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IDENTIFICATION OF ENDEMIC *CURCUMA ALBIFLORA* THW. FROM CURCUMA SPECIES GROWN IN SRI LANKA BY DNA BARCODING METHOD

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ABSTRACT: Curcuma is an important genus in traditional medicine. Five plants are reported in Sri Lanka; *C. albiflora*, *C. aromatica*, *C. longa*, *C. zedoaria*, and *C. oligantha*. Since the phylogenetic analysis of gene sequences and combining with complete genomic sequences helps to identify the genetic basis of plants, the current study was conducted. Standard CTAB method with little modifications was used for the extraction and purification. Extracted DNA was amplified using universal primers for matK genes in chloroplast genome by PCR (polymerase chain reaction). Sequenced fragments were analyzed and used for DNA barcoding. DNA barcodes {Accession Numbers: *C. aromatica* (GU180412), *C. albiflora* (KF 521885), *C. longa* (JQ409688), *C. oligantha* (JQ409715), *C. zedoaria* (JQ409703)} were used for analysis. *C. albiflora* appeared as a different group as per the Neighbor-Joining method and therefore, it can be identified as a new group. The matK gene of *C. albiflora* and other species showed, totally 222 variable sites. Therefore, the matK gene was an appropriate DNA barcode for identifying *C. albiflora* from remaining Curcuma plants grown in Sri Lanka.

INTRODUCTION: Curcuma is a genus of family Zingiberaceae, many species are important in Sri Lankan traditional medicine. Since the morphological similarity of *Curcuma species*, pharmacognostical evaluation is useful in the identification of *Curcuma* species. DNA sequencing is one of the reliable methods in biological identification [1]. DNA barcode of each species belongs distinct pattern, especially MaturaseK (matK) gene of the chloroplast, can be used to show as separate group [2, 3]. Phylogenetic analysis of the matK coding and noncoding regions is used to derive relationship among genera [4].

QUICK RESPONSE CODE

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Biological diversity in plant species is evidenced by a phylogenetic tree. The main objective of this study was to differentiate *Curcuma* species grown in Sri Lanka using DNA barcode. Due to the stability of the DNA pattern of each species, the authentication by DNA barcode is a reliable method to identify similar plant raw materials. DNA barcoding method in identification of authentic raw-material is a reliable method since it is based on the macromolecular level which is not mutating by external environment factors [5].

MATERIAL AND METHODS: DNA extraction, the analysis was done according to methodology explained by Wijayasiriwardene [3]. Multiple alignments were performed using a built-in CLUSTAL W and aligned sequences were edited by molecular evolutionary genetics analysis (MEGA) version 7.0. Evolutionary analyses were conducted in MEGA7 [6]. Tajima test statistic [7] was performed according to Nei [8].

The phylogenetic tree was obtained using the Neighbor-Joining method [8]. The sum of branch length was computed using the maximum composite likelihood method [9]. The genetic distance was calculated based on the Kimura-2-parameter model [10]. All positions containing gaps and missing data were eliminated by multiple alignments.

RESULTS AND DISCUSSION: The alignment of the matK gene of combined nucleotide sequence showed 1610 conserved sites, 222 variable sites, zero parsim-info sites, and 220 singleton sites. Nucleotide compositions of uncoded, 1st, 2nd, and 3rd codon positions were reported in **Table 1**.

TABLE 1: NUCLEOTIDE COMPOSITION OF matK GENE OF *CURCUMA* SPECIES

	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos#1	T-2	C-2	A-2	G-2	Pos#2	T-3	C-3	A-3	G-3	Pos#3
C. oligantha	40.2	16.6	31.1	12.1	717.0	44	12.1	32.6	11.7	239.0	36	23.8	23.0	16.7	239.0	41	13.8	37.7	7.9	239.0
C. albiflora	37.0	18.0	29.1	15.8	859.0	35	20.3	30.1	15.0	286.0	36	19.9	27.3	17.1	286.0	41	13.9	30.0	15.3	287.0
C. zedoaria	37.6	14.8	32.5	15.1	1832.0	37	13.6	35.2	14.2	611.0	34	20.0	28.2	18.0	611.0	42	10.8	34.3	13.1	610.0
C. longa	37.6	14.9	32.4	15.1	1832.0	37	13.7	35.0	14.2	611.0	34	20.0	28.2	18.0	611.0	42	11.0	34.1	13.1	610.0
C. aromatica	38.5	16.2	30.3	15.1	816.0	34	18.4	34.9	12.5	272.0	34	20.5	27.8	17.6	273.0	47	9.6	28.0	15.1	271.0
Avg.	37.9	15.7	31.5	14.9	1211.2	37	15.1	34.1	13.8	403.8	34	20.5	27.4	17.7	404.0	42	11.5	33.2	13.1	403.4

The matK gene of *C. albiflora* and other species showed, totally 222 variable sites. *C. albiflora* matK gene showed a total of 859 nucleotide positions, but only *C. oligantha* showed less than 800 nucleotide positions **Table 1**. Pairwise distances of *Curcuma species* were mentioned in **Table 2**.

TABLE 2: ESTIMATES OF PAIRWISE DISTANCES BETWEEN matK SEQUENCES

	1	2	3	4
C. aromatic	0.0000			
C. albiflora	0.3723	0.0000		
C. oligantha	0.0012	0.3433	0.0000	
C. longa	0.0000	0.3428	0.0025	0.0000
C. zedoaria	0.0000	0.3428	0.0033	0.0011

Thymine nucleotide percentage of *C. oligantha* of matK was significantly higher (44%) at the uncoded position than other four species. Phylogenetic tree of the matK gene of Curcuma was shown in **Fig. 1**. The optimal tree with the sum of branch length was 0.0135. There were a total of 522 positions in the final dataset.

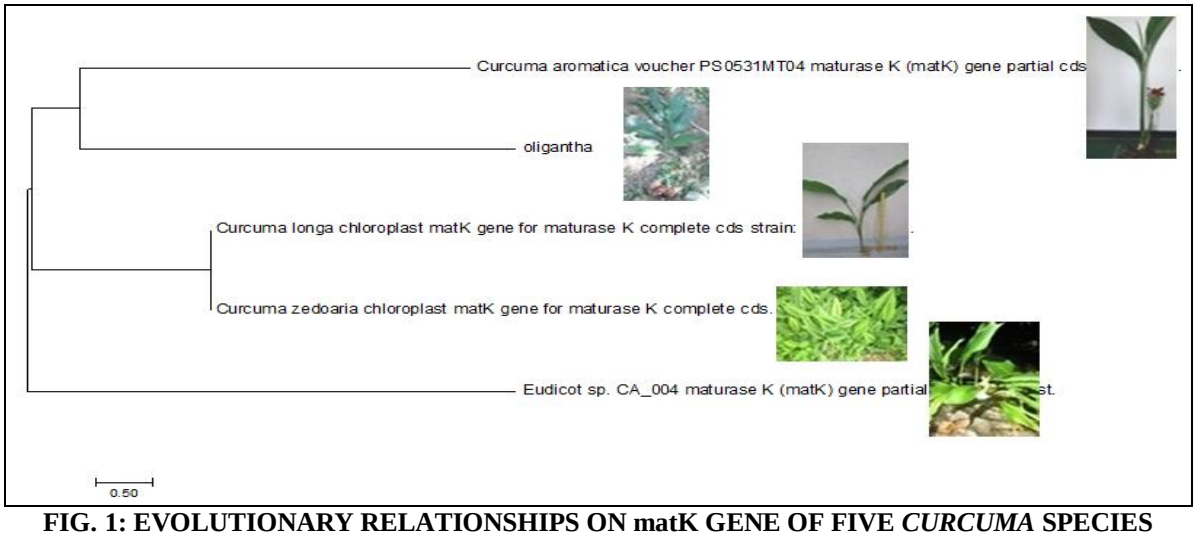

FIG. 1: EVOLUTIONARY RELATIONSHIPS ON matK GENE OF FIVE *CURCUMA* SPECIES

The estimated value of the shape parameter for the discrete gamma distribution was 0.8978. Mean evolutionary rates in these categories were 0.09, 0.33, 0.67, 1.21, 2.70 substitutions per site. The nucleotide frequencies were A = 29.62%, T/U = 39.85%, C = 18.11%, and G = 12.42%. There were a total of 264 positions in the final dataset.

Results of Tajima's Neutrality test were mentioned in **Table 3**.

TABLE 3: RESULTS FROM TAJIMA'S NEUTRALITY TEST

m	s	ps	Θ	π	D
5	83	0.314394	0.150909	0.125758	-1.264783

m = number of sequences, S = Number of segregating sites, ps = S/n, Θ = ps/a1, π = nucleotide diversity, and D is the Tajima test statistic

As per the results of Tajima's Neutrality Test, 83 number of segregating sites and nucleotide diversity of 0.1257 was found, which was different from species (212 segregating sites and 0.1823 of nucleotide diversity) claimed as Harankaha [3]. The maximum pairwise distance between *C. albiflora* and with other species which were studied **Table 2**.

From the results of the phylogenetic tree, *C. albiflora* was separately clustered into one group, while *C. zedoaria* and *C. longa* were clustered into one group and *C. aromatica* and *C. oligantha* into another group **Fig. 1**. Therefore, the matK gene was an appropriate DNA barcode for identifying *C. albiflora* from Curcuma plants grown in Sri Lanka.

CONCLUSION: Phylogenetically, *C. albiflora* appears as a different group as per the Neighbor-Joining method and therefore, it can be identified as a new group. Therefore, DNA barcoding study provided reliable proof to identify *C. albiflora* from other *Curcuma species.*

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REFERENCES:

1. Kumar BR: DNA representation: DNA Sequencing – Methods and Applications. In Tech Janeza Trdine, Rijeka, Croatia 2012.
2. Selvaraj S, Sarma RK and Sathishkumar R: Phylogenetic analysis of chloroplast matK gene from Zingiberaceae for plant DNA barcoding. Bioinformation 2008; 3(1): 24-27.
3. Wijayssiriwardene TDCMK, Herath HMIC and Premakumara GAS: Identification of endemic Curcuma albiflora Thw. by DNA barcoding method. Sri Lankan Journal of Biology 2017; 2(1): 23-30.
4. Kress WJ, Linda M and Prince KJ: The phylogeny and a new classification of the gingers (Zingiberaceae): evidence from molecular data. American Journal of Botany 2002; 89(10): 1682-96.
5. Yu X, Xie Z, Wu J, Tao and Xu X: DNA barcoding identification of Kadsurae caulis and Spatholobi caulis based on internal transcribed spacer 2 region and secondary structure prediction. Pharmacognosy Magazine 2016; 12(2): 165-9.
6. Kumar S, Stecher G and Tamura K: MEGA7: Molecular Evolutionary Genetics Analysis version 7 for bigger datasets, Molecular Biology and Evolution 2015.
7. Tajima F: Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. Genetics 1989; 123: 585-95.
8. Nei MS: The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 1987; 4: 406-25.
9. Tamura K, Nei M and Kumar S: Prospects for inferring very large phylogenies by using the neighbour-joining method. Proceedings of the National Academy of Sciences (USA) 2004; 11030-35.
10. Kimura M: A simple method for estimating the evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 1980; 16: 111-20.

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