The application of barcode DNA

by Sundari Su

Journal of Physics: Conference Series

The application of barcode DNA rbcL gene for identification of medicinal plants: red jabon and gofasa

The application of barcode DNA rbcL gene for identification of medicinal plants: red jabon and gofasa

Sundari[1], Khadijah[2], A.M Jayali[2], N.H Sukamto[3]

[1] Department Biology education, Faculty of Teacher and Training education, Khairun University Ternate,Indonesia
[2] Department Chemistry education, Faculty of Teacher and Training education, Khairun University Ternate,Indonesia
[3] Department Chemistry, Faculty of Mathematic and Natural Science, Hasanudin University Makasar, Indonesia
*Corresponding author: sundari@unkhair.ac.id

Abstract. Red jabon and gofasa plants has potential as a medicinal plant. One of the potential drugs of these two plants as anti-cholesterol purpose of this study was to identify the red jabon and gofasa plants in Ternate Island using rbcL gene of DNA barcode. The method in this study consists of total DNA isolation using ZymoBiomic (Zymo Research DNA Extraction) Kit, DNA amplification using rbcL gene, sequencing and BLASTn analysis. Based on barcode DNA rbcL, the red jabon plants has similarities 99% *Cephalanthus occidentalis, Mitragyna speciosa and Ocherreuclear sp*. for gofasa plants has 99% resemblance with *Vitex glabrata, Vitex megapotamica, Vitex trifolia and Vitex negundo* at NCBI database. The application of rbcL barcode DNA is effective to identify plants at the family level up to the genus.

1. Introduction

Red jabon (*Anthocephalus macrophyllus*) is a fast-growing type of local plant with a broad spectrum of uses. In some places in Indonesia, this type has begun to be developing and planted by the community. Red jabon wood includes softwood with low to medium density. According to jabon red wood belongs to the strong wood class I to II. The height of the red jabon tree can reach 40 meters with a round and perpendicular stem reaching 70% - 80% with a stem circumference reaching more than 150 cm (diameter of more than 50 cm) [1]. Red jabon is a pioneer plant that is light tolerant, can live in the lowlands to an altitude of 50-1000 m above sea level [2,3].

Gofasa or bitti belong to the Verbenaceae family, the Vitex genus and species: *Vitex cofassus* Reinw. ex Blume. This tree is of medium to large size and can reach heights up to 40 meters. The stem usually without tire and diameter can reach 130 cm, deep grooved and clear, the wood is solid and pale. The wood is classified as moderate to heavy, strong, durable and does not contain silica and wet wood scented like skin [4,5]. In general, this type always blooms every year after five years with pollination assisted by insects, most likely bees [6]. One of the potential plants is the Bitti wood plant (*Vitex cofassus*) which is a typical Sulawesi endemic plant and the wood is the superior wood of South Sulawesi. Bitti wood (*Vitex cofassus*) has not been widely reported. The people of South Sulawesi generally only use Bitti wood (*Vitex cofassus*) as a building material. Another potential that can be developed in the utilization of Bitti wood (*Vitex cofassus*) is to use it as a plant that has a toxic effect

on cancer cells in this case is the bark of the skin has some pharmacological effects, especially as anticancer [7].

One approach used to identify plants is DNA barcodes. DNA barcode is a tool that can be used to quickly identify species [8,9]. DNA barcodes that are often used in plants generally are chloroplast DNA (cpDNA). The DNA sequence that has the opportunity to be used as a DNA barcode is the rbcL gene. The rbcL gene is about 1400 bp long so it provides many characters for phylogenetic studies [10]. The role of the rbcL gene that encodes the RuBisCO protein is thought to cause this gene sequence to have a low mutation level compared to other barcode genes in cpDNA so that the level of similarity between species is quite high [11]. This low level of mutation provides benefits for in-depth study of intraspecies genetic and phylogenetic variations. This study aims to identify red jabon and gofasa plants found on the island of Ternate with the rbcL gene application.

2. Material And Methode

2.1 DNA isolation and Amplification of Fragmen rbcL Gene

DNA isolation carried out using DNA Presto TM Mini gDNA kit KIT (Geneaid) kit. The amplification process uses MyTag Red Mix (Bioline). RbcL gene amplification with the primary sequence forward rbcLaF (5'-ATG CCA CAA ACA GAG ACT AAA GC-3') and reverse sequence ie rbcLaR (5'-GTA AAA TCA AGT CCA CCR CG-3'. 30 mL PCR reaction mixture (MyTag Red Mix, Primary, ddH2O, and DNA templates). The PCR process was carried out under denaturation at 95 ^{0}C, annealing at 55 ^{0}C, and extension at 72^{0}C and post extension at 72^{0}C. PCR products were purified using Zimoclean TM gel DNA recovery KIT (Zimo research). Bidirectional sequencing uses 1st Base Malaysia services.

2.2 Phylogenetic Analysis

DNA alligment with MEGA 5 program, BlASTn DNA samples with DNA sequences in GenBank, DNA sequencing from GenBank, and Phylogenetic Neighbor Joint (NJ) tree construction using MEGA 5 program [12].

3. Result And Discussion

3.1 DNA Isolation

Isolation of DNA is an early stage to perform the PCR process. Whole genome DNA is isolated from the leaves and stems of clove plants. The results of isolation obtained two whole genome DNA. To determine the profile and quality of DNA, agarose electrophoresis and quantitative measurements of DNA were carried out. Results of measurements of concentration and purity of DNA obtained (Table 1).

Table 1. Quantitative Result of DNA Isolation

No	Sample name	Conc (ng/ml)	A260/280	A260/230	Volume (microlit)
1	Red jabon (A)	3,3	1,51	0,17	30
2	Gofasa (B)	52,1	1,89	1,36	30

The results of the quantitative analysis revealed that from two samples of clove plants had a good DNA concentration with purity close to 1.8. DNA amplification was perform using the rbcL gene

3.2 The Amplification of Fragmen Gen rbcL from Red jabon and gofasa plant

The results of rbcL gene amplification from clove plant samples tested by electrophoresis presented in Figure 1.

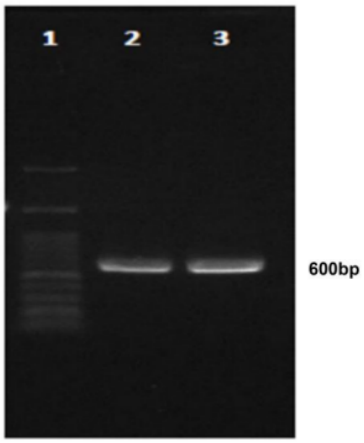

Figure 1. Profil of rbcL gene amplification on Red jabon (A) and gofasa (B) plants from Ternate

The amplification of the rbcL gene showed DNA bands with a size of ± 600 bp. A and B samples was well-amplified rbcL primer. Furthermore, an analysis of BLASTn (NCBI gene bank) was conduct to find out that red jabon was identical to *Cephalanthus occidentalis, Mitragyna speciosa and Ochreinauclea* sp. For gofasa plants, it has 99% similarity with *Vitex glabrata, Vitex megapotamica Vitex trifolia and Vitex negundo* NCBI database. DNA barcode application is enought effective to identify plants at the family to genus level.

3.3 Phylogenetic Analysis

In order to identify phylogenetic analysis, selected samples were comparing with data from the genebank of BLAST search results at NCBI. From this phylogenetic analysis can know the position of taxon from the sample of red jabon and gofasa plants. Data from kinship analysis (phylogenetic) shown in Figure 2.

The results of phylogenetic analysis using Neighbor Join (NJ) method note that the rbcL gene can used to clarify taxon positions in the identification of a species. A specimen from a different area can be together on the same cluster [9]. Phyllogenous trees show the relationship of species based on genetic similarities. jabon red plant samples (A) are located far from the cluster members in phylogenetic trees while gofasa (B) is located next to the cluster with vitex glabrata.

The results showed that the identification of jabon Merah and gofasa plants was effective. Red jabon is identical to *Cephalanthus occidentalis*, *Mitragyna speciosa* and *Ochreinauclea* sp. For gofasa plants, it has a 99% similarity with *Vitex glabrata, Vitex megapotamica Vitex trifolia* and *Vitex negundo* NCBI database. Technically, the rbcL gene can amplified with a high success rate with one or two universal primers. It was further stated that when compared to other barcode gene candidates, the rbcL gene has a high success rate of bidirectional sequencing (CBOL sequencing with forward and reverse primers) [10,13]

One of the key proteins encoded by cpDNA is ribulose-1,5-biphosphate carboxylase-oxygenase (abbreviated as RuBisCO), which participates in carbon fixation in photosynthesis. Chloroplast DNA (cpDNA) is circular in shape with a size range of 85-2000 kilobasa (kb). cpDNA controls the production of transfer RNA (tRNA), ribosomal RNA (rRNA), and most of the protein found in chloroplasts organelles. The characteristics of cpDNA, namely: (1) having a small genome and a stable structure, (2) a more conservative genome with a low average nucleotide substitution and (3) the genome does not undergo recombination and uniparental inheritance. Thirty codes for photosynthetic protein complexes forming subunits, namely for photosystem I, photosystem II, ribulose-1,5-biphosphate carboxylase-oxygenase, cytochrome b6-f complex, and ATP synthase [14].

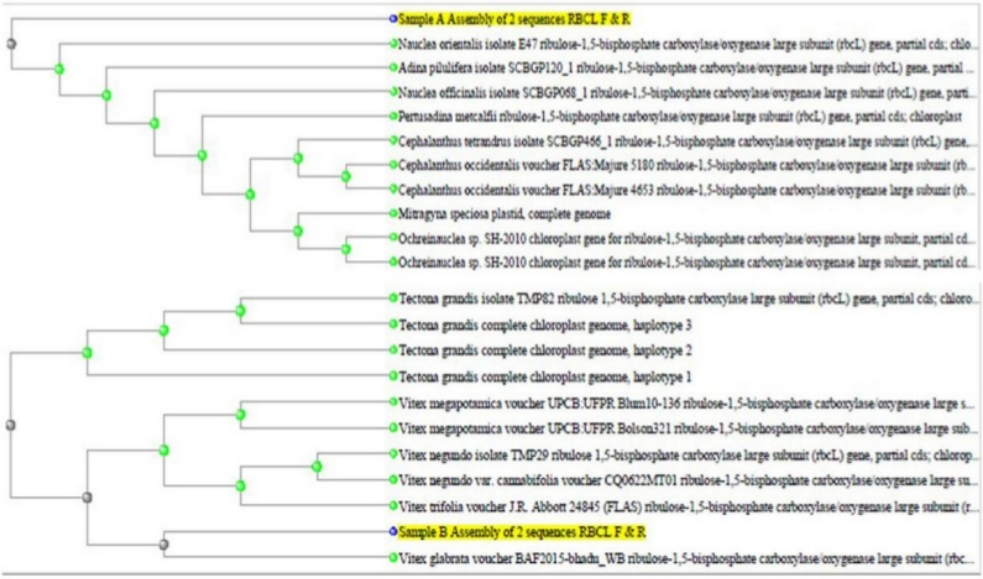

Figure 2. Phylogenetic analysis of samples of red jabon and gofasa

4. Conclusion

Red jabon and gofasa plants successfully amplified with amplicon size of 600 bp. Furthermore, the results of BLASTN analysis revealed that the sequence has a 99% similarity of *Cephalanthus occidentalis, Mitragyna speciosa and Ochreinauclea sp*. For gofasa plants, it has a 99% similarity with *Vitex glabrata, Vitex megapotamica Vitex trifolia and Vitex negundo* NCBI database.

Acknowledgments

We like to thanks to the Kementerian Ristek DIKTI Indonesia for providing funding Hibah PEKERTI for the research.

Reference

[1] BPTH 2011Sulawesi *Anthocephalus macrophyllus* (Roxb.) Miq. Quick Inforamtion of seed. No 126

[2] Burley A L, Enright N J and Mayfield M M 2011 Demographic response and life history of traditional forest resource tree species in a tropical mosaic landscape in Papua New Guinea *Journal of Forest Ecology & Management* **262** 750-758

[3] Seran D 1987 Trial on the cultivation of Vitex cofassus Reinw. in the lowland area of Andi Pangerang Pettarani Forest Complex, Pare-Pare, South Sulawesi Province. *Jurnal Penelitian Kehutanan (Ujung Pandang)* **1** 9-16.

[4] Anonim 2014 Detil data Vitex cofassus Reinw. ex Blume. *http://www.proseanet. org/florakita/browser.php?docsid=862*.

[5] Lemmens R H M J, Soerianegara I, Wong W C 1995 Plant Resources of South-east Asia Timber trees: minor commercial timbers *Backhuys Publishers* Leiden **5** 2-4

[6] Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S 2009 Agroforestree Database:a tree reference and selection guide version 4.0 *(http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp)*.

[7] Herna´ndez M M 1999 Biological activities of crude plant extracts from *Vitex trifolia* L. (Verbenaceae), *J . of Ethnopharmacol Jurnal*

[8] 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

[9] Che J, Chen HM, Yang JX, Jin JQ, Jiang K,Yuan ZY, Murphy RW, dan Zhang YP 2012 Universal COI primers for DNA barcoding amphibians *Mol Ecol Res* **12** 247-258

[10] Consortium Barcode of Life (CBOL) 2009 A DNA Barcode for Land Plants *PNAS* **106** 31

[11] Asahina H, Shinozaki J, Masuda K, Morimitsu Y, 2010 Identification of medicinal *Dendrobium* species by phylogenetic analyses using matK and rbcL sequences. *J. Nat. Med.* **64** 133-138

[12] Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods *Mol. Biol. Evol.* **28** 2731-2739.

[13] Fazekas A J, Burgess K S, Kesanakurti P R, Graham S W, Newmaster, S G, Husband B C, Hajibabaei M and Barrett S C H 2008 Multiple multilocus DNA barcodes from the plastid genome discriminate plant species equally well *PlosOne **3*** 7 e2802

[14] Tamarin R H 2001 Principles of Genetics (Seventh Edition). *Massachusetts: The McGraw-Hill Companies*

The application of barcode DNA

ORIGINALITY REPORT

%19	%19	%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	repository.unair.ac.id Internet Source	%7
2	mafiadoc.com Internet Source	%3
3	iopscience.iop.org Internet Source	%2
4	repository.uin-malang.ac.id Internet Source	%2
5	jurnal.fkip.uns.ac.id Internet Source	%1
6	www.geneticsmr.com Internet Source	%1
7	hdl.handle.net Internet Source	%1
8	journals.plos.org Internet Source	%1
9	hal.archives-ouvertes.fr Internet Source	<%1

EXCLUDE QUOTES	ON	EXCLUDE MATCHES	OFF
EXCLUDE BIBLIOGRAPHY	ON		