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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 *mat*K 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. *Mat*K successfully amplified with 245 bp in length. *Mat*K 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 *mat*K with short sequence is not efficience 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

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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 (*mat*K and *rbc*L) and non-coding regions of the plastid gene (*psb*K-*psb*I, *trn*H-*psb*A, *rpo*C1 and *rpo*B) 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).

*mat*K is ones of DNA barcodes that can be used in plant. *mat*K 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 *mat*K gene approximately is 1500 bp. Since 2003, the *mat*K 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 storedin 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 *mat*K DNA barcodding 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 $MgCl_2$; 0.2 mM of each primer and 0.6 µg of DNA. Primer pair used in this study was consisting of forward primer *mat*K 1F 5' ATATCCGCTTATATTTCAGGAGT 3' and reverse primer *mat*K 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.

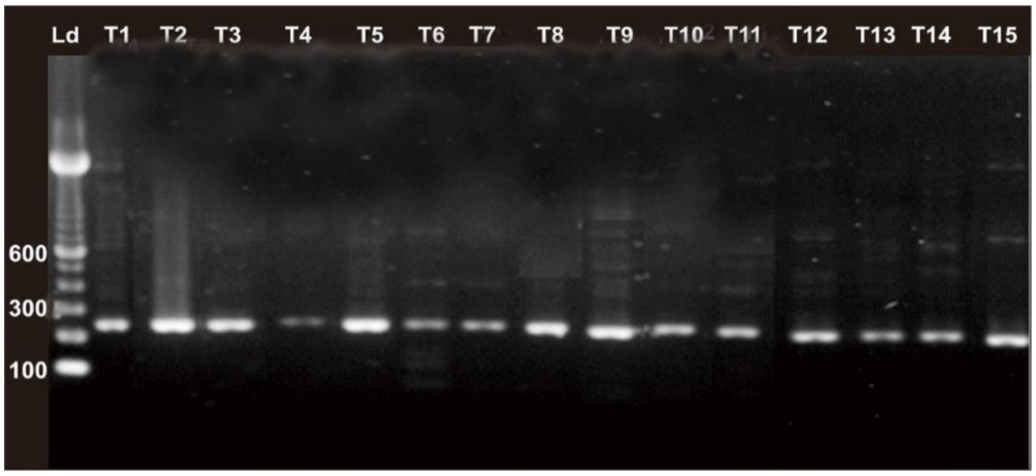
3. Data Analysis

The sequence data of *mat*K 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 *mat*K with NCBI queries and to know the effectiveness of identification using *mat*K, The BLASTN at NCBI was performed (Altschul et al, 1990; Morgulis et al, 2008; Zang et al, 2000).

RESULTS AND DISCUSSION

*mat*K 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 catagorized as partial structure of the *mat*K gene (Group et al, 2009).



Note: Ld= DNA Ladder T1=Cinta, 2. T2= Urat, T3= Mentega, T4= Luri, T5= Boso T6= Coklat, T7= Gosi, T8= Pondak, T9= Biasa, T10= Gajah kuning, T11=Pare, T12=Rua, T13=Biji mati, T14= Udi, T15=Ratem

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

Amplified DNA successfully sequenced and has 71.3% of sequencing success. Ones of standarts the gene catagorized as DNA barcoding is easy to be amplified and

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sequenced (Shen et al, 2017). In this study only 71% of *mat*K which succesfully amplified can be sequenced. This result indicate that *mat*K is enough difficult to be sequenced. Neverthelss the results of sequencing are catagorized as high quality because of the results of QV20+ which raise more than 80% (Figure 2).

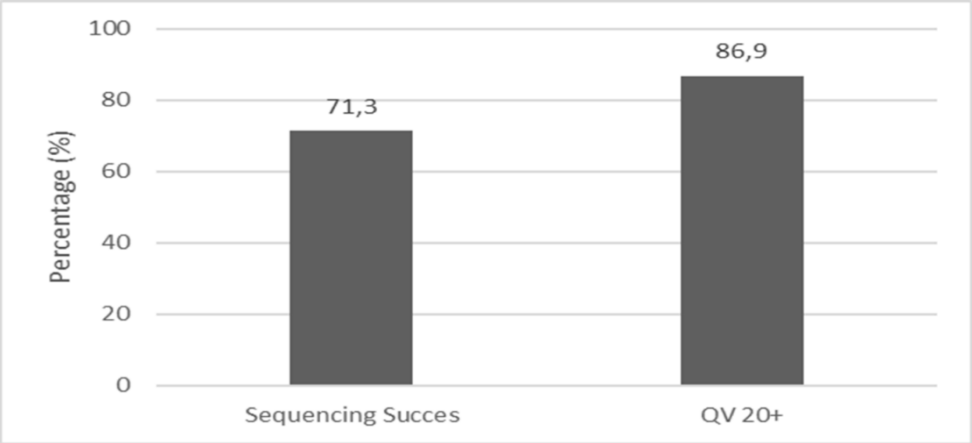

Figure 2. Sequencing success and Quality Value (QV20+) of *mat*K analysed by sequence scanner software 2.0.

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 *mat*K using BLAST NCBI is cannot be accepted as DNA barcodding requirement since their query cover were not reached 100% (Table 1).

Table 1. Results BLAST search on NCBI genebank *mat*K gene local durian in Ternate

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

Identification using BLAST which showed that local durian in Ternate Island come from genus of *Durio* and *Neseea* which both of them are belong to family of Bombacaceae also has low e-value. *mat*K 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 *mat*K 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 *mat*K barcode has a high amplification success rate for many species and is easy to sequence (Hollingsworth, 2011). Nevertheless, *mat*K 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 (*trn*L-*trn*F and *mat*K) 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 *mat*K from local durian samples of Ternate were succesfully amplified and sequenced. m*at*K have low sequencing success and high-quality value. Nevertheless, *mat*K 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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REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of molecular biology*, *215*(3), 403-410.

Barthet MM. 2006. Expression and function of the chloroplastencoded gene matK [Dissertation]. Virginia: Virginia Polytechnic Institute.

Central Bureau of Statistics. (2014). North Maluku In Figures: BPS North Maluku. pp 88-96.

Dick, C.W., & Kress, W.J. (2009). Dissecting tropical plant diversity with forest plots and a molecular toolkit. *BioScience*, *59*(9), 745-755.

Group, C.P.W., Hollingsworth, P.M., Forrest, L.L., Spouge, J.L., Hajibabaei, M., Ratnasingham, S., & Fazekas, A.J. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, *106*(31), 12794-12797.

Hollingsworth, M.L., Andra C., Forrest, L.L., Richardson, J., Pennington, R.T., Long, D.G., & Hollingsworth, P.M. (2009). Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. *Molecular ecology resources*, *9*(2), 439-457.

Hollingsworth, P.M., Graham, S.W., & Little, D.P. (2011). Choosing and using a plant DNA barcode. *PloS one*, *6*(5), e19254.

Kolondam, B. J., Lengkong, E., Polii M.J., Pinaria, A., & Runtunuwu, S. (2012). DNA barcode of payus limondok orchid (Phaius tancarvilleae) based on the rbcL and matK genes. *Jurnal Bioslogos*, *2*(2), 55-62.

Kuzmina ML, Johnson KL, Barron HR, Herbert PDN. 2012. Identification of vascular plants of Churchill, Manitoba, using a DNA barcode library. BMC Ecology 12:1-11. http:// dx.doi.org/10.1186/1472-6785-12-25

Ministry of Agriculture. (2014). Outlook Durian Commodity, Jakarta; Agricultural Data and Information System Center of the Secretariat General of Agriculture. pp 38-42

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T.L., Agarwala, R., & Schäffer, A.A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics*, *24*(16), 1757-1764.

Muller, K.F., Borsch, T., & Hilu, K.W. (2006). Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: contrasting matK, trnT-F, and rbcL in basal angiosperms. *Molecular phylogenetics and evolution*, *41*(1), 99-117.

Newsmaster SG, Ragupathy S. (2009). Testing plant barcoding in a sister species complex of pantropical Acacia (Mimosoideae, Fabaceae). *Molecular Ecology Resources*, 9:172-180. http:// doi: 10.1111/j.1755-0998.2009.02642.x

Santoso, P.J. (2010). Lai durian Colorful Attractive Flesh; Export Potential. Tropical Fruit Plant Research Center. pp 36-41.

Shen, Y.Y., Chen, X., & Murphy, R.W. (2013). Assessing DNA barcoding as a tool for species identification and data quality control. *PLoS One*, *8*(2), e57125.

Siew, G.Y., Ng, W.L., Salleh, M.F., Tan, S.W., Ky, H., Alitheen, N.B. M., & Yeap, S.K. (2018). Assessment of the Genetic Variation of Malaysian Durian Varieties using Inter-Simple Sequence Repeat Markers and Chloroplast DNA Sequences. *Pertanika Journal of Tropical Agricultural Science*, *41*(1), 321-332.

Siregar, M. (2006). Species diversity of local fruit trees in Kalimantan: problems of conservation and its development. *Biodiversitas Journal of Biological Diversity*, *7*(1), 94-99.

Soltis, P.S., Soltis, D.E., & Doyle, J.J. (1992). Molecular systematics of plants. *Chapman & Hall:* New York & London, 434, 1-13.

Sundari & Tolangara A.R. (2014). The study of taxonometric and philogenetic of local durian variety of Ternate and Jailolo in North Moluccas Province, Indonesia. *International Journal of Engineering Research and Development*, 10 (8), 52-57.

Sundari, Arumingtyas, E.L., Hakim, L., Azrianingsih, R. (2015). Morphological variation of local durian (*Durio zibethinus Murr*.) on the Ternate Island*, Proceedings of International Biology and Life Science Conference* (ICOLIB), 10 (2), 52-57.

Sundari, Arumingtyas, E.L., Hakim, L., Azrianingsih, R., Wahyudi, D. (2017). Genetic variability of local durian (*durio zibethinus murr*.) In Ternate island based on RAPD markers. *Plant Cell Biotechnology and Molecular Biology Journa,l* 18 (1&2), 68-75.

Xue, C.Y., & li, D.Z. (2011). Use of DNA barcode sensu lato to identify traditional Tibetan medicinal plant Gentianopsis paludosa (Gentianaceae). *Journal of Systematics and Evolution*, *49*(3), 267-270.

Zang, H., Jue, J. P., & Mukherjee, B. (2000). A review of routing and wavelength assignment approaches for wavelength-routed optical WDM networks. *Optical networks magazine*, *1*(1), 47-60.