded striking results, with 99% of the genetic variation occurring within populations and only 1% occurring among them. This suggests that the genetic diversity within individual populations is far greater than the

differences observed between the populations, which could imply a high degree of genetic homogeneity across the groups sampled. (Szczecińska *et al.,* 2016). Such a finding could reflect gene flow between populations or relatively recent divergence, where genetic differences have not yet accumulated at the population level. The estimated variation among populations was calculated at 0.763 while the variation within populations was considerably higher at 137.379, reinforcing the notion that within-population diversity is the dominant contributor to the overall genetic variation observed. These results are consistent with Wang *et al*. (2020), who revealed Demography and natural selection have shaped genetic variation in the widely distributed conifer Norway spruce (*Picea abies*) indicating that genetic variation in many plant species, especially those with a wide geographic distribution, often manifests more strongly within populations rather than between them.

The diversity indices presented in Table 6 provide further understanding of the level of genetic variation present among the Jatropha populations. Both populations showed a total of 2000 alleles, indicating the presence of substantial genetic variation within the sampled materials. The values obtained for the mean number of effective alleles were almost identical between the two populations (1.909 for population 1 and 1.904 for population 2), suggesting that the observed genetic variation is distributed fairly evenly across the groups. This pattern implies that the populations have retained a considerable amount of genetic diversity and do not show strong indications of genetic erosion or a recent population bottleneck (Lonsinger *et al*., 2018).

The Shannon's Information Index, which is commonly used to estimate the amount of genetic variation within a population (Konopiński, 2020), showed very similar values between the two groups, with 0.667 recorded for population 1 and 0.665 for population 2. The similarity in these values suggests that both populations have comparable levels of genetic diversity and complexity, which may contribute to their ability to respond to environmental changes. Similarly, the observed diversity values (0.474 for population 1 and 0.473 for population 2) indicate that a considerable amount of genetic variation is present within both populations (Morris *et al*., 2014). The slightly higher unbiased diversity estimates (0.499 and 0.496) further suggest that the populations have maintained their genetic variation, with no strong evidence of diversity loss resulting from genetic drift (Kitikidou *et al*., 2024).

The phylogenetic analysis of the rbcL gene sequences revealed that the studied samples were grouped into two distinct clusters, with *Cnidoscolus aconitifolius* forming one group (Group I) and *Jatropha curcas* forming another (Group II). This separation suggests that, although the two species may occupy similar environments and share some ecological features, they are genetically

distinct from each other. The observed clustering pattern reflects the evolutionary differences between the species and agrees with the findings of Ha *et al*. (2019), who reported considerable genetic variation among members of the Euphorbiaceae family. A more detailed observation of the phylogenetic tree showed that some level of variation exists among samples within the same major clusters. The formation of smaller branches within these groups suggests that closely related populations may still possess unique genetic differences. These variations could have developed over time due to factors such as geographical isolation, adaptation to different environmental conditions, or limited movement of genes between populations (Heinicke *et al*., 2017). This pattern reflects the process of population differentiation, where populations that become separated may gradually acquire their own genetic features as they respond to local conditions and reduced gene flow (Avise *et al*., 2009).

The clade values obtained from the phylogenetic analysis ranged from 0 to 3 and provided an indication of the level of relatedness among the sequences studied. Samples that grouped together with a clade value of 2 showed a higher degree of similarity, suggesting that these sequences are more closely related and may share a relatively recent evolutionary history. However, samples with a clade value of 3 displayed greater sequence differences, indicating a higher level of genetic separation. These differences may have developed as a result of factors such as environmental adaptation, genetic drift, or reduced gene exchange between populations. The pattern of clustering observed in the tree highlights the genetic complexity present among the samples and supports the suitability of the rbcL gene as a useful marker for studying genetic variation and evolutionary relationships (Singh *et al*., 2021).

The analysis of the rbcL gene sequences from the 40 Jatropha samples revealed a wide range of genetic variation among the materials studied. The detection of 229 segregating sites and 685 mutation events shows that the sequences contain numerous genetic differences, indicating considerable diversity within the population. This level of variation is further supported by the high haplotype diversity value (0.999) and nucleotide diversity value (0.70410), suggesting that the sampled Jatropha individuals possess a rich genetic background. The AMOVA results showed that most of the genetic variation (99%) was found among individuals within the populations, while only 1% of the variation occurred between populations. This pattern suggests that the two populations are not strongly separated genetically and may share a similar evolutionary background. The close similarity in diversity measures, including Shannon's information index and observed diversity, also indicates that both populations maintain comparable levels of genetic variability. The phylogenetic analysis provided additional evidence by grouping the samples into two major

clusters, with *Cnidoscolus aconitifolius* and *Jatropha curcas* forming separate genetic groups. These groupings reflect differences in their genetic makeup while also showing the relatedness among samples within each species. The findings demonstrate that the studied Jatropha materials contain substantial genetic diversity, which could be valuable for conserving existing genetic resources and supporting future breeding and improvement efforts.

Conflicts of interest

The authors declare that they have no financial or personal conflicts that could have influenced this work.

References

Akinkuolie, T. A., Ogunbode, T. O. and Adekiya, A. O. (2025). Resilience to climate-induced food insecurity in Nigeria: A systematic review of the role of adaptation strategies in flood and drought mitigation. Frontiers in Sustainable Food Systems, 8:1490133.

Alemu, A., Åstrand, J., Montesinos-López, O. A., Isidro y Sánchez, J., Fernández-Gónzalez, J., Tadesse, W., Vetukuri, R. R., Carlsson, A. S., Ceplitis, A., Crossa, J., Ortiz, R. and Chawade, A. (2024). Genomic selection in plant breeding: Key factors shaping two decades of progress. Molecular Plant, 17:552-578.

Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon, 7:e08093.

Avise, J. C. and Ayala, F. J. (Eds.). (2009). Genetics and ecological speciation. *In* In the Light of Evolution: Volume III: Two Centuries of Darwin. National Academy of Sciences, pp.61-72. Available from: https://www.ncbi.nlm.nih.gov/books/NBK219728/

Babil, Y. B. (2021). Environmental, economical, and social implications of *Jatropha*: A systematic review. Journal of Resources Development and Management, 74:2422-8397.

Balasubramanian, S. P., Cox, A., Brown, N. J. and Reed, M. W. (2004). Candidate gene polymorphisms in solid cancers. European Journal of Surgical Oncology (EJSO), 30:593-601.

Bidyananda, N., Jamir, I., Nowakowska, K., Varte, V., Vendrame, W. A., Devi, R. S. and Nongdam, P. (2024). Plant genetic diversity studies: Insights from DNA marker analyses. International Journal of Plant Biology, 15:607-640.

Buckler, E. S. and Thornsberry, J. M. (2002). Plant molecular diversity and applications to genomics. Current Opinion in Plant Biology, 5:107-111.

Ciappina, A. L., Ferreira, F. A., Pereira, I. R., Sousa, T. R., Matos, F. S., Melo-Reis, P. R., Gonçalves, P. J., Bailão, E. F. L. and Almeida, L. M. (2017). Toxicity of *Jatropha curcas* L. latex in *Allium cepa* test. Bioscience Journal, 3:1295-1304.

Cork, J. M. and Purugganan, M. D. (2005). High-diversity genes in the *Arabidopsis* genome. Genetics, 170:1897-1911.

de Boer, H., Rydmark, M. O., Verstraete, B. and Gravendeel, B. (Eds.). (2022). *Molecular identification of plants:* From sequence to species. Pensoft Publishers.

Edem, U. L., Osuagwu, A. N., Edu, N. E., Agbor, R. B., Emeagi, L. I., Uno, U. U., Ekerette, E. E. and Udensi, O. O. (2025a). Genetic insights into the African yam bean (*Sphenostylis stenocarpa* (Hochst. ex A. Rich.) Harms) revealed through the ribulose-1,5-bisphosphate carboxylase/oxygenase large (*rbcL*) gene. Journal of Food Legumes, 38:236-245.

Edem, U. L., Osuagwu, A. N., Edu, N. E., Agbor, R. B., Emeagi, L. I., Oluwatobi, C. D., Uno, U. U. and Ekerette, E. E. (2025b). Unveiling the evolutionary history and structural dynamics of *Dioscorea bulbifera* L. and congeners through *matK* gene *in silico* analysis. Tropical Journal of Natural Product Research, 9:2220.

Edem, U. L., Osuagwu, A. N., Edu, N. E., Emeagi, L. I., Agbor, R. B., Nsungo, N. E., Uno, U. U. and Ekerette, E. E. (2024). Assessing genetic diversity in squash pumpkin (*Cucurbita moschata*) through computational analysis of plastid genes. Tropical Journal of Natural Product Research, 8:9310-9314.

Edu, N., Uzoma, L., Edem, U., Obua, P., lsorshe, I. and Ogbaji, H. (2025). Phytochemical profiling and bioactive compound variation in *Jatropha* landraces from Nigeria: Implications for agricultural and medicinal applications. Tropical Journal of Pharmaceutical Research, 24:385-393.

Francis, G., Edinger, R. and Becker, K. (2005). A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India: Need, potential and perspectives of *Jatropha* plantations. Natural Resources Forum, 29:12-24.

Govindaraj, M., Vetriventhan, M. and Srinivasan, M. (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical

perspectives. Genetics Research International. https://doi.org/10.1155/2015/431487Digital Object Identifier

Guo, G., Tang, L. and Xu, Y. (2024). Genetic diversity and population genetic structure of *Jatropha curcas* L. accessions from different provenances revealed by amplified fragment-length polymorphism and inter-simple sequence repeat markers. Forests, 15:1575.

Ha, J., Shim, S., Lee, T., Kang, Y. J., Hwang, W. J., Jeong, H., Laosatit, K., Lee, J., Kim, S. K., Satyawan, D., Lestari, P., Yoon, M. Y., Kim, M. Y., Chitikineni, A., Tanya, P., Somta, P., Srinives, P., Varshney, R. K. and Lee, S. H. (2019). Genome sequence of Jatropha curcas L., a non-edible biodiesel plant, provides a resource to improve seed-related traits. Plant Biotechnology Journal, 17:517-530.

Heinicke, M. P., Jackman, T., Bauer, A. M. and others. (2017). The measure of success: Geographic isolation promotes diversification in Pachydactylus geckos. BMC Evolutionary Biology, 17:1-15.

Ichikawa, K., Miyake, C., Iwano, M., Sekine, M., Shinmyo, A. and Kato, K. (2008). Ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit translation is regulated in a small subunit-independent manner in the expanded leaves of tobacco. Plant Cell Physiology, 49:214-225.

Islam, A. K. M. A., Yaakob, Z. and Anuar, N. (2011). *Jatropha*: A multipurpose plant with considerable potential for the tropics. Scientific Research and Essays, 6:2597-2605.

Jiang, G. F., Hinsinger, D. and Strijk, J. (2016). Comparison of intraspecific, interspecific and intergeneric chloroplast diversity in Cycads. Scientific Reports, 6:31473.

Kaushik, S., Kaushik, S. and Sharma, D. (2019). Functional genomics. In S. Ranganathan, M. Gribskov, K. Nakai, and C. Schönbach (Eds.), Encyclopedia of Bioinformatics and Computational Biology. Academic Press, pp.118-133.

Kitikidou, K., Milios, E., Stampoulidis, A., Pipinis, E. and Radoglou, K. (2024). Using biodiversity indices effectively: Considerations for forest management. Ecologies, 5:42-51.

Komolafe, C. J., Adetoyinbo, I. I., Alao, F. O. and Ogunsola, J. F. (2024). Phytochemical analyses and antibacterial activities of *Jatropha tanjorensis* J. L. Ellis and Saroja leaves extract against clinical pathogens. Asian Journal of Applied Chemistry Research, 15:9-23.

Konopiński, M. K. (2020). Shannon diversity index: A call to replace the original Shannon's formula with an unbiased estimator in population genetics studies. PeerJ, 8:e9391.

Lateef, D. (2015). DNA marker technologies in plants and applications for crop improvements. Journal of Biosciences and Medicines, 3:7-18.

Li, Y., Haseneyer, G., Schön, C. C., *et al.* (2011). High levels of nucleotide diversity and fast decline of linkage disequilibrium in rye (*Secale cereale* L.) genes involved in frost response. BMC Plant Biology, 11:6.

Lonsinger, R. C., Adams, J. R. and Waits, L. P. (2018). Evaluating effective population size and genetic diversity of a declining kit fox population using contemporary and historical specimens. Ecology and Evolution, 8:12011-12021.

Montes Osorio, L. R., Torres Salvador, A. F., Jongschaap, R. E., Azurdia Perez, C. A., Berduo Sandoval, J. E., Trindade, L. M., Visser, R. G. and van Loo, E. N. (2014). High level of molecular and phenotypic biodiversity in *Jatropha curcas* from Central America compared to Africa, Asia, and South America. BMC Plant Biology, 14:77.

Morris, E. K., Caruso, T., Buscot, F., Fischer, M., Hancock, C., Maier, T. S., Meiners, T., Müller, C., Obermaier, E., Prati, D., Socher, S. A., Sonnemann, I., Wäscheke, N., Wubet, T., Wurst, S. and Rillig, M. C. (2014). Choosing and using diversity indices: Insights for ecological applications from the German Biodiversity Exploratories. Ecology and Evolution, 4:1155-1169.

Mudau, F. N., Chimonyo, V. G. P., Modi, A. T. and Mabhaudhi, T. (2022). Neglected and underutilised crops: A systematic review of their potential as food and herbal medicinal crops in South Africa. Frontiers in Pharmacology, 12:809866.

Murthy, N., Anand, S. R. and Prithviraj, S. K. (2020). Underutilized potential crops for food and nutritional security under changing climate. Mysore Journal of Agricultural Sciences, 54:1-14.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G. and Baloch, F. S. (2017). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Agriculture and Environmental Biotechnology, 3:261-285.

Openshaw, K. (2000). A review of *Jatropha curcas*: An oil plant of unfulfilled promise. Biomass and Bioenergy, 19:1-15.

Osuagwu, A. N. and Edem, U. L. (2020). Evaluation of genetic diversity in aerial yam (*Dioscorea bulbifera* L.) using simple sequence repeats (SSR) markers. Agrotechnology, 9:202.

Peakall, R. and Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research, An update. Bioinformatics, 28:2537-2539.

Pecina-Quintero, V., Anaya-López, J. L., Zamarripa-Colmenero, A., Núñez-Colín, C. A., Montes-García, N., Solís-Bonilla, J. L. and Jiménez-Becerril, M. F. (2014). Genetic structure of *Jatropha curcas* L. in Mexico and probable centre of origin. Biomass and Bioenergy, 60:147-155.

Pezoa, I., Villacreses, J., Rubilar, M., Pizarro, C., Galleguillos, M. J., Ejsmentewicz, T., Fonseca, B., Espejo, J., Polanco, V. and Sánchez, C. (2021). Generation of chloroplast molecular markers to differentiate *Sophora toromiro* and its hybrids as a first approach to its reintroduction in Rapa Nui (Easter Island). Plants, 10:342.

Pipan, B., Sinkovič, L., Neji, M., Janovská, D., Zhou, M. and Meglič, V. (2023). Agro-morphological and molecular characterization reveal deep insights in promising genetic diversity and marker-trait associations in *Fagopyrum esculentum* and *Fagopyrum tataricum*. *Plants*, 12:3321.

Qi, H., Sun, X., Wang, C., Zheng, D., *et al.* (2023). Geographic isolation causes low genetic diversity and significant pedigree differentiation in populations of *Camellia drupifera*, a woody oil plant native to China. Industrial Crops and Products, 192:116026.

Quan, J., Li, Y., Yang, Y., Yang, T., Sha, Y., Cai, Y., Jiao, T., Wu, J. and Zhao, S. (2021). Population genetic diversity and genetic evaluation models reveal the maternal genetic structure and conservation priority characteristics of indigenous cattle in China. Global Ecology and Conservation, 32:e01903.

Saitou, N. and Nei, M. (1987). The Neighbor-Joining Method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4:406-425.

Salgotra, R. K. and Chauhan, B. S. (2023). Genetic diversity, conservation, and utilization of plant genetic resources. Genes, 14:174.

Salim, M. N., Masyitha, D., Harris, A., Balqis, U., Iskandar, C. D., Hambal, M. and Darmawi. (2018). Anti-inflammatory activity of *Jatropha curcas* Linn. latex in cream formulation on CD68 expression in mice skin wound. Veterinary World, 11:99-103.

Sangale, P., Jadhav, V. and Jadhav, G. (2022). A review on *Jatropha curcas*. International Journal for Research Trends and Innovation, 7:115.

Sharma, A. K., Gangwar, M., Kumar, D. and Tripathi, Y. B. (2016). Phytochemical characterization, antimicrobial activity, and reducing potential of seed oil, latex, machine oil, and presscake of *Jatropha curcas*. Avicenna Journal of Phytomedicine, 6:366-375.

Shi, X., Hannon, N. M. and Bloom, A. J. (2025). Carboxylation and oxygenation kinetics and large subunit (*rbcL*) DNA sequences for Rubisco from two ecotypes of *Plantago lanceolata* L. that are native to sites differing in atmospheric CO_2 levels", Plant, Cell & Environment, 48:3159-3170.

Singh, L., Dixit, P., Srivastava, R. P., Pandey, S., Singh, A., Verma, P. C. and Saxena, G. (2021). Phylogenetic and evolutionary relationships in selected Pinus species using rbcL and matK chloroplast genes. Open Journal of Plant Science, 6:64-68.

Soltis, P. S. and Soltis, D. E. (2003). Applying the bootstrap in phylogeny reconstruction. Statistical Science, 18:256-267.

Szczecińska, M., Sramko, G., Wołosz, K. and Sawicki, J. (2016). Genetic diversity and population structure of the rare and endangered plant species *Pulsatilla patens* (L.) Mill in East Central Europe. PLOS One, 11:e0151730.

Tamura, K., Nei, M. and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America, 101:11030-11035.

Tinpun, K., Nakpheng, T., Padmavathi, A. R. and Srichana, T. (2020). *In vitro* studies of *Jatropha curcas* L. latex spray formulation for wound healing applications. Turkish Journal of Pharmaceutical Sciences, 17:271-279.

Trent, R. J. (2012). DNA genetic testing. In R. J. Trent (Ed.), Molecular Medicine (4th ed.). Academic Press, pp.81-115.

Van Esse, H. P., Reuber, T. L. and van der Does, D. (2020). Genetic modification to improve disease resistance in crops. New Phytologist, 225:70-86.

Vandepitte, K., Valdés-Rodríguez, O. A., Sánchez-Sánchez, O., De Kort, H., Martinez-Herrera, J., García-Pérez, E., De Meyer, T., Pérez-Vázquez, A., Muys, B. and Honnay, O. (2019). High SNP diversity in the non-toxic indigenous *Jatropha curcas* germplasm widens the potential of this upcoming major biofuel crop species. Annals of Botany, 124:645-652.

Waheeda, K., Kitchel, H., Wang, Q. and Chiu, P. L. (2023). Molecular mechanism of Rubisco activase: Dynamic assembly and Rubisco remodeling. Frontiers in Molecular Biosciences, 10:1125922.

Wang, H. Z., Feng, S., Lu, J., Liu, J. J., *et al.* (2009). Phylogenetic study and molecular identification of 31 *Dendrobium* species using inter-simple sequence repeat (ISSR) markers. Scientia Horticulturae, 122:440-447.

Wang, X., Bernhardsson, C. and Ingvarsson, P. K. (2020). Demography and natural selection have shaped genetic variation in the widely distributed conifer Norway spruce (*Picea abies*). Genome Biology and Evolution, 12:3803-3817.

Yong, W. T. L., Mustafa, A. A., Derise, M. R. and Rodrigues, K. F. (2024). DNA barcoding using chloroplast *matK* and *rbcL* regions for the identification of bamboo species in Sabah. Advances in Bamboo Science, 7:100073.

Zhao, L., Cai, Z., Li, Y. and Zhang, Y. (2024). Engineering Rubisco to enhance CO_2 utilization. Synthetic and Systems Biotechnology, 9:55-68.

(Received: 25 April 2025, Revised: 7 January 2026, Accepted: 10 January 2026)