Adv. Hort. Sci., 2026 40(1): 47-60

DOI: 10.36253/ahsc-18571
https://oaj.fupress.net/index.php/ahs

AHS
Advances in Horticultural Science

DNA barcoding-based assessment of genetic variation in selected Southwest Nigerian medicinal *Senna* species (Caesalpinoideae: Fabaceae)

OPEN ACCESS

O.F. Akinyemi (*), E.T. Obiwole, T.O. Bamigboye, O.O. Fawibe, A.S. Oyelakin

Pure and Applied Botany, College of Biosciences, Federal University of Agriculture Abeokuta, P.M. Box 2240, Abeokuta, Ogun State, Nigeria.

(*) Corresponding author:
akinyemiof@funaab.edu.ng

Citation:
AKINYEMI O.F., OBIWOLE E.T., BAMIGBOYE T.O., FAWIBE O.O., OYELAKIN A.S., 2026 - *DNA barcoding-based assessment of genetic variation in selected Southwest Nigerian medicinal senna species (Caesalpinoideae: Fabaceae).* - Adv. Hort. Sci., 40(1): 47-60.

ORCID:
AOF: 0000-0003-1910-0813
OET: 0000-0002-9071-8619
BTO: 0009-0002-4038-147X
FOO: 0000-0003-4522-4828
OAS: 0000-0003-0045-4224

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 3 September 2025

Accepted for publication 30 December 2025

Key words: Characterization, DNA sequences, molecular analysis, trnH-psbA intergenic spacer region.

Abstract: *Senna* species in Southwest Nigeria possess significant medicinal and economic potential yet remain underutilized. This study aimed to employ DNA barcoding to characterize twenty *Senna* accessions and assess their genetic variation. Seeds from twenty accessions comprising six species (*S. hirsuta, S. obtusifolia, S. alata, S. siamea, S. acutifolia,* and *S. occidentalis*) were collected from Southwest Nigerian states. Fresh leaf samples underwent DNA extraction, PCR amplification of the psbA-trnH intergenic spacer region, and Sanger sequencing. Molecular analyses using Bioedit, MEGA 11, and NCBI BLAST yielded high-quality DNA sequences (312-414 bp). BLAST searches confirmed species identities with exceptional accuracy. Multiple alignments revealed inter-specific variations and genus-specific conserved regions. Genetic distance analysis showed moderate diversity (0.000-0.074) between species. Base composition analysis revealed characteristic A-T rich patterns: thymine showed highest variance, followed by adenine, while cytosine and guanine exhibited lower variation. Conserved sequences provided molecular evidence of monophyletic relationships within *Senna*, while base composition variations reflected evolutionary divergence and environmental adaptation. These findings establish a molecular framework for the identification and conservation of Nigerian Senna species, supporting their development for medicinal and agricultural applications.

1. Introduction

Senna Mill is a genus of plants belonging to the subfamily Caesalpinioideae of the family Fabaceae. The genus name was derived from the Arabic sanā, describing plants whose leaves and pods have cathartic and laxative properties (Monkheang, 2011). Originally, Hutchinson (1964) moved the subfamilies (Caesalpinioideae, Papilionioideae, and Mimosoideae) to the rank of families and placed them in order Leguminales. This division was primarily based on the

AHS - Firenze University Press
ISSN 1592-1573 (on line) - 0394-6169 (print)

characteristics of the corolla and the stamens (Dutta, 2001; Judd *et al*., 2002). However, more recent phylogenetic studies of many legume groups have unambiguously demonstrated the inadequacies of the tribal classifications proposed by Polhill and Raven (1981), Polhill (1994), and Lewis *et al*. (2005) because of the non-monophyly of most of the traditionally recognised tribes (LPWG, 2013). Currently, based on the phylogenetic structure of the family Leguminosae, the Legume Plant Working Group (LPWG, 2017) recognised six sub-families within Leguminosae (Fabaceae), and Caesalpinioideae was included.

The genus *Senna* (Fabaceae) contains around 300 species distributed pantropically across the Americas, Africa, Asia, and Oceania. The key morphological features include the lack of bracteoles on floral pedicels, clavoform to pyramidal extrafloral nectaries, and indehiscent fruits appearing as cylindrical pods or compressed legumes (Correia and Conceição, 2017). The genus is divided into six sections: Astroites, Chamefistula, Paradictyon, Peiranisia, Psilorhegma, and Senna (Irwin and Barneby, 1982).

In Nigeria, three *Senna* species hold particular ethnomedicinal and pharmacological significance. *Senna alata*, known as «Asunwon oyinbo» by the Yoruba, treats typhoid, diabetes, malaria, asthma, and skin infections (Oladeji *et al*., 2020), with decoctions used across regions for wounds, respiratory infections, and diarrhea. *Senna occidentalis* («Sanga-sanga» in Hausa, «Akidiagbara» in Igbo) serves as an antimalarial (Daskum *et al*., 2019) and remedy for hepatitis and liver diseases (Ibrahim *et al.,* 2022). *Senna siamea* leaves combine with *Carica papaya* and *Cymbopogon citratus* to combat malaria fever (Adewole *et al*., 2024). Pharmacological studies confirm antimicrobial (Oladeji *et al*., 2020; Tamasi, 2021), antimalarial (Daskum *et al*., 2019), hepatoprotective (Ibrahim *et al*., 2022), antioxidant properties (Atanu *et al.,* 2022), and multiple activities including antibacterial, antifungal, anticancer, and antidiabetic effects, attributed to secondary metabolites (Oladeji *et al.,* 2020).

DNA barcoding is one of the molecular techniques employed in recent times to resolve new evolutionary and taxonomical queries in plant diversity studies (Arif *et al.,* 2010). Molecular identification of plant species currently plays a key role in the assessment of conserving biodiversity especially in the phase of many plant species going into extinction (de Boer *et al*., 2022). DNA barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed-upon position in the genome (Kress and Erickson, 2008; Arif *et al.,* 2010; de Vere, 2015; Kenfack *et al.*, 2022). Accurate species identification is a prerequisite for conducting numerous basic and applied studies on monitoring and conserving natural resources, blocking the traffic of endangered and invasive species, as well as controlling the quality of pharmaceutical and food products. In this context, molecular markers are indispensable tools for measuring the diversity of plant species (Pang *et al*., 2012).

The *trnH-psbA* intergenic spacer region is a non-coding region located between the trnH and psbA genes in the chloroplast genome and described as a diagnostic barcode in distinguishing plant species. The trnH-psbA intergenic spacer is widely recognized as the most commonly used non-coding universal genetic marker in plant DNA barcoding and molecular phylogenetic studies, due to its high variability across plant species (CBOL, 2009; Hao *et al*., 2010; Hollingsworth *et al*., 2011; Monkheang *et al*. 2011; Pang *et al*., 2012; Feng *et al*., 2018; Loera-Sanchez *et al*., 2020; Hassan, 2023). Hassan (2023) evaluated a DNA-based assay that examined the resolution and sensitivity of the trnH-psbA intergenic spacer region as a DNA barcoding marker. Their findings suggest that these genetic markers could provide a novel method for understanding the evolutionary relationships and classification of closely related *Prunus* species.

The trnH-psbA region is widely used for plant DNA barcoding due to its variability and ease of amplification, offering significant discriminatory power for species identification and inferring genetic relationships (Damthongdee *et al*., 2024). It has been crucial in resolving inter-specific relationships and identifying cryptic diversity among closely related taxa (Hussain *et al*., 2019). In Fabaceae, trnH-psbA and other chloroplast markers (matK and rbcL) effectively resolve generic and species-level relationships, particularly in regions with inadequate molecular datasets (Bruneau *et al*., 2013). Although *Senna* is underrepresented in molecular studies from Nigeria, analogous research in legumes has established the importance of generating barcoding data for conservation planning and sustainable use.

Research on medicinal and economically valuable plants across Africa and Asia demonstrates that

integrating trnH-psbA data provides a robust framework for taxonomy, phylogeny, biogeographic tracing, and population structure analysis. Comparative chloroplast genome studies including the trnH-psbA region have identified mutational hotspots that serve as informative molecular markers for species discrimination and phylogenetic inference (Dong *et al*., 2021).

DNA barcoding in medicinal legumes like *Flemingia* and *Sophora* effectively resolves relationships in understudied regions, establishing baselines for population genetics and evolutionary studies (Duan *et al*., 2025; Wei *et al.,* 2025 b). Sub-Saharan investigations using DNA barcoding have revealed substantial intra-specific genetic diversity correlated with ecological and geographical factors (Akinro *et al*., 2019). A DNA barcode dataset for Nigerian *Senna* species could similarly uncover cryptic diversity and inform both in situ and ex situ conservation strategies.

Generating trnH-psbA molecular data from Nigerian *Senna* species would enable taxonomic clarification and accurate species identification (Zhao *et al*., 2021), baseline data for phylogeographic and population genetic analyses to identify unique genetic resources (Shi *et al.*, 2022), and reference barcodes supporting enforcement against adulteration and overharvesting for sustainable utilization (Wei *et al*., 2025 a).

Some molecular markers have been used for phylogenetic studies in *Senna* species (Mao *et al.,* 2017; Eldemerdash *et al*., 2022; Azeez *et al*., 2024). Previous studies of *Senna* in Nigeria examined seven species but with fewer accessions per species (Azeez *et al.,* 2024). However, studies have not reported the use of trnH-psbA intergenic spacer region in the barcoding of members of species of *Senna* in Nigeria. This study employs DNA barcoding using the trnH-psbA intergenic spacer to characterize twenty *Senna* accessions in Southwest Nigeria and assess their genetic diversity and phylogenetic relationships, addressing the region's rich biodiversity and the economic significance of these medicinal plants.

2. Materials and Methods

Plant data collection

Field collection of accessions of *Senna* species seeds was undertaken at various locations within the senatorial districts of Ogun, Oyo, and Ekiti States, South Western Nigeria. The coordinates of the sites are specified in Table 1. The collected *Senna* seeds included: six accessions of *Senna hirsute*, six accessions of *S. obtusifolia*, two accessions of *S. alata*, an accession of *S. siamea* and *S. acutifolia*, and four

Table 1 - Senatorial districts and coordinates of Senna species studied

S/N	Plant species	Senatorial district	State	Coordinate latitude N	Coordinate longitude E
1	*Senna hirsuta*	Ekiti south	Ekiti	7°37'30.94068"	3°54'39.70728"
2	*S. acutifolia*	Ekiti south	Ekiti	7°33'10.00332"	3°53'56.193"
3	*S. obtusifolia*	Ekiti south	Ekiti	7°32'56.45976"	3°53'41.00172"
4	*S. obtusifolia*	Ogun west	Ogun	6°52'46.18484"	3°11'27.08412"
5	*S. hirsuta*	Ogun west	Ogun	6°52'52.64400"	3°11'25.26170"
6	*S. occidentalis*	Ogun west	Ogun	6°53'15.486"	3°11'43.86688"
7	*S. hirsuta*	Oyo south	Oyo	7°23'45.7944"	3°51'29.93256"
8	*S. occidentalis*	Oyo south	Oyo	7°23'57.80292"	3°51'24.4152"
9	*S. hirsuta*	Ekiti north	Ekiti	7°33'5.02812"	3°53'47.51628"
10	*S. obtusifolia*	Ekiti north	Ekiti	7° 33'16.6554"	3°54'8 .35236"
11	*S. obtusifolia*	Ogun east	Ogun	7°37'30.89712"	3°54'40.14396"
12	*S. alata*	Ogun east	Ogun	7° 37'30.89712"	3°54'40.14396"
13	*S. hirsuta*	Ogun east	Ogun	7°37'24.73536"	3°54'50.53608"
14	*S. obtusifolia*	Oyo north	Oyo	7°35'30.2928"	3°53'37.221"
15	*S. hirsute*	Oyo north	Oyo	7°35'30.2766"	3°53'37.17384"
16	*S. occidentalis*	Oyo north	Oyo	7°33'12.3408"	3°54'0.36756"
17	*S. obtusifolia*	Ogun central	Ogun	7°13'54.57288"	3°26'1.9345"
18	*S. alata*	Ogun central	Ogun	6°52'46.18484"	3°11'2.08412"
19	*S. occidentalis*	Oyo town	Oyo	7°35'30.282"	3°53'37.35348"
20	*S. siamea*	Ekiti south	Ekiti	7°933'8.87796"	3°53'54.1878"

accessions of *S. occidentalis*. The collected seeds of each accession were planted in 10 L planting pots filled with humus soil and wet daily at the experimental plot of CAEDESE Federal University of Agriculture Abeokuta. Fresh leaves were collected from each plant accession and taken to Inqaba Biotec West Africa in Ibadan for DNA extraction and quantification, PCR amplification, and Sanger sequencing.

DNA extraction

Genomic DNA was extracted from each of the fresh leaf tissues of *Senna hirsute* (6), *S. obtusifolia* (6), *S. alata* (2), *S. siamea* and *S. acutifolia*, and *S. occidentalis* (4), following the protocols of Quick-DNA Plant/Seed Kits (Zymo Research, Catalogue No.D6020, California USA).

DNA quantification

The quality and quantity of the extracted DNA were measured using a nanodrop (Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer). The system was blanked using 2 µL of DNA Elution Buffer. Afterwards 2 µL of the DNA was placed on the pedestal and measured. The concentration (ng/µL), A260/280 ratio and A260/230 ratio of the sample were noted.

PCR amplification

PCR total reaction volume was optimised to 25 µL using PCR master mix 12.5 µL (according to One Taq Quick-Load 2X Master Mix) (NEB, Catalogue No. M0486), 0.5 µL of 10 µM forward and reverse primers, 10.5 µL Nuclease-free water, and 1 µL genomic DNA as a template for the reaction. The reaction mixture was votexed and spun, then placed in the thermal cycler. PCR were performed by subjecting the samples to thermal cycling conditions following Eppendorf Mastercycler nexus gradient 230: initial denaturation at 95°C for 5 minutes followed by 35 cycles, denaturation at 95°C for 30 seconds, annealing at 50°C for 1 minute and 68°C for 90 secs with a final 10 mins extension step at 68°C and hold at 4°C. The primer sequences used for the amplification of trnH-psbA include:

psbA: 5'-GTTATGCATGAACGTAATGCTC-3' (Sang *et al*., 1997);

trnH: 5'-CGCGCATGGTGGATTCACAATCC-3' (Tate and Simpson, 2003).

Gel electrophoresis

After PCR amplification, 2 µL of PCR product was run on 1% agarose gel, stained with 1 µL of safe view Ethidium Bromide (EtBr) (10 mg/ml), and photographed using a gel documentation system (E-BOX, Vilber Lourmat, Italy). PCR products (amplicons) were cleaned using an enzymatic method (ExoSAP).

Sanger sequencing

The fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions (https://www.nimagen.com/products/Sequencing/Capillary-Electrophoresis/BrilliantDye-Terminator-Cycle-Sequencing-Kit/). The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up-Kit (Catalogue No. D4053). The cleaned products were injected on the Applied Biosystems ABI 3500XL Genetic Analyser with a 50 cm array, using POP7 (https://www.thermofisher.com/order/catalog/product/4406016, and sequence data collected).

Molecular data analysis

The sequences obtained were quality checked and edited using FinchTV version 1.4.0. The alignments were edited with BioEdit sequence alignment editor version 7.2.5 (Hall, 1999). The evolutionary history was inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) according to Sneath and Sokal (1973). The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed according to Felsenstein (1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (Felsenstein, 1985). The quantitative assessment of genetic relationships among the trnH-psbA genes of *Senna species* was done using genetic distance matrix. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004) and are expressed in units of the number of base substitutions per site. This analysis involved 20 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 409 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021).

3. Results

Considerable variations were revealed among the DNA sequences of members of *Senna* species studied when they were aligned using the Multiple ClustalW algorithm in Bioedit software (Fig. 1). There is a significant variable region at the start of the sequence from positions 1 to 35. Specific variation in plant sample sequences 8_PSBA and 9_PSBA was observed at position 23; a small variable region occurs at positions 71 and 72, and a single variable position at 165. Meanwhile, a longer stretch of variable sequences was observed at the regions 151 to 165, and a large variable region at regions 200-

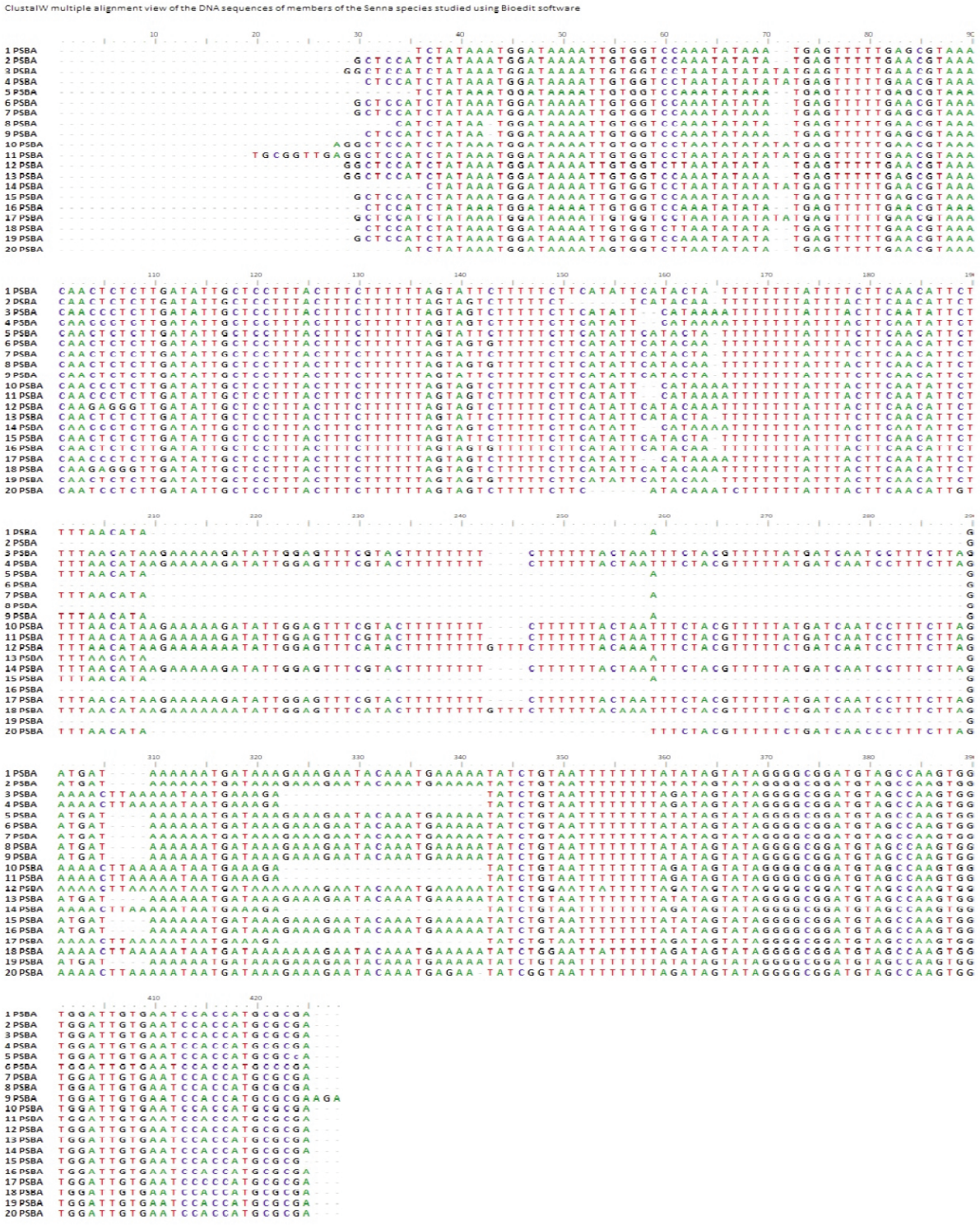

Fig. 1 - ClustalW multiple alignment of the DNA sequences of members of Senna species studied using Bioedit software.

289. Another substantial variable region is at positions 306 to 343, and lastly, variations are seen at the end of the sequence (positions 426 and 427). This implies the analyzed *Senna* species are unique individual species that exhibit significant variation or divergence.

Conserved regions identified in the DNA sequence alignment (Fig. 2) indicate shared genetic similarities among the *Senna* species, providing molecular evidence for their taxonomic grouping within the genus. They represent regions of DNA that have remained unchanged or very similar across different species within the genus *Senna*.

BLAST description of hits studied using MEGA software

The results from the Basic Local Alignment Search Tool (BLAST), a bioinformatics tool used to compare nucleotide or protein sequences against a sequence database and determine the statistical significance of matches, are presented in Table 2. The base pair lengths for the aligned DNA sequences of *Senna* species studied range from 312 to 414 bp. The results of the database sequences show very high-quality matches overall, with percent identity ranging from 99.02% to 100%, query coverage between 95-100%, and very low E-values (ranging from 0.0 to 4e-155).

Fig. 2 - Muscle multiple alignment of the DNA sequences of members of Senna species studied using MEGA software.

Table 2 - BLAST description of hits from the National Centre for Biotechnology Information (NCBI) and sequences producing significant alignments

S/N	Plant sample sequence	Base pair	Description of matched organism	Scientific name	Max score	Total score	Query cover %	E value	% Identity	Accession length	Alignment score	GenBank Accession
1	1_PSBA	323	*Senna hirsutaPsbA gene, partial cds*	*Senna hirsuta*	568	568	96	2,00E-157	99.68	349	>200	KC150886.1
2	2_PSBA	312	*Senna occidentalis chloroplast, complete genome*	*Senna occidentalis*	560	560	99	4,00E-155	99.35	159994	>200	OR478159.1
3	3_PBSA	390	*Senna tora voucher JKTM-1-000064 tRNA-His (trnH) gene*	*Senna tora*	710	710	98	0	100	426	>200	KP058332.1
4	4_PBSA	388	*Senna tora voucher JKTM-1-000064 tRNA-His (trnH) gene*	*Senna tora*	706	706	99	0	99.74	426	>200	KP058332.1
5	5_PSBA	323	*Senna hirsutaPsbA gene, partial cds*	*Senna hirsuta*	580	580	97	3,00E-161	100	349	>200	KC150886.1
6	6_PSBA	318	*Senna occidentalis isolate SCBGP509_1 photosystem II*	*Senna occidentalis*	568	568	99	2,00E-157	99.05	339	>200	KP095340.1
7	7_PBSA	329	*Senna hirsutaPsbA gene, partial cds*	*Senna hirsuta*	588	588	97	2,00E-163	100	349	>200	KC150886.1
8	8_PBSA	313	*Senna occidentalis isolate SCBGP509_1 photosystem II*	*Senna occidentalis*	547	547	97	3,00E-151	99.02	339	>200	KP095340.1
9	9_PSBA	330	*Senna hirsutaPsbA gene, partial cds*	*Senna hirsuta*	575	575	96	1,00E-159	99.37	349	>200	KC150886.1
10	10_PSBA	391	*Senna tora voucher JKTM-1-000064 tRNA-His (trnH) gene*	*Senna tora*	708	708	99	0	99.74	426	>200	KP058332.1
11	11_PBSA	399	*Senna tora voucher JKTM-1-000064 tRNA-His (trnH) gene*	*Senna tora*	706	706	96	0	99.74	426	>200	KP058332.1
12	12_PBSA	414	*Senna alata chloroplast, complete genome*	*Senna alata*	747	747	99	0	99.51	159176	>200	NC_065665.1
13	13_PSBA	330	*Senna hirsutaPsbA gene, partial cds*	*Senna hirsuta*	582	582	95	9,00E-162	100	349	>200	KC150886.1
14	14_PSBA	382	*Senna tora voucher JKTM-1-000064 tRNA-His (trnH) gene*	*Senna tora*	697	697	99	0	99.74	426	>200	KP058332.1
15	15_PBSA	328	*Senna hirsutaPsbA gene, partial cds*	*Senna hirsuta*	593	593	98	4,00E-165	100	349	>200	KC150886.1
16	16_PBSA	317	*Senna occidentalis isolate SCBGP509_1 photosystem II*	*Senna occidentalis*	568	568	99	2,00E-157	99.36	339	>200	KP095340.1
17	17_PSBA	389	*Senna tora voucher JKTM-1-000064 tRNA-His (trnH) gene,*	*Senna tora*	704	704	99	0	99.48	426	>200	KP058332.1
18	18_PSBA	412	*Senna alata chloroplast, complete genome*	*Senna alata*	747	747	100	0	99.51	159176	>200	NC_065665.1
19	19_PBSA	318	*Senna occidentalis isolate SCBGP509_1 photosystem II*	*Senna occidentalis*	573	573	99	5,00E-159	99.68	339	>200	KP095340.1
20	20_PBSA	352	*Senna siamea chloroplast, complete genome*	*Senna siamea*	638	638	98	2,00E-178	100	148437	>200	MN525772.1

This indicates highly significant matches between the query (aligned DNA sequences of *Senna species* studied) and that of the database with a consistent alignment score of >200. Notable matches occurred in KC150886.1 accession, which appears multiple times as a top match (plant codes PSBA 1, 5, 7, 9, 13, 15). KP058332.1 is another common match (plant codes PSBA 3, 4, 10, 11, 14, 17). Two plant codes (PSBA 12 and 18) matched to NC_065665.1, with the longest accession length of 159,176 bp. Best matches were observed in PBSA 3, 5, 7, 15, and 20, they achieved 100% identity with their matches. PSBA 12 and 18 had the highest max scores (747) while PSBA 18 achieved 100% query coverage. These results suggest that the sequences are very well conserved, and the sequencing quality was high. The consistent high scores and low E-values indicate that these matches are highly reliable or significant and not due to chance. The plant codes were identified as *Senna hirsute, S. occidentalis, S. tora, S. alata,* and *S. siamea* among the matches. A taxonomically significant observation emerged where field-identified *S. obtusifolia* and *S. acutifolia* accessions returned *S. tora* and *S. occidentalis* identifications, respectively.

Genetic distance matrix of the DNA sequences based on trnH-psbA intergenic spacer regions of members of Senna species studied

Estimates of the evolutionary divergence between DNA sequences of various members of the *Senna* species are presented in Table 3. The genetic distance values range from 0.000, indicating no

divergence, to a higher divergence value 0.074, which suggests significant evolutionary differences. This variation provides insights into how closely related or distantly related these species are. The lack of divergence was observed between the same species (PSBA 10 and 11; 1 and 13, 1 and 15, and others) irrespective of their locations. Higher divergence value observed between PSBA 1 and 17; 5 and 17 implies they are more distantly related as depicted also in the phylogenetic tree. Low divergence values observed range from 0.017 (between PSBA 2 and 13; 2 and 15) to 0.030 (between 10 and 17 PSBA). Divergence values were consistently high across all comparisons with 12 PSBA (*S. alata*). 5PSBA showed the greatest divergence (0.073), while 20 PSBA exhibited the lowest divergence (0.05). This range of 0.05- 0.073 suggests significant phylogenetic differentiation between the plant accessions. This implies that *S. alata* is distantly related to all other members of *Senna* species studied. Notwithstanding, it is more closely related or have identical DNA sequences with *S. alata* (18PSBA) from another location.

The phylogenetic tree analysis, using UPGMA based on the trnH-psbA intergenic spacer region, depicting four clades, is presented in figure 3. It shows the evolutionary relationships between and among different *Senna* species studied. *S. hirsuta*

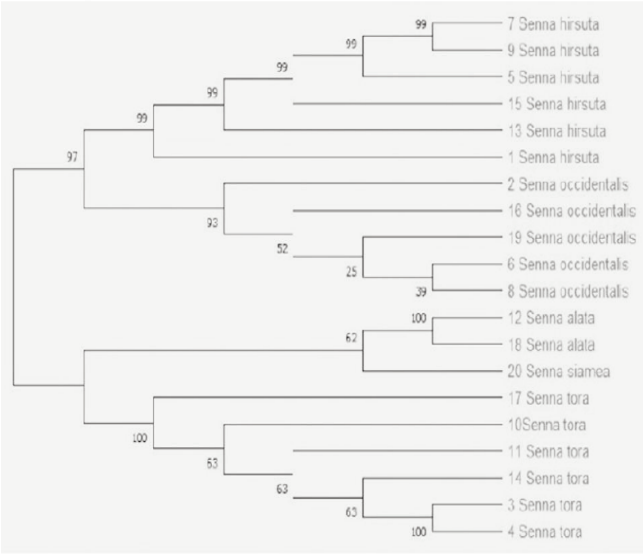

Fig. 3 - Phylogenetic relationships among members of Senna species studied using UPGMA based on trnH-pbsA gene sequences, depicting four clades.

Table 3 - Evolutionary divergence between DNA sequences based on trnH-psbA intergenic spacer regions of members of Senna species studied

	1	10	11	12	13	14	15	16	17	18	19	2	20	3	4	5	6	7	8	9
1																				
10	0.07																			
11	0.07	0																		
12	0.073	0.06	0.06																	
13	0	0.068	0.068	0.071																
14	0.07	0	0	0.062	0.07															
15	0	0.069	0.069	0.072	0	0.071														
16	0.021	0.057	0.057	0.06	0.02	0.058	0.021													
17	0.074	0.003	0.003	0.063	0.072	0.003	0.072	0.06												
18	0.073	0.06	0.06	0	0.072	0.062	0.072	0.06	0.064											
19	0.021	0.056	0.056	0.06	0.02	0.058	0.02	0	0.06	0.06										
2	0.018	0.054	0.054	0.057	0.017	0.055	0.017	0.003	0.058	0.057	0.003									
20	0.067	0.061	0.061	0.05	0.067	0.061	0.067	0.053	0.065	0.05	0.053	0.051								
3	0.07	0	0	0.06	0.068	0	0.069	0.057	0.003	0.06	0.056	0.054	0.061							
4	0.07	0	0	0.06	0.069	0	0.069	0.057	0.003	0.06	0.057	0.054	0.061	0						
5	0	0.07	0.07	0.073	0	0.071	0	0.021	0.074	0.073	0.021	0.018	0.067	0.07	0.07					
6	0.024	0.06	0.06	0.063	0.024	0.062	0.024	0.003	0.064	0.063	0.003	0.007	0.057	0.06	0.06	0.024				
7	0	0.069	0.069	0.072	0	0.07	0	0.02	0.072	0.072	0.02	0.017	0.067	0.069	0.069	0	0.024			
8	0.021	0.057	0.057	0.061	0.021	0.058	0.021	0	0.061	0.061	0	0.003	0.054	0.057	0.057	0.021	0.003	0.021		
9	0	0.069	0.069	0.072	0	0.071	0	0.021	0.073	0.072	0.021	0.017	0.067	0.069	0.069	0	0.024	0	0.021	0

1= *Senna hirsute*, 2= *S. occidental*, 3= *S. tora*, 4= *S. tora*, 5= *S. hirsute*, 6= *S. occidentalis*, 7= *S. hirsute*, 8= *S. occidentalis*, 9= *S. hirsute*, 10= *S. tora*, 11= *S. tora,* 12= *S. alata*, 13= *S. hirsute*, 14= *S. tora*, 15= *S. hirsute*, 16= *S. occidentalis*, 17= *S. tora*, 18= *S. alata*, 19= *S. occidentalis,* 20= *S. siamea.*

forms one distinct clade (PSBA 7, 9, 5, 15, 13, 1), *S. occidentalis* forms another clade (PSBA 2, 16, 19, 8, 6), *S. tora* clusters together (PSBA 10, 11, 14, 3, 4) while *S. alata* (PSBA 12, 18) and S. *siamea* (PSBA 20) form smaller groups. Bootstrap values reveal high confidence values (97-100%) at many major branching points. Some lower values (25-63%) indicate less certain relationships in those branches. The 99% values in the *S. hirsuta* clade suggest very strong confidence in the relationship within the taxa, and also support for the phylogenetic groupings while 39-52% bootstrap values in the *Senna occidentalis* clade indicate uncertainty and weak support for phylogenetic groupings. The tree shows clear separation between major species. Two main branches were observed: one is leading to *S. hirsuta/S. occidentalis* and another to *S. alata/S. siamea/S. tora*. Terminal branches with the same species accessions indicate recent common ancestry.

Nucleotide composition of some members of Senna species DNA sequences studied

The frequencies of each of the Nucleotide compositions and their total means in the DNA sequences of the members of the *Senna species* studied using MEGA 11 software are presented in Table 4. The Base composition patterns show Thymine has the highest variance ranging from 35.8% (2 PSBA) to 40.5% (14 PSBA), while Adenine has the second highest variance ranging from 31.0 % (11 PSBA) to 35.0% (5 and 8 PSBA). Cytosine ranges from 12.5% to 14.1%, and Guanine shows less variation ranging from 14.6% (4 PSBA) to 16.2% (20 PSBA). The mean total of Thymine, Cytosine, Adenine and Guanine is 38.1, 13.3, 33.3 and 15.4%, respectively, and the grand mean total for nucleotide composition for the twenty DNA sequences is 333.9%. Total A+T content is 71.4% (33.3% A + 38.1% T) consistently across all sequences while, total G+C content is 28.7% (15.4% G + 13.3% C). These imply that the nucleotide composition shows an AT-rich bias for *Senna species.* Sequence length varies from the shortest with 293 bases (2 PSBA) to the longest with 395 bases (12 PSBA). Notable length clusters were observed in ~300 bases (PSBA 1,2,5,6,8,16,19) and ~370 bases (PSBA 3,4,10,14,17).

Table 4 - Nucleotide frequencies of the DNA sequences of members of Senna species studied

Plant code	T (U)	C	A	G	Total
1 PSBA	37.5	12.5	34.9	15.1	304
2 PSBA	35.8	13.3	34.8	16	293
3 PSBA	40.2	13.7	31	15.1	371
4 PSBA	40.4	13.8	31.2	14.6	369
5 PSBA	37.6	12.5	35	14.9	303
6 PSBA	36.1	13.4	34.8	15.7	299
7 PSBA	37.1	13.2	34.5	15.2	310
8 PSBA	36.4	12.6	35	16	294
9 PSBA	37	13.2	34.7	15.1	311
10 PSBA	40.1	13.7	31.2	15.1	372
11 PSBA	40	13.7	30.5	15.8	380
12 PSBA	38.2	12.7	33.4	15.7	395
13 PSBA	37	13.2	34.4	15.4	311
14 PSBA	40.5	13.2	31.4	14.9	363
15 PSBA	37.2	13.3	34.3	15.2	309
16 PSBA	36.2	13.1	34.9	15.8	298
17 PSBA	40.3	14.1	30.8	14.9	370
18 PSBA	38.4	12.7	33.6	15.3	393
19 PSBA	36.1	13	34.8	16.1	299
20 PSBA	36.9	13.8	33	16.2	333
Mean	38.1	13.3	33.3	15.4	333.9

All frequencies are given in percent (%).
T (U)-Thyamine (Uracil), C- Cytosine, A- Adenine and G-Guanine.

4. Discussion and Conclusions

The gaps and variations observed in the ClustalW multiple alignments of the DNA sequences of members of the *Senna* species studied indicate genetic differences between the *Senna* species. The presence of multiple variable gene positions suggests these species have diverged enough to accumulate distinct genetic changes. The variations observed could represent different evolutionary paths, adaptations to different environments, and species-specific genetic characteristics. ClustalW identified these variations through multiple sequence alignment and the gaps indicate where insertions or deletions (indels) have occurred in some species relative to others. The presence of both short (single position) and long (spanning multiple positions) variable regions suggests a complex evolutionary history. Hassan (2023) suggested that trnH-psbA intergenic spacer regions as DNA barcoding markers could provide a novel method for understanding the evolutionary relationships and classification of closely related *Prunus* species. On the contrary, Olsson *et al*. (2022) observed the non-suitability of matK and psbA-trnH for species identification and phylogenetic analysis in closely related pines.

The BLAST result provides a strong molecular evidence for accurate species identification (99.02-100%) and high-quality genetic data, essential for taxonomic and evolutionary studies of *Senna* species using trnH-psbA intergenic spacer region. There is significant length polymorphism in the trnH-psbA chloroplast DNA region, with sequences ranging from 312 to 414 base pairs across the studied *Senna* species*;* Hassan (2023) also observed a varied length of base pairs in his study. The sequence length variation may be due to evolutionary studies in *Senna*. The psbA-trnH sequence lengths obtained in this study (312-414 bp) align closely with Indian populations (341-384 bp), where this marker demonstrated higher sequence length variation compared to conserved coding regions like rbcL (682/705 conserved sites) and matK (726/785 conserved sites) (Mishra *et al*., 2018).

The significant evolutionary differences observed between DNA sequences based on trnH-psbA intergenic spacer regions of various members of *Senna* species studied signify genetic divergence, which allows them to adapt to specific local environmental conditions. As populations evolve separately, they can develop unique traits that enhance their survival and reproductive success in distinct habitats (Chung *et al*., 2023). Higher genetic divergence typically correlates with increased genetic variation within a species. This variation is essential for adaptability, as it provides a broader pool of traits that can be selected for in response to changing environmental conditions. Genetic divergence can also influence phenotypic plasticity, the ability of a single genotype to produce different phenotypes in response to varying environmental conditions. Populations that have diverged genetically may exhibit different levels of plasticity, affecting how they respond to environmental changes. The lack of divergence between the same species collected from different locations may perhaps imply they are more closely related and that their sequences are not affected by changes in the environmental conditions. It confirms the existence of conserved regions in the sequence alignment.

The genetic distance among the studied *Senna* species (0.000-0.074) indicates moderate diversity, lower than that reported in other continental studies. Asian *Senna* species show substantially higher genetic variation, with Indian *S. italica* displaying intra-specific divergence of 0.77-16.03% and *S. auriculata* and *S. tora* sharing 0.14% maximum identity (Mishra *et al*., 2018). Analysis of 14 Thai *Senna* species using the psbA-trnH region demonstrated successful species discrimination comparable to our findings (Monkheang *et al*., 2011), validating the marker's utility across geographic populations.

The phylogenetic tree reveals evolutionary intra-specific and inter-specific diversity, and relationships in the species studied. This is demonstrated as *S. hirsuta* appears to be the most divergent group. *S. tora* shows internal variation but clear species cohesion, while *S. alata* and *S. siamea* appear to be more closely related to each other than to other species. The genetic distinctness observed in *S. hirsuta* accessions forms a well-supported clade with high bootstrap values (99%). This shows a significant genetic differentiation from other *Senna* species and occupies a distinct position at the top of the phylogenetic tree. The internal structure contains multiple subgroups (PSBA 7, 9, 5 vs. 15, 13, 1) which shows higher intra-genetic variation compared to other species and demonstrates a complex evolutionary history within the species. Evolutionarily, it may represent an earlier divergence from other *Senna species* or suggest longer independent evolutionary history. More so, it could indicate adaptation to different ecological niches and possibly might have undergone more extensive genetic changes over time.

Moreover, previous Nigerian studies from Ile-Ife using matK and rbcL markers (546-843 bp) reported similar phylogenetic relationships, particularly the close association between *S. occidentalis* and *S. hirsuta* (Azeez *et al*., 2024), corroborating our findings. Azeez *et al.* (2024) recorded chromosome counts of x=18 in *S. podocarpa* and *S. obtusifolia*, x=20 in *S. occidentalis*, x=22 in *S. alata* and *S. hirsuta*, and x=24 in *S. siamea* and *S. sophera*, demonstrating karyotypic diversity within Nigerian populations. However, the comparatively lower genetic diversity in Southwest Nigerian populations relative to Asian counterparts suggests recent colonization events, geographic isolation, or adaptation to uniform environmental conditions within the study region.

Notwithstanding, the intra-variation observed in the *S. tora* (PSBA 3, 4, 10, 11, and 14) show some genetic differences among them. This is seen in the slight branching patterns within the *S. tora* clade. Bootstrap values of 63% between some *S. tora* accessions indicate minor genetic differences. Despite these differences, all *S. tora* cluster together

in one distinct group. They form a monophyletic clade (sharing a common ancestor). There is a clear separation from other *Senna* species and strong bootstrap support (100%) for some branches. Genetic distances between *S. tora* accessions are relatively small. This pattern suggests that *S. tora* maintains its distinct species identity, shows normal population-level genetic diversity, all accessions are correctly identified as *S. tora* and natural variation exists within the species but not enough to question species boundaries. Thus, the phylogenetic tree has aided in resolving evolutionary relationships within the genus. It also supports the genetic distance data and provides a clear visual representation of the evolutionary relationships and divergence patterns within these *Senna* species. The closeness of *S. alata* and *S. siamea* might suggest species with potentially similar medicinal properties.

In contrast, the *S. occidentalis* clade displayed moderate to low bootstrap values (39-52%), these lower confidence values suggest greater intra-specific diversity within *S. occidentalis* studied, possibly reflecting multiple introduction events from different source populations, longer establishment history allowing accumulation of mutations, or potential historical gene flow with related species. The bootstrap values below the conventional 70% threshold indicate that additional molecular markers (ITS, matK, rbcL) are needed to clarify relationships within this clade and test for possible cryptic diversity or reticulate evolution.

Additionally, the nucleotide frequencies explain the phylogenetic relationships earlier discussed, with sequence length and base composition differences potentially reflecting evolutionary divergence patterns. The characteristic A-T richness (71.4%) observed in this study is consistent with typical chloroplast intergenic spacer regions globally, reflecting conserved evolutionary patterns within the genus. The A-T rich pattern provides pbsA-trnH intergenic spacer region as a valuable molecular markers for species identification and supports the taxonomic classification of these *Senna* species (Ravi *et al*., 2008). This is evidence in 99-100% BLAST identity, clear separation of all species and high confidence (99-100% bootstrap for *S. hirsute*). Functionally, A-T richness influences DNA stability and melting temperature, and may impact DNA replication efficiency.

Furthermore, the moderate genetic diversity observed in the *Senna* species (0.000-0.074) reflects adaptive evolutionary responses to environmental pressures (Alzahrani *et al*., 2020), enabling plants to adapt to climatic fluctuations (Li *et al*., 2022), though lower diversity compared to Asian species may constrain responses to rapid environmental changes (Mishra *et al*., 2018). Genetically diverse *Senna* species contain important metabolites with diverse pharmacological activities (Elbashir *et al*., 2021), and intra-specific genetic variation suggests potential variation in sennoside concentrations among Nigerian accessions similar to Indian studies (Kumar *et al*., 2024).

Meanwhile, the moderate genetic divergence observed may reflect evolutionary adaptation within similar ecological zones across Southwest Nigeria, contrasting with broader environmental gradients in Asian populations, and while psbA-trnH provides adequate species discrimination, future phylogenetic studies could benefit from multi-locus approaches combining this marker with nuclear markers to enhance resolution of population structure and evolutionary relationships (Mishra *et al*., 2018).

Nonetheless, market analysis in India revealed substantial adulteration in *Senna* herbal products (Seethapathy *et al*., 2015), highlighting the importance of DNA barcoding for authentication, while successful discrimination of Thai *Senna* species using trnH-psbA fragment polymorphism (Monkheang *et al.,* 2011) demonstrates the practical application of this marker for rapid species identification across geographic regions.

In addition, the presence of Shared conserved sequences provide molecular evidence for the monophyletic nature of *Senna* within Fabaceae subfamily Caesalpinioideae, validating genus-level separation from *Cassia* sensu stricto established by Irwin and Barneby (1982), while genetic distances (0.000-0.074) reveal moderate inter-specific divergence consistent with species-level differentiation and corroborate previous Nigerian studies documenting karyotypic diversity (x=18 to x=24) and the close relationship between *S. occidentalis* and *S. hirsuta* (Azeez *et al*., 2024), demonstrating concordance between cytogenetic, molecular, and morphological evidence characteristic of integrative taxonomy.

The amplified psbA-trnH intergenic spacer region reveals significant variations among the DNA sequences of *Senna* species studied. The presence of shared conserved sequences provides the molecular evidence of the monophyletic nature of these taxa

within *Senna*, while base composition variations reflect evolutionary divergence and adaptation of these species to different environmental pressures. These findings provide the molecular framework for Nigerian *Senna* species identification and conservation, supporting their potential development for medicinal and agricultural applications.

Acknowledgements

We acknowledge the Institutional Based Research (IBR) grant from TetFund and the support from Federal University of Agriculture Abeokuta that enabled us to conduct this research.

References

ADEWOLE M.A., SALAWU K.M., TIJANI A.Y., ADEBAYO J.O., CHUKWUEMEKA C.N., 2024 - *Nutritional, phytochemical, and antimicrobial properties of* Senna siamea *leaves*. - Heliyon, 10(23): e40323.

AKINRO L., ADESOYE A., FASOLA T.R., 2019 - *Genetic diversity in cola acuminata and cola nitida using rapd primers.* - Genetika, 51(1): 227-236.

ALZAHRANI D.A., YARADUA S.S., ALBOKHARI E.J., ABBA A. 2020 - *Complete chloroplast genome sequence of* Barleria prionitis*, comparative chloroplast genomics and phylogenetic relationships among* Acanthaceae. - BMC Genomics, 21: 393.

ARIF I.A., BAKIR M.A., KHAN H.A., AL FARHAN H.A., AL HOMAIDAN A.A, BAHKALI A.H., SADOON M.A., SHOBRAK M., 2010 - *A brief review of molecular techniques to assess Plant diversity*. - Int. J. Mol. Sci*.,* 11(5): 2079-2096.

ATANU F.O., NWONUMA C.O., AGBOOLA T.M., OKAIYETO K., BATIHA G.E., HETTA H.F., 2022 - *Hydroethanolic extracts of* Senna alata *leaves possess antimalarial effects and reverse haematological and biochemical perturbation in* Plasmodium berghei-*infected mice.* - Natural Product Communications, 17(8).

AZEEZ S.O., MATTHEW J.O., ABRAHAM O.G., BABAYEMI O.S., IKHINMWIN I.V. 2024 - *Morpho-cytological studies and DNA barcoding in genus* Senna (Fabaceae, Caesalpinoideae). - Phytotaxa, 637(2): 165-176.

BRUNEAU A., DOYLE J.J., HERENDEEN P.S., HUGHES C.E., KENICER G., LEWIS G.P., MACKINDER B.A., PENNINGTON R.T., SANDERSON M.J., WOJCIECHOWSKI M.F., BOATWRIGHT J., BROWN G.K., CARDOSO D., CRISP M.D., EGAN A.N., FORTUNATO R.H., HAWKINS J.A., KAJITA T., KLITGAARD B., WYK B.V., 2013 - *Legume phylogeny and classification in the 21st century: Progress, prospects and lessons for other species rich clades*. - Taxon, 62(2): 217-248.

CBOL, PLANT WORKING GROUP, 2009 - *A DNA barcode for land plants*. - Proc. Nat. Acad. Sci., 106: 12794-12797.

CHUNG M.Y., MERILÄ J., LI J., MAO K., LÓPEZ-PUJOL J., TSUMURA Y., CHUNG M.G., 2023 - *Neutral and adaptive genetic diversity in plants: An overview*. - Front. Ecol. Evol., 11: 1116814.

CORREIA C.L., CONCEIÇÃO A.D., 2017 - *The genus* Senna *Mill. (Leguminosae: Caesalpinioideae) in a fragment of the Ecological Station Raso da Catarina, Bahia.* - Acta Sci. Biol. Sci., 39(3): 357.

DAMTHONGDEE A., SAENGPHO C., WIYA C., BAKA A., CHANTHAMRONG K., UE-AREE P., CHAOWASKU T., 2024 - Drepananthus khaosoi *sp. nov. (annonaceae) from southern Thailand, with molecular phylogenetic reconstructions*. - Anales Jardín Botánico Madrid, 81(1): e144.

DASKUM A.M., GODLY C., QADEER M.A., LING L.Y., 2019 - *Effect of Senna occidentalis (Fabaceae) leaves extract on the formation of β-hematin and evaluation of* in vitro *antimalarial activity*. - Int. J. Herbal Med., *7*(3): 46-51.

DE BOER H., RYDMARK M.O., VERSTRAETE B., GRAVENDEEL B. 2022 - *Molecular identification of plants: From sequence to species.* - Pensoft Publishers, Sofia, Bulgaria, pp. 387.

DE VERE N., RICH T.C., TRINDER S.A., LONG C., 2015 - *DNA barcoding for plants*. - Methods Mol Biol. 1245: 101-118.

DONG S., YING Z., YU S., WANG Q., LIAO G., GE Y., CHENG R., 2021 - *Complete chloroplast genome of* Stephania tetrandra *(Menispermaceae) from Zhejiang province: Insights into molecular structures, comparative genome analysis, mutational hotspots and phylogenetic relationships*. - BMC Genomics, 22: 880.

DUAN N., RU D., LIU B., 2025 - *Comparative chloroplast genomes of sophora species: identification of variable DNA markers and phylogenetic relationships within the genus*. - BMC Plant Biol., 25: 1308.

DUTTA A.C., 2001 - *Botany for Degree Students*. - Oxford University Press, Oxford, UK, pp. 720.

ELBASHIR S.M.I., DEVKOTA H.P., WELI A.M., AL-HARRASI A., 2021 - *The genus* Senna *(Fabaceae): A review on its traditional uses, botany, phytochemistry, pharmacology and toxicology*. - J. Ethnopharmacology, 272: 113919.

ELDEMERDASH M.M., EL-SAYED A.S.A., HUSSEIN H.A., TELEB S.S., SHEHATA R.S., 2022 - *Molecular and metabolic traits of some Egyptian species of* Cassia *L. and* Senna *Mill (Fabaceae-Caesalpinioideae).* - BMC Plant Biol., 22: 205.

FELSENSTEIN J., 1985 - *Confidence limits on phylogenies: An approach using the bootstrap*. - Evol., 39: 783-791.

FENG S., JIAO K., ZHU Y., WANG H., JIANG M., WANG H., 2018 - *Molecular identification of species of* Physalis *(Solanaceae) using a candidate DNA barcode: the*

chloroplast psbA-trnH intergenic region. - Genome, 61(1): 15-20.

HALL T.A., 1999 - *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT*. - Nucleic Acids Symp. Ser., 41: 95-98.

HAO D.C., CHEN S.L., XIAO P.G., 2010 - *Sequence characteristics and divergent evolution of the chloroplast psbA-trnH noncoding region in gymnosperms*. - J. Appl. Genetics, 51(3): 259-273.

HASSAN A.H.M., 2023 - *DNA barcode of TRNH-PSBA, a promising candidate gene for efficient identification of bitter and sweet almond and related species.* - Egypt. J. Desert Res., 73(1): 265-281.

HOLLINGSWORTH P.M., WRAHAM G.S., LITTLE D.P., 2011 - *Choosing and using a plant DNA barcode*. - PLoS One, 6: e19254.

HUSSAIN A., POTTER D., KIM S., HAYAT M.Q., BOKHARI S.A.I., 2019 - *Molecular phylogeny of* Artemisia *(Asteraceae-Anthemideae) with emphasis on undescribed taxa from Gilgit-Baltistan (Pakistan) based on nrDNA (ITS and ETS) and cpDNA (psbA-trnH) sequences.* - Plant Ecol. Evol., 152(3): 507-520.

HUTCHINSON J., 1964 - *The genera of flowering plants (Angiospermae)*. - Clarendson Press, Oxford, UK, pp. 659.

IBRAHIM S., FAGBEMI K.A., ADETUNJI C.O., OLOKE J.K., BOROKINI T.I., IORTSUUN D.N., UBA A., 2022 - *Traditional medicinal plants used for treating emerging and re-emerging viral diseases in northern Nigeria*. - J. Ethnopharmacology, 295: 115425.

IRWIN H.S., BARNEBY R.C., 1982 - *The American Cassiinae : A synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the New World*. - Memoirs New York Bot. Garden, 35(1): 1-454.

JUDD W.S., CAMPBELL C.S., KELLOGG E.A., STEVENS P.F., DONOGHUE M.J., 2002 - *Plant systematics. A phylogenetic approach*. - Sinauer, Sunderland, MA, USA, pp. 576.

KENFACK D., ABIEM I., CHAPMAN H., 2022 -*The efficiency of DNA barcoding in the identification of Afromontane forest tree species*. - Diversity, 14(4): 233.

KRESS W.J., ERICKSON D.L., 2008 - *DNA barcodes: Genes, genomics, and bioinformatics.* - Proc. Natl. Acad. Sci. USA, 105(8): 2761-2762.

KUMAR A., CHAUHAN R.S., SEMWAL R., VENUGOPAL S., KUMAR V., CHANDRA S., KUMAR U., MISHRA A.P., SINGH N., LAL R.K., 2024 - *Estimation of genetic diversity utilizing gene-targeted SCoT markers and morpho-chemotypic analyses in* Senna alexandrina *Mill. (*Senna*).* - Ecolog. Genetics Genomics, 30: 100252.

LEWIS G., SCHRIRE B., MACKINDER B., LOCK M., 2005 - *Legumes of the World*. - Royal Botanic Gardens, Kew, Richmond, UK, pp. 577.

LI Y., ZHANG X., FANG Y., GAO J., WANG C., LI X., 2022 - *Genetic diversity and structure of* Cassia obtusifolia *and* Senna tora *revealed by SSR markers.* - Genetic Resources Crop Evol., 69: 2789-2801.

LOERA-SANCHEZ M., STUDER B., KÖLLIKER R., 2020 - *DNA barcode trnH-psbAis a promising candidate for efficient identification of forage legumes and grasses*. - BMC Res. Notes, 13: 35.

LPWG, LEGUME PHYLOGENY WORKING GROUP, 2013 - *Legume phylogeny and classification in the 21st century: Progress, prospects and lessons for other species-rich clades*. - Taxon, 62: 217-248.

LPWG, LEGUME PHYLOGENY WORKING GROUP, 2017 - *A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny.* - Taxon, 66(1): 44-77.

MAO R., XIA P., HE Z., LIU Y., LIU F., ZHAO H., HAN R., LIANG Z., 2017 - *Identification of seeds based on molecular markers and secondary metabolites in* Senna obtusifolia *and* Senna occidentalis. - Bot Stud., 58(1): 43.

MISHRA P., SHUKLA A.K., SUNDARESAN V., 2018 - *Candidate DNA barcode tags combined with high resolution melting (Bar-HRM) curve analysis for authentication of* Senna alexandrina *Mill. with validation in crude drugs.* - Front. Plant Sci., 9: 283.

MONKHEANG P., SUDMOON R., TANEE T., NOIKOTR K., BLETTER N., CHAVEERACH A., 2011 - *Species diversity, usages, molecular markers and barcode of medicinal* Senna *species (Fabaceae, Caesalpinioideae) in Thailand.* - J. Med. Plants Res., 5(26): 6173-6181.

OLADEJI O.S., ADELOWO F.E., AYODELE D.T., ODELADE K.A., 2020 - *Ethnobotanical description and biological activities of S*enna alata. - Evidence-Based Complementary and Alternative Medicine, 2580259.

OLSSON S., GIOVANNELLI G., ROIG A., SPANU I., VENDRAMIN G.G., FADY B., 2022 - *Chloroplast DNA barcoding genes matKand psbA-trnHare not suitable for species identification and phylogenetic analyses in closely related pines.* - iForest, 15: 141-147.

PANG X., LIU C., SHI L., LIU R., LIANG D., LI H., 2012 - *Utility of the trnH-psbA Intergenic Spacer Region and Its Combinations as Plant DNA Barcodes: A Meta-Analysis*. - PLoS ONE, 7(11): e48833.

POLHILL R.M., 1994 - *Classification of the Leguminosae*, pp. xxxv-xlviii. - In: BISBY F.A., J. BUCKINGHAM, and J.B. HARBOURNE (eds.). *Phytochemical dictionary of the Leguminosae. Volume 1. Plants and their constituents.* CRC Press, Chapman and Hall, London, UK, pp. 112.

POLHILL R.M., RAVEN P.H., 1981 - *Advances in legume systematics, part 1.* - Royal Botanic Gardens, Kew, Richmond, UK, pp. 1050.

RAVI V., KHURANA J.P., TYAGI A.K., KHURANA P., 2008 - *An update on chloroplast genome*. - Plant System. Evol., 271: 101-122.

SANG T., CRAWFORD D.J., STUESSY T.F., 1997 - *Chloroplast DNA phylogeny, reticulate evolution and biogeography of* Paeonia *(Paeoniaceae).* - Am. J. Bot., 84: 1120-1136.

SEETHAPATHY G.S., GANESH D., SANTHOSH KUMAR J.U., SENTHILKUMAR U., NEWMASTER S.G., RAGUPATHY S., UMA SHAANKER R., RAVIKANTH G., 2015 - *Assessing product adulteration in natural health products for laxative yielding plants,* Cassia*,* Senna*, and* Chamaecrista*, in Southern India using DNA barcoding.* - Int. J. Legal Med., 129(4): 693-700.

SHI X., XU W., WAN M., SUN Q., CHEN Q., ZHAO C., SUN K., SHU Y., 2022 - *Comparative analysis of chloroplast genomes of three medicinal* Carpesium *species: genome structures and phylogenetic relationships.* - PLoS ONE, 17(8): e0272563.

SNEATH P.H.A., SOKAL R.R., 1973 - *Numerical taxonomy*. *The principles and practice of numerical classification.* - W.H. Freeman, San Francisco, CA, USA, pp. 573.

TAMASI A.A., SHOGE M.O., ADEGBOYEGA T.T., CHUKWUMA E.C., 2021 - *Phytochemical analysis and* in-vitro *antimicrobial screening of the leaf extract of Senna occidentalis (Fabaceae)*. - Asian J. Natural Product Biochem., 19(2): 58-65.

TAMURA K., NEI M., KUMAR S., 2004 - *Prospects for inferring very large phylogenies by using the neighbor-joining method*. - Proc. Natl. Acad. Sci. USA, 101(30): 11030-11035.

TAMURA K., STECHER G., KUMAR S. 2021 - *MEGA 11: Molecular evolutionary genetics analysis version 11.* - Mol. Biol. Evol., 38(7): 3022-3027.

TATE J.A., SIMPSON B.B., 2003 - *Paraphyly of* Tarasa *(Malvaceae) and diverse origins of the polyploid species*. - Syst. Bot., 28: 723-737.

WEI F., LIN Y., TANG D., LIANG Y., QIN S., 2025 b - *Comparative analysis of complete chloroplast genomes of* Flemingia prostrata *and* Flemingia macrophylla*, two commonly used medicinal plants in southern china.* Frontiers in Plant Science Volume 16: 1591427. https://doi.org/10.3389/fpls.2025.1591427

WEI S., YING J., LU M., LI J., HUANG Y., WU Z., NEVILL P.G., LI P., JIN X., LU Q., 2025 a - *Plastome comparison and phylogenomics of chinese endemic* Schnabelia *(Lamiaceae): Insights into plastome evolution and species divergence.* - BMC Plant Biol., 25: 600.

ZHAO K., LI L., QUAN H., YANG J., ZHANG Z., LIAO Z., LAN X., 2021 - *Comparative analyses of chloroplast genomes from 14* Zanthoxylum *species: identification of variable dna markers and phylogenetic relationships within the genus.* - Front. Plant Sci., 11: 605793.