

The Maturase K Region of Kebak, A Javanese Cultural Flora

Poppy Rahmatika Primandiri^{1,3}, Muhammad Rifqi Hariri², Tutut Indah Sulistiyowati^{1,3} and Agus Muji Santoso^{1,3}

¹ Biology Education, Universitas Nusantara PGRI Kediri, Kediri, Indonesia
² Research Center for Plant Conservation and Botanic Gardens, National Research and Innovation Agency (BRIN), Indonesia

³ Research Centre of Biodiversity, Universitas Nusantara PGRI Kediri, Kediri, Indonesia agusmujisantoso@unpkediri.ac.idl

Abstract. Kebak is a historically significant plant in the Javanese cultural activity Ngunggahne Beras. During field collection, several plants are morphologically indistinguishable, demanding the use of DNA sequences to back up the results of morphological identification. This research aims to describe the maturase k sequence and generate the first DNA barcode sequence of local kebak plants. The DNA was extracted from the leaves, amplified using matK universal primers, and sequenced. The morphological study identifies the kebak plant as Ficus fulva Reinw. ex Blume, a member of the Moraceae, the fig family. This species' native range extends from northeast India to Malesia. This plant was distributed from Sumatra to Moluccas in Indonesia. It is a tree that performs well in the moist tropical biome. A length of 730 base pairs, partial region, of the matk Genes' sequence was obtained. The sequence comparison through BLAST revealed that the sequence for Ficus fulva was unavailable, but it is 99.72% like Ficus esquiroliana H. Lév., a synonym for Ficus triloba Buch. -Ham. ex Voigt. Compared to four other Ficus species found in Java, the matK sequence revealed six diverse sites, suggesting that this region is predominantly conserved.

Keywords: conservation, cultural plant, kebak, matK.

1 Introduction

Indonesia has cultural diversity. *Ngunggahne Beras* is a traditional ceremony in Kediri, East Java, that still takes place today. This tradition is carried out before the wedding ceremony [1]. This traditional ceremony aims to ensure that the bride and groom receive blessings from God. The traditional ceremony uses *kebak* leaves. *Kebak* leaf means *kebak ing pambudi* means full of wisdom. It is expected that fortune will continue to flow and gain good wisdom [2].

Previous research reported that *kebak* is very rarely found but is still needed in traditional ceremonies. *Kebak* plant is the least plant planted (7%) and the least plant obtained from other areas (2%) [1]. However, *kebak* can still be conserved because

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people consider kebak to be a mystical plant and they also teach their descendants not to play with parts of the kebak plant.

Identification of plant morphology reveals that *kebak* belongs to the Ficus group. For the conservation and cultivation of *kebak*, various supporting data are needed, one of which is valid data about the taxonomic identity of *kebak*. However, the characteristics of *kebak* leaves contrast with those of other Ficus groups. Identification by molecular means is needed to support morphological identification.

Methods for identifying living species have developed from morphological to molecular identification based on short pieces of DNA called DNA barcodes [3]. The advantage of DNA barcoding is its speed and ability to identify physically indistinguishable plant species, as well as whether an organ under study is missing or damaged. Furthermore, molecular identification can be conducted by anyone, even if they are not taxonomists [4]. *Kebak* identification using DNA barcoding research is relatively uncommon.

Some short DNA sequences, such as the *rbcL*, *matK*, ITS, and *psbA-trnH* intergenic spacer, have been designed as DNA barcodes for plant molecular identification [5,6,7]. The CBOL (*Consortium for the Barcode of Life*) recommended *rbcL* and *matK* as standard barcodes [8]. Due to its higher level of precision at the species level, the *matK* gene is more widely used in diverse studies than the *rbcL* gene [9]. *MatK* is a gene that encodes the maturase K enzyme and is found in the chloroplast genome of plants. This gene experiences high mutation and quick evolution, making it less conservative than the *rbcL* gene, the sequence is more variable, and it also can differentiate some Angiosperm species [10].

2 Method

The plant materials used in this study were parts of *kebak* tree. The 3–5 leaves from the shoots were freshly collected from Semen Village, Kediri Regency, East Java, Indonesia. Main primer that used in this research were MatK 472F (5'-CCC RTY CAT CTG GAA ATC TTG GTT C-3') dan 1248R (5'-GCT RTR ATA ATG AGA AAG ATTTCT GC-3').

Total plant DNA extraction was carried out using the Multisource Genomic DNA Miniprep Kit (Axygen) according to the manual instructions. Polymerase Chain Reaction (PCR) using Ready-to-Load Master Mix (Solis Biodyne) with thermocycler temperature settings starting with initial denaturation at 95°C for 2 minutes, then continuing for 35 cycles [95°C for 30 seconds, 52°C for 30 seconds, and 72°C for 1 minute]. Sequencing and sequence data were analyzed with NCBI BLAST.

3 Results and Discussion

The morphological study identifies the *kebak* plant as *Ficus fulva* Reinw. ex Blume, a member of the Moraceae, the fig family. This species native range extends from northeast India to Malesia. This plant was distributed from Sumatra to Moluccas in Indonesia. It is a tree that performs well in the moist tropical biome.

This research has obtained the matK *kebak* gene sequence with a gene length of 730 bp (see Fig. 1). A length of 730 base pairs, partial region, of the matK gene sequence was obtained. The length of the *matK* gene sequence that was successfully amplified varied quite widely. In this study, we only used a partial *matK* gene whose length was only around 700–900 bp according to the primer attachment regions *matK-r* and *matK-1-f*. Each primary design has benefits and drawbacks. Although various primers have been developed, they are frequently unsuccessful for some species [11]. This is because intraspecies genetic variation is very small. In addition, to determine intraspecies variation more accurately, two or more combinations of loci are needed so that they can produce more significant data [12, 13].

TCTTCTTTGCATTTATTACGGTTTTTTCTTCACGACTATTATAATTGGAATAGTTTTAGTATTCCAAAATAATATTTC 100 110 120 130 140 - - - I TTTCAAAAAGTAATCTAAGATTATTCTTGTTCTTATATAATTCTCATGTTTTTGAATACGAATCCATCTTACTTT TTCTACGTAACCAATCTTCTCCATTTACGATTAACATCTTCTGGGGGGCTTTTTTGAGCGAATAATATTTCTATGGAAAAATA AAACATCCCCTAGAAGAAGTCTTTCCCAATGATTTTCCCGACTAGCTTCTTCGAGGATTTCTTCATGCATTATGT 330 340 350 360 TAGATATCAAGGAAAATCAATTCTGACTTCAAAAGATACGCCCCCTTTTTCATGAATAAATGGAGATATTACCTTGTCCTTT 500 510 520 530 540 |....|...... TTGGGCTATCTTTCAAATATGCAAATAAATCTTTCAGTAGGAGGCGAGTCAAATGTTAGAAAATTCATTTCTAATGGATAA TCCTATGAAGAAGATTGATACATTAGTTCCAATTAGTCCTCTGATTGGATCGTTGGCTAAAATGCGATTTTGTAACGTAT TAGGACATCCCGTTAGTAGTCGACCTGGCCTGATTCATCGGACTTTGGTATATTATCGGACCGATTTGGCGTATATATGCGAC AATCTTTCTC

Fig. 1. matK kebak gene sequence

The *matK* gene is gene highly variable in the chloroplast genome of plants that may be used to identify and verify plant species. *MatK* encodes a maturase-like protein that aids in the splicing of its own and other genes in chloroplasts [14,15]. The *matK* gene has been used for molecular identification and phylogenetic analysis of citrus [16], Fabaceae [17], and some species in the genus Morus [18]. In this study, The use of *matK* as a DNA barcode confirmed the taxonomic position of *kebak*, which had previously been recognized visually by Universitas Nusantara PGRI Kediri.

This confirmation helps to increase public awareness that the *matK* sequence may be utilized for plant molecular identification. The following are the benefits of plant molecular identification using DNA barcoding: (1) anyone, taxonomist or non-taxonomist, can perform plant molecular identification; (2) unlike morphological characters, the DNA sequence or DNA barcode is not influenced by the environment;

and (3) molecular identification is simple and quick if a DNA sequence database of target species is provided [19]. Until now, the *matK* of *kebak* database in GenBank was unavailable. As a result, this study is unique in that it improved the DNA sequence database of *kebak* in a public database.

	1	10	20	30	40	50	60	70	80	90	100
Sample	тсттст	TTGCATTTAT	TACGGTTTTT	TCTTCACGAC	TATTATAAT	GGAATAGTT	TAGTATTCCA	ААТАААТАТ	ATTTCTTTTT	TTCAAAAAG	ТААТС
F.fulva	TCTTCT	TTGCATTTAT	TACGGTTTTT	TCTTCACGAC	TATTATAAT	GGAATAGTT	TAGTATTCCF	AATAAATAT	ATTICTTIT	TTCAAAAAG	TAATC
F.fistulosa	TCTTCT	TTGCATTTAT	TACGGTTTTT	TCTTCACGAC	TATTATAAT	I GGAATAGTT	TATTATTCCF	AATAAATAT	ATTTCTATTT	TTCAAAAAAG	TAATC
F.variegata	TETTET	TTGCATTTAT	TACGGTTTTT	TCTTCACGAC	TATTATAAT	GGAATAGTT	TATTATTCCF	ААТАААТАТ	ATTTCTATTT	TTCAAAAAG	TAATC
	101	110	120	130	140	150	160	170	180	190	200
Comple	тоосот	тоттеттетт	сттототоот	тетертетт	ттеротосс			стооссоот	CTTCTCOTTC		
F.fulva	TAAGAT	TATTCTTGTT	CTTATATAAT	TCTCATGTT	TTGAATACGA	ATCCATCTT	ACTITITCTAC	GTAACCAAT	CTTCTCATTTA	CGATTAACA	TCTTC
F.hispida	CAAGAT	TATTCTTGTT	CTTATATAAT	TCTCATGTT	TTGAATACG	ATCCATCTT	ACTITITCTAC	GTAACCAAT	CTTCTCATTTA	CGATTAACA	TCTTC
F.tistulosa F.variegata	CAAGAT	TATTCTTGTT	CTTATATAAT	TCTCATGTT	TTGAATACGA	ATCCATCTT	ACTITITCIAC	GTAACCAAT	CTTCTCATTTA	ССАТТААСА	TETTE
	201	210	220	230	240	250	260	270	280	290	300
	1	+	+	+	+	+	+	+	+	+	1
Sample E fulua	TGGGGG	CTTTTTTGAG	CGAATATATT	TCTATGGAAF	IAATAAAACA IAATAAAACA	ICCCGTAGAA ICCCGTAGAA	GAAGTCTTTG	TAATGATTT	TCCGACTAGCT	TATEGTICT	TCGAG
F.hispida	TGGGGG	CTTTTTGAG	CGAATATATT	TCTATGGAAF	AATAAAACA	CCCGTAGAA	GAAGTCTTTG	TAATGATTT	TCCGACTAGCT	TATGGTTCT	TCGAG
F.fistulosa	TGGGGG	CTTTTTTGAG	CGAATATATI	TCTATGGAAA	AATAAAACA	ICCCGTAGAA		TAATGATTT	TCCGACTAGCT	TATEGTICT	TCGAG
F.variegata	166666			ICIHIGGHH	IHH I HHHHLH	ILLLGIHGHH	3HH61C11161	THHIGHTT	ILLUHLIHULI	THIGHTLI	ICONO
	204	24.0	200	220	240	250	200	270	200	200	400
	301	310	320	330	340	350	360 +	370	+	390	400
Sample	GATTTC	TTCATGCATT	ATGTTAGATA	ITCAAGGAAAA	ITCAATTCTG	ICTTCAAAAG	ATACGCCTCTI	TTCATGAAT	AAATGGAGATA	ITTACCTTGT	CCTTT
F.fulva F bispida		TTCATGCATT	ATGTTAGATA	itchhgghhhf Itcaaggaaaf	ITCHHITCIGI ITCAATTCIGI	ICTTCHHHHGI ICTTCAAAAG	THUGCUTUT	TTCATGAAT	HAATGGAGATA	TTACCTTGT	
F.fistulosa	GATTTC	TTCATGCATT	ATGTTAGATA	TCAAGGAAAA	TCAATTCTG	CTTCAAAAG	TACGCCTCTI	TTCATGAAT	AAATGGAGATA	TTACCTTGT	CCTTT
F.variegata	GATTTC	TTCATGCATT	ATGTTAGATA	itcaaggaaaa	ITCAATTCTG	icttcaaaagi	ATACGCCTCTI	TTCATGAAT	AAATGGAGATA	ITTACCTTGT	CCTTT
	401	410	420	430	440	450	460	470	480	490	500
Sample	TATGGC	AATGTCAGTT	TTCTGTGTG	TCTCAACCAG	GAAGGATGTA	таталасса	аттатосалас	ATTCCCTCA	GCTTTTTGGGC	TATCTTCA	ААТАТ
F.fulva	TATGGG	AATGTCAGTT	TICIGIGIG	TCTCAACCA	GAAGGATGT	TATAAACCA	ATTATGCAAAC	ATTCCCTCA	GCTTTTTGGGC	TATCTTTCA	AATAT
F.fistulosa	TATGGC	AATGTCATTT	TTCTGTGTG	TCTCAACCA	GAAGGATGTA	TATAAACCA	ATTATGCAAAC	ATTCCCTCA	GCTTTTTGGGC	TATCTTTCA	AATAT
F.variegata	TATGGC	AATGTCATTT	TTCTGTGTGG	TCTCAACCAC	GAAGGATGTA	TATAAACCA	ATTATGCAAAC	ATTCCCTCA	GCTTTTTGGGC	TATCTTTCA	AATAT
	501	510	520	530	540	550	560	570	580	590	600
Sample	GCAAAT	AAATCTTTCA	IGTAGTACGGA	GTCAAATGTT	AGAAAATTCA	ITTTCTAATG	GATAATTCTAT	GAAGAAGAT	TGATACATTAG	TTCCAATTA	GTCCT
F.fulva	GCAAAT	AAATCTTTCA	GTAGTACGGA	IGTCAAATGT1	AGAAAATTCA	ITTTCTAATG	GATAAT <mark>g</mark> ctat	GAAGAAGAT	TGATACATTAG	TTCCAATTA	GTCCT
F.fistulosa	GCAAAT	AAATCTTTCA	IGTAGTACGGA	IGTCAAATGC1	AGAAAATTCE	ATTTCTAATG	GATAAT <mark>G</mark> CTAT	сннсннсн і Саабаабат	TGATACATTAG	TTCCAATTA	GTCCT
F.variegata	GCAAAT	AAATCTTTCA	GTAGTACGGA	IGTCAAATG <mark>C</mark> 1	AGAAAATTCA	ITTTCTAATG	GATAAT <mark>g</mark> ctat	GAAGAAGAT	TGATACATTAC	TTCCAATTA	GTCCT
	601	610	620	630	640	650	660	670	680	690	700
Sample	CTGATT	GGATCGTTGG	ставааться	ATTTTGTAA	GTATTAGGA	ATCCCGTTA	STAAGTCGACO	тебестент	TCATCGGATTI	TGATATTAT	22892
F.fulva	CTGATT	GGATCGTTGG	CTAAAATGCO	ATTTTGTAAC	GTATTAGGA	ATCCCGTTA	GTAAGTCGACO	TGGGCTGAT	TCATCGGATTI	TGATATTAT	CGACC
F.hispida	CTGATT	GGATCGTTGG	CTAAAATGCO	ATTTTGTAAC	GTATTAGGAU	CATCCCGTTA	STAAGTCGACO	TGGGCTGAT	TCATCGGATTI	TGATATTAT	CGACC
F.variegata	CTGATT	GGATCGTTGG	CTAAAATGCO	ATTTTGTAAC	GTATTAGGA	ATCCCGTTA	GTAAGTCGACC	TGGGCTGAT	TCATCGGATTI	TGATATTAT	CGACC
	701	710	720 72	27							
Sample	1										
Jample	COTTO	сстототото	COC000TCTT	т							
F.fulva	GATTTG GATTTG	CGTATATATG CGTATATATG	CAGAAATCTT Cagaaatctt	TT TT							
F.fulva F.hispida	GATTTG GATTTG GATTTG	CGTATATATG CGTATATATG CGTATATATG	CAGAAAATCTT CAGAAAATCTT CAGAAAATCTT								

Fig. 2. Aligned Ficus *matK* gene sequences

The sequence comparison through BLAST revealed that the sequence for *Ficus fulva* was unavailable, but it is 99.72% similar to *Ficus esquiroliana* H.Lév., a synonym for *Ficus triloba* Buch.-Ham. ex Voigt. Compared to four other Ficus species found in Java, the *matK* sequence revealed eight diverse sites, suggesting that this region is

predominantly conserved. The difference between the nucleotide base sequences of *ke-bak* plants and others was located at sites 59, 82, 101, 256, 405, 414, 535, and 562 (see Fig. 2).

Differences in nucleotide sequences can be caused by mutations. Based on the characteristics of the differences in nucleotide sequences, it is most likely caused by nucleotide base sequence substitution mutations, either of the transition or transversion type. The findings in this study are in line with the results of previous research showing that variations in Trypsin Inhibitor (TI) gene sequences in several numbers of plants resulting from *Jatropha curcas* crosses are also more likely to be caused by nucleotide base substitutions [20].

The results of this research show that valid plant identification can help local governments formulate plant conservation strategies and policies that have potential. Plant potential does not only focus on its potential as a source of alternative food ingredients, a source of alternative board materials and textile fibers, and a source of medicinal ingredients. However, it also focuses on conserving plants that have cultural value for indigenous peoples. Previous research reported that compiling a digital-based rare plant database requires valid plant identity data [21].

This research data can be useful to support conservation efforts for rare plants in Kediri, East Java, Indonesia. In particular, there is data on the identity of the *kebak* plant as one of the cultural plants of the Javanese tribe. Apart from that, the results of this research can also be used as a context for developing biodiversity learning with a green economy at the school or higher education level. The results of previous research reveal that developing worksheets based on project-based learning using the surrounding context can improve students' high-level thinking [22], collaboration skills [23], and also students' creativity [24].

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