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UTILITY OF *MATK* GENE TO ASSESS EVOLUTIONARY RELATIONSHIP OF GENUS *MANGIFERA* (ANACARDIACEAE) IN INDONESIA AND THAILAND

TOPIK HIDAYAT[1,2,*], ADI PANCORO[3], and DIAH KUSUMAWATY[2]

[1] *Department of Biological Science, Faculty of Bioscience and Bioengineering, Universiti Teknologi Malaysia (UTM), Malaysia*
[2] *Department of Biology Education, Indonesia University of Education (UPI), Bandung, Indonesia*
[3] *School of Life Sciences and Technology, Institute Technology of Bandung, Indonesia*

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ABSTRACT

MaturaseK (*mat*K) gene of chloroplast DNA has served as an appropriate candidate to be a DNA marker in angiosperms. Using this marker, 19 species of genus *Mangifera*, one of the ecologically important crop, collected from Indonesia and Thailand were analyzed. Phylogenetic analysis using parsimony method revealed that the gene could clasify *Mangifera* into three major groups, namely group I, II, and III. Moreover, the *mat*K gene can identify *Mangifera* species originated from Thailand. Although this classification system is different with the previous classification system, it can provide a new information on the current status of *Mangifera* taxonomy. Further result exhibited that DNA sequences of the *mat*K of two *Mangifera* species (*M. laurina* dan *M. macrocarpa*) are different between Indonesia and Thailand specimens.

Keywords: DNA barcode, *Mangifera*, *mat*K gene, parsimony, phylogenetic analysis

INTRODUCTION

The genus *Mangifera* L., one of the most important plant groups in deciduous forest and wet tropical rainforests including mountain forests, is one of the largest genera of the family Anacardiaceae to which approximately 69 species have already been described. The genus is mostly distributed in the tropical parts of Asia (India, Burma, Sri Lanka, Thailand, South Tropical China, Malaysia, Indonesia, Papua New Guinea, the Philippines, the Solomon Islands) but also in the Pacific Islands (Kostermans & Bompard 1993). In spite of their economical importance, phylogenetic relationships among species within the genus have been poorly understood due to their extremely complicated vegetative and reproductive organs.

E-mail: topik28@yahoo.com or topik@fbb.utm.my

Previously, Marchand (1869), Pierre (1897), and Kostermans and Bompard (1993) have revealed classification systems for the genus based upon floral characters. However, these characters were extremely complicated in the genus and subjected to parallelism (Yonemori *et al.* 2002), suggesting many taxonomic and phylogenetic problems still remain unresolved. Given the shortcomings of these characters, data obtained from nucleotide substitutions of appropriate molecules are preferable for clarifying phylogenetic relationships (e.g., Moritz & Hillis 1996).

Methods for clarifying relationships in species or group organisms by using DNA sequences have been proposed and initiated recent years (Kress *et al.* 2005). MaturaseK gene of chloroplast genome served as potentially usable DNA regions to flowering plants. The *mat*K gene is frequently choosen by plant systematists because the region is a single copy gene and has enough variable sites of nucleotide substitution. Recently, the *mat*K gene has been widely used in phylogenetic inferences of various groups of plant (e.g. Ito *et al.* 1999; Ferguson & Sang 2001; Raymond *et al.* 2002; Ebihara *et al.* 2005; Hidayat *et al.* 2005).

Phylogenetic analysis to clarify phylogenetic relationships among members of genus *Mangifera* have been carried out using DNA sequences of the *mat*K gene. An understanding of the evolutionary relationships in this group may contribute to the field of plant systematics or ecology.

MATERIALS AND METHODS

A total of 19 species of *Mangifera* was collected from Indonesia and Thailand, plus two species of *Bouea*. Two members of genus *Bouea* (M9 and M13) were used as outgroup in phylogenetic analysis based on previous research this genus was sister group to *Mangifera* (Yonemori *et al.* 2002). Detailed information of the plant is summarized in Table 1.

DNA genome was extracted from fresh materials (young leaf) using QIAGEN Dneasy Mini Plant Kit with slight modification. Amplification was conducted using four primers as shown in Figure 1. Table 2 provides detailed information on sequences of primer pairs.

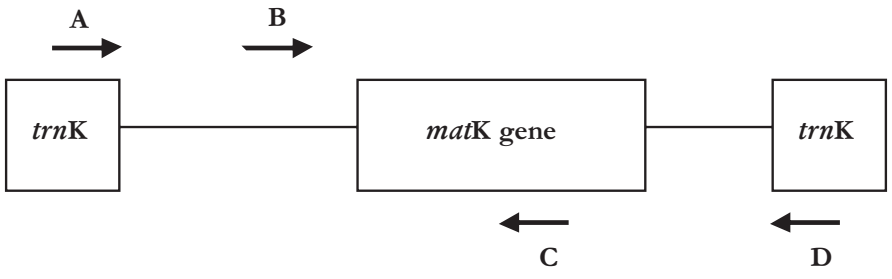

Figure 1. Strategy of amplification and sequencing of the *mat*K gene. A=*trn*K-5F, B=TAA-09F, C=TAA- 09R, dan D=*trn*K-2R. Two internal primers (B and C) were designed for this study.

Table 1. Plant materials, their geographic origins and codes used in this study

Species	Origin	Code
Mangifera altissima Blanco var bingloe	Indonesia	M18
Mangifera applanata Kosterm.	Indonesia	M14
Mangifera foetida Lour.	Indonesia	M17
Mangifera gedebe Miq.	Indonesia	M10
Mangifera indica L.	Indonesia	M11
Mangifera laurina Bl.	Indonesia	M7
Mangifera macrocarpa Bl.	Indonesia	M3
Mangifera odorata Griff.	Indonesia	M16
Mangifera spp	Indonesia	M12
Mangifera rufocostata Kosterm.	Indonesia	M8
Mangifera similis Auct.	Indonesia	M2
Mangifera caesia Jack ex Wall	Indonesia	M5
Mangifera casturi Kosterm.	Indonesia	M15
Mangifera macrocarpa Bl.	Thailand	S1
Mangifera conchinchinensis Englar	Thailand	S6
Mangifera flava Evrard	Thailand	S3
Mangifera gracilipes Hook.f.	Thailand	S2
Mangifera caloneura Auct.	Thailand	S5
Mangifera laurina Bl.	Thailand	S7
Bouea oppositifolia (Roxb.) Meiss	Indonesia	M13
Bouea macrophylla Griff.	Indonesia	M9

Table 2. Primers used in this study

Name	Sequences
trnK-5F	5' TGGGTTGCTAACTCATGG 3'
trnK-2R	5' AACTAGTCGGATGGAGTAG 3'
TAA-09F	5'GGTTTTCCCATGAGTAGATTATCG 3'
TAA-09R	5' CGAAGTAGACGAAGCTCTTGG 3'

For amplification, we used primer pairs A and D, whereas all primers used once sequencing. PCR (Polymerase Chain Reaction) reaction included buffer PCR (1x), $MgCl_2$ (2-3mM), primers (@ 0,5 mM), enzyme Taq polymerase (1 U/uL), dNTPs Mix (1,6 mM), and DNA template (100-150 ng/uL). PCR was conducted according to Hidayat *et al*. (2005). PCR cycles include 1 cycle at 94°C (predenaturation) for 5 minutes; 30 cycles at 94°C (denaturation) for 30 seconds, 49°C (*annealing*) for 30 seconds, and 72°C (extension) for 2 minutes; and ended with 1 cycle at 72°C (final extension) for 8 minutes. PCR products were cloned into pGEM-T Easy (Promega) before sending them to Macrogen (Korea) for sequencing.

DNA sequences obtained from the *mat*K gene were aligned with Clustal X (Thompson *et al*. 1997) and then adjusted manually. Phylogenetic analyses based on the maximum parsimony criterion was performed using PAUP* version 4.0b10 (Swofford 1998). All characters were equally weighted and unordered (Fitch 1971). All the data sets were analysed by the heuristic search method with tree bisection-reconnection (TBR) branch swapping and the MULTREES option ON, ten replications of random addition sequences with the stepwise addition option, and all most parsimonious trees (MPTs) were saved. Evaluation of internal support of clades was conducted by the bootstrap analysis (Felsenstein 1985) utilizing 1000 replicates with TBR branch

swapping and the MULTREES option OFF. Number of steps, consistency indices (CI) and retention indices (RI) were calculated on one of the MPTs in each analysis with the TREE SCORES command in PAUP*.

RESULTS AND DISCUSSION

DNA extraction can be done using various types of DNA sources such as leaf, stem, flower, and seed. In this research, young leaf was used for DNA extraction to minimize contamination that can inhibit PCR amplification. High level of concentration (600 ng/uL in average) with good ratio ($\pm$ 1.750) was obtained. Size and border of *mat*K gene for *Mangifera* were determined through comparative analysis in genebank (www.ncbi.nlm.nig.gov). The results indicated that size of *mat*K gene in *Mangifera* is about 1500 bp.

Multiple alignment analysis was performed by using ClustalX (Thompson *et al.* 1997). The aligned *mat*K comprised 1,601 characters (Fig. 2). Of these, 1,429 were constant and 51 were potentially informative. Reconstruction of phylogenetic tree (Fig. 2) using PAUP resulted in 23 MPTs with a length of 121 steps, CI of 0.852, and RI of 0.739.

The tree (Fig. 3) demonstrated that the genus was monophyletic and split into three major groups. Monophyletic nature of *Mangifera* was supported by character of stoma, anomositic (Hidayat, unpublished data).

The three major groups found in this study is not consistent with previous classification system by Mukherjee (1953), Kostermans and Bompard (1993) based upon morphological characters, and even Yonemori *et al.* (2002) on the basis of DNA sequences of internal transcribed spacer (ITS) region. The number of plant materials used in this study is likely to be insufficient (only 19 out of 69 recognized species). Further phylogenetic analysis is, therefore, needed using more extensive sampling.

However, this study has provided new information on taxonomy of *Mangifera*. As depicted in Figure 2 *M. applanata*, *M. macrocarpa* (from Indonesia), and *M. altissima* were united (Group I), whereas *M. laurina* (form Thailand), *M. casturi, M. odorata,* and *M. indica* were closely related (Group II). Group III was housed by the rest of species, and *Mangifera* species which is originated from Thailand was placed within Group III (Fig. 2). Unfortunately, no single synapomorphic character is found to support each group.

Moreover, this research has revealed that there are variations of *mat*K in *M. laurina* and *M. macrocarpa* which come from Indonesia and Thailand. As seen in Figure 2, *M. laurina* (from Thailand) was separated from that of Indonesia (Group III; Thailand specimen in Group II). Similar situation has been found in *M. macrocarpa*: Thailand in Group III and Indonesia in Group I. Different nature between these two countries has driven the mutation in *mat*K, but this does not lead to shift the morphology. These are related with the ability of plant to adapt to the environment changes (Evans 1975).

As mentioned, *mat*K gene is highly conserved (e.g. Ebihara *et al.* 2005; Hidayat *et al.* 2005). Mutation rate in this kind of gene is very slow. This is reflected by the small number of informative characters (only 51 from a total 1,601 characters) to build the

tree. As a consequence, bootstrap value in most branches of the tree are less than 50. Similar condition was found in other angiosperms (e.g. Raymond *et al.* 2002; Ebihara *et al.* 2005; Hidayat *et al.* 2005). Further analysis based on the phylogenetic scheme presented here will shed more light on overlooked characters.

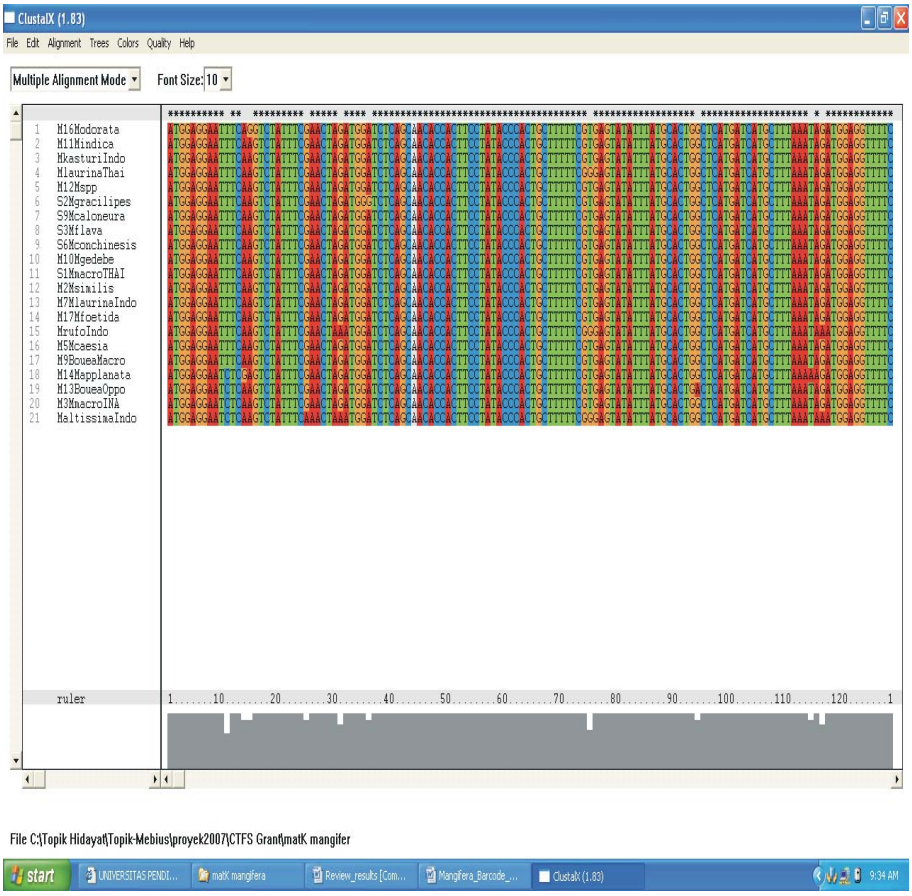

Figure 2. Alignment process using ClustalX shows the level of homology (*)

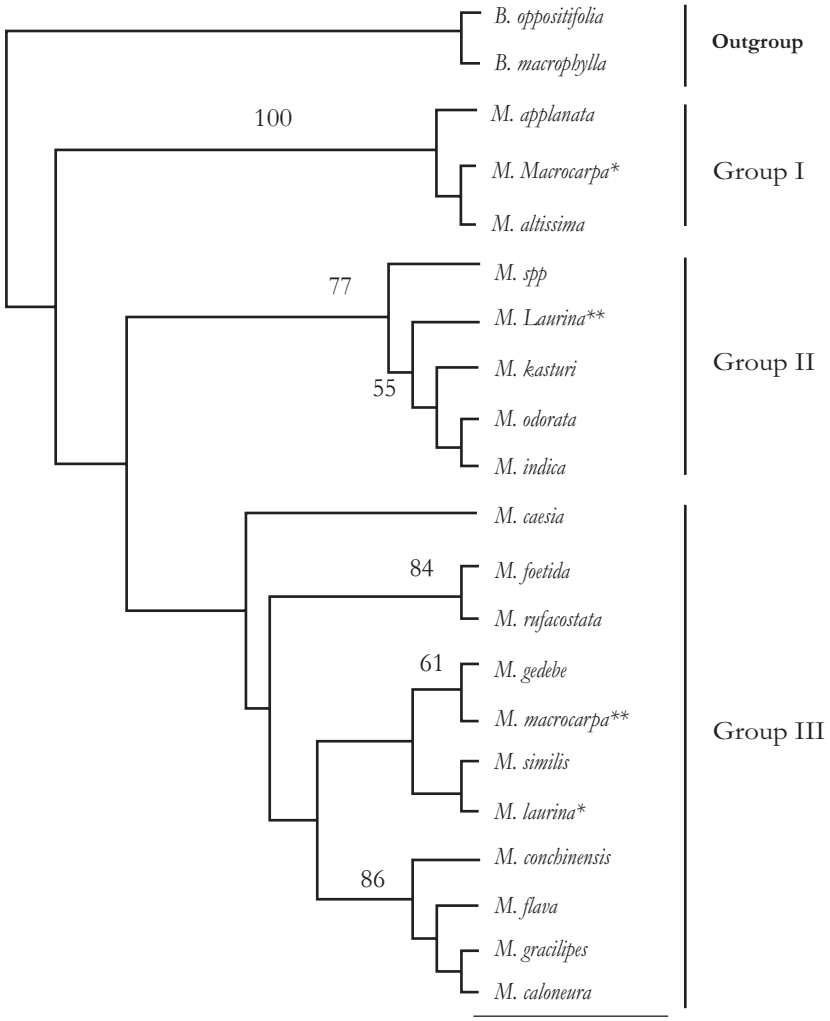

Figure 3. One of the 23 MPTs of *Mangifera* based on *mat*K gene. Bootstrap value of >50 are shown above each branch. * = Indonesia specimen; **= Thailand specimen. Species inside the box are originated from Thailand.

CONCLUSIONS

This study demonstrated that the *mat*K gene classified the *Mangifera* into three major groups. Furthermore, the *mat*K identified *Mangifera* species that is originated from Thailand. The *mat*K gene in the two species, namely *M. laurina* and *M. macrocarpa*, was different between Indonesia and Thailand specimens. This study is subjected to be preliminary, so it is suggested that further researches employing another DNA region with more extensive sampling should be conducted in the future.

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