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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 phylogenetic analysis of gene sequences and combining with complete genomic sequences helps to identify 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, 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 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 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]. Biological diversity in plant species is evidenced by phylogenetic tree.

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The main objective of this study was to differentiate *Curcuma* species grown in Sri Lanka using DNA barcode. Due to stability of 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 macromolecular level which is not mutating by external environment factors [5].

MATERIAL AND METHODS: DNA extraction, 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 Kimura-2-parameter model [10]. All positions containing gaps and missing data were eliminated by multiple alignments.

RESULTS AND DISCUSSION: The alignment of 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 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. aromatica	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 uncoded position than other four species. Phylogenetic tree of matK gene of Curcuma were 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.

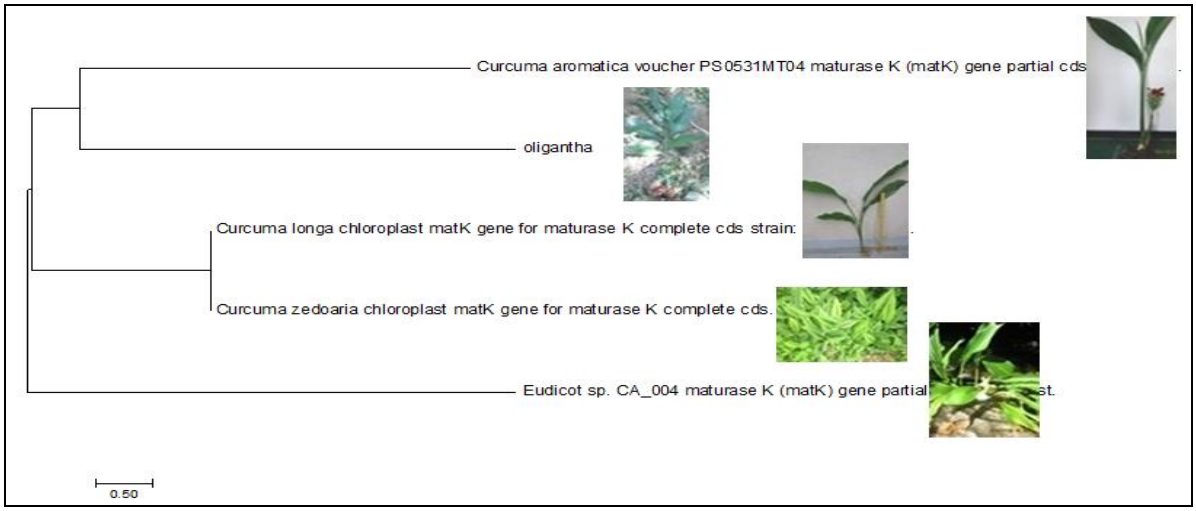

FIG. 1: EVOLUTIONARY RELATIONSHIPS ON matK GENE OF FIVE *CURCUMA* SPECIES

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 were found, which was different from species (212 segregating sites and 0.1823 of nucleotide diversity) claimed as Harankaha [3]. The maximum pairwise distane between *C. albiflora* and with other species which were studied **Table 2**.

From the results of 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, 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 a reliable proof to identify *C. albiflora* from other *Curcuma species*.

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