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MORPHOLOGICAL CHARACTERIZATION AND SCREENING OF UNIVERSAL DNA BARCODES FOR SPECIES IDENTIFICATION OF *MORINGA* OLEIFERA AND *MORINGA* STENOPETALA

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ABSTRACT

The genus *Moringa* comprises 13 species that differ morphologically, genetically, and phytochemically, with variation influenced by environmental conditions and cultivation practices. *M. oleifera* is the most widely distributed species, while *M. stenopetala* has gained interest due to *ITS* medicinal properties and amino acid profile. After processing, the two species cannot be distinguished morphologically, reinforcing the need for molecular markers. This study characterized *M. oleifera* and *M. stenopetala* using morphology and a screening of universal DNA barcodes in samples cultivated in northern Paraná State, Brazil. Leaflet and pinna numbers per leaf differed significantly between species. The combination of *psbA* and *ITS-2* proved to be an effective barcode for distinguishing *Moringa* species. Phylogenetic analyses using *matK, psbA, rbcL*, and *ITS-2* inferred the origin of *M. oleifera* accessions, but this was not possible for *M. stenopetala* due to limited public molecular data. This work contributes to DNA barcoding efforts by identifying markers that support a global bioidentification system for both species.

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INTRODUCTION

The genus *Moringa*, belonging to the family *Moringa*ceae, is monogeneric within the Dicotyledoneae and comprises only 13 species: *Moringa arborea* Verdc., *M. borziana* Mattei, *M. drouhardii* Jum., *M. hildebrandtii* Engl., *M. longituba* Engl., *M. pygmaea* Verdc., *M. rivae* Chiov., *M. ruspoliana* Engl., *M. peregrina* (Forssk.) Fiori, *M. stenopetala* (Baker f.) Cufod., *M. concanensis* Nimmo, and *M. oleifera* Lam. (Olson and Carlquist, 2001; Abd Rani *et al*., 2018). Despite the limited number of species within the genus, their distinct morphological tra*ITS* and environmental adaptations confer remarkable phenotypic variation to the family. This diversity is particularly evident in arid and semi-arid regions of Africa and Asia, which are considered the centers of origin of these species (Olson and Carlquist, 2001; Abd Rani *et al*., 2018; Boopathi and Abubakar, 2021). Within the genus, *M. oleifera*, native to northern India and the Himalayan region (Paliwal and Sharma, 2011), is widely used for *ITS* nutritional, medicinal, and industrial properties across Asia, Africa, and South America (Leone *et al*., 2015). This species adapts well to tropical and subtropical climates, both dry and humid, with annual precipitation ranging from 760 to 2,500 mm and temperatures between 18 and 28 °C (Palada, 1996; Nouman *et al*., 2014). In Brazil, *M. oleifera* was introduced in the 1960s as an ornamental and melliferous plant, later spreading to the arid and semi-arid regions of northeastern Brazil due to *ITS* hardiness, rapid growth, and drought tolerance (Marinho *et al*., 2016; Tavares Filho *et al*., 2020). *M. stenopetala,* domesticated in the lowlands of eastern Ethiopia and Kenya, is widely cultivated throughout tropical and subtropical regions. It is adapted to arid and semi-arid climates, typically found at elevations between 1,000 and 1,800 m (Jiru *et al*., 2006; Gebregiorgis *et al*., 2012), with annual rainfall ranging from 250 to 1,500 mm and temperatures between 25 and 35 °C (Tefera *et al*., 2023). Known as African *Moringa* or cabbage tree (Leone *et al*., 2015; Boopathi and Abubakar, 2021), this species exhib*ITS* rapid growth and reaches reproductive maturity within 30 months (Boopathi and Abubakar, 2021). It has a high capacity for water storage in *ITS* stem, which enhances *ITS* adaptation to semi-arid environments (Boopathi and Abubakar, 2021). Similar to *M. oleifera, M. stenopetala* is highly valued for *ITS* versatility, serving as both human food and animal feed. This recognition is attributed to *ITS* high protein concentration in leaves (~28.02%) (Ntshambiwa *et al*., 2023), and the presence of significant levels of essential amino acids, in higher concentrations than those found in *M. oleifera* (Kim and Kim, 2019). Comparative biochemical analyses of *M. oleifera* and *M. stenopetala* leaves reveal similarities in fat, fiber, crude protein, and ash contents; however, *M. stenopetala* shows higher moisture content relative to dry matter (Ntshambiwa *et al*., 2023). Like *M. oleifera, M. stenopetala* has a long history of traditional medicinal use, exhibiting antihyperglycemic, anticancer, anticholesterolemic, hypotensive, antibacterial, antifungal, and antiviral activities (Hadis *et al*., 2020; Abdu *et al*., 2023). Research on *M. oleifera* and *M. stenopetala* has primarily focused on their medicinal properties, amino acid composition, feed formulation, and water treatment applications, among others (Akram *et al*., 2022; Santhanam *et al*., 2022; Melaku, 2023; Shahbaz *et al*., 2024; AbdEl-Halim, 2025; Eltawila *et al*., 2025; Kumar *et al*., 2025). Using advanced genomic methodologies, Indian researchers have assembled the genome of *M. oleifera* var. Bhagya to identify genes associated with drought stress regulation (Shyamli *et al*., 2021) and performed transcriptome analyses to identify candidate genes of medicinal importance (Pasha *et al*., 2020).

Morphologically, *M. oleifera* and *M. stenopetala* are phenotypically similar in leaflets, leaves, and seeds, and share high chemical and genetic similarity (Hamada *et al*., 2024). Although the two species can be differentiated by their leaf pinnation, this characteristic is ineffective in processed samples (Wetters *et al*., 2024). As an alternative to overcome such limitations, DNA-based molecular markers are essential tools for species identification, as they complement traditional taxonomy by detecting specific genetic variations, allowing precise classification of organisms (Boopathi and Raveendran, 2021). Among these, DNA barcoding enables accurate organism identification independent of developmental stage and serves to clarify taxonomic classifications (Boopathi and Raveendran, 2021). DNA barcoding involves specific DNA segments located in coding and non-coding regions within a taxonomic group which presents sufficient interspecific variation to discriminate among species or higher taxonomic group (Paz *et al*., 2011). In animals, the mitochondrial gene **COI** (cytochrome c oxidase), used by Paul Hebert, enabled the identification of 13,320 species based on sequence data, defining it as the first DNA barcode marker and establishing the core of a global bioidentification system for animals (Hebert *et al*., 2003).

In plants, however, mitochondrial genes are unsuitable for barcoding due to their slow evolutionary rate (Wang *et al*., 2022). Consequently, studies have focused on identifying nuclear or chloroplast genes that can serve as effective plant barcodes (Cho *et al*., 2004; Chase *et al*., 2005). In 2009, during the Third International Barcode of Life Conference, consensus was reached that the chloroplast genes ***rbcL*** and *matK* would form the core of a global plant barcode system, while *trnH-psbA* (chloroplast) and *ITS* (nuclear) regions would serve as complementary barcode markers (Wang *et al*., 2022; Letsiou *et al*., 2024). Within the genus *Moringa*, several barcode regions have been successfully applied for species discrimination: *rbcL* in Egyptian genotypes (Rayan, 2019), *ITS2* in Malaysian genotypes (Nasarodin *et al*., 2023), and *psbA-trnH* in genotypes collected in Germany (Wetters *et al*., 2024).

These studies have proven effective in distinguishing species within the genus and even among *M. oleifera* individuals (Hassan *et al*., 2020). In Brazil, two *Moringa* species are found: *M. oleifera***,** the most widespread, and *M. stenopetala***.** In the northwestern region of Paraná State, *M. oleifera* is predominantly cultivated, primarily for urban landscaping or in agroforestry systems. Two *M. stenopetala* specimens are also present, but their origin remains unknown, and they have not yet been studied. Given the limited information available on *Moringa* species identification, particularly in Brazil, this study aimed to characterize *M. oleifera* and *M. stenopetala* morphologically and to screen universal DNA barcoding regions (*matK, psbA, rbcL*, and ***ITS-2***) for accurate species identification.

MATERIAL AND METHODS

Plant material

Plants of *Moringa* ***oleifera*** (M1, M2, M3, M4, and M6) and *Moringa* ***stenopetala*** (M5 and M7) cultivated at the Iguatemi Experimental Farm (FEI) of the State University of Maringá (UEM), located in the district of Iguatemi, Paraná, Brazil (23°25′S, 51°57′W, 550 m a.s.l.), were used in this study.

Morphological characterization: Considering that the *Moringa* plants at FEI varied in age from 2 to 18 years, only adult individuals were selected for morphological analysis. One plant from each species (*M. oleifera* – M1 and *M. stenopetala* – M5), both approximately 18 years old, were evaluated. Morphological characterization followed the parameters described by Hassanein and Al-Soqeer (2018), including:(a)plant height (m), measured from the soil surface to the apex;(b) stem diameter (m), measured 5 cm above the soil surface and calculated using the formula$DC = \frac{CC}{\pi}$, where DC is the stem diameter (m), CC is the stem circumference (m), and π = 3.14;(c) canopy area (m²), calculated by multiplying the canopy projection in the north–south (N–S) direction by that in the east–west (E–W) direction;(d) leaf length (cm), measured from the node to the tip of the entire leaf;(e) number of leaflets per leaf;(f) number of pinnae (sub-leaflets) per leaf; and(g) leaf area (cm²), estimated by multiplying length by width of each pinna, with the sum of all pinnae representing the total leaf area.

Genomic DNA extraction: Leaf samples were collected from ***five*** *M. oleifera* and two *M. stenopetala* individuals. Sampling followed the procedure described by Khankhum *et al*. (2017), in which leaves were stored in glass jars containing silica gel and kept at 4 °C for 15 days for dehydration. Subsequently, samples were stored at −20 °C until DNA extraction genomic DNA was isolated using the extraction protocol of Dellaporta (1983) with modifications adapted from Ojuederie *et al*. (2013). The quality of extracted DNA was assessed by electrophoresis on 0.8% agarose gel, and DNA quantity was determined using a NanoDrop™ spectrophotometer (Thermo Fisher Scientific).

PCR amplification and sequencing: Polymerase chain reaction (PCR) was performed to amplify and analyze four DNA barcoding loci, including three chloroplast genes, ***rbcL*** (Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit), *matK* (Maturase K), and ***psbA*** (Photosystem II protein D1), and one nuclear region, ***ITS-2*** (Internal Transcribed Spacer 2) (Letsiou *et al*., 2024). Amplification was carried out following Ahmed (2019), using the primer pairs listed in Table 1.

PCR reactions were prepared in a total volume of 25 µL containing 50–75 ng of genomic DNA, 1× Taq polymerase buffer (50 mM KCl, 10 mM Tris-HCl pH 7.5), 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 µM of each primer, and 1 U of Taq DNA polymerase. The thermal cycling profile consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 48 °C for 40 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The obtained amplicons were separated on 1.5% agarose gels stained with Sybr Safe (0.02%) and subsequently visualized using a Loccus L-PIX photodocumentation system. A molecular weight marker (NZYtech Ladder IV, 20–500 bp) was used. PCR products containing specific amplicons of the ***rbcL, matK, psbA***, and ***ITS-2*** genes were sent to GoGenetic (Curitiba, Paraná, Brazil) for purification and sequencing using the Sanger method.

Sequence analysis: Nucleotide sequences obtained for the *rbcL*, *matK*, *psbA*, and *ITS-2* genes from both *Moringa* species were compared with reference sequences available in the NCBI database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic inference was performed in MEGA X software (Kumar *et al*., 2018), employing the MUSCLE algorithm (Edgar, 2004) for multiple sequence alignment. Polymorphism analyses, including the number of informative sites, number of polymorphic sites (S), and the average number of nucleotide differences per sequence pair (k), were obtained using DnaSP v6 (Rozas *et al*., 2017).

RESULTS AND DISCUSSION

Morphological characterization: Morphological analyses of the two *Moringa* species (Figure 1A–F) revealed that *M. stenopetala* exhibited a shorter plant height than *M. oleifera*; however, it presented greater canopy area and stem diameter (Figure 1G). These morphological differences may be associated with the speciesadaptation to local edaphoclimatic conditions. *M. stenopetala*, possibly due to lower adaptation to these conditions, underwent several pruning events during *ITS* development to promote recovery. Nevertheless, this management practice stimulated the growth of lateral branches and consequently increased stem thickening, resulting in a larger trunk diameter and shorter plant height compared with *M. oleifera*.

M. oleifera exhibited five times more leaflets per leaf compared with *M. stenopetala* (Figure 2A and B). Typically, *M. oleifera* has more leaflets per leaf than *M. stenopetala*, a pattern also observed in other studies, such as that conducted by Hamada *et al.* (2024), who compared the two species genetically, morphologically, and phytochemically. Those authors reported that *M. oleifera* had an average of 9-11 leaflets per leaf, while *M. stenopetala* had 5-7. Leaflet length in *M. oleifera* and *M. stenopetala* ranged from 1.6-2.1 cm and 1.6-3.5 cm, respectively. The leaflet lengths of *M. oleifera* observed in this study were consistent with those reported by Hamada *et al.* (2024) for *M. oleifera* plants from Egypt, which averaged 1.5-2.3 cm in length. In contrast, leaflet lengths in *M. stenopetala* ranged from 1.5 to 2.0 cm in their study. In the present work, the average values were slightly higher but remained within the confidence interval.

Overall, in plants cultivated at FEI/UEM/Brazil, *M. oleifera* presented a greater number of smaller leaflets per leaf than *M. stenopetala*, which had fewer but larger ones. The number of pinnae per leaf was higher in *M. stenopetala*, with an average of 1.6 additional pinnae, which, together with larger leaflet dimensions (length and width), resulted in a greater total leaf area for this species, as shown in Figure 1. Therefore, both the number of leaflets per leaf and the number of pinnae per leaf stood out as the main morphological tra*ITS* distinguishing the two *Moringa* species cultivated at FEI/UEM/Brazil.

Amplification quality of the ***rbcL, matK, psbA,*** *and* ***ITS-2*** *genes:* All four genes evaluated in this study were successfully amplified in all seven genotypes analyzed (Figure 3). The obtained fragments showed approximate lengths of 350 bp for ***ITS-2,*** 850 bp for ***matK***, 550 bp for ***rbcL***, and between 430 and 500 bp for ***psbA***. Similar results were reported for *Moringa* by Alkaraki *et al.* (2021), who obtained amplicons of 676 bp and 724 bp for the ***rbcL*** and ***matK*** genes, respectively, in ***M. peregrina***; Nasarodin *et al.* (2023) observed fragment sizes of 643 bp for ***rbcL*** and 404 bp for ***ITS-2*** in *M. oleifera****;*** and Wetters *et al.* (2024) reported amplicons of 381 bp and 320 bp for the ***psbA*** gene in *M. oleifera* and ***M. peregrina***, respectively. Among the markers tested, the electrophoretic profile of the ***psb A*** gene revealed the most evident differences between the species, with *M. oleifera* genotypes exhibiting a band of approximately 430 bp, while *M. stenopetala* showed a band around 500 bp. The ***psbA*** gene is located in the plastid (chloroplast) genome and encodes the D1 protein, which is essential for photosynthesis in plants (Williams-Carrier *et al*., 2025). In contrast, the ***ITS-2, matK***, and ***rbcL*** genes produced amplicons with either no or less noticeable differences in fragment size, and therefore initially demonstrated lower taxonomic resolution between the analyzed *M. oleifera* and *M. stenopetala* genotypes.

Sequence analysis: All amplicon sequences from the four genes (***rbcL, matK, psbA***, and ***ITS-2***) obtained from the seven analyzed genotypes were aligned within each gene locus to assess which marker would be most efficient in identifying *M. oleifera* and *M. stenopetala*, as a result, standing out as a suitable DNA barcode. Following sequencing and alignment, the amplicon lengths obtained for the ***ITS-2*** gene ranged between 237 and 354 bp. This variation was attributed to random occurrences of insertions and deletions (indels) distributed throughout the entire sequence, regardless of species. For instance, in genotype M5 of *M. stenopetala*, a consecutive deletion of 83 nucleotides was observed, the largest gap detected (Figure 4A). For the *rbcL* and *matK* genes, the main discrepancies were observed in the initial and terminal regions of the sequences. When each gene was analyzed separately, the amplicons from the seven genotypes showed an average similarity of 98.54% and 96.42%, respectively, differing only by single-nucleotide substitutions (SNPs) or indels, which are typically associated with interspecific differentiation (Figure 4B and 4D). Specifically for *M. stenopetala* (M5 and M7), a 15-bp gap was detected in the *matK* gene. In comparison with the previously described results, the *psbA* gene stood out in differentiating *M. oleifera* from *M. stenopetala*. Sequencing and alignment revealed amplicons ranging from 430 to 513 bp, with two gaps of five nucleotides each in *M. stenopetala* and three longer gaps in *M. oleifera* of 7, 67, and 10 nucleotides. In addition to the high intraspecific conservation observed within this gene locus, a total of eight SNPs and one indel were identified when comparing *M. oleifera* and *M. stenopetala* genotypes (Figure 4C).

To quantify genetic differences and compare the resolution power of the marker genes, the alignments were analyzed based on informative sites (excluding gaps), resulting in 563 sites for *rbcL*, 415 for *psbA*, 817 for *matK*, and 234 for *ITS-2*. Among the evaluated genes, *ITS-2* presented the highest number of polymorphic sites (S = 88) and the highest mean number of nucleotide differences per sequence pair (k = 39.10), followed by *matK* (S = 60; k = 24.10), *psbA* (S = 23; k = 10.33), and *rbcL* (S = 19; k = 8.62). Based on these results, *ITS-2* demonstrated a high discriminatory power between the analyzed species, while *matK* and *psbA*, although showing lower polymorphism rates, still exhibited adequate levels for species distinction. Conversely, the *rbcL* gene showed the lowest interspecific differentiation capacity, although the obtained values were similar to those reported by Rayan (2019) when using the same gene to analyze phylogenetic relationships between *M. oleifera* and *M. peregrina* (k = 7.08%).

In this context, when the amplicon sequences were clustered using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, the *ITS-2* and *psbA* genes proved to be the most efficient in distinguishing *M. oleifera* (M1, M2, M3, M4, and M6) from *M. stenopetala* (M5 and M7), which were grouped into entirely separate and distinct clusters from *M. oleifera* (Figure 5). The *matK* and *rbcL* genes also separated the species into distinct groups; however, *M. stenopetala* genotypes remained within the larger *M. oleifera* cluster. These results reinforce the potential of the *ITS-2* and *psbA* genes for interspecific differentiation, suggesting their usefulness as DNA barcoding markers in phylogenetic studies involving *Moringa* species, particularly for the bioidentification of *M. oleifera* and *M. stenopetala*. Notably, the *psbA* gene, one of the most variable noncoding plastid loci, exhib*ITS* a high level of interspecific discrimination (Hollingsworth *et al*., 2011) and, together with the nuclear *ITS-2* gene, stands out as a complementary barcode for plant DNA barcoding (Wang *et al*., 2022; Letsiou *et al*., 2024).

Phylogenetic analysis: The sequences of the genes ***ITS-2***, ***matK***, ***psbA***, and ***rbcL*** were aligned using ***BLASTn*** against *Moringa* sequences deposited in public biological databases. The alignments of all four genes showed identity percentages greater than 80% and ***e-values***< 1e−5, indicating high statistical significance and biological relevance (Table 2). The alignments performed for *M. oleifera*, regardless of the gene analyzed, resulted in matches within the species in 100% of cases. However, exceptionally, for the *matK* gene sequences of *M. oleifera*, identity percentages were also observed with *M. peregrina* (NC_070088.1). In contrast, M5 and M7 (*M. stenopetala*) showed high identity percentages with *M. oleifera* in

Table 1. Primer sequences used for amplification of *ITS-2, matK, psbA,* and *rbcL* genes

Genes	*Primer sequences* (5'→3')	
	Forward	Reverse
ITS-2	ATGCGATACTTGGTGTGAAT	TCCTCCGCTTATTGATATGC
matK	ACCCAGTCCATCTGGAAATCTTGGTTC	CGTACAGTACTTTTGTGTTTACGAG
psbA	CGCGCATGGTGGATTCACAATCC	GTTATGCATGAACGTAATGCTC
rbcL	ATGTCACCACAAACAGAGACTAAAGC	GTAAAATCAAGTCCACCRCG

Table 2. Accession numbers of *ITS*-2, *matK*, *psbA*, and *rbcL* sequences identified in the NCBI database for *Moringa* spp. and the percentage of identity with the amplicon sequences from *M. oleifera* and *M. stenopetala* accessions

Gene	*M. oleifera*					*M. stenopetala*	
	M1	M2	M3	M4	M6	M5	M7
ITS-2	KT737757.1 (89,07 %)	MT176045.1 (84,0 %)	MT176051.1 (84,99 %)	MT827803.1 (84,46 %)	KT737764.1 (84,76 %)	KT737797.1 (82,41 %)	MT176045.1 (92,63 %)
matK	MK726020.1 (96,14 %)	MK726020.1 (98,69 %)	MK726020.1 (98,81 %)	MK726020.1 (98,57 %)	MK726020.1 (97,92 %)	KY697380.1 (97,60 %)	KY697380.1 (98,32 %)
rbcL	MK726020.1 (99,63 %)	MK726020.1 (99,12 %)	MK726020.1 (99,46 %)	MK726020.1 (99,64 %)	MK726020.1 (99,46 %)	JX091929.1 (99,11 %)	AF405249.1 (98,76 %)
psbA	MK726020.1 (99,03 %)	MK726020.1 (99,75 %)	MK726020.1 (99,75 %)	MK726020.1 (99,28 %)	MK726020.1 (99,03 %)	MT916803.1 (99,48 %)	MT916803.1 (99,47 %)

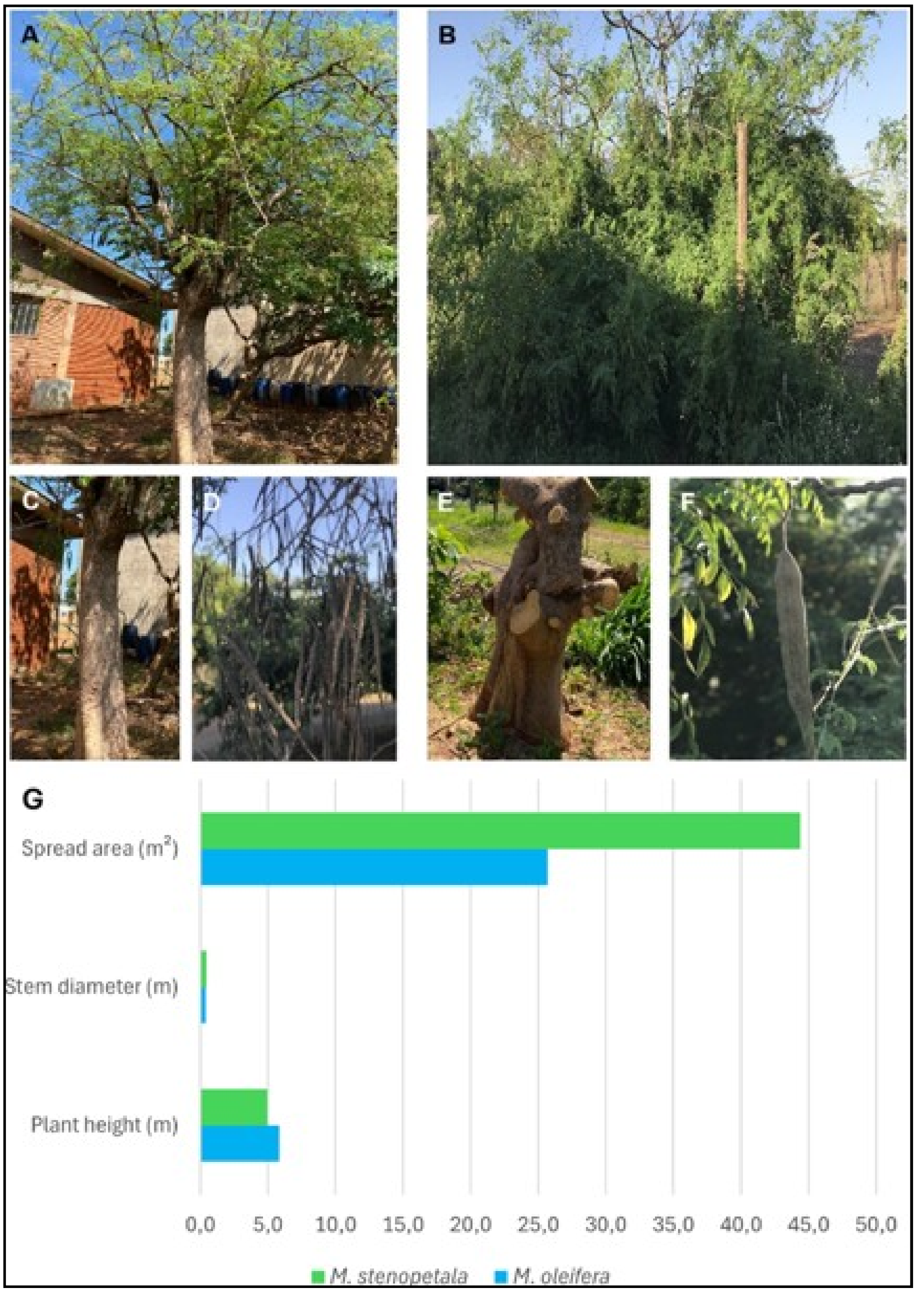

Figure 1. *Moringa* plants analyzed, highlighting trunk and fruit: A, C, and D represent *M. oleifera*, while B, E, and F correspond to *M. stenopetala*. (G) Results of analyses related to canopy area, stem diameter, and plant height.

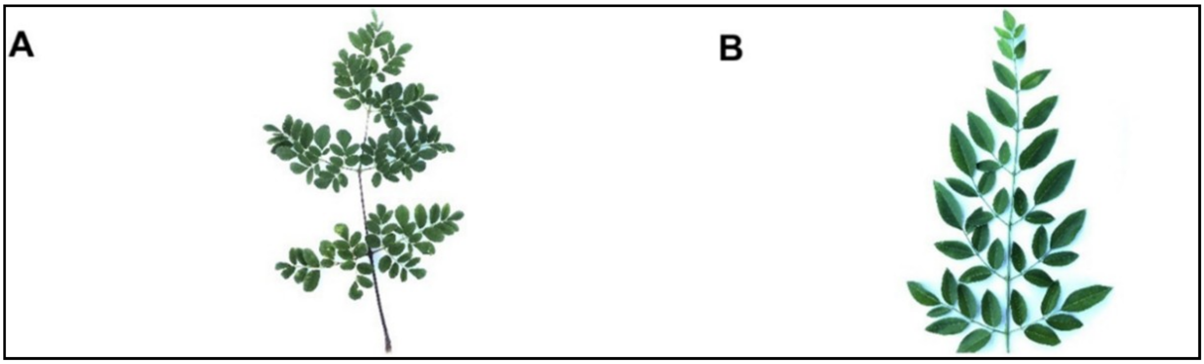

Figure 2. Leaves of *M. oleifera* **(A) and leaves of** ***M. stenopetala*** **(B).**

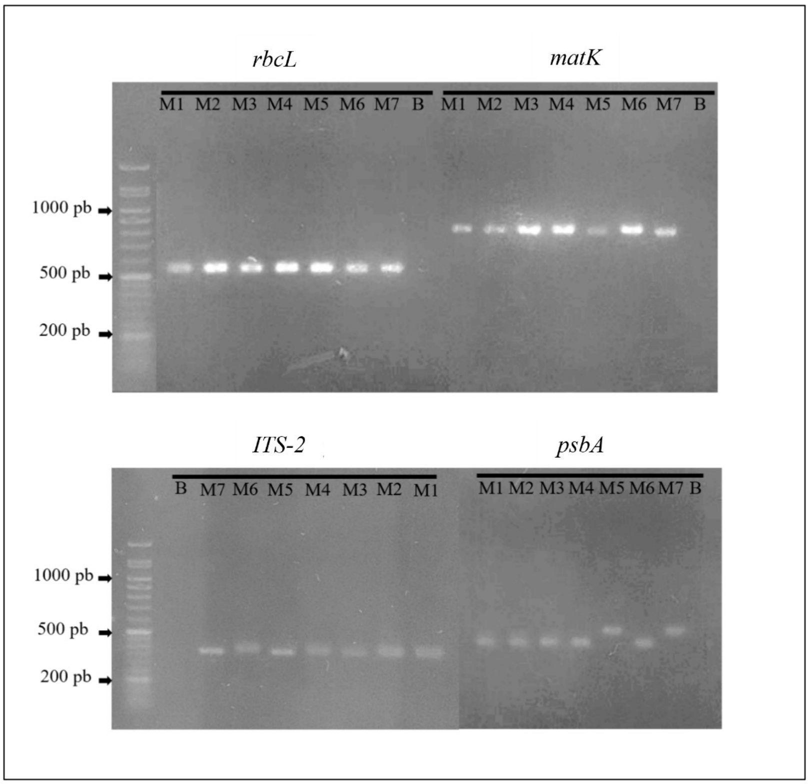

Figure 3. Amplification of the four genes used as DNA barcoding markers (*rbcL, matK, psbA*, and *ITS-2*) in five genotypes of *Moringa oleifera* (M1, M2, M3, M4, and M6) and two genotypes of *Moringa stenopetala* (M5 and M7). Sample B corresponds to the negative PCR control.

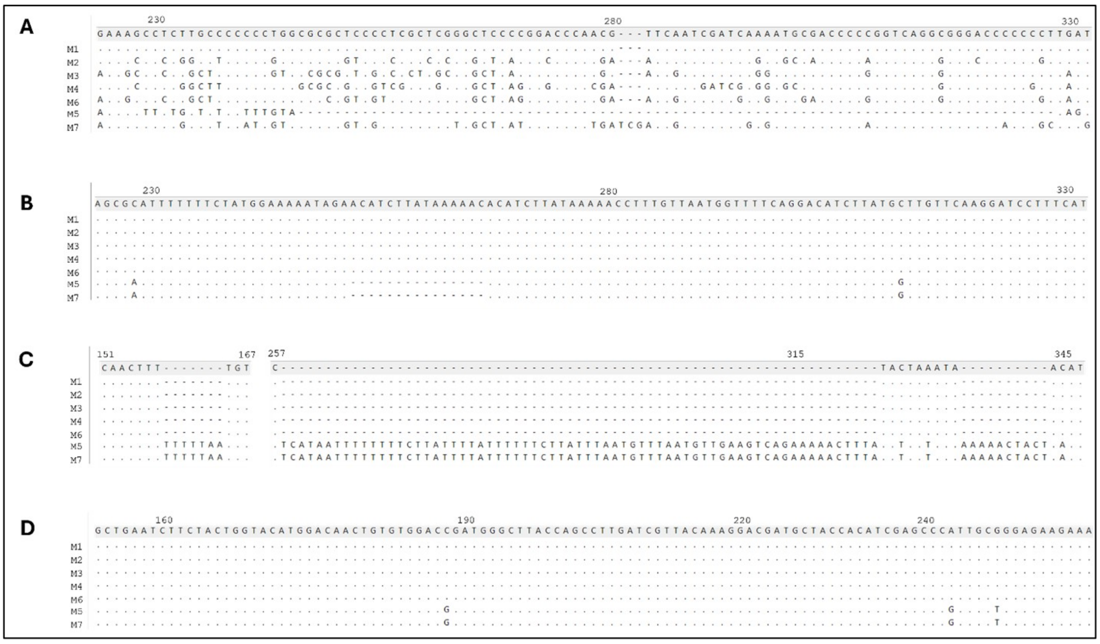

Figure 4. Alignment of *M. oleifera* **(M1, M2, M3, M4, and M6) and** ***M. stenopetala*** **(M5 and M7) genotypes using the MUSCLE algorithm: (A) *ITS-2*; (B) *matK*; (C) *psbA*; (D) *rbcL*.**

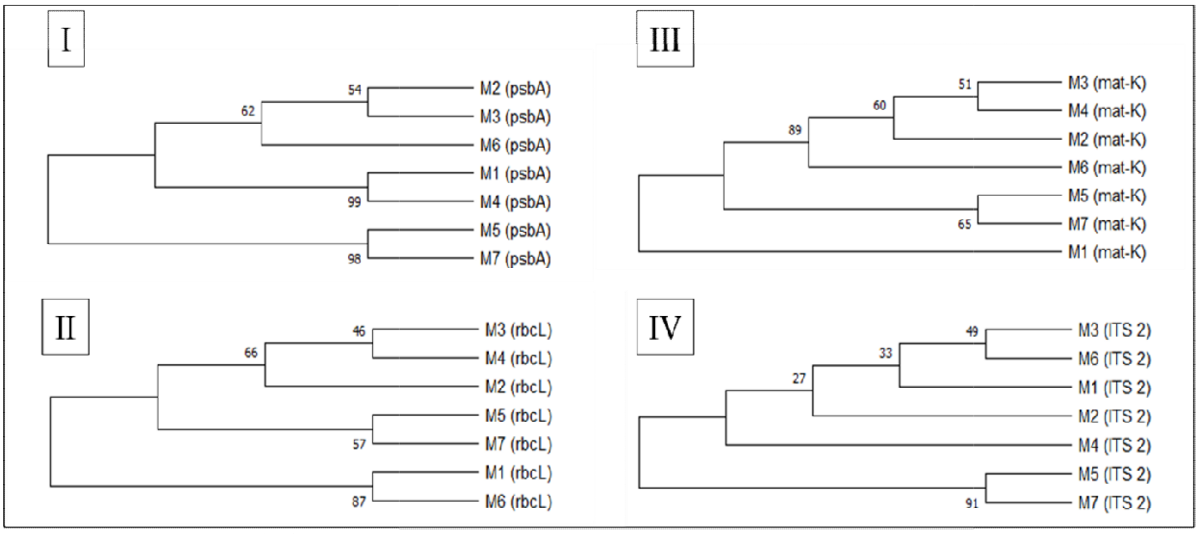

Figure 5. Clustering of *M. oleifera* (M1, M2, M3, M4, and M6) and *M. stenopetala* (M5 and M7) genotypes using the UPGMA method based on the genes: I, *psbA*; II, *rbcL*; III, *matK*; *IV, ITS-2*

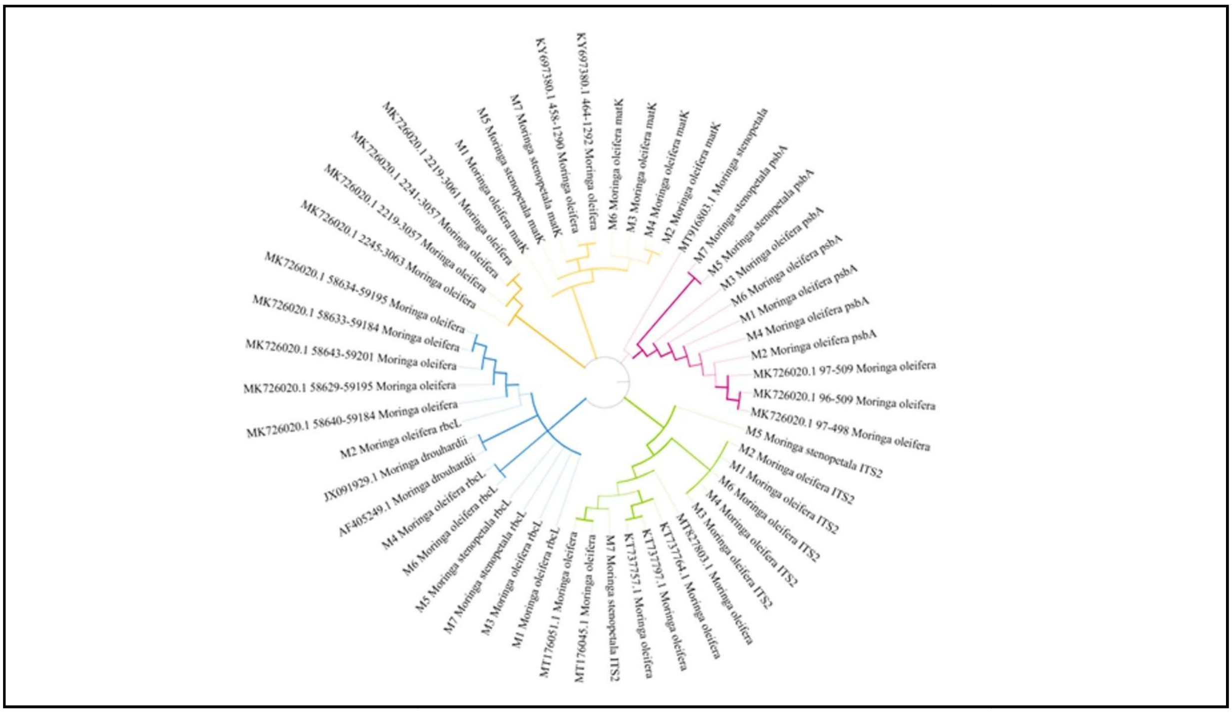

Figure 6. Phylogenetic tree based on sequences of the *psbA* (pink), *ITS-2* (green), *rbcL* (blue), and *matK* (yellow) genes, including *Moringa* genotypes analyzed in this study (M1-M7) and sequences obtained from NCBI. The alignment was performed using the MUSCLE algorithm. Branches in bold represent groupings with bootstrap values greater than 80%

50% of cases, followed by *M. drouhardii* (25%) and *M. stenopetala* (25%). The phylogenetic analysis, constructed from the sequences of *matK*, *psbA*, *rbcL*, and *ITS-2*, together with homologous accessions available in public databases, allowed the inference of the origin and phylogenetic relationships of the *Moringa* samples evaluated in this study. Although the overall tree topology suggests some separation between groups, this distinction was not consistently supported by all markers used (Figure 6). For the *psbA* gene, M5 and M7 clustered with the *M. stenopetala* sequence (MT916803.1), whereas in the other genes these accessions grouped with *M. oleifera*. The other five isolates (M1, M2, M3, M4, and M6) consistently grouped with reference sequences of *M. oleifera* from India (KT737764) and Saudi Arabia (MT176045, MT176051) across all genes analyzed. *M. oleifera* is recognized as native to northwest India and eastern Pakistan, being widely cultivated and dispersed by human-mediated routes to various tropical regions, including South America (Sharma *et al*., 2024). The similarity pattern between the sequences deposited in databases and the study samples indicates a strong genetic proximity to genotypes from the Asian center of origin, suggesting that the analyzed material derives from introductions or cultivations originating in this region. Historically, *M. oleifera* was introduced to different tropical regions due to its nutritional and medicinal value (Sharma *et al*., 2024). This process of dispersal and domestication contributed to the intense gene flow observed among populations, resulting in low genetic divergence between accessions from different geographic origins. Supporting this hypothesis, Sharma *et al*. (2024) reported that approximately 86% of genetic variation in *M. oleifera* occurs within populations and only 19% between them, indicating that genetic groupings of the species do not follow a clear geographic pattern. On the other hand, the M5 and M7 samples of *M. stenopetala* were associated with *M. oleifera* in the *rbcL*, *matK*, and *ITS-2* genes, indicating a more complex phylogenetic pattern, since *M. stenopetala* is recognized as a species native to East Africa (Kidane *et al*., 2024). This clustering may be explained by the predominance of Asian *M.*

oleifera genotypes in reference databases, which tends to bias the alignments toward this species. Another plausible hypothesis is that the African-origin material may have undergone hybridization or introgression events during domestication and dissemination processes, resulting in the sharing of plastidial and nuclear haplotypes among closely related species (Ojeda-López *et al*., 2020). Furthermore, the small number of *M. stenopetala* specimens included in the study limits the robustness of phylogenetic inferences and may contribute to the inconsistent positioning of these samples across different markers. The combined analysis of amplification, alignment, and clustering results indicated that the *psbA* gene exhibited the highest discriminatory power between *M. oleifera* and *M. stenopetala*. In addition to the marked difference in sequence size and the consistent separation into distinct groups, the alignment obtained from this gene was the only one to provide specific identification of *M. stenopetala* for the M5 and M7 samples. These results reinforce the potential of *psbA* as an efficient DNA barcode for intra- and interspecific distinction in the *Moringa* genus, even considering previous reports that insertions and deletions (INDELs) in this region could compromise precise sequence identification (Tnah *et al*., 2019).

CONCLUSION

Morphological analyses were effective in differentiating the two *Moringa* species studied, identifying characteristics, mainly foliar, that distinguished *M. oleifera* from *M. stenopetala*. Among the loci analyzed, *psbA* and *ITS-2* demonstrated high potential as molecular DNA barcodes for the *Moringa* genus, allowing efficient discrimination between *M. oleifera* and *M. stenopetala* cultivated in Brazil. Considering the difficulty in finding reliable DNA barcodes for plant species, as plastidial and mitochondrial genes evolve at a slow rate, the present study makes a significant contribution by identifying DNA barcode markers that can be integrated into a global system for bioidentification of *M. oleifera* and *M. stenopetala* species.

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