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Short Communication

Genetic kinship of tongkat langit banana (*Musa troglodytarum* L.) from Moluccas based on *rbcL* gene sequence

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Abstract

The present study was conducted to investigate the genetic kinship of tongkat langit banana population from Moluccas which was out-grouped into *Musa fehi*, *Ensete glaucum*, *Ensete superbum* dan *Ensete ventricosum* (NCBI). The results of phylogenetic analysis showed that *tongkat langit* banana and *Musa fehi* formed sub-clade I with similarity value of 99-100%. *Ensete genus* formed clade II with similarity value of 98-99%.

Key words: Genetic, kinship, tongkat langit banana, *Moluccas*, *rbcL* gene sequence

Tongkat langit banana (*Musa troglodytarum* L.) belongs to Australimusa, the family group of Musaceae, genus *Musa* with a pseudo-stem which is composed by vagina.People in Moluccas make use of tongkat langit bananaas an alternative to side dish, cake ingredient, juice and baby food. The distinguished feature of this banana is that it contains isoprenoid or carotenoid which can be enzymatically converted into vitamin A inside human body. Some phenotypic features of tongkat langit banana have appeared to be unstable and prone to modification. Therefore, the study of evolutionary development is worth doing through genetic characteristics approach to provide more consistent information (Moritz et al. 1996; Wu et al. 2010).

The genetic investigation of banana kinship has been conducted by previous researchers using various DNA sequence as a molecular marker. The *rbcL* (cpDNA) is one of the markers which is regularly utilized across varied plants groups, including bananas, to understand plants diversity and to satisfy inquiries on phylogenetic kinship. The *rbcL* can be universally functioned to provide sequence data for phylogenetic analysis on different kinds of higher level plants. The *rbcL* is a piece of chloroplast DNA molecule of which substitute rate of nucleotide is slower than the nuclear gene (Bausher et al. 2006).

This study was designed to investigate the genetic kinship of tongkat langit banana population which can be found in Moluccas with *rbcL* marker. The results of this research are expected to provide information about the germ plasm diversity of tongkat langit banana, the tendency of population genetic flow, and the conserved genetic resources which can be acted as elders in plant breeding.

Young leaves were taken from tongkat langit banana (*Musa troglodytarum*) as the research material. They were collected from ten different Moluccas islands with four different out-groups (NCBI) and different origin. DNA extract was obtained through CTAB method (Ferdous et al. 2012). The amplification used the *rbcL*. Primer pair employed was *rbcL*-aF and rbcL 724R. PCR product was sequenced by automatic sequencer machine (ABI prism 3730 XL) in Macrogen Korea. Multiple alignment between *rbcL* sequences was done by Mega 6.0 software which was later continued to phylogenetic reconstruction method.

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Maximum Parsimony, Maximum likelihood and Neighbor Joining considered the most stable bootstrap value. Changes in nucleotide bases were examined by bio-edit software. dnasp5 software was used to recognize the invariable sites, variable sites andsites position.

The analysis result using maximum parsimony method indicated that the tree topology had the polytomy quality in sub-clade I with bootstrap value of 72. The species *Ensete ventricosum* belonged to more primitive out-group with bootstrap value of 83 (Fig. 1). The polytomy of this tree showed that *rbcL* gene sequence will be compatible to intra-genus and intra-species kinship study. Fig. 1 displays that bootstrap value on sub clade I and II has reached 72 and 83, which means that the arrangement of both clade has been positioned in its consistency so that there is a little possibility that rearrangement will occur.

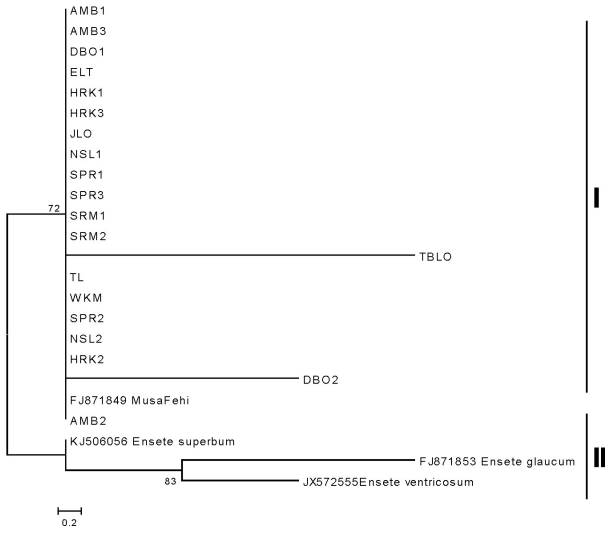

Fig. 1. Phylogenic tree of Maximum parsimony from 20 tongkat langit banana samples found in Moluccas and three samples of Ensete banana (from the out-group) based on rbcL gene sequence

The phylogenic tree resulted from maximum parsimony analysis produced a hypothesis of tongkat langit bananaintra-species kinship in Moluccas according to the rbcL similarity sequence. Tongkat langit banana which comes from different places on sub clade I, except the DBO1 and TBLO, have genetically reached 100% similarity level despite their different phenotypic appearance (Hiariej et al. 2015). Based on the rbcL sequence similarity level on sub clade I, tongkat langit banana which comes from various areas in Moluccas and Musa fehi from China were concluded to belong to M. troglodytarum species. Two samples taken from Tobelo (TBLO) and Dobo (DBO2), in separable way, are almost similar to the species since there is hardly any difference between them (1%) if they should be separated as one individual taxon. The rbcL gene sequence can distinguish three species from genus Ensete (sub clade II) which come from three different areas *viz*., Ensete superbum (India), Ensete glaucum (China) and Ensete ventricosum (South Africa). The three species, however, are 98-99% similar to one another. In other words, only 1% genetic gap is found. The similarity value of *Ensete glaucum* compared to TBLO and DBO; *Ensete ventricosum* and TBLO is 98%. This was resulted from a substitution of transition or trans-version which changed the nucleotide composition.

Group/sub-clade I on which tongkat langit banana from Moluccas existsis also merged with Musa fehi (Ning et al. 2009) from China (NCBI, 2013). According to the map which recorded the spread of tongkat langit banana, Musa fehi probably come from the Pacific. Tongkat langit banana is also named Musa fehi or Musa fei. The widest distribution area of this species is on the Pacific. It also has various local names (Simmond 1960; Ploetz et al. 2007). The consistency of gene composition with widespread geographical area indicates that the gene can not easily change. As a result, the similarity value of tongkat langit banana is very high even though it is found in many different locations. Unlike the result of sample diversity analysis on phenotypic features, low gene variation of tongkat langit banana was reported by well-conserved *rbcL* gene sequence (Hiariej et al. 2015). Changes in phenotypic features can occur without any follow up changes from previous genetic transformation. However, the in equality phenomen on of genotype and phenotype variation in this study could be due to the use of *rbcL* gene sequences as marker. It was only a small fraction of the genetic information sequence possessed by tongkat langit banana. Therefore, it is very advisable for future researcher to make use another sequence marker and take into consideration the effectiveness of genotype changes of tongkat langit banana.

The results of genetic kinship analysis provided important notes in making decision to conserving the germ plasm of tongkat langit banana from Moluccas. In setting conservation strategies to preserving tongkat langit banana, basic information needed is diversity,

kinship, and phylogenetic as well as the potential resources of the germ plasm either from phenotypic or genotypic features. According to the rbcL gene sequence, tongkat langit banana from Moluccas has genetic diversity on TBLO and DBO2. The population which is diverse genetically has the chance to adapt to new pressure, such as climate change, habitat destruction due to human activities or natural disasters. Conservation is important since it can maintain the germ plasm as the gene resources available to breeding effort.

Based on small genetic diversity found in tongkat langit banana and the limitation of its distribution area, one of long-term conservation strategies recommended is to preserve the population in its natural habitat (in situ). Tongkat langit banana is not mostly cultivated by the people so that the existence is more potentially threatened. Another threat to the local banana is that there is a tendency that people like to plant another kind of banana which can be sold in the market. Thus, there should be an attempt made to add economic value to tongkat langit banana, such as diversifying food and crafts that are popular among the society. Besides, it is also necessary to establish an ex situ conservation by relocating the banana to a safer place and preserving the germ plasm of tongkat langit banana in a botanical garden run by the conservation institution and also to put the protected species in an in-vitro culture.

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