



Molecular identification and phylogenetic analysis of *Corchorus olitorius* using psbA-trnH plastid DNA region

K. A. Abdulkareem^{*1}, M. A. Abdulkareem¹, K. O. Sidiq¹, A. Bello¹, S. B. Tsoho², M. M. Danzak², A. O. Koiki³, M. A. I. Audu⁴ and O. T. Mustapha¹

¹Department of Plant Biology, University of Ilorin, P.M.B. 1515, Nigeria.

²Department of Biology, Nigerian Army University, P.M.B. 1500, Biu, Nigeria

³Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria

⁴Federal University of Technology, Minna, Nigeria

ARTICLE INFORMATION

MS No. JOBAS_2026_005

Received: 17/08/2025

Revised: 15/10/2025

Accepted: 26/12/2025

Published: 15/02/2026

Keywords:

Corchorus olitorius
Molecular identification
Phylogeny
DNA barcoding
psbA trnH

*Corresponding author:

abdulkareem.ak@unilorin.edu.ng

Tel: +2348035607708

ORCID:

0000-0001-5329-6456

ISSN: 3121-8210

ABSTRACT

Corchorus olitorius L. (commonly known as jute mallow) is a widely utilised leafy vegetable valued for its nutritional and medicinal benefits. It exhibits considerable genetic diversity, making it an important crop for breeding programmes and conservation efforts. However, to achieve this, accurate identification is required. Morphological methods of identification are found to be insufficient as it often fails to reveal the intra-specific differences and evolutionary relationships existing between closely related taxa. In contrast, Molecular methods of identification such as DNA barcoding are accurate and reliable for species identification and elucidating evolutionary relationship existing between closely related taxa. This study aimed to utilize DNA barcoding as a molecular technique to identify *C. olitorius* using the psbA-trnH plastid DNA region. DNA was extracted from leaf tissues, amplified and sequenced. BLAST analysis was conducted on the NCBI Genbank database, followed by multiple sequence alignments. Maximum Likelihood method was employed to infer phylogeny using MEGA11 software. Results revealed the psbA-trnH region produced a DNA barcode of 359 bp sequence. Also, BLAST similarities ranged from 90% to 100% across 21 Genebank accessions. Phylogenetic analysis revealed a strong evolutionary relationship between *C. olitorius*, *C. orinocensis*, and *C. cunninghamii* supported by high bootstrap values. This research provides DNA barcodes for molecular identification of *C. olitorius* as well as information about its evolutionary relationships with closely related taxa. Such information will aid plant breeding, conservation, and biosystematics endeavours.

1.0 Introduction

Corchorus olitorius L. (family *Malvaceae*), commonly known as "Jew's mallow" or "Jute Nalta", is widely used as a vegetable in Asia and Africa [1]. *Corchorus olitorius* offers several health benefits and contains high levels of dietary fibre which aids digestion

while promoting healthy bowel movements. It is an indispensable ingredient in many traditional Nigerian dishes due to its unique flavour, and numerous traditional benefits [2]. It has been reported that the leaves of *Corchorus* are an excellent source of protein, minerals, vitamins, certain hormone precursors [3] and a wide range of bioactive

compounds such as glycosides, phenolics, flavonoids, tannins, saponins, sterols, triterpenoids, ionones, fatty acids, and carbohydrates [4].

The proper identification and evaluation of biologically important plant families play a key role in understanding the evolutionary history of many important plant species [5]. This enables researchers to discover the similarities and differences among different plant families and the need for conservation and crop improvement endeavours to reduce the loss of valuable plant species, and to ensure food security. Due to environmental changes and genetic erosion, some valuable genetic resources have already become extinct [6].

However, for crop improvement and plant resource conservation to be successful, adequate and appropriate identification and characterization are essential. Among the several methods used for the identification of plant as well as its characterisation, the molecular methods (that involve analysing plant DNA and understanding their genetic make-up), unlike the morphological methods (which involve identifying and characterising plants based on their physical characteristics) offer accurate and reliable means of plant identification [7]. Traditional identification methods mainly rely on the observation and comparison of morphological and ecological characteristics. However, this method appears inadequate when faced with issues such as significant morphological variation and difficulty in distinguishing closely related species [8].

With the advent of molecular techniques, researchers can quickly and accurately identify plant species and varieties by using PCR technology with universal primers, Random Amplified Polymorphic DNA (RAPD) and DNA barcoding [9]. DNA barcoding is a species identification tool that uses a short section of the genome that provides a genetic signature of the species. The main advantage of this novel technique is that it requires a small sample of tissue [10]. DNA barcoding serves as a fast and standardised technique for identifying and categorising organisms, providing researchers with a reliable molecular tool for taxonomy [11,12]. Research on the genetic variability and phylogenetic relationship of *C. olitorius* have been reported [13-15]. The adoption of molecular markers such as simple sequence repeats (SSRs), RAPD for plant identification is however common. However, information on the use of DNA barcoding with the specific use of *psbA-trnH* plastid DNA region for identification of *C.*

olitorius is inadequate in literature.

This study aimed to molecularly identify *C. olitorius* using *psbA-trnH* plastid DNA region and assess its phylogenetic relationship. Globally, efforts are underway to create DNA barcodes for various life forms and make these datasets publicly accessible to support biodiversity research, conservation and sustainable use [16]. Information from this study will aid plant breeding, taxonomic and conservation efforts.

2.0 Materials and Methods

2.1 Sample Preparation and DNA Isolation

Seeds of *C. olitorius* with accession NGB00195 were used in the study. The seeds were collected from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan. The seeds were planted in planting pot containing loamy soil for 14 days. Young leaf samples of *C. olitorius* were used. Fresh leaf samples were rinsed with distilled water and then homogenised using a clean mortar and pestle. DNA was isolated using the Qiagen DNeasy plant mini kit following the manufacturers instruction.

2.2 Amplification Primers

The DNA barcode region was amplified using *psbA* and *trnH* primers. The primers sequences employed in this study were designed and validated in our earlier study [17] and were synthesised by Inqaba Biotech Ltd. (Pretoria, South Africa). The sequences of the primer are listed in Table 1

Table 1: Amplification Primers

Primer	Sequence
PSBA	GTTATGCATGAACGTAATGCTC
TRNHF 05	CGCGCATGGTGGATTACAATCC

2.3 Amplification and Sequencing for DNA Barcoding Marker

The PCR was carried out with a total reaction volume of 30 µl in a 96-well thermocycler (Eppendorf, Germany). The reaction mixture consisted of 20-50 ng of genomic DNA, 10X PCR buffer, 2.5 µM dNTPs, 5 pmol primers and 1µ of Taq DNA polymerase (Genet.Bio. Korea. The thermocycler condition was an initial denaturation at 95°C for 5 minutes, and 35 cycles of denaturation at 95°C for 30 seconds. Annealing was at 55°C for 30 seconds, and extension was at 72°C for 1 minute. The final

extension was at 72°C for 5 minutes. The amplicon verification was carried out on a 1% agarose gel and was purified with EZ- 10 Spin Column PCR product purification Kit (Bio Basic Inc. Ontario, Canada). The PCR setup for cycle sequencing reaction was done in a total reaction volume of 10 µl, and the thermocycler conditions for cycle sequencing reaction are an initial denaturation at 96°C for 1 minute in 1 cycle. This was followed by 30 cycles of denaturation at 96°C for 10 seconds, and annealing at 50°C for 5 seconds. The extension for the reaction was at 60°C for 4 minutes, and the final extension was at 60°C for 7 minutes. The amplicon was purified with EZ-10 Spin Column PCR product purification Kit (Bio Basic Inc. Ontario, Canada). The DNA sample was sequenced using the protocols reported in our earlier work [17]

2.4 Data Analysis

The obtained forward and reverse sequences were grouped using the SeqTrace 9.0 tool and were submitted to the NCBI (National Center for Biotechnology Information) for BLAST (Basic Local Alignment Search Tool) analysis. The query cover of the sequences was identified considering E value $<1 \times 10^{-5}$ and maximum hits (99 or 100%) with species in the reference database of the NCBI (www.ncbi.com). Phylogenetic analysis was conducted using MEGA11 [18]. The phylogenetic trees were inferred using the maximum likelihood method based on the Tamura Nei model [18]. The topologies of the phylogenetic trees were evaluated using the bootstrap resampling method with 1000 replicates.

3.0 Results

The DNA sequence obtained for *C. olitorius*, using psbA-trnH DNA region was 359 base pairs (bp) in length. The sequences are provided in Supplementary material (see supplementary 1).

The BLAST search similarity obtained from the NCBI website ranged from 90 – 100%. BLAST analysis of the sequences obtained from *C. olitorius* using the psbA-trnH region, two species in the *Corchorus* genus namely; *C. orinocensis* and *C. cunninghamii* proving psbA-trnH markers high resolution at higher taxonomic levels. Other notable species from genus *Abutilon*, *Microsis*, *Apeiba* and *Triumfetta* were also recorded. Table 2 shows the BLAST analysis for the psbA-trnH barcode region of the consensus: *C. olitorius* against other accessions showing the accession

number, botanical name, genus and family.

Phylogenetic analysis using the maximum likelihood method revealed clear clade formation. The consensus *C. olitorius* sequence clustered with *C. orinocensis* (HG963671.1) and *C. cunninghamii* (KU564658) in a monophyletic clade supported by a strong bootstrap value (99%). The consensus sequence also shared a paraphyletic relationship with a clade containing two (2) members of the *Apeiba* genus namely *A. tibourbou* (HG963880.1) and *A. glabra* (FJ038986.2) with a bootstrap value of 97.4% (Figure 1). The consensus: *C. olitorius* also formed a polyphyletic association with members of the *Microcos* genus (*M. coriacea*, *M. paniculata* and *M. cinnamomifolia*), *Triumfetta cana*, *T. lappula*, *Abutilon theophrasti*, *A. pakistanicus*, *A. friticosum*, *Alcea rosea*, *Catostemma fragrans* and *Magnoliophyta environmental*. All evolutionary relationships were supported by a strong bootstrap value (>70%). The outgroup was identified as *Magnoliophyta environmental* (HG964043.1) validating the tree topology

4.0 Discussion

The molecular identification and phylogenetic study of *C. olitorius* using the psbA-trnH plastid DNA region has provided significant insights into its phylogenetic relationships within the members of the family *Malvaceae*. DNA barcode Sequences of *C. olitorius* were successfully generated using the psbA-trnH marker. The DNA barcodes serve as a promising candidate for identification of *C. olitorius* as occurrence of members of the *Corchorus* genus from BLAST results revealed its higher discriminatory power. This efficiency of the psbA-trnH has been reported in several studies. [19] described it as the most promising coding plastid DNA barcode among the bitter and sweet almond at low taxonomic levels. Also, [20] reported that, DNA barcode *trnH-psbA* is a promising candidate for efficient identification of forage legumes and grasses.

Results from the phylogeny tree placed the consensus: *C. olitorius* in a monophyletic group, together with two members of *Corchorus* genus namely *C. cunninghamii* and *C. orinocensis* with accession numbers KU564658.1 and HG963671.1 respectively. This occurrence indicates the close evolutionary relationship existing between them. This is in line with the finding of [21] where *C. olitorius* occurred in a monophyletic group with another member of the same genus. Also, the paraphyletic grouping with

Apeiba and *Triumfetta* species (97 – 100% bootstrap support value) aligns with their shared family *Malvaceae* [22]

The tree exhibited high bootstrap values (>70%) for most branches, indicating reliable phylogenetic relationships. For example: KP095705.1_*Triumfetta cana* and OK632003.1_*Triumfetta sp.* formed a monophyletic clade with 100% bootstrap value and MZ423505.1_*Abutilon pakistanicum* and OR188579.1_*Abutilonfriticosum* cluster together with 99% support. In the tree, the consensus, *C. olitorius* formed a monophyletic clade with HG963671.1_*Corchorus orinocensis*

and KU564658.1_*Corchorus cunninghamii*, supported by a high bootstrap value of 99%. This finding is corroborated by [23] where it was reported that, when the bootstrap probability is high for most branches, the tree is considered to be reliable

The quality of the DNA sequence obtained from *C. olitorius* using the psbA-trnH primer is critical for accurate phylogenetic analysis. High-quality sequences are characterised by clear, unambiguous base calls and minimal noise in the chromatograms [24]. In this study, the presence of ambiguous bases (denoted as "N" in the sequence) suggests potential

Table 2: BLAST search of the psbA-trnH barcode region of *C. olitorius*

S/No.	Accession numbers	Botanical name	Genus	Family
1	HG963671.1	<i>Corchorus orinocensis</i>	<i>Corchorus</i> sp.	<i>Malvaceae</i>
2	KU564658.1	<i>Corchorus cunninghamii</i>	<i>Corchorus</i> sp.	<i>Malvaceae</i>
3	HG964059.1	<i>Magnoliophyta environmental</i>	<i>Magnoliophyta</i> sp.	<i>Malvaceae</i>
4	HG963658.1	<i>Triumfetta lappula</i>	<i>Triumfetta</i> sp.	<i>Malvaceae</i>
5	LC737732.1	<i>Microcos cinnamomifolia</i>	<i>Microcos</i> sp.	<i>Malvaceae</i>
6	HG964043.1	<i>Magnoliophyta environmental</i>	<i>Magnoliophyta</i> sp.	<i>Malvaceae</i>
7	OK632003.1	<i>Triumfetta sp.</i>	<i>Triumfetta</i> sp.	<i>Malvaceae</i>
8	HG963880.1	<i>Apeiba tibourbou</i>	<i>Apeiba</i> sp.	<i>Malvaceae</i>
9	KX377383.1	<i>Microcos paniculate</i>	<i>Microcos</i> sp.	<i>Malvaceae</i>
10	MW589552.1	<i>Grewia serrulata</i>	<i>Grewia</i> sp.	<i>Malvaceae</i>
11	KP095705.1	<i>Triumfetta cana</i>	<i>Triumfetta</i> sp.	<i>Malvaceae</i>
12	FJ038986.2	<i>Apeiba glabra</i>	<i>Apeiba</i> sp.	<i>Malvaceae</i>
13	KC667886.1	<i>Microcos coriacea</i>	<i>Microcos</i> sp.	<i>Malvaceae</i>
14	MF786182.1	<i>Catostemma fragrans</i>	<i>Catostemma</i> sp.	<i>Malvaceae</i>
15	MH197410.1	<i>Abutilon theophrasti</i>	<i>Abutilon</i> sp.	<i>Malvaceae</i>
16	MZ423509.1	<i>Abutilon theophrasti</i>	<i>Abutilon</i> sp.	<i>Malvaceae</i>
17	MZ423505.1	<i>Abutilon pakistanicum</i>	<i>Abutilon</i> sp.	<i>Malvaceae</i>
18	OR188579.1	<i>Abutilon friticosum</i>	<i>Abutilon</i> sp.	<i>Malvaceae</i>
19	MF348839.1	<i>Alcea rosea</i>	<i>Alcea</i> sp.	<i>Malvaceae</i>
20	LC737732.1	<i>Microcos cinnamomifolia</i>	<i>Microcos</i> sp.	<i>Malvaceae</i>
21	CONSENSUS	<i>C. olitorius</i>	<i>Corchorus</i> sp.	<i>Malvaceae</i>

Supplementary 1: DNA sequences of *C. olitorius* obtained from psbA-trnH plastid DNA region

NNGAAGCTCCATCTACNAACGNCTAAGACTTTGGTTTTAGNGTATACGAGTTTTTAAAAATAA
 AGGAGCAATGCCAATACTCNTGATAGAACAAGAAATTGGTTATTGCTCCTTTATTTAGTAGTCC
 TTCCNTTCGTTACATTTTGTACATAAGTTTTTTTTTTCTTTATTTTATTTTANCCCCCTCTAT
 CCNATTTTACCTTTNCCATANAAAATAAAAAAAGGNTTTTAAATGAGNTTGATTTGTTTTGT
 TGGCTTTTATGTTAGNNTTGNATTATACGNCTTTTATGGNCTAACTAATAATAAAATTTTA
 TAAATTTATAAAAAAATATATATAAACGAAAANAGGNN

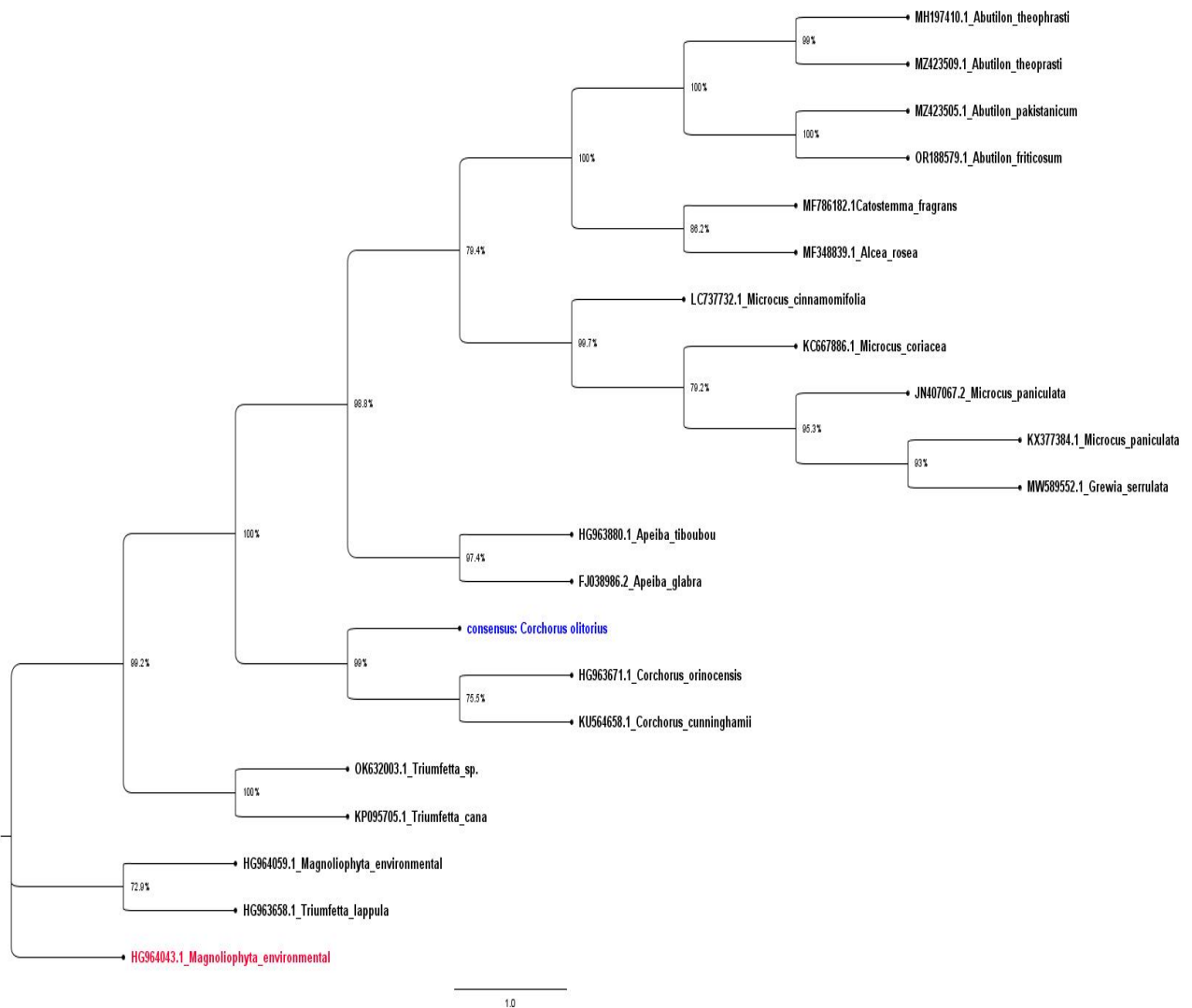


Figure 1: Phylogenetic tree of *Corchorus olitorius* using Maximum Likelihood method

issues with sequencing quality or regions of low complexity. However, the results obtained from the BLAST and phylogeny tree supported by strong bootstrap values indicates the reliability of the DNA sequences for identification of *C. olitorius*. Also, the reported sequence length for *C. olitorius* was 359 base pairs (bp). This aligns with the expected

amplicon size for the psbA-trnH intergenic spacer region, which typically ranges between 300–600 bp in angiosperms [25]. The consistency in length indicates successful amplification of the target region.

The BLAST analysis confirmed the sequence's alignment with the psbA-trnH intergenic spacer, a widely used plastid

barcode for plants [16]. The high similarity (90–100%) with other *Corchorus* species in GenBank supports the sequence's authenticity. The psbA-trnH marker's utility for species-level discrimination is evident, but its resolution for higher taxonomic levels remains limited [24]. Integrating nuclear markers (e.g., ITS) could improve phylogenetic accuracy [26].

In conclusion, the study provides valuable contribution to the phylogenetic relationships and molecular identification of *C. olitorius* using the psbA-trnH plastid DNA region. The emergence of species in the *Corchorus* genus as well as its robust clade support in the phylogenetic tree validate this marker for specific level identification. Also, the DNA sequence barcode from this study could serve as reference sequence for identification of *C. olitorius* species which will aid species identification and conservation purposes. However, the presence of ambiguous bases and reliance on a single chloroplast region highlights the need for complementary molecular markers to enhance taxonomic resolution.

Future research should incorporate broader sampling and multi loci approach for a broader genetic resolution.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Disclosure of source of funding/grant

None

Authors Contributions

KAA – Conceptualization, Supervision and Resources; MAA - Data curation; KOS - Formal analysis and Writing -original draft, review & editing; AB - Formal analysis and Writing - original draft, review & editing; SBT – Methodology; MMD – Methodology; AOK – Formal analysis and Writing – review & editing; MAIA – Writing -review & editing; OTM – Supervision, Resources; and Writing – review & editing.

References

- Gupta, S., Kumar, J., Mishra, P., Singh, A.K., & Maral, A. (2023). Morphological and pharmacological study of herbal medicine: *C. olitorius* L. *Journal of Pharmacognosy and Phytochemistry*, 12(2): 84-87.
- Adeoye, I.B., Iliasu, K.B., Akinbile, H.T., & Adeigbe, F.O. (2023). Consumers' preference and attitude towards dried *C. olitorius* L. in Ibadan Metropolis, Oyo State, Nigeria. *Asian Journal of Agricultural and Horticultural Research*, 10(4): 455 – 463.

- Akter, S.A., Satter, M., Shahin Ahmed, K., Biswas, S., Abdul Bari, M., Das, A., Ahsanul Karim, M., Saha, N., Hossain, H., Islam, S., Jahan Tisa, K., & Swaraz, A.M. (2024). Nutritional composition, bioactive compounds, and pharmacological activities of tossa jute sprout (*Corchorus olitorius* L.): A potential functional food. *Food Bioscience*, 60: 104324. <https://doi.org/10.1016/j.fbio.2024.104324>
- Biswas, A., Dey, S., Huang, S., Deng, Y., Birhanie, Z.M., Zhang, J., Akhter, D., Liu, L., & Li, D. (2022). A comprehensive review of *C. capsularis* and *C. olitorius*: A source of nutrition, essential phytoconstituents and pharmacological activities. *Antioxidants*, 11(7): 1358. <https://doi.org/10.3390/antiox11071358>
- Pamarthi, R. K., Nagaraju, S., Prasad, K., Pavan, K. M., Nayar, E. R., Panda, R. R., & Bhatt, K. C. (2025). Plant taxonomy and its role in studies of plant genetic resources. In: Tripathi, K., Gupta, V., Krishnappa, G., Kumari, J., & Singh, G. P. (Eds.), *Textbook of Plant Genetic Resources*. pp. 7–43. Springer Nature Singapore Pte Ltd., Singapore. https://doi.org/10.1007/978-981-96-5024-8_2
- Salgotra R.K., & Chauhan B.S., (2023). Genetic diversity, conservation, and utilization of plant genetic resources. *Genes*, 14(1): 174. <https://doi.org/10.3390/genes14010174>
- Chac, L.D., & Thinh, B.B. (2023). Species identification through DNA barcoding and its applications: A Review. *Biology Bulletin*, 50(6): 1143–1156. <https://doi.org/10.1134/s106235902360229x>
- Yang, G., Zhang, L., Meng, C., & Sui, Y. (2024). Application of molecular biology techniques in plant identification. *Journal of Life Sciences and Agriculture*, 1(3): 86–90. <https://doi.org/10.62517/jlsa.202407313>
- Azizi, M.M.F., Lau, H.Y., & Abu-Bakar, N. (2021). Integration of advanced technologies for plant variety and cultivar identification. *Journal of Biosciences*, 46(4): 91. <https://doi.org/10.1007/s12038-021-00214-x>
- Trujillo-Argueta, S., Del Castillo, R.F., & Velasco-Murguía, A. (2022). Testing the effectiveness of rbcLa DNA-barcoding for species discrimination in tropical montane cloud forest vascular plants (Oaxaca, Mexico) using BLAST, genetic distance, and tree-based methods. *PeerJ*, 10: e13771.

11. Khan, M.Q., Khalil, A.T., Rehman, K., & Shinwari, Z.K. (2015). Searching for DNA barcodes in plants. *Global Journal of Biotechnology and Biochemistry*, 10 (2-10): 1-10.
12. Onstein, R.E., Carter, R.J., Xing, Y., Richardson, J.E., & Linder, H.P. (2015). Do Mediterranean-type ecosystems have a common history? –Insights from the Buckthorn family. *Rhamnaceae Evolution*, 69(3): 756-771.
13. Benor, S., Demissew, S., Hammer, K., & Blattner, F.R. (2011). Genetic diversity and relationships in *Corchorus olitorius* (Malvaceae s.l.) inferred from molecular and morphological data. *Genetic Resources and Crop Evolution*, 59(6): 1125–1146. <https://doi.org/10.1007/s10722-011-9748-8>
14. Fang, S., Zhang, L., Qi, J., & Zhang, L. (2020). *De novo* assembly of chloroplast genomes of *Corchorus capsularis* and *C. olitorius* yields species-specific InDel markers. *The Crop Journal*, 9(1):216–226. <https://doi.org/10.1016/j.cj.2020.05.010>
15. Elwakil, H.D., Gomaa, S.E.F.M., Zaitoun, A., Abd El-kader, B., & Abdelsalam, N. (2023). Morphological, biochemical and barcoding analysis of different Egyptian Jew's mallow (*Corchorus olitorius* L.) Landraces. *Journal of the Advances in Agricultural Researches*, 28(3): 582-596. <https://doi.org/10.21608/jalexu.2023.223436.1147>
16. CBOL Plant Working Group. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, 106(31): 12794-12797.
17. Abdulkareem K.A., Ajibade, Y.A. Yusuff, R.A. Bello, A., Sidiq, K.O., Olayinka, B.U., Lateef, A.A., Kareem, I., & Danzaki, M.M. (2025). DNA barcoding of *Adansonia digitata* using multigenic regions (ITS, rbcL, rpoC1 and psbA-trnH)". *Scientific Journal of the University of Zakho* 13(3): 302-314. <https://doi.org/10.25271/sjuoz.2025.13.3.1511>.
18. Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3): 512-526.
19. 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. *Egyptian Journal of Desert Research*, 73(1): 265-281
20. Loera-Sánchez, M., Studer, B., & Kölliker, R. (2020). DNA barcode trnH-psbA is a promising candidate for efficient identification of forage legumes and grasses. *BMC Research Notes*, 13(1): <https://doi.org/10.1186/s13104-020-4897-5>
21. Fang, S., Zhang, L., Qi, J., & Zhang, L. (2020). *De novo* assembly of chloroplast genomes of *Corchorus capsularis* and *C. olitorius* yields species-specific InDel markers. *The Crop Journal*, 9(1):216-226. <https://doi.org/10.1016/j.cj.2020.05.010>
22. Zou, Y., Zang, Z., Zeng, Y., Hu, H., Hao, Y., Huang, S., & Li, B. (2024). Common methods for phylogenetic tree construction and their implementation in R. *Bioengineering*, 11(5): 480. <https://doi.org/10.3390/bioengineering11050480>
23. Katsura, Y., Stanley, C.E., Kumar, S., & Nei, M. (2017). The reliability and stability of an inferred phylogenetic tree from empirical data. *Molecular Biology and Evolution*, 43(3), 718-723. <https://doi.org/10.1093/molbev/msw272>
24. Kress, W.J., Wurdack, K.J., Zimmer, E.A., & Weigt, L.A. (2005). Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences*, 102: 8369-8374
25. Shaw, J., Lickey, E.B., Schilling, E.E., & Small, R.L. (2007). Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *American Journal of Botany*, 94(3): 275-288.
26. Li, X., Yang, Y., Henry, R. J., Rossetto, M., Wang, Y., & Chen, S. (2021). Plant DNA barcoding: From gene to genome. *Biological Reviews*, 96(1): 247-268.