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Verification of Maman (*Cleome gynandra* (L.) Briq.) from Riau Based on *matK* and *trnL-trnL-trnF Intergenic Spacer*

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Abstract. Maman is a traditional plant frequently used as food by people in Melayu Rokan of Riau Province. Morphological detection indicates that the scientific name of maman is *Cleome gynandra* L. The objective of this study is to confirm the taxonomic status of maman from Riau based on *matK* and *trnL-trnL-trnF intergenic spacer* (*IGS*) sequences. Methods utilized in this study included sampling, DNA extraction, PCR, electrophoresis, sequencing, and data analysis. The fresh leaves were picked up from Rokan Hulu Regency, Riau Province. In this study, the *matK* and *trnL-trnL-trnF IGS* sequences of maman with a length of 754 bp and 938 bp, respectively, had been obtained. The BLASTn analysis based on both sequences showed that maman had 100% similarity to *Gynandropsis gynandra*. *Cleome gynandra* was synonymous with *G. gynandra*. There were 49 nucleotide variations, 16 critical nucleotides, and there were no indels in *matK*. Meanwhile, in *trnL-trnL-trnF IGS* there were 181 nucleotide variations, 13 critical nucleotides, and 61 indels. In conclusion, this study succeeded in confirming the taxonomic status of maman from Riau as *C. gynandra* syn. *G. gynandra.* The result enriched the abundance of DNA barcode sequences of this plant in GenBank which is useful for the molecular identification.

Keywords: *Cleome gynandra*; DNA barcode; maman from Riau; *matK*; *trnL-trnL-trnF intergenic spacer*.

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INTRODUCTION

Riau Province has unique geographic and environmental conditions, which are reflected by lots of wet land, peat, nutrient-poor soil, acidic and dry soil. Consequently, plants that grow in Riau Province are also unique and have many benefits, for instance, they serve as sources of food and medicine for the local Riau community such as maman plant. Morphological detection of maman indicates that its scientific name is *Cleome gynandra* (L.) Briq. In other regions in Indonesia, *C. gynandra* is known as mamang or lenglengan (Restusari et al., 2022; Restusari et al., 2023).

Cleome gynandra are herbaceous plants originating from Africa and Asia and are found growing in tropical climates such as Indonesia. In several regions of the world, *C. gynandra* is also known as *African spider-flower, African cabbage, cat's-whiskers, shona cabbage, spider plant, spider wisp, bastard-mustard* (Shilla et al., 2019), and tikawoch (Yimer et al., 2023). Shouthwestern Ethiopian people (Yimer et al., 2023) and Shurugwi people in Zimbabwe (Maroyi, 2013) use the *C. gynandra* leaves as a source of traditional food. Besides that, this plant is used by Songkhla and Krabi peoples in Thailand as skin disease medicine (Neamsuvan & Bunmee, 2016).

Study about botanical, morphological, and physiological characteristics of *C. gynandra* has been performed by Wasonga et al. (2015), Shilla et al. (2016), Shilla et al. (2019), Raju & Rani (2016). Moreover, researches related to its phytochemical (Pillai & Nair, 2013; Ranjitha et al., 2013), genome size and ploidy (van der Bergh et al., 2014; Omondi et al., 2017) have also been conducted. But, study about DNA barcoding of this plant is still limited.

DNA barcoding is a technique for molecular organism identification using DNA barcode. The

DNA barcode itself is a short DNA sequence whose location has been discovered in the genome. This technique will make it easier to identify organisms, including plants, quickly, precisely, and accurately (Kress, 2017). Another advantage of this technique is that it helps identify plants which organs are incomplete when identification is carried out and is very important for people who do not have plant taxonomic expertise (Roslim et al., 2023). Furthermore, The DNA barcoding can be used to track an organism (Rahayu & Jannah, 2019) and forensic botanical analysis of endangered species (Kress, 2017).

The DNA barcode can be derived from nuclear (nDNA), chloroplast (cpDNA), and mitochondrial (mtDNA) genomes (Wanda et al., 2021). The DNA barcodes from cpDNA are considered more suitable for use in identifying plant species because cpDNA has variation and a high degree of discrimination in plants (Shafqat et al., 2020). Examples of chloroplast DNA barcodes that are widely used are the *matK* and *trnL-trnL-trnF Intergenic Spacer* (*IGS*) (Herman et al., 2023).

The *matK* encodes maturase-K that functions in pre-mRNA splicing (Mustafa et al., 2018). It has higher mutations and undergoes more rapid evolution compared to *rbcL*. The *matK* also has the ability to differentiate several species in Angiosperms (Patwardhan et al., 2014) such as Sangihe Nutmeg (*Myristica fragrans*) (Tallei & Kolondam, 2015), flowering plants in Sumatra (Amandita et al., 2019), *Metroxylon sagu* (Abbas et al., 2020), andaliman (*Zanthoxylum acanthopodium* DC) from North Sumatra (Suriani et al., 2021), and some rare fruits in West Kalimantan (Candramila et al., 2023). Meanwhile, the *trnL-trnL-trnF IGS* sequence includes the intron region of the *trnL(UAA)* gene and the spacer between the *trnL(UAA)* and *trnF(GAA)* genes. The *trnL-trnL-trnF IGS* is a non-coding region and has a higher frequency of evolution and mutation than the coding region, so it is well used in carrying out evolutionary studies and identification between species (Matiz-Ceron et al., 2022). In addition, the *trnL-trnL-trnF IGS* is easy to be amplified, relatively short in size, and has a fairly good ability to differentiate between species (Roslim et al., 2021). The *trnL-trnL-trnF IGS* has been applicated in some plant like *Benstonea* sp. (Roslim, 2017), *Kalanchoe x laetivirens* (Roslim et al., 2021), *Phalaenopsis* (Mursyidin et al., 2021), and some species in Solanaceae and Fabaceae (Herman et al., 2023).

Therefore, this study aims to verify the scientific name of maman from Riau using two DNA barcodes such as *matK* and *trnL-trnL-trnF IGS*. This research is important to enrichment the DNA barcodes of this plant in public database and supporting the identification of this plant using DNA barcoding technique.

METHODS

Materials and Procedures

The fresh maman leaves used in this study were picked up from Serombau Indah Village, Rambah Hilir District, Rokan Hulu Regency, Riau Province. The primer pairs used to amplify *trnL-trnL-trnF IGS* region are B49317_F2: CGA AAT CGG TAG ACG CTA CG and A50272_R3: ATT TGA ACT GGT GAC ACG AG (Herman et al., 2023); and *matK* is matK-413F-1: TAA TTT ACR ATC AAT TCA TTC AAT ATT TCC and matK-1227R-3: GAR GAT CCR CTR TRA TAA TGA AAA AGA TTT (Heckenhauer et al., 2016).

Field observation was conducted to characterize of the morphological characteristics from three plants of maman from Riau. The characterization was performed referring to Mashamaite et al. (2022). Maman fresh leaves were then used to extract the total DNA using the Genomic DNA Mini Kit Plant (Geneaid). The total DNA solution was then amplified using the primer pairs for *matK* and *trnL-trnL-trnF IGS*, with the PCR component referring to the study by Herman et al. (2023). The PCR technique following Herman et al. (2023) that began with one cycle of pre-PCR at 95℃ for 3 minutes, followed by 35 cycles consisting of denaturation (45 seconds at 95℃), annealing or primer attachment (45 seconds at 49.2℃ for *trnL- trnL-trnF IGS* and 45 seconds at 47.5℃ for *matK*), and primer elongation (90 seconds at 72℃). The PCR process was ended with one cycle for 10 minutes at 72℃.

The success of the PCR process was examined using the electrophoresis technique. Electrophoresis was carried out on 1% agarose gel added with 3 µl ethidium bromide in 1X TBE buffer, at 50 volts for 45 minutes. After that, a minimum of 40 µl of PCR product solution and 30 µl of primer per PCR product in different tubes were packed and sent to PT. Genetika Science Indonesia as an intermediary, for further sequencing to be carried out at First Base Laboratories, Malaysia (Roslim et al., 2021).

Data Analysis

The DNA sequences obtained from

sequencing using forward and reverse primers were then aligned using the BioEdit version 7.0 application. After that, the sample sequences were matched with the sequences in the GenBank database using the BLASTn (Basic Local Alignment Search Tool nucleotide) program. A total of 10 accessions that appeared in the BLASTn analysis were used to create dendrograms using the MEGA (Molecular Evolutionary Genetic Analysis) application version 6.0 (Roslim et al., 2021).

RESULTS AND DISCUSSION

The Maman from Riau Description

The morphological characteristics of maman from Riau were as follows: plant height reaches about 80 cm, with digitate leaf blade shape, finger compound leaf type (palmatus), alternating leaf arrangement, apiculate leaf tip, arrowhead leaf base (sagittate), the shape of the leaf edge was flat (entirely), the leaf surface was smooth (glabrous), green in color and had glandular hairs on the petiole. The inflorescence of this plant was unique because it reached about 10 cm in length with an unlimited compound inflorescence (racemose). Flowers had a long stalk with 4 sepals, 4 petals, 6 long purple filamentous stamens, and 1 pistil with a purple rounded (oval) ovule. The sepals were ovoid in shape and the petals were yellowish-white. The fruit was a legume with a rounded (oval) shape called a long-stemmed capsule, and thin-textured valves with glandular hairs. The unripe fruit was green then turned yellow when ripe and brown when dried before bursting (when the fruit cracked it released seeds). The seeds were brown when the fruit was not ripe and would be gray to black when the fruit was ripe and broken (Figure 1).

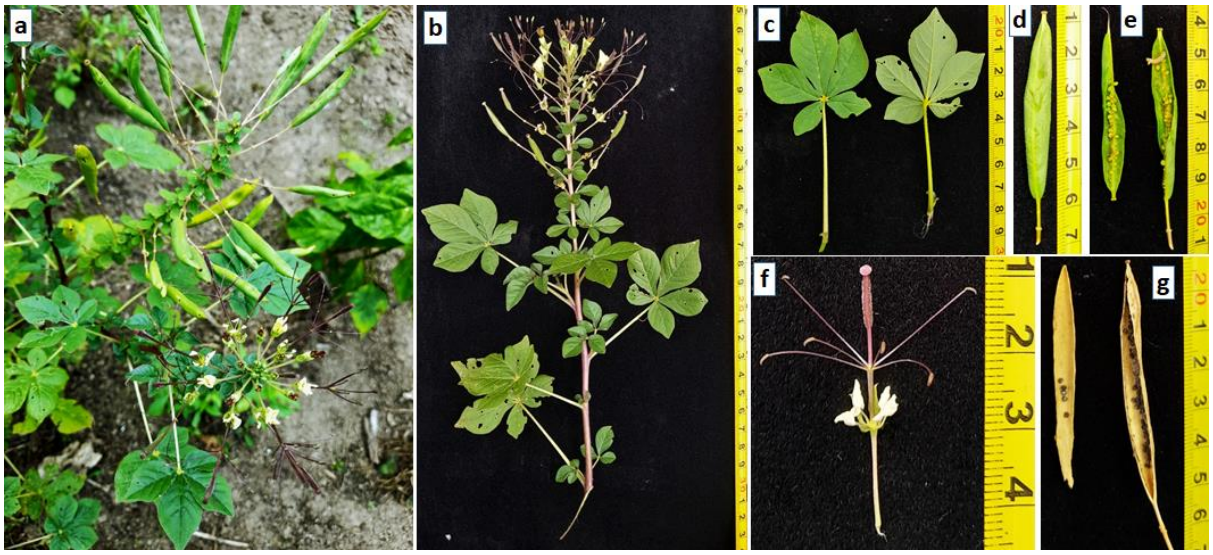

Figure 1. Maman plant. (a) habitus, (b) branching, (c) leaves, (d) fruit, (e) yellow seeds inside immature fruit skin, (f) inflorescence, (g) black seeds inside mature fruit skin.

Maman from Riau has been used by Riau people as a special Riau food called "Jeruk Maman". This food is made by fermenting maman leaves using the salting method resulting in a sour taste. This taste arises due to the appearance of lactic acid produced by the bacteria *Lactobacillus fermentum* and *Lactococcus lactis* (Irakoze et al., 2023) *L. plantarum*, *L. futsaii*, *L. paralimentarius* and *P. pentosaceus* (Ajibola et al., 2023). Study of *C. gynandra* in Africa revealed that this plant had chromosome numbers of 2n = 34 and the size of the genome was about 2.31 to 2.45 pg/2C (Omondi et al., 2017).

The Analysis of Maman *matK* Sequence

In this study, the maman *matK* sequences with a size of 754 bp were obtained and registered in GenBank with accession numbers OQ174531, OQ174532, and OQ174533 (Figure 2). The BLASTn analysis results based on *matK* sequences showed that maman had a high similarity to species from the Cleomaceae family. The highest similarity (100%) was found in *Gynandropsis gynandra* and *C. gynandra*, with a query cover value of 100% and an E-value of zero. The lowest similarity (96.42%) was to *Sieruela schimperi* with a query cover value of 100% and an E-value of zero (Table 1).

Figure 2. The *matK* sequences of maman from Riau.

Table 1. The BLASTn analysis based on the maman *matK* sequence.

Description	Query Cover (%)	E-value	Identity (%)	Accession
Gynandropsis gynandra	100	0.0	100.00	NC_054276.1
Cleome gynandra	100	0.0	100.00	EU371812.1
Sieruela usambarica (syn. *C. usambarica*)	100	0.0	96.82	KF923162.1
Rorida quinquenervia (syn. *C. quinquenervia*)	100	0.0	96.68	KF923150.1
Sieruela iberidella (syn. *C. iberidella*)	100	0.0	96.68	KF923138.1
Cleome viscosa (syn. *Corynandra viscosa*)	100	0.0	96.68	EU371806.1
Sieruela briquetii (syn. *C. briquetii*)	100	0.0	96.55	KF923127.1
Cleome droserifolia (syn. *R. droserifolia*)	100	0.0	96.55	EU371794.1
Polanisia dodecandra	100	0.0	96.55	AY483234.1
Sieruela schimperi (syn. *C. schimperi*)	100	0.0	96.42	KF923152.1

In the *matK* sequence, 49 nucleotide variations were found. The variations were caused by substitutional mutations and 16 of them were critical nucleotides at the bases numbers 37, 46, 88, 63, 252, 313, 316, 324, 400, 481, 536, 544, 559, 647, 735, and 751. The critical nucleotides in maman from Riau were TATCGCGCTTATCAAT while in other accessions were CGCACAATCGGATGCA. In this study there were no deletion mutations in the *matK* sequences (Table 2).

Table 2. The nucleotide variations based on *matK*

Nucleotide number (vertically)

No	37	46	52	55	88	97	137	146	160	161	163	168	184	229	236	252	265	267	276	280	286	313	316	324	342	358	379	400	425	439	452	464	481	504	536	544	559	577	591	592	618	636	647	707	715	721	735	747	751
1	T	A	C	G	T	A	A	G	A	T	C	T	G	A	C	G	G	C	G	C	T	C	G	C	A	A	C	T	G	A	A	G	T	C	A	T	C	A	T	A	T	G	A	G	G	C	A	C	T
2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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6	C	G	.	.	C	T	.	T	.	.	A	.	.	.	.	C	.	.	.	.	.	A	A	T	.	G	T	C	.	.	.	A	G	T	G	A	T	.	.	.	.	A	G	C	.	.	C	.	A
7	C	G	.	.	C	G	.	T	G	.	A	G	T	.	T	C	.	.	C	.	.	A	A	T	.	.	.	C	.	G	.	.	G	.	G	A	T	T	.	.	.	.	G	.	.	.	C	.	A
8	C	G	.	.	C	G	.	.	.	.	A	G	.	T	.	C	.	.	.	.	.	A	A	T	.	G	.	C	A	.	.	A	G	T	G	A	T	.	.	.	.	A	G	.	.	T	C	.	A
9	C	G	A	.	C	G	.	T	.	.	A	G	T	.	.	T	.	T	.	.	.	A	A	T	.	G	.	C	.	.	.	.	G	.	G	A	T	T	.	.	A	.	G	.	.	.	C	.	A
10	C	G	.	.	C	T	.	T	.	.	A	.	.	.	.	C	A	.	.	.	.	A	A	T	.	G	T	C	.	.	.	A	G	T	G	A	T	.	A	.	.	A	G	C	.	.	C	.	A
11	C	G	.	.	C	G	.	T	G	.	A	G	T	.	T	C	.	.	C	.	.	A	A	T	.	.	.	C	.	G	.	.	G	.	G	A	T	T	.	.	.	.	G	.	A	.	C	.	A
12	C	G	.	A	C	.	.	T	.	G	A	G	T	.	.	C	.	.	.	T	.	A	A	T	G	.	.	C	.	.	G	.	G	.	G	A	T	.	.	C	.	.	G	.	.	.	C	T	A
13	C	G	.	.	C	G	C	T	.	.	A	G	.	.	.	C	.	.	.	.	G	A	A	T	.	G	.	C	A	.	.	A	G	T	G	A	T	.	.	.	.	A	G	.	.	T	C	.	A

No. 1. *Cleome gynandra DIR107*, 2. *C. gynandra DIR108*, 3. *C. gynandra DIR109*, 4. *Gynandropsis gynandra*, 5. *C. gynandra*, 6. *Sieruela usambarica*, 7. *Rorida quinquenervia*, 8. *S. iberidella*, 9. *C. viscosa*, 10. *S. briquetii*, 11. *C. droserifolia*, 12. *Polanisia dodecandra*, 13. *S. schimperi*. (*)The vertical number shows the nucleotide position referring to *C. gynandra DIR107*. Dots (.) show that the nucleotide in that position is the same as in *C. gynandra DIR107*. Nucleotides in the yellow boxes are critical nucleotides for the maman from Riau identifying.

The phylogenetic tree showed that three individual maman studied (*C. gynandra* DIR107, 108, and 109) were in the same group as *G. gynandra* and *C. gynandra* with bootstraps values of 100%, separated from the other accessions studied (Figure 3). This result indicated that mamans from Riau were more closely related to *G. gynandra* and *C. gynandra* than other *Cleome* species.

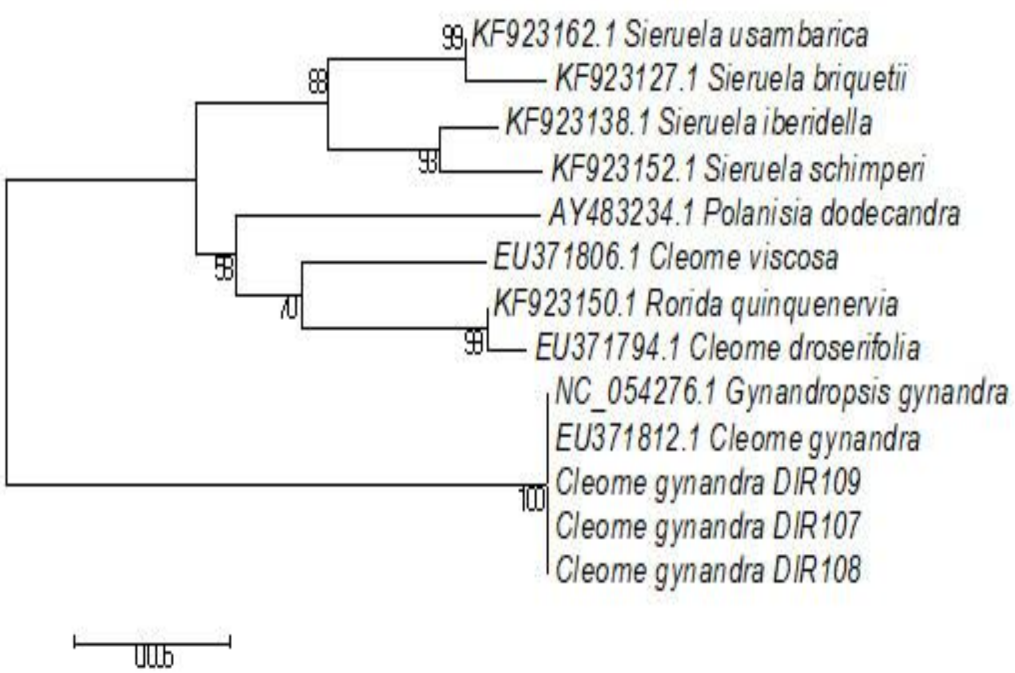

Figure 3. Phylogenetic tree based on *matK* sequences with *Neighbour-Joining* method and 1000 multiple bootstraps.

The Analysis of Maman *trnL-trnL-trnF Intergenic Spacer* Sequence

The *trnL-trnL-trnF IGS* sequences from the maman plants have been obtained with a length of 938 bp and registered in GenBank with accession numbers OQ174518 and OQ174519 (Figure 4). BLASTn analysis results indicated that maman had similarity to several species from the Cleomaceae and Capparaceae families. The highest similarity was to *G. gynandra* with the identity value of 100%, query cover value of 100% and E-value of zero, while the lowest similarity was to *C. domingensis* with the identity value of 93.70%, query cover of 98% and E-value of zero (Table 3).

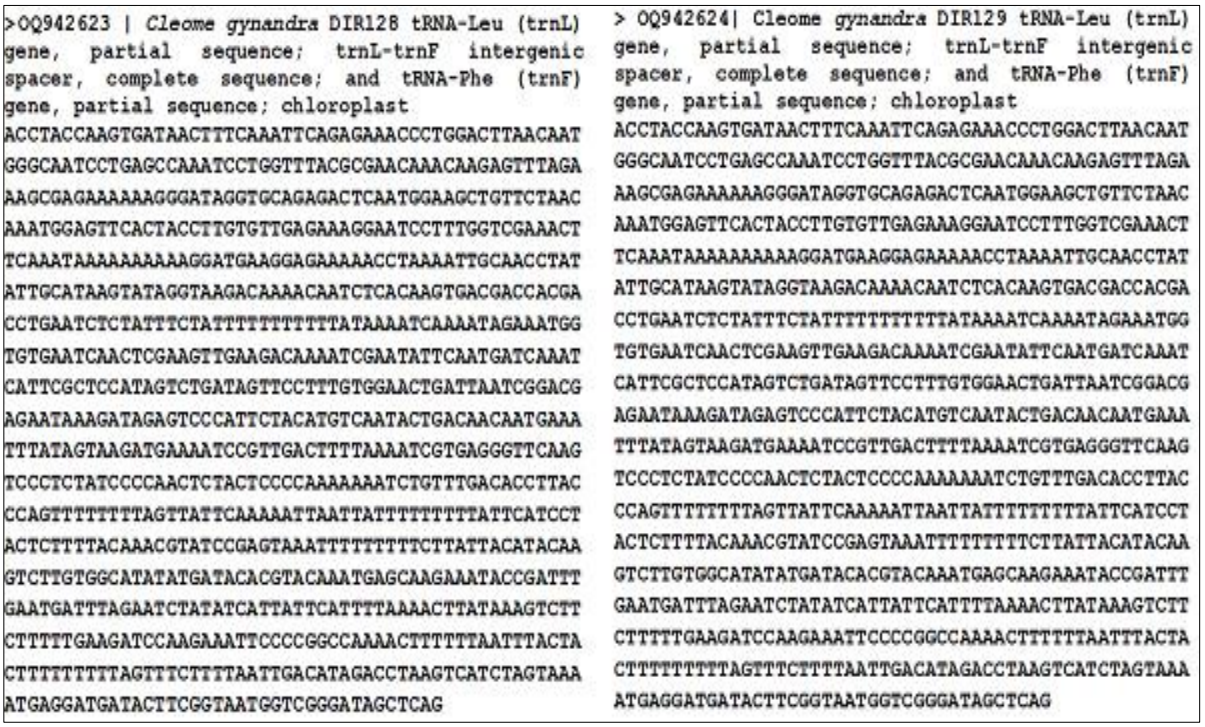

Figure 4. The maman *trnL-trnL-trnF intergenic spacer* sequences.

Table 3. The BLASTn analysis result of the maman *trnL-trnL-trnF intergenic spacer* sequence.

Description	Family	Query Cover (%)	E-value	Identity (%)	Accession
Gynandropsis gynandra	Cleomaceae	100	0.0	100.00	NC_054276.1
Tarenaya hassleriana	Cleomaceae	100	0.0	96.28	NC_034364.1
Cleome aculeata	Cleomaceae	98	0.0	96.53	AY122438.1
Cleome viridiflora	Cleomaceae	97	0.0	96.09	AY122441.1
Cleome spinosa	Cleomaceae	97	0.0	95.87	DQ649093.1
Cleome chrysantha	Cleomaceae	100	0.0	94.70	NC_053524.1
Cleome monophylla	Cleomaceae	98	0.0	95.04	AY122440.1
Podandrogyne chiriquensis	Cleomaceae	98	0.0	94.65	AY122450.1
Cadaba virgata	Capparaceae	98	0.0	94.58	AY122415.1
Cleome domingensis	Cleomaceae	98	0.0	93.70	AY122439.1

In the *trnL-trnL-trnF IGS* sequence, 181 nucleotide variations were found and 13 (7%) of them were critical nucleotides, namely at the base numbers 42, 197, 291, 294, 347, 360, 371, 401, 433, 436, 646, 652 and 827. The critical nucleotides in the maman from Riau were CGCGCGCATCATT while in other accessions were AAAAATTTATCCC. Moreover, in contrast to the *matK* sequence, the *trnL-trnL-trnF IGS* sequence contained 61 (34%) indels mutations (Figure 5). Phylogenetic tree based on the *trnL-trnL-trnF IGS* sequence showed that maman from Riau (*C. gynandra* DIR128 and DIR129) formed one group with *G. gynandra* (Figure 6).

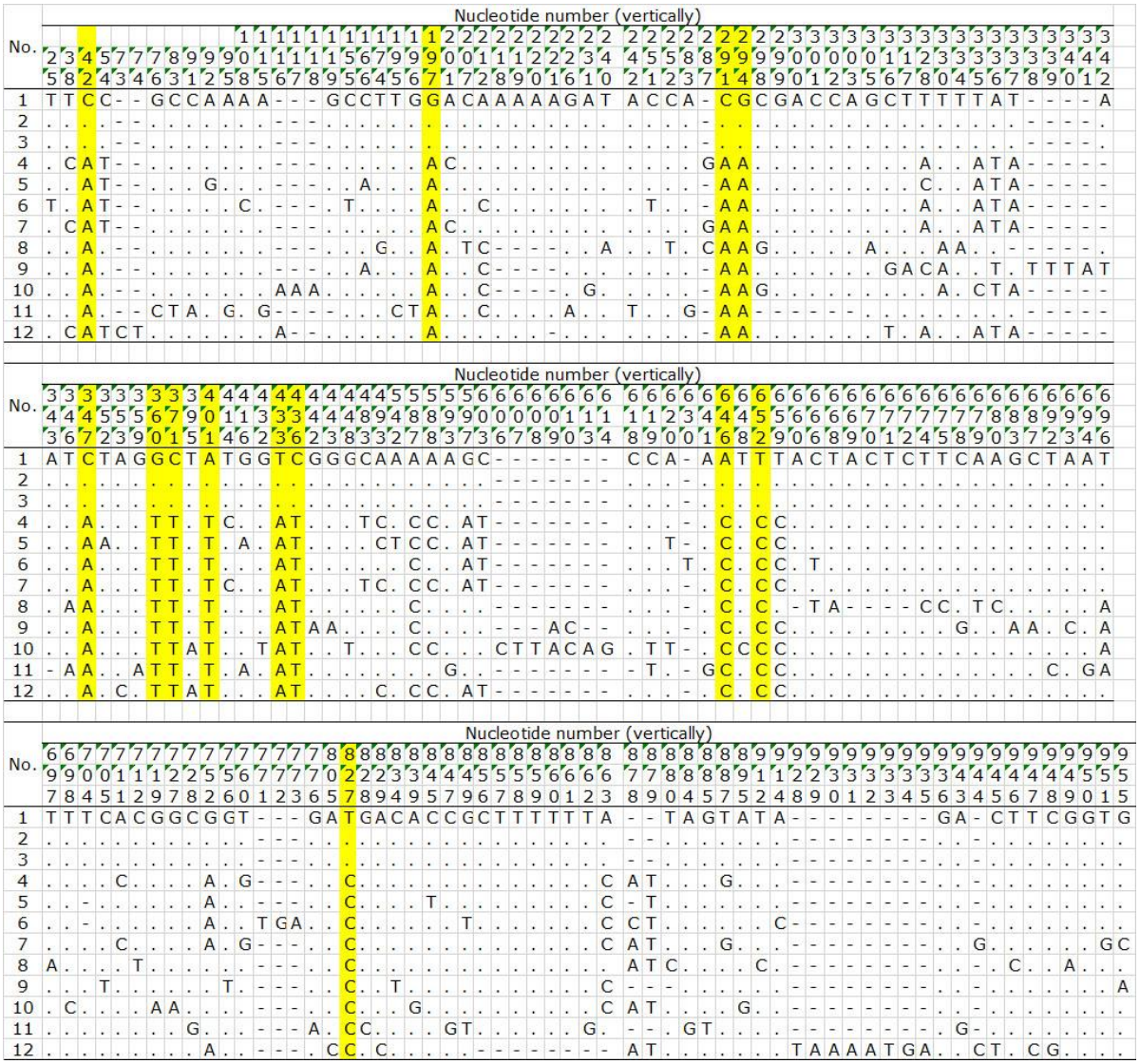

Figure 5. Nucleotide variations on *trnL-trnL-trnF intergenic spacer* sequence.
No. 1. *C. gynandra* DIR128, 2. *C. gynandra* DIR129, 3. G. gynandra, 4. Tarenaya hassleriana, 5. *C. aculeata*, 6. *C. viridiflora*, 7. *C. spinosa*, 8. *C. chrysantha*, 9. *C. monophylla*, 10. *Podandrogyne chiriquensis*, 11. *Cadaba virgata* and 12. *C. domingensis*. Dots (.) show that nucleotide in that position is the same as in *C. gynandra* DIR128. Nucleotides in the yellow boxes are critical nucleotides for the maman from Riau identifying.

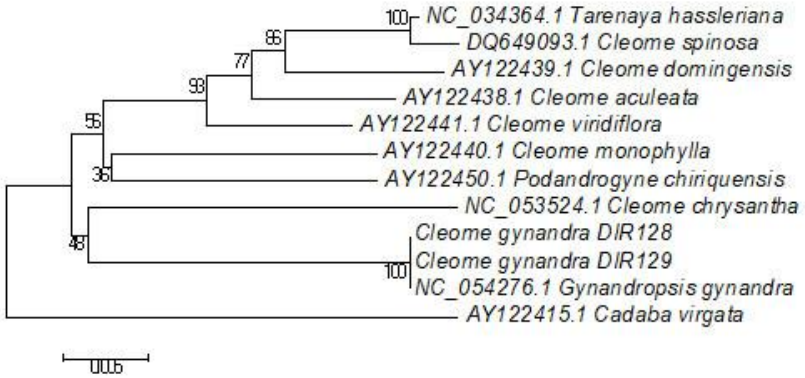

Figure 6. Phylogenetic tree based on *trnL-trnL-trnF intergenic spacer* sequences with *Neighbour-Joining* method and 1000 multiple bootstraps.

Determining the scientific of a plant species using BLASTn analysis must pay attention to several parameters such as query cover (%) and identity (%) which must reach 100% and the E-value equals zero (Roslim et al., 2016). Query cover is a sample sequence that will be compared to the all entries in GenBank database. The E-value or expectation value represents the number of different alignments with scores equivalent to or better than S that is expected to occur in a database search by chance. The lower the E-value is the more significant the alignment and score are. The identity represents that at the same positions in an alignment, the two sequences have the same residues. Query cover and identity are expressed as a percentage (%) (Fassler & Cooper, 2011).

In this study, those values were achieved (Table 1 and Table 3). There were resemblances between the BLASTn analysis results based on the *matK* and *trnL-trnL-trnF IGS* sequences that the maman from Riau had 100% similarity to *G. gynandra*. But, the *C. Gynandra trnL-trnL-trnF IGS* sequence did not appear in the BLASTn result of the maman *trnL-trnL-trnF IGS* sequence. This results indicated that the *C. Gynandra trnL-trnL-trnF IGS* sequence has never been reported and deposited in the GenBank database. Moreover, the results indicated that the species name of maman from Riau had been successfully verified using two DNA barcodes namely *matK* and *trnL-trnL-trnF IGS* as *C. gynandra* syn. *G. gynandra*.

Furthermore, *Cleome gynandra* has the synonymous name to *G. gynandra* (Mashamaite et al., 2022). Many species in genus *Cleome* have synonymous name such as *C. fritzscheae* syn. *C. iberidella*, *C. subcordata* Steud. syn. *C. monophylla*, *C. vahliana* Fresen. syn. *C. brachycarpa* Vahl ex DC., *C. diversifolia* Hochst. & Steud. ex T. Anderson syn. *C. brachycarpa* Vahl ex DC (Roalson & Hall, 2017).

In this study, the *matK* and *trnL-trnL-trnF IGS* are able to distinguish maman from other species. This is because the two barcodes have high variation in the form of substitution and indels mutations. Those variations give rise to critical nucleotides which are the hallmarks of maman. Substitutions and indels mutations occur in *trnL-trnL-trnF IGS* but in *matK* only substitution occurs (Table 2, Figure 5). Moreover, the discrimination of maman from other species studied is supported by phylogenetic trees constructing by the *matK* and *trnL-trnL-trnF IGS* sequences (Figure 3, Figure 6). Similar result also occurs in DNA barcoding analysis in *Benstonea* sp. (Roslim, 2017) and family Solanaceae and Fabaceae (Herman et al., 2023). Moreover, this research could enrich the maman DNA barcode database in public database. The DNA barcode database may support the maman molecular identification.

CONCLUSIONS

The use of two DNA barcodes, namely *matK* and *trnL-trnL-trnF IGS*, has succeeded in verifying the scientific name of maman from Riau as *C. gynandra* syn. *G. gynandra*. The length of the maman *matK* sequence is 754 bp and *trnL-trnL-trnF IGS* ones is 938 bp. The *matK* sequence contains more critical nucleotides (16 bases) than *trnL-trnL-trnF IGS* ones (13 bases). Moreover, there are no indels in *matK* sequence but there are 61 indels in *trnL-trnL-trnF IGS*. Both the DNA barcodes can be used for *C. gynandra* syn. *G. gynandra* identifying by using DNA barcoding technique.

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REFERENCES

Abbas, B., Kabes, R. J., Mawikere, N. L., Ruimassa, R. M. R., & Maturbong, R. A. (2020). DNA barcode of *Metroxylon sagu* and others palm species using *matK* gene. *Biodiversitas*, *21*(9), 4047-4057. DOI: 10.13057/biodiv/d210916.

Ajibola, O. O., Thomas, R., & Femi Bakare, B.F. (2023). Selected fermented indigenous vegetables and fruits from Malaysia as potential sources of natural probiotics for improving gut health. *Food Science and Human Wellness*, *12*(5), 1493-1509.

Amandita, F. Y., Rembold, K., Vornam, B., Rahayu, S., Siregar, I. Z., Kreft, H., & Finkeldey. (2019). DNA barcoding of flowering plants in Sumatra, Indonesia. *Ecology and Evolution, 9,* 1858-1868. DOI: 10.1002/ece3.4875.

Candramila, W., Fajri, H., Mardiyyaningsih, A. N., Firmansyah, R., & Akbar, D. (2023). Taxonomic confirmation of rare fruits in West Kalimantan using rbcL and matK sequences. *Biosaintifika: Journal of Biology & Biology Education, 15*(1), 20-25. DOI: http://dx.doi.org/

10.15294/biosaintifika.v15i1.40284.

Fassler, J., & Cooper P. (2011). *BLAST Glossary*. In: BLAST® Help [Internet]. Bethesda (MD): National Center for Biotechnology Information (US).

Heckenhauer, J., Barfuss, M. H., & Samuel, R. (2016). Universal multiplexable *matK* primers for DNA barcoding of angiosperms. *Applications in Plant Science, 4*(6), 1-7.

Herman, Al-Khairi, H., Wansyah, A. R., Asmania, Utami, R. I., Anzaelina, D. N., Oktaviano, Z., Lestari, W., Adiwirman, & Roslim, D. I. (2023). The ability of *matk* and *trnL-trnL-trnF intergenic spacer* to discern certain species accessions of Solanaceae and Fabaceae. *SABRAO Journal of Breeding and Genetics*, *55*(1): 97-106.

Irakoze, M. L., Wafula, E. N., & Owaga, E. E. (2023). Effect of lactic acid fermentation on phytochemical content, antioxidant capacity, sensory acceptability and microbial safety of African Black Nightshade and African Spider Plant Vegetables. *Bacteria*, *2*(1), 48–59. https://doi.org/10.3390/bacteria2010004

Kress, J. W. (2017). Plant DNA barcodes: applications today and in the future. *Journal of Systematics and Evolution, 55*(4), 291-307.

Maroyi, A. (2013). Use of weeds as traditional vegetables in Shurugwi District, Zimbabwe. *Journal of Ethnobiology and Ethnomedicine*, *9*(6), 1-10.

Mashamaite, C. V., Manyevere, A., & Chakauya, E. (2022). *Cleome gynandra*: A wonder climate-smart plant for nutritional security for millions in semi-arid areas. *Frontiers in Plant Science*, *13*, 1003080. doi: 10.3389/fpls.2022.1003080.

Matiz-Ceron, L., Reyes, A., & Anzola, J. (2022). Taxonomical evaluation of plant chloroplastic markers by Bayesian Classifier. *Frontiers in Plant Science*, *12*, 782663. doi: 10.3389/fpls.2021.782663

Mursyidin, D. H., Ahyar, G. M. Z., Saputra, A. W., & Hidayat, A. (2021). Genetic diversity and relationships of Phalaenopsis based on the rbcL and trnL-F markers: in silico approach. *Biosaintifika: Journal of Biology & Biology Education*, *13*(2), 212-221.

Mustafa, K. M., Ewadh, M. J., Al-Shuhaib, M. B. S., & Hasan, H. G. (2018). The in silico prediction of the chloroplast *maturase K* gene polymorphism in several barley varieties. *Agriculture (Pol'nohospodarstvo)*, *64*(2018), 3-16.

Neamsuvan, O., & Bunmee, P. (2016). A survey of herbal weeds for treating skin disorders from Southern Thailand: Songkhla and Krabi Province. *Journal of Ethnopharmacology, 193*, 574-585.

Omondi, E. O., Debener, T., Linde, M., Abukutsa-Onyango, M., Dinssa, F. F, & Winkelmann, T. (2017). Mating biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. *Plant Breeding, 136*(4), 578-589.

Patwardhan, A., Ray, S., & Roy, A. (2014). Molecular markers in phylogenetic studies: A Review. *Journal of Phylogenetics Evolutionary Biology*, *2*(2), 131.

Pillai, L. S., & Nair, B. R. (2013). Proximate composition, mineral elements and anti-nutritional factors in *Cleome viscosa* L. and *Cleome burmanni* W. & A. (Cleomaceae). *International Journal of Pharmacy and Pharmaceutical Sciences, 5*(1), 384-387.

Rahayu, D. A., & Jannah, M. (2019). *DNA Barcode Hewan dan Tumbuhan Indonesia.* Jakarta Selatan: Yayasan Inspirasi Ide Berdaya.

Raju, A. J. S., & Rani, D. S. (2016). Reproductive ecology of *Cleome gynandra* and *Cleome viscosa* (Capparaceae). *Phytologia Balcanica, 22*(1), 15-28.

Ranjitha, J., Vijiyalakshmi, S., Anand, M., & Bhagiyalakshmi, M. (2013). Biological assay of in vitro antioxidant and antibacterial activity of the whole plant material *Cleome gynandra* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 4(3), 97-102.

Restusari, L., Fitri, Mulyani, S., Ningrum, E. D., Farahiyati, B., & Putri, I. M. (2022). Edukasi khasiat dan pemanfaatan tanaman maman pada masyarakat di Desa Sedinginan Kabupaten Rokan Hilir. *PITIMAS: Journal of Community Engagement in Health, 1*(3), 64-69.

Restusari, L., Muharni, & Fitri. (2023). Activities of fermented maman (*Cleome gynandra* L.) on blood sugar level in hyperglycemic white rats. *Acta Scientific Nutritional Health*, 7(7), 85-90.

Roalson, E. H., & Hall, J. C. (2017). New generic concepts for African Cleomaceae. *Systematic Botany*, *42*(4), 925–942.

Roslim, D. I. (2017). Identification of Pandan Plant (*Benstonea* sp) from Riau, Indonesia Using Three DNA Barcodes. *SABRAO Journal of Breeding and Genetics, 49*(4), 346-360.

Roslim, D. I., Herman, Adiwirman, & Lestari, W. (2023). *Barkoding DNA (Jilid 1)*. Yogyakarta: Penerbit Karya Makmur.

Roslim, D. I., Khumairoh, S., & Herman. (2016). Confirmation of tuntun angin (*Elaeocarpus*

floribundus) taxonomic status using *matK* and *ITS* sequences. *Biosaintifika: Journal of Biology & Biology Education*, *8*(3), 392-399.

Roslim, D. I., Putra, Y. O., Dewi, Y. M., Bago, Y., Sitohang, H., Herman, Fitmawati, & Sofiyanti, N. (2021). First record of five DNA barcodes on nothospecies cocor bebek (*Kalanchoe* × *laetivirens*). *SABRAO Journal of Breeding and Genetics*, *53*(2), 263-377.

Shafqat, M., Qasim F., Nasrullah, U., Ahmad, M., Hussain, M., Ali, H., Noreen, S., & Qasim, M. W. (2020). DNA barcoding in plants and current molecular issues. *Scholars International Journal of Biochemistry*, *03*(03), 53-58.

Shilla, O., Abukutsa-Onyango, M. O., Dinssa, F. F., & Winkelmann, T. (2016). Sed dormancy, viability, and germination of *Cleome gynandra* (L.) Briq.: A review. *African Journal of Horticultural Science*, *10*, 42-52.

Shilla, O., Dinssa, F. F., Omondi, E. O., Winkelmann, T., & Abukutsa-Onyango, M. O. (2019). *Cleome gynandra* L. origin, taxonomy and morphology: A review. *African Journal of Horticultural Research, 14*(32), 1568-1583.

Suriani, C., Prasetya, E., Harsono, T., Manurung, J., Prakasa, H., Handayani, D., Jannah, M., & Rachmawati, Y. (2021). DNA barcoding of andaliman (*Zanthoxylum acanthopodium* DC) from North Sumatra Province of Indonesia using *maturase K* gene. *Tropical Life Science Research, 32*(2), 15-28.

Tallei, T. E., & Kolondam, B. J. (2015). DNA barcoding of Sangihe Nutmeg (*Myristica fragrans*) using *matK* gene. *Hayati Journal of Biosciences*, *22*(1), 41-47.

van den Bergh, E., Kulahoglu, C., Brautigam, A., Hibberd, J. M., Weber, A. P., Zhud, X., & Schranz, M. E. (2014). Gene and genome duplications and the origin of C4 photosynthesis: Birth of a trait in the Cleomaceae. *Current Plant Biology, 1*, 2-9.

Wanda, I. F., Djulita, N. R., & Chikmawati, T. (2021). Molecular phylogenetics od Malesian *Diospyros* (Ebenaceae) based *trnL-F Spacer* sequences. *Biodiversitas*, *22*(9), 4106-4114.

Wasonga, D.O., Ambuko, J. L., Chemining'wa, G. N., Odeny, D. A., & Crampton, B. G. (2015). Morphological characterization and selection of spider plant (*Cleome gynandra*) accessions from Kenya and South Africa. *Asian Journal of Agricultural Sciences, 7*(4), 36-44.

Yimer, A., Forsido, S. F., Addis, G., & Ayelign, A. (2023). Nutritional composition of some wild edible plants consumed in Southwest Ethiopia. *Heliyon*, *9*(6): e16541.