

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Characterization of *rbcL* gene sequences in tongkat langit banana (*Musa troglodytarum* L.) from Maluku.

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ABSTRACT

The characterization of *rbcL* gene sequences in tongkat langit banana from Maluku with genus *Musa* and genus *ensete* (NCBI) as the main *outgroup* was conducted to explore diversity in nucleotides and haplotype, to examine the ratio of nucleotide substitutions, and to determine variable sites. The results of characterizing the *rbcL* gene sequences using DNAsP version 5.10.01 validated the nucleotide diversity value as 0.00204. The haplotype characterization was performed by Network version 4.6 and the results indicated diversity value of 0,57 which was derived from 6 out of 24 haplotypes samples analyzed. Substitution polymorphism was spread among tongkat langit species and also the outgroup. Transversion substitution, however, was found higher in tongkat langit banana from Maluku. There were 10 variable sites discovered with varied site position comprising singleton two variant, tree variant, and four variant.

Keywords: characterization, *rbcL* gene sequences, tongkat langit, from Maluku.

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INTRODUCTION

Maluku is located in the eastern part of Indonesia. According to the spreading map of tongkat langit, this species is mostly found in Maluku. However, hardly ever is survey conducted to prove its location and if there is any, it is only focused on some places. These surveys have indicated some morphological character differences found in tongkat langit. Tongkat langit obtained from some locations in Maluku has been proved to contain carbohydrate, protein, vitamin, mineral and varied crude fiber (Hiariej unpublished). Therefore, people in Maluku often make use of this fruit as an alternative to their staple food. The morphological differences found in tongkat langit result from carotenoids contained inside the fruit [11]

The exploration of some Moluccas islands on which tongkat langit can be found showed the phenotypic variation responses [4]. Genetically, the ability of plants to grow properly on an area is determined by the genetic composition carried by their genotype. The genetic composition of tongkat langit from Maluku can be analyzed through marking the *rbcL* (cpDNA) gene molecular. This molecular marker is often used among plants species including bananas to understand their phylogenetic kinship [5]. The characteristics of *rbcL* gene are universal. They can provide sequence data for a genetic analysis, but compared to the nuclear genes, the non synonymous nucleotide substitution rate of *rbcL* gene is considered much slower.

The characterization of *rbcL* gene sequences in tongkat langit from Maluku with a comparison sequence (NCBI) has produced phylogenetic tree topology under maximum parsimony criterion which formed two clads [5]. Further characterization analysis conducted covered identifying the haplotype diversity, nucleotide substitution ratio, and variable sites tongkat langit banana can be found.

MATERIALS AND METHODS

The DNA taken from 20 survey collection samples was extracted through CTAB method [1] and amplified by *rbcL* primers sequenced in Macrogen Korea. Alignment between sequences was compared to the alignment of the outgroup sequences [8] code FJ871849.1, FJ871853.1, KJ506056.1, JX572555.1 [7], [9], [12]. The alignment was characterized by software Mega 6.0, further analyzed by software bioedit, dnasp5 and Network.

RESULTS AND DISCUSSION

Data analysis conducted to earn the haplotype diversity parameter, transition substitution ratio, transversion and variable sites was performed using software Mega 6.0 software bioedit, dnasp5 and Network. The results of *rbcL* gene sequences characterization successfully identified 6 haplotypes. The *rbcL* gene sequences among tongkat langit bananas which belong to genus *Musa* (ingroup) and genus *Ensete* (outgroup) kept the haplotype diversity value of 0.5794. Haplotype 1, 2, 3 were the haplotype groups which differentiated genus *Musa* from genus *Ensete*. Tongkat langit banana from Maluku and *Musa fehi* from China which belong to genus *Musa* formed haplotype 1 while haplotype 2 and 3 were identified to be the property of tongkat langit banana TBLO and DBO2. The *rbcL* gene sequences from genus *Ensete* were divided into three haplotypes based on its geographical characterization. They were haplotype 4, 5, and 6. Each of them was originally from South China, India, and South Africa (Figure 1).

According to the haplotype network above, it is clearly seen that only *Musa fehi* from the outgroup of *Australimusa* joined the same group as tongkat langit from Maluku. This is in line with the idea that tongkat langit (*Musa troglodytarum* L) is also called *Musa fehi* or *Musa fei* [10]. Haplotype 1 was categorized as conserved sequence of which its gene composition consistency indicates that the gene will be constantly unchanged even though it is spread throughout different areas with different phenotype properties [4],[5]. In addition, haplotype 2 and 3 also belong to the same group of tongkat langit from Maluku (Tobelo dan Dobo) but with different sequence compared to Haplotype 1. Similarly, Genus *Ensete* with distinct geographical property showed sequence variation on haplotype 4, 5 and 6. The *rbcL* gene sequences characterization presented polymorphism sites, haplotype groups, sites position as follows H2:38, 39, 67 Tobelo; H3: 439,485 Dobo2; H4:317, 242 China; H5:7 India and China; H6:89,323 China and Africa.

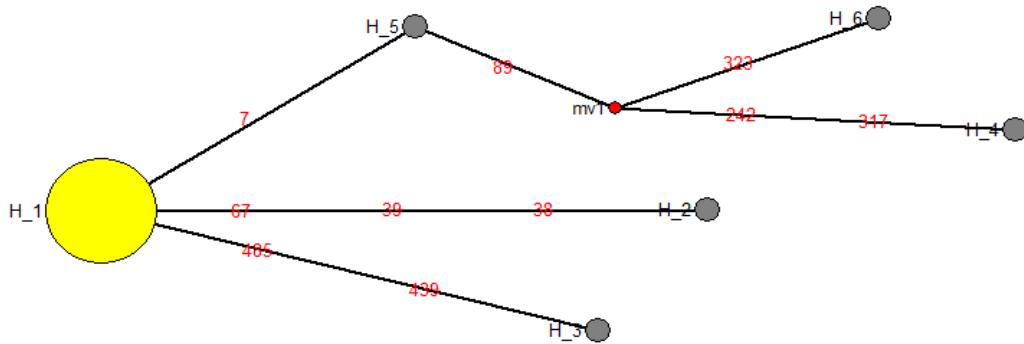


Figure 1: The Haplotype network of *rbcL* gen sequences in tongkat langit and its outgroup (NCBI, 2013)

Note: Hap-1: 19 [AMB1,AMB2, AMB3, DBO1,ELT, HRK1, HRK3,JLO, NSL1, SPR1, SPR3, SRM1, SRM2,TL, WKM, FJ871849 *Musa fehi*, SPR2, NSL2, HRK2]; Hap-2: 1 [TBLO]; Hap-3: 1 [DBO2]; Hap-4: 1 [FJ871853 *Ensete glaucum*]; Hap-5: 1 [KJ506056 *Ensete superbum*; Hap-6: 1 [JX572555 *Ensete ventricosum*]

Based on the results of sequences characterization, it was found that nucleotide diversity of ingroup and outgroup samples was 0.00204 with small nucleotide composition variation. The nucleotide composition of tongkat langit from TBLO varied on C (20.5) and A (27.4) while the composition of tongkat langit from DBO2 varied on T (29.7) and G (22.4). Furthermore, *Musa fehi* from China which belongs to the outgroup had the same nucleotide composition as that of tongkat langit found in some locations in Maluku. The outgroup nucleotide composition particularly of genus *Ensete glaucum*, *Ensete superbum*, each varied on A (26.8,27.0) and G (22.8,22.60) while *Ensete ventricosum* varied on T (U) (29.7), A (26.8), G (22.8). The nucleotide diversity of tongkat langit population from Maluku and their outgroup [8]. as explained in the above has been drawn in the phylogenetic tree under maximum parsimony which clarifies the difference between genus *Musa* and genus *Ensete* on two sub clads [5]. This nucleotide composition indicates genetic diversity of tongkat langit from Maluku and also indirectly impacts the population, community, and ecosystem [6].

From the results of *rbcL* gene sequences characterization in tongkat langit from genus *Ensete* (*outgroup*), ten variable (polymorphic) sites were recognized. Variable sites are areas in which at least two kinds of nucleotides can be found. A variable site consists of singleton and parsimony informative site. A singleton comprises singleton two, three and four variants. The results of *rbcL* gene sequences characterization demonstrated singleton variable site 8, and singleton variable sites (two variants) with sites positions 38, 39, 67, 242, 317, 323, 439, 485.

Table 1: Singleton variable sites and Parsimony informative sites of *rbcL* genes sequences in ingroup and outgroup

singleton variable sites	Parsimony informative sites	sites position	Location
A-C		242	China
T-G		317	China
T-A		38	Tobelo
A-T		39	Tobelo
C-A		67	Tobelo
G-A		439	Dobo2
A-T		485	Dobo2
A-G		323	Africa
A-C-C		7	India and China
C-T-T		89	China

Parsimony informative sites are the sites in which at least two kinds of nucleotides can be found with two-time appearance. The parsimony informative sites consist of parsimony informative two, three and four variants. It means that on a particular position, they appear at the same time. This result showed that there were two parsimony informative sites (two variants) found with site positions 7 and 89. The *rbcL* gene sequences provided information that there were 10 mutations, 8 singleton variable sites (two variants) and one parsimony (two variants). More detailed information is displayed in Table 1

Based on the table, it can be seen that none of the ingroup samples has the parsimony informative sites two variants while the site can only be found among the outgroup. The ingroup samples only have the singleton variable site two variants. There are two different kinds of nucleotide found insingleton variable site two variants. On this position, one taxon can be diffed from another. Mean while, there are two different kinds of nucleotide found in parsimony informative sites which can appear at least twice.

Mutations occurred in the *rbcL*gene sequences identified in tongkat langit group from Maluku and also its outgroup. The characterization of gene sequences showed following details: T-A (1), A-T (2), C-T (3), C-A (1), T-G (1) with transversionin Tobelo and Cina as the mutation category. The transition category in Dobo, China and Africa indicated mutation as follows: C-T (2), G-A (1) and A-G (1). Alkali substitution on *rbcL* gene sequences of the ingroup and outgroup describes non synonymous substitution with 13 mutations occurred. Transversion substitution taken place in the *rbcL* gene sequences of the ingroup except in the DBO2 population presents average transition substitution. On the other hand, in the outgroup, transversion substitution as well as transition occurred concurrently. The total number of transversion substitution was 8 and of transition substitution was 5. The characterization of haplotype diversity, nucleotide composition and diversification, nucleotide substitution ratio, genetic as well as phenotypic kinship has provided basic information for the conservation decision making. There is a need to conserve tongkat langit found in Maluku since its low genetic diversity makes it hard to adapt to new tension [3]. The lost genetic type will reduce the ability of the species to adapt to the environment changes (Frakham, 1999). In line with the idea, Fei banana found in Maluku needs to be preserved.

ACKNOWLEDGEMENT

The authors would like to thank Indonesian Directorate General of higher education for the research funding and the local government of Maluku, Dr. Stepanus M, M. Si for his approval of survey funding and ArikArubil Fatinah, S. Si for their technical support.

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