



The Maturase K Region of Kebak, A Javanese Cultural Flora

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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 Malaysia. 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

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 ATTCT 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-l-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].

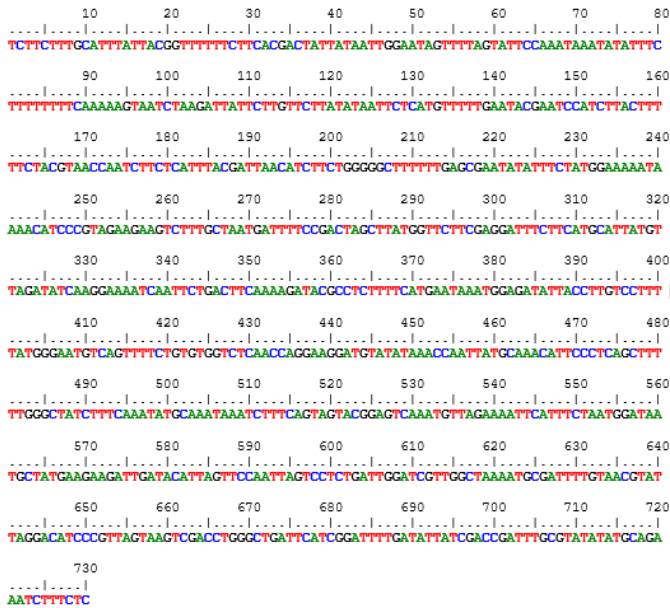


Fig. 1. *matK kebak* gene sequence

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.

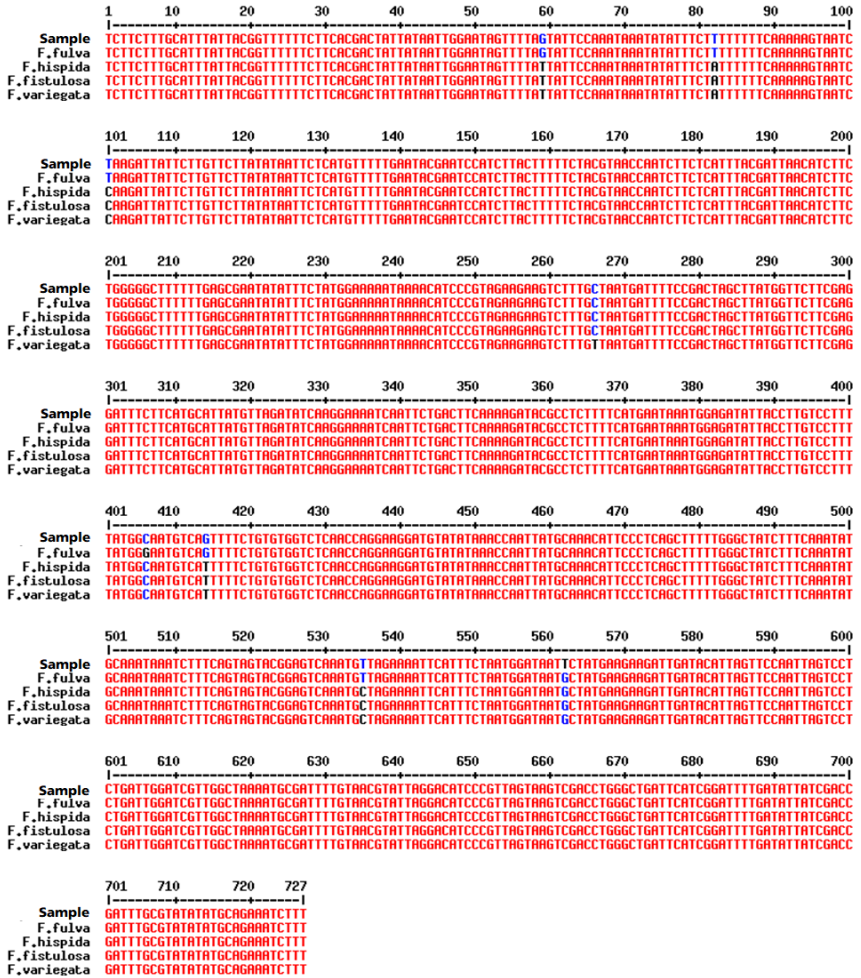


Fig. 2. Aligned *Ficus matK* gene sequences

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 *kebak* 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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