Analysis of *matK*, *rbcL* and *trnL-trnF* Intergenic Spacers on Durik-Durik (*Syzygium* sp)

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**Abstract.** *Syzygium* sp. with the local name of durik-durik, that grows in Riau Province, Indonesia, is one of many adapted plants to flooding stress in floodplain ecosystems, and plays a role for survival of the ecosystems. This study reports analysis of three DNA barcodes, i.e. *matK*, *rbcL* and *trnL-trnF* intergenic spacer on this plant. The fresh leaves of this plant were picked up from floodplain ecosystem in Pelalawan, Riau Province for DNA extraction requiring. In this study had been obtained DNA sequences of *matK*, *rbcL* and *trnL-trnF* intergenic spacer with the size of 686 bp, 641 bp and 828 bp, respectively. The BLASTn analysis based on those sequences showed that none of the accessions of the *Syzygium* genus were 100% similar to *Syzygium* sp. The phylogenetic tree showed that *Syzygium* sp. formed one group with the same species of *Syzygium* and separated from other genera in Myrtaceae family. These results confirmed that durik-durik was a member *Syzygium*. Those sequences had been deposited in GenBank and could be used for molecular identification of this plant.

1. **Introduction**

DNA barcode is a short sequence of genome in all organisms [1], that today it has been widely used for the purposes of molecular identification. For example, there are three DNA barcodes, i.e. *matK*, *rbcL* and *trnL-trnF* intergenic spacer, that has been used for plant molecular identification [2,3,4,5,6]. Those sequences are on plant chloroplast genome. The *matK* and *rbcL* sequences encode maturase K protein and ribulose bisphosphate carboxylase large chain respectively, while *trnL-trnF* intergenic spacer does not encode protein and only spacer between two genes such as *trn-L* and *trn-F* genes. Compared to *matK* and *rbcL*, the *trnL-trnF* intergenic spacer is easy to mutate and its variation very high among plants so that it can be better distinguish plant species [2].

Those DNA barcode sequences of *Syzygium* sp. are not yet available in GenBank and this can inhibit molecular identification of this plant. Molecular identification has many advantages compared to conventional identification based on morphological data such as 1) plants that are incomplete or damaged by organs can still be identified, 2) plants can be identified at any stage of development, and 3) molecular identification does not require expertise in taxonomic fields or in other words molecular identification can be carried out by everyone [1].

Molecular identification can be performed easily and quickly if DNA barcode sequence data is available in public databases such as GenBank and can be accessed easily [7,5,6]. Therefore, this study reports the first the tree DNA barcodes namely *matK*, *rbcL*, and *trnL-trnF* intergenic spacer on
Syzygium sp. The three sequences can later be utilized by the public for molecular identification of this species.

2. Methodology

2.1. Materials

There are about six Syzygium sp. in Kajuik Lake located in Langgam, Pelalawan Regency, Riau Province, Indonesia. The leaves of this plant were taken for analysis. The primer pairs used for the amplification of the *matK* and *rbcL* genes were designed based on the conserved regions of both genes in the *Syzygium* genus sequentially extracted from the GenBank database. Moreover, the primer pairs for the amplification of the exon *trnL* region and the *trnL-trnF* intergenic spacers were designed based on [8] (Table 1).

| Primers             | 5′--------------------------------------------3′ | Annealing Temperatures (°C) | Regions          |
|---------------------|----------------------------------------------|----------------------------|------------------|
| Sz-matK-F           | CTT GGT TCA AAC CCT TCG CTA                 | 53.40                      | maturase K       |
| Sz-matK-R           | TGAATCAGCCCCGGGTCGGTT                       |                            |                  |
| Sz-rbcL-F1          | GTGTTGGATTTCAAGCTGGTG                       | 51.95                      | ribulose         |
| Sz-rbcL-R1          | AGGGCCTCGGCAAAATACGA                       |                            | bisphosphate     |
| B49317_F2           | CGAAATCCTGGTAGACGCTACG                      | 51.80                      | carboxylase      |
| A49855_R2           | GGGGATAGGGGACTTGAGA                        |                            |                  |
| B49873_F3           | GGTCTCAAGTCCCTCTATCCCC                     | 50.60                      | trnL-trnF exon   |
| A50272_R3           | ATTTGAACTGGTGACACGAG                      |                            | trnL-trnF intergenic spacer |

2.2. Methods

The total DNA molecule was extracted from fresh leaves using the DNeasy plant mini kit (Qiagen). The presence of total DNA was determined by electrophoresis technique. The total DNA molecule was then amplified using PCR technique. PCR components included 1X PCR buffers (plus Mg²⁺), 0.1 mM dNTPs, 2.4 μM forward primer, 2.4 μM reverse primer, 2 U Dream Taq DNA polymerase (Thermo Scientific), 1 ng total DNA, and aquabidestilata up to 50 μl of total PCR volume [9]. The PCR procedure was performed as follows: 1 cycle at 94°C for 5 minutes, followed by 35 cycles consisting of three stages: denaturation at 94°C for 45 seconds, annealing at annealing temperature (Table 1) for 45 seconds, and elongation at 72°C for 1 minute. The process resumed at 1 cycle at 72°C for 10 minutes. The PCR products were then migrated on agarose gel electrophoresis.

The PCR products are packaged for shipment to PT Genetika Science located in Jakarta and then sent to 1st Base Malaysia for purification and two-way sequencing. The sequencing was conducted using the primer pairs for PCR (Table 1). The sequences obtained were then analysis using BioEdit version 7.0.0 [10] software, BLASTn (Basic Local Alignment Search Tool) program at http://www.ncbi.nlm.nih.gov/BLAST [11] and MEGA version 6.06 (Build #: 6140226) software [12]. The DNA sequences from *Eugenia uniflora* and *Psidium guajava* which are also members of the Myrtaceae family were used as outgroups. The DNA sequences other than *Syzygium* sp. DNA sequence used for phenograms construction were taken from the GenBank database (ncbi.nlm.nih.gov).

3. Results and Discussion

The total DNA of *Syzygium* sp. had been obtained (Figure 1) with good concentration and quality for use in the PCR process. The PCR products of the target genes such as *matK*, *rbcL*, *trnL* exon and *trnL-trnF* intergenic spacer had been obtained with the size of 700 bp, 650 bp, 600 bp, and 250 bp,
respectively (Figure 1). These fragments were thick single bands that were fine for sequencing purposes.

![Image](https://example.com/image.png)

**Figure 1.** The total DNA (1) and DNA fragments of matK (2), rbcL (3), the trnL exon (4), and trnL-trnF intergenic spacer (5) of Durik-durik (Syzygium sp) that were migrated on 1.2% agarose gel in 1X TBE buffer. 1 kb DNA Ladder (Thermo Scientific).

3.1. The matK Sequence Analysis of Syzygium sp.

The sequenced Syzygium sp. matK fragments produced a DNA sequence of 686 nucleotides. The sequence had been registered and released in GenBank with accession number KY503028.1. The BLASTn analysis of the Syzygium sp sequence indicated that matK sequence from Syzygium sp matched 99% resemblance to most species of the Syzygium genus. This result was supported by high max score and the total score (1308), high query cover value (100%), and low value (0.0) (Table 2).

| Description                  | Max score | Total score | Query cover | E value | Ident | Accession     |
|------------------------------|-----------|-------------|-------------|---------|-------|---------------|
| Syzygium tenuiflorum         | 1308      | 1308        | 100%        | 0.0     | 99%   | DQ088614.1    |
| Syzygium ngoyense            | 1308      | 1308        | 100%        | 0.0     | 99%   | DQ088596.1    |
| Syzygium lateriflorum        | 1308      | 1308        | 100%        | 0.0     | 99%   | DQ088585.1    |
| Syzygium auriculatum         | 1308      | 1308        | 100%        | 0.0     | 99%   | DQ088562.1    |
| Syzygium laetum              | 1302      | 1302        | 100%        | 0.0     | 99%   | KT936451.1    |
| Syzygium buxifolium          | 1302      | 1302        | 100%        | 0.0     | 99%   | HQ427387.1    |
| Syzygium hancei              | 1302      | 1302        | 100%        | 0.0     | 99%   | HQ415316.1    |
| Syzygium levinei             | 1302      | 1302        | 100%        | 0.0     | 99%   | HQ415313.1    |
| Syzygium sandwicense         | 1302      | 1302        | 100%        | 0.0     | 99%   | DQ088606.1    |
| Syzygium bungadinnia         | 1302      | 1302        | 100%        | 0.0     | 99%   | DQ088568.1    |

The matK sequences analysis of Syzygium sp., some species of the Syzygium genus, and outgroups (Psidium guajava and Eugenia uniflora) indicated 39 variations or different nucleotides in the form of substitution among them. Nucleotides number 471 and 636 were critical nucleotides that distinguished Syzygium sp. with other analysed accessions (Table 3). In that positions, Syzygium sp. had Adenine (A) and Guanine (G) nucleotides respectively, whereas the other accessions in that positions had nucleotides Cytosine (C) and Thymine (T), respectively (Table 3).
Table 3. Nucleotide differences on matK sequences.

| Accessions      | Nucleotide number* |
|-----------------|--------------------|
| S. sp.          | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. tenuiflorum  | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. ngoyense     | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. lateriflorum | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. auriculatum  | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. laetum       | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. buxifolium   | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. hancei       | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. levinei      | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| S. sandwicenseG | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| P. guajava      | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |
| E. uniflora     | C T G T T C T A C T A A C T C G G T A C G C T G G A C G C G C A G C A G A C |

*The nucleotide numbers arranged vertically show nucleotide positions referring to Syzygium sp sequence. Dots (.) indicate that the nucleotide on particular position was the same as the one of Syzygium sp sequence. Nucleotides in the box and bold are the critical nucleotides for the identification of Syzygium sp sequence.

Dendrogram based on the matK sequence showed that Syzygium sp. formed a group similar to the fellow members of the Syzygium genus (Group 1), separated from outgroups (Group II) (Figure 2). Phenogram also showed that the Syzygium sp. had a closer genetic relationship with S. tenuiflorum with the difference at two critical nucleotides (Table 3).

Figure 2. Dendrogram constructed based on the matK sequences using Neighbor Joining method with 1000 bootstrap.
3.2. The rbcL Sequence Analysis of Syzygium sp.
The rbcL sequence obtained from Syzygium sp. was 641 bp in size. This sequence had been registered and released in GenBank with accession number KY503029.1. The BLASTn analysis showed that the rbcL sequence from Syzygium sp. had a similarity of up to 100% with S. balsameum, but this result was not supported by the high query cover value (the query cover value was only 98%). On the other hand, some species of the genus Syzygium had a 100% query cover value but the similarity value was only 99% (Table 4).

| Description                  | Max score | Total score | Query cover | E value | Ident  | Accession   |
|------------------------------|-----------|-------------|-------------|---------|--------|-------------|
| Syzygium globiflorum         | 1227      | 1227        | 100%        | 0.0     | 99%    | KJ440001.1  |
| Syzygium hancei              | 1221      | 1221        | 100%        | 0.0     | 99%    | KJ439998.1  |
| Syzygium rowlandii           | 1221      | 1221        | 100%        | 0.0     | 99%    | KC627942.1  |
| Syzygium balsameum           | 1215      | 1215        | 98%         | 0.0     | 100%   | KR530084.1  |
| Siphoneugena guilfoyleana    | 1215      | 1215        | 99%         | 0.0     | 99%    | KF981269.1  |
| Eugenia feijoi               | 1215      | 1215        | 100%        | 0.0     | 99%    | GQ428586.1  |
| Eugenia macrocalyx           | 1215      | 1215        | 99%         | 0.0     | 99%    | FJ038134.1  |
| Myrcianthes fragrans         | 1215      | 1215        | 100%        | 0.0     | 99%    | U26328.2    |
| Pimenta pseudocaryophyllus   | 1211      | 1211        | 99%         | 0.0     | 99%    | KF981267.1  |
| Syzygium cumini              | 1211      | 1211        | 99%         | 0.0     | 99%    | GQ870669.3  |
| Syzygium maire               | 1210      | 1210        | 100%        | 0.0     | 99%    | KT626830.1  |
| Syzygium austroyunnanense    | 1210      | 1210        | 98%         | 0.0     | 99%    | KR530072.1  |
| Lophomyrtus bullata          | 1210      | 1210        | 99%         | 0.0     | 99%    | JQ933392.1  |

An analysis on the rbcL sequences presented 34 variations of nucleotides. The variations included substitution, insertion and deletion and no critical nucleotides distinguished Syzygium sp. from compared accessions. The grouping analysis based on the rbcL sequence indicated that Syzygium sp. belongs to one group (Group I) with fellow members of the Syzygium genus and distinguished from other species outside the genus of Syzygium (Group II) which is still a Myrtaceae family (Figure 3).
3.3. The Intergenic Spacer trnL-trnF Intergenic Spacer Sequence Analysis of Syzygium sp.

The trnL-trnF intergenic spacer sequence of Syzygium sp. had been obtained with size of 828 bp. The sequence had been registered and released in GenBank with accession number MG6836254. The BLASTn analysis based on the sequence showed that Syzygium sp. had 94% -99% similarity with some species of the Syzygium genus and other genus in the Myrtaceae family. The query cover values were ranging 70% -100% and the E-values were low such as 0.0 (Table 5).

Table 5. The alignment analysis using BLASTn on the trnL-trnF intergenic spacer sequence of Syzygium sp.

| Description                  | Max score | Total score | Query cover | E value | Ident | Accession     |
|------------------------------|-----------|-------------|-------------|---------|-------|---------------|
| Syzygium pseudoformosum      | 1329      | 1329        | 85%         | 0.0     | 99%   | KU853255.1    |
| Syzygium ridleyi             | 1325      | 1325        | 85%         | 0.0     | 99%   | KU853246.1    |
| Syzygium nemestrinum         | 1317      | 1317        | 85%         | 0.0     | 99%   | KU853252.1    |
| Allosyncarpia ternata        | 1313      | 1313        | 100%        | 0.0     | 95%   | KC180806.1    |
| Eucalyptus diversicolor      | 1313      | 1313        | 100%        | 0.0     | 95%   | KC180795.1    |
| Corymbia henryi              | 1302      | 1302        | 100%        | 0.0     | 94%   | KP015032.1    |
| Corymbia citriodora          | 1302      | 1302        | 100%        | 0.0     | 94%   | KP015031.1    |
| Syzygium sayeri              | 1185      | 1185        | 82%         | 0.0     | 98%   | KC428619.1    |
| Syzygium cryptophlebium      | 1090      | 1090        | 81%         | 0.0     | 97%   | KC428618.1    |
| Syzygium pachyphyllum        | 1075      | 1075        | 70%         | 0.0     | 99%   | KU853250.1    |
Analysis of the trnL-trnF intergenic spacer sequences showed 145 variations of nucleotides in which 53 of them were critical nucleotides that differentiated Syzygium sp. from other comparable species (data not shown). The grouping analysis based on the trnL-trnF intergenic spacer sequences indicated the same grouping pattern to the matK and rbcL sequences. In other words, Syzygium sp. formed a similar group to the same species of the Syzygium genus and separated from other species of the Myrtaceae family (Figure 4). These results indicated that Syzygium sp. was closely related to the species of the Syzygium genus.

![Figure 4. Dendrogram constructed based on the trnL-trnF intergenic spacer sequences using Neighbor Joining method with 1000 bootstrap.](image)

4. Results and Discussion
The DNA Barcode is a short-term DNA sequence of about 500 to 1500 bp that can be used to identify organisms [1,13,14,15,16]. In addition, the DNA barcode has also been applied for phylogenetic analysis in plants, animals, and microorganisms [17,18,19,20]. The ordo of Myrtales consists of 9 families, and one of them is the Myrtaceae family which is composed of 132 genus and 5920 species [21,22]. Syzygium Gaertn as one of the members of the Myrtaceae family consists of a group of woody plants with the largest number of species compared to other flowering plant genus. The number of species in this genus ranges from 1200-1800 species [23]. However, the amount of DNA sequence data from the genus Syzygium available in the public database is limited, i.e. 309 for matK, 252 for rbcL, and 18 for trnL-trnF intergenic spacers (updated by March 4, 2018). In line with this, [24] have also reported the limited amount of DNA sequence data from the Syzygium genus based on the sequence of ITS and psbA-trnH intergenic spacer regions. Thus, the availability of DNA sequence data in public databases becomes very important for the molecular identification using DNA barcoding techniques. Therefore, the absence of sequences of matK, rbcL, and trnL-trnF intergenic spacers in GenBank databases of 100% resemblance and 100% query cover with sequences of matK, rbcL, and trnL-trnF intergenic spacers from Syzygium sp. indicates that the sequences obtained in this study are the first reported from this plant.

Two critical nucleotides were found in the matK and 52 critical nucleotides in the trnL-trnF intergenic spacer that definitely determine Syzygium sp. On the other hand, no critical nucleotides were found in the rbcL sequence. These results indicate that the DNA barcodes of matK and trnL-trnF intergenic spacer are more varied than the rbcL sequence and both (matK and trnL-trnF intergenic spacer) can serve as a differentiator for plant species.
A slightly different result was found by [25] when identifying the Pandan plant (Benstonea sp) from Riau using the same three DNA barcodes. It was found some critical nucleotides that identify Pandanus sp in the three DNA barcodes. There was 1 critical nucleotide on the sequence of matK and rbcL and there were 2 nucleotides found in the trnL-trnF intergenic spacer sequence.

The DNA barcodes are the promising sequence to identify plant species, but this depends on the availability of these sequences in the public database. Therefore, the availability of abundant DNA sequences in the public database is essential for the identification of organisms using DNA barcoding technique. This study has been obtained and deposited three DNA barcodes namely matK, rbcL, and trnL-trnF intergenic spacer on Syzygium sp. in GenBank. These sequences are very useful for molecular identification of this plant species.

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