USING SHORT SEQUENCE matK GENE AS BARCODE DNA FOR IDENTIFICATION OF DURIO Sp IN TERNATE ISLAND

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ABSTRACT

The Barcode of Life Consortium (CBOL) recommended a standard method for the identification of plant species using matK and rbcL barcoding gene. This study was aimed to evaluate the efficiency of matK DNA barcoding for identification of local durian (Durio sp.) from Ternate Island. Total 15 local durian has been used in this study. Whole genom DNA was isolated by Geneid plant DNA kit and then successfully amplified by the Polymerase Chain Reaction (PCR) technique using specific primer. MatK successfully amplified with 245 bp in length. MatK has sequencing success (71.3%) and relative high of Quality value 20+ (86%). BLAST analysis of the sequence showed that local durian in Ternate are identifies as Durio zibethinus and Neesia malayana with query cover 97%-99%. It could be concluded that matK with short sequence is not efficiency for durian identification. The recommended in this studies for using of molecular markers with sequence lengths above 500 bp would be more effective for the identification of cryptic species Durio sp. and other.

Keywords: Identification, Local Durian, matK Gene, Ternate Island

INTRODUCTION

Durian (Durio zibethinus Murr.) is one of the favorite tropical fruits in Indonesia (Ministry of Agriculture, 2014). One effort to build the good image of durian archipelago is by utilizing the local genetic resources of durians relatives scattered in various regions (Santoso, 2010; Dick & Kress, 2009). Local durian is the name for durian variant (Durio spp) growing in various regions in Indonesia (Siregar, 2006). Durian Ternate is one of durian variant (Durio spp.) that grows naturally (wild) at Ternate Island from seeds with hereditary ownership. Ternate Island is one of durian production centers in North Maluku province besides Tidore and Jailolo islands in West Halmahera Island. Local durian production in Ternate reaches 3.15 tons per year (Central Bureau of Statistics, 2014).

The genetic diversity of local durians on the Ternate Island has not been reported so far. One of the parameters of the genetic diversity of local durians on the Ternate Island is the presence of local names of durian variants given by the local community. The local name is based on the morphological variation, durian owner’s name and the growing location. Seven groups of morphology diversity based on the taxonometric analysis were found in local durian in Ternate. The highest similarity
was between durian Udi and Sina (Sundari & Tolangara, 2014; Sundari et al, 2015). Furthermore, Sundari et al, (2017) reported a high polymorphism values in local durians in Ternate based on RAPD analysis. The up-to-date information about genetic diversity will contribute fundamentally to the local durian genetic resource breeding program.

To raise the popularity of tropical fruit including local durian, more attention and work are needed. The development of durian commodity should not only focus on the quantity but also should to focus on the quality aspect according to the national standard of SNI durian. Durian breeding is the solutions for this problem. In other side determination of which Durian should be crossed is the some of the most recent problem. Molecular marker using DNA barcodes is ones of the solutions of these problems.

The Consortium for the Barcode of Life (Group et al, 2009) recommends to use of several universal barcode DNAs, including Internal Transcribed Spacer (ITS), two plastid genes (matK and rbcL) and non-coding regions of the plastid gene (psbK-psbl, trnH-psbA, rpoC1 and rpoB) for plant identification (Group et al, 2009; Hollingsworth, 2009). DNA barcode has many advantages in identification of specimens due to its high accuracy in identification. Furthermore, DNA Barcode has been used in several application such as for ecological surveys, identification of cryptic taxons, and confirmation of plant species (Xue & Li, 2011).

matK is ones of DNA barcodes that can be used in plant. matK is a gene encoding the maturase enzyme of sub-unit K for photosynthesis in plant chloroplast genome (Soltis et al, 1992). In general, the length of nucleotide sequence of matK gene approximately is 1500 bp. Since 2003, the matK gene has been used as a standard coding for the plant DNA barcode. This barcode is widely used in many species plant identification studies based on its accuracy and specificity at the genus level. Nevertheless, plant DNA barcoding sometimes is only applicable in one plant and not applicable in other plant. There are currently no publications found scientific knowledge of the identity of plant DNA barcodes durian (Durio zibethinus.), which has been storeedin BOLD (Barcode of Life Database) Systems (www.boldsystems.org). Therefore, it is necessaryconducted research on barcode sequences Durian DNA as inventory data Molecular identity of local durians in North Maluku. Therefore this study aimed to evaluate the efficiency of matK DNA barcoding for identification of local durian (Durio spp) from Ternate Island.

**METHOD**

1. DNA Isolation

Total DNA Isolation Total of 15 local durians were used in this study (Table 1). These durian were collected from several distric from Ternate island. Total DNA was isolated from 100 mg of durian young leaves. DNA isolation procedure was performed according to the manual instructions supplied by Geneid DNA miniprep kit (Brand-country). The kit apply a column purification technique which is capable to extract the total plant DNA. Plant cells was lyzed by grinding in lysis buffer and proteinase K. Furthermore, the precipitation protein and waste material were separated by centrifugation at 13,000 rpm for 5 min. The supernatant was passed through a silica membrane column. The total DNA was washed from residual protein and salt then was eluted in eppendof 1.5 ml and incubated at -20° C for 8 hours.
2. Polymerase Chain Reaction (PCR)

DNA amplification cocktail was prepared using PCR Master Mix (Intron-company) in 40 μL of total volume containing of 1.25 units of Taq DNA polymerase, 0.2 mM of each dNTP, 1.5 mM MgCl₂; 0.2 mM of each primer and 0.6 μg of DNA. Primer pair used in this study was consisting of forward primer matK 1F 5’ ATATCCGCTTATATTTCAGGAGT 3’ and reverse primer matK 1R 5’ GAACTAGTCGGATGGAGTAG 3’ (Muller et al, 2006). The PCR reaction was done under condition as follow: initial denaturation at 95°C for 2 minutes then continued 35 cycles containing of denaturation: 95°C, 30 sec; primer anealing 50°C, 30 seconds, and DNA extension: 72°C, 50 sec. The PCR product then electrophoresed using 1% agarose gel and visualized using UV-transilluminator. Sequencing was done by Malaysia’s 1st Base service provider using ABI PRISM® 310 Genetic Analyzer.

3. Data Analysis

The sequence data of matK gene was analyzed using ABI sequence scanner v.10 program. Sequencing success and Quality value 20+ (QV20+) was performed by this software. The percentage of sequencing success was calculated using formula: % Sequencing success = total sequenced DNA/total amplified DNA x 100%. Whereas the percentage QV20+ was calculated using formula: % QV20%= Total Nucleotide with QV20+/sequencing success x 100%.

Furthermore to know the homology of matK with NCBI queries and to know the effectiveness of identification using matK, The BLASTN at NCBI was performed (Altschul et al, 1990; Morgulis et al, 2008; Zang et al, 2000).

RESULTS AND DISCUSSION

matK gene were successfully amplified with 245 bp in size (Figure 1). The 245 bp matK gene section was the best result of sequencing and sequence alignment. These amplicon is categorized as partial structure of the matK gene (Group et al, 2009).

![Figure 1](https://i.imgur.com/3Q5Q5Q5.png)

*Figure 1.* PCR product of partial matK gene generated from 15 local durians from Ternate Island

Amplified DNA successfully sequenced and has 71.3% of sequencing success. Ones of standards the gene categorized as DNA barcoding is easy to be amplified and
sequenced (Shen et al, 2017). In this study only 71% of matK which successfully amplified can be sequenced. This result indicate that matK is enough difficult to be sequenced. Nevertheless the results of sequencing are categorized as high quality because of the results of QV20+ which raise more than 80% (Figure 2).

Figure 2. Sequencing success and Quality Value (QV20+) of matK analysed by sequence scanner software 2.0.

Basic Local Alignment Search Tool (BLAST) technique using NCBI (www.ncbi.nlm.nih.gov) has been done. Local durians in Ternate Island are identified as Durio zibethinus and some of them are identified as Neesia malayana (Table 1). Nevertheless, identification with matK using BLAST NCBI is cannot be accepted as DNA barcoding requirement since their query cover were not reached 100% (Table 1).

Table 1. Results BLAST search on NCBI genebank matK gene local durian in Ternate

<table>
<thead>
<tr>
<th>Code</th>
<th>Lokal name</th>
<th>Identified</th>
<th>% Query Cover</th>
<th>E Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Cinta</td>
<td>Durio zibethinus</td>
<td>87%</td>
<td>7e-75</td>
</tr>
<tr>
<td>T2</td>
<td>Urat</td>
<td>Durio zibethinus</td>
<td>98%</td>
<td>4e-86</td>
</tr>
<tr>
<td>T3</td>
<td>Mentega</td>
<td>Neesia malayana, Durio zibethinus</td>
<td>99%</td>
<td>4e-71</td>
</tr>
<tr>
<td>T4</td>
<td>Luri</td>
<td>Durio zibethinus</td>
<td>97%</td>
<td>8e-84</td>
</tr>
<tr>
<td>T5</td>
<td>Boso</td>
<td>Durio zibethinus</td>
<td>98%</td>
<td>2e-90</td>
</tr>
<tr>
<td>T6</td>
<td>Coklat</td>
<td>Durio zibethinus</td>
<td>97%</td>
<td>3e-88</td>
</tr>
<tr>
<td>T7</td>
<td>Gosi</td>
<td>Durio zibethinus</td>
<td>98%</td>
<td>1e-87</td>
</tr>
<tr>
<td>T8</td>
<td>Pondak</td>
<td>Durio zibethinus</td>
<td>97%</td>
<td>1e-86</td>
</tr>
<tr>
<td>T9</td>
<td>Biasa</td>
<td>Neesia malayana, Durio zibethinus</td>
<td>97%</td>
<td>4e-71</td>
</tr>
<tr>
<td>T10</td>
<td>Gajah kuning</td>
<td>Durio zibethinus</td>
<td>99%</td>
<td>2e-74</td>
</tr>
<tr>
<td>T11</td>
<td>Pare</td>
<td>Durio zibethinus</td>
<td>90%</td>
<td>4e-87</td>
</tr>
<tr>
<td>T12</td>
<td>Rua</td>
<td>Durio zibethinus</td>
<td>90%</td>
<td>6e-85</td>
</tr>
<tr>
<td>T13</td>
<td>Biji mati</td>
<td>Durio zibethinus</td>
<td>98%</td>
<td>1e-86</td>
</tr>
<tr>
<td>T14</td>
<td>Udi</td>
<td>Durio zibethinus</td>
<td>98%</td>
<td>2e-74</td>
</tr>
<tr>
<td>T15</td>
<td>Ratem</td>
<td>Durio zibethinus</td>
<td>99%</td>
<td>4e-76</td>
</tr>
</tbody>
</table>

Identification using BLAST which showed that local durian in Ternate Island come from genus of Durio and Neesea which both of them are belong to family of Bombacaceae also has low e-value. matK barcode which is proved can be utilized for plant identification (Kolondam et al, 2012) is not proved in local durian in Ternate Island.
The discrimination power of barcode gene matK has been widely published and recommended for use in plant identification (Hollingsworth, 2011). Although it has not best discrimination power at the species level but the matK barcode has a high amplification success rate for many species and is easy to sequence (Hollingsworth, 2011). Nevertheless, matK is not applicable for identification of Local Durian in Ternate.

One of the reasons is the short sequence used in this study. This is appropriate of statement Siew et al., (2018) said that two cpDNA regions (trnL-trnF and matK) were successfully amplified for assess genetic diversity of Malaysian durian varieties, but showed no variation in their DNA sequences.

The development of DNA barcode is very important for plant identification and to retain plant’s identity. Genetic diversity assessment is also important of species that are endemic, rarely found, or endangered, because it helps in plant conservation. According Newsmaster & Raguphaty (2009) that matK has significant variation and can be used for DNA barcode in nutmeg family.

Many genes used in plant systematics are in substitution rate spectrum, representing genes that evolve rapidly or slowly. Which genes to be used, is usually determined by the level of phylogenetic analysis conducted by researcher. Each region in a gene has strengths and weaknesses. Good quality sequences, for example, can be found in the rbcL (ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit) and atpB (ATPase beta-subunit), but these sequences have a low level of differentiation of species because they are highly conserved among plant groups, therefore its resolution is only good in the level of family and above.

Sequences that have high degree of species differentiation are trnH-psbA (chloroplast intergenic spacer) and matK (maturase K), because it evolves so quickly that provides enough character to analyze evolution below family level (Barthet, 2006; Hollingsworth, 2011). The matK gene is considered to evolve rapidly, due to the fact that the gene has a high degree of substitution and its sequence is more varied than other genes (Barthet, 2006). However, a group of researchers at the Consortium for the Barcode of Life (Cbol) recommends two loci combination, rbcL and matK, as standard DNA barcode for plants. These two regions in chloroplast DNA were chosen because of having high degree of differentiation between species (Bafeel et al. 2011). Furthermore, these two genes play important role in phylogenetic reconstruction for land plants (Kuzmina et al, 2012).

**CONCLUSIONS**

The partial sequences of matK from local durian samples of Ternate were successfully amplified and sequenced. matK have low sequencing success and high-quality value. Nevertheless, matK is not suitable as Plant DNA barcodes for local durian in Ternate Island.

For recommended should be included in future studies use of molecular markers with sequence lengths above 500 bp would be more effective for the identification of cryptic species *Durio* spp. and other.

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