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Phylogenetic analysis of *Mangifera* from central region of Sumatra using *trn*L-F intergenic spacer

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Abstract. *Fitmawati, Fauziah R, Hayati I, Sofiyanti N, Inoue E, Matra DD. 2017. Phylogenetic analysis of* Mangifera *from central region of Sumatra using trnL-F intergenic spacer. Biodiversitas 18: 1035-1040. Mangifera* from Central Sumatra has an unique ability to adapt to the high rainfall regions. This genus is therefore as a potential germplasm source in Sumatra. The aim of this study was to analyze and compare the nucleotide sequences of *Mangifera* in the central region of Sumatra based on gene loci of *trn*L-F intergenic spacer. The initial step of research was conducted by isolating DNA from leaves sample of *Mangifera* based on CTAB method. Sequences of gene loci of *trn*L-F were analyzed with Maximum Parsimony and Neighbour Joining methods through PAUP* Program version 4.0b10. From the results of sequences analysis with Maximum Parsimony method, it was obtained a monophyletic group of *Mangifera* consisted of two main clades which separated *M. kemanga* from five other species of *Mangifera*, i.e. *M. foetida*, *M. odorata*, *M. laurina* and *Mangifera* sp. Parsimony analysis also revealed that the common ancestor of *M. kemanga* is the first species appeared in the central region of Sumatra. The result of Neighbour Joining analysis showed *M. laurina* had the longest genetic distance among examined species and might be assumed as the most primitive species of *Mangifera*. *Mangifera laurina* and *M. odorata* were closely related as well as *M. foetida* and *M. odorata*. The results of research also revealed and supported the genus *Mangifera* as a monophyletic group.

Keywords: Central region of Sumatra, cpDNA, *Mangifera*, phylogenetic analysis, *trn*L-F intergenic spacer sequences

INTRODUCTION

The uniqueness of mango species (*Mangifera* spp.) from Sumatra is derived from its ability to grow and adapt to environment of high rainfall. Variations of habitat types and their interactions with genetic factors will result the diversity of mango species that is different with the other regions in Indonesia. Mangoes that grow and spread in Sumatra has superiority on their flowers that are either resistant against high rainfall or adapt to wet climate. The advantages of these properties can be used to assemble an appropriate quality of seeds cultivated in Sumatra, especially in the central region of Sumatra. In the area of existing plantations, mango does not show encouraging results since the flowers fall down when rain came, as a result of the seedling generally comes from areas with low rainfall. In fact, it is completely different from wild mangoes that tend to be not affected or still bear on high rainfall. In other words, wild mango has a resistance to high rainfall than mango crop being grown (Fitmawati et al. 2013). However, along with the high rate of deforestation continued, especially the last two decades, the forest fires in natural forest and forest transition areas also keeps continuing to increase every year and will be impacted by the genetic erosion of mango species in natural stands (BPS 2010).

In the central region of Sumatra, since 2011 to 2013 it had been conducted a discovering of the wild and cultivated mango species, and estimated that there were 10 species of mango collected. From these ten species, six species of mango were identified as cultivated and semi-cultivated, namely *Mangifera indica* Lour., *M. laurina* Bl., *M. foetida* Lour., *M. odorata* Griff., *M. sumatrana* Miq. and *M. zeylanica* (Bl.) Hooker f. Meanwhile, four other species which were consisted of *M. kemanga* Bl., *M. torquenda* Kosterm., *M. quadrifida* Jack. and *Mangifera* sp. were expected as new species or new records that are rarely found in Sumatra, but they had still an unclear taxonomic status. The diversity of mango (*Mangifera*) in Indonesia is an important and interesting aspect to be studied as the mango's improvement program relies heavily on the available genetic diversity (Fitmawati et al. 2013).

The morphological characters have long been used in many studies of phylogenetic but complicated the continuity of morphological characters in defining the evolutionary relationships. The utilization of the molecular approaches based on DNA sequences is more informative to support and strengthen the morphological character data. The development of molecular biology techniques in this century is to facilitate the assessment of genetically relationship taxa (h).

Identification is conducted based on the morphological characters to make the species barriers encountered some obstacles (Kostermans and Bompard 1993). The vegetative characters of mango in nature are continually available, while the generative characters are hard to be found at the time of exploration since it does not coincide with the season of flowering and fruiting in different regions. By providing the potential of wild mango as a source of crossing over aspects in repairing the characters of cultivated mango crops which are more adapted to high rainfall in the future, and the importance of securing the rarely (endangered) mango status from an extinction, it is, therefore, necessary to discover information and comprehend the investigation of the species of mangoes in Sumatra based on the molecular techniques that are today's rapidly developed, namely DNA Barcoding system, to obtain specific characteristics of each species.

The large morphological plasticity of *M. laurina* and its related species make them hard to be defined, which are resulted in frequent status changes due to their polyembryonic characters, open pollination system, and the number of allotetraploid chromosomes (2n=40) (Litz 2004) with a high compatibility between cultivars and in its genera. Markers of chloroplast DNA (cpDNA), that are widely used is *trn*L-F intergenic spacer, are a part of the cpDNA non-coding region, the region which more varied than the coding region, so this makes it more suitable to be used in uncovering the evolutionary relationships among the lower level of taxa (Bayer et al. 2000). Some studies in non-coding regions of chloroplast showed a higher variation and it was frequently mutated (Baldwin et al. 1995) in the forms of transversion, transition, insertion, and deletion. The use of molecular marker of cpDNA is to reveal diversity, to discover the genetic relationship with the basis of evolution, also, to clarify the status of *Mangifera* in the central region of Sumatra which has never been conducted before.

Some phylogeny studies of plants based on cpDNA markers were reported, such as in *Morus* (Weiguo et al. 2005) and *Cucumis* (Chung et al. 2006; Chung et al. 2007). The cpDNA markers provided data for reconstructing phylogeny among families of flowering plants (Kajita 1998). The sequences of *trn*L-F region of cpDNA are also frequently used in the phylogenetic studies at generic and specific levels (Alejanro et al. 2005; Barfuss et al. 2005; Shaw et al. 2005). Therefore, this platform is commonly used in the phylogenetic studies because they are easily isolated, purified, characterized and cloned. The *trn*L-F region of cpDNA is naturally conservative with a low rate evolution (Bayer et al. 2000). This region is more varied than the sequences of the coding region (Bayer et al. 2000). This analysis is expected to show higher nucleotide variation in closely related species or the extent intraspecies, which will discover the distinguishing characteristics among species in the genus of *Mangifera*. Overall, this study was aimed to analyze phylogenetic relationship from 10 species of *Mangifera* using cpDNA marker of *trn*L-F region.

MATERIALS AND METHOD

Plant material

Leaf samples of six mangoes species of *Mangifera indica*, *M. laurina*, *M. foetida*, *M. odorata*, *M. sumatrana* and *M. zeylanica*, as well as one out group: *Bouea macrophylla*, were collected from the central region of Sumatra. The list of plant material used could be seen in Table 1.

DNA extraction

Whole genome DNA were isolated from the leaf of each plant after being soaked in aquadest by using the CTAB method of Doyle and Doyle (1987), with a slight modification by soaking a leaf in demineralization water for 24 hours before isolation.

A total of 0.15 g of leaf samples was crushed using a sterile mortar with the addition of 0.6-0.8 ml of buffer extraction [10% CTAB, 0.5 M EDTA (pH 8.0), 1 M Tris-HCl (pH 8.0), 5 M NaCl, 1% β-mercaptoethanol]. The solution was homogenized and then incubated at a temperature of 65°C for 1 hour and then added by 0.7 ml of chloroform, followed by centrifugation at 11,000 rpm for 10 minutes. The supernatant was transferred into sterile Eppendorf tube for 2 ml and added to 500 mL of cold alcohol and then incubated overnight in the freezer. The solution was centrifuged for 10 minutes at 11,000 rpm. The liquid phase was discarded and the solid phase (pellet) was air-dried and stored in 100 µL of TE solution (1 M Tris-HCl pH 8.0, 0.5 M EDTA pH 8.0, distilled water).

Table 1. Plant species of *Mangifera* and their origin

Species	Origin	Coordinate
Mangifera foetida Lour	West Sumatra	N $00^0$17'59.5" E $100^0$27'00.0"
Mangifera indica Lour	Riau	N $00^0$27'04.9" E $101^0$25'14.7"
Mangifera laurina Bl	Jambi	N $01^0$35'16.8" E $103^0$25'24.8"
Mangifera odorata Griff	Riau	N $00^0$20'27.0" E $101^0$08'10.6"
Mangifera zeylanica (Bl.) Hooker f.	Riau	N 00˚30'28,5" E 101˚24'56,0"
Mangifera sumatrana Miq.	Riau	N $00^0$27'28.0" E $101^0$22'51.4"
Mangifera sp.	Riau	N $00^0$20'27.0" E $101^0$08'10.6"

Amplification and sequencing

The genomic DNA was amplified by using universal primer E and primer F (Small et al. 2005) for the entire *trn*L-F intergenic spacer region. The reaction mixture (50 µL) was contained DreamTaq Buffer 10x, 2 mM of each dNTP Mix, 25 pmol of each primer, 20-50 ng genomic DNA, 1 unit of DreamTaq DNA Polymerase and nuclease-free-water. Thirty-five cycles of PCR were conducted by using Thermal Cycle under following profiles: pre-denaturation at temperature of 95°C for 4 m, followed by 35 cycles with reaction condition of denaturation at temperature of 95°C for 30 s, annealing at temperature of 48.6°C for 30 s, and extension at 72°C for 1 m 30 s, then the PCR process was ended with the process of post-extension at temperature of 72°C for 10 m.

The PCR products were visualized in agarose gel 1% using electrophoresis machine with TAE buffer (Tris Base, acetate glacial acid, EDTA) at a voltage of 100 volts for 45 minutes. The amplified products were then purified by PCR Clean-Up or Gel Extraction depend on Visualization results for Single Pass DNA Sequencing. The PCR products were sequenced at Laboratorium Biotechnology of Tokyo University of Agriculture and Technology.

The purification of PCR products was performed by PCR Clean-Up and gel extraction. Sequencing was conducted by DNA Sequencing Single Pass. A forward primer which is sequencing the reaction was conducted with BigDye® Terminator v3.1 of cycle sequencing kit following the standard protocol (Applied Biosystems 2010). Analysis of DNA sequences was conducted by an automatic sequencer after extra-fluorescence ddNTPs was eliminated with Centri-Sep Spin Column (Applied Biosystems 2010).

Phylogenetic analysis

DNA sequences of *Mangifera* were aligned with ClustalW Multiple Allignment in Bioedit software (Thompson et al. 1997). Analysis of phylogenetic tree reconstruction based on the method of MP and NJ was carried out with the program of PAUP* version 4.0 (Swofford 2002). NJ analysis was performed by using a model of evolution HKY85 (Hasegawa et al. 1985) with 1000 bootstrap replications to test the strength of each branch of tree cladogram obtained with a frequency of >50%.

RESULTS AND DISCUSSION

***trn*L-F intergenic spacer sequences analysis**

The aligned length of *trn*L-F DNA spacer of six *Mangifera* species and outgroup species were 313 nucleotides. The sequences consisted of a total of 91 bp constant characters; 163 variable characters were uninformative; and 59 bp were informative characters. From 313 nucleotides, there were 82 conserved sites, 222 variable sites, and 161 singleton sites. The alignment results showed that a gap in the sequence was caused by insertion and deletion (Table 1). There were 11 indels sites in *M. foetida*, *M. indica* and the outgroup of *B. macrophylla*, 12 indels in *M. kemanga*, 13 indels in *M. laurina*, 30 indels in *Mangifera* sp. and 41 indels in *M. odorata.* This phenomenon affected the regulation of gene expression. Although a number of changes in these sequences was very small while compared to the changes in the core genome (SABioscience 2008), it was able to provide important information in describing the process of evolution since cpDNA was inherited maternally or uniparentally where the changes in nucleotide (s) took place for a very long time (Hancock 2003), in contrast to the changes on nucleotides base occurred in the core of DNA were inherited biparentally.

The average frequency of nucleotides on *trn*L-F sequences was 31.4% (T), 18.0% (C), 30.6% (A) and 20.1% (G). These sequences were rich in AT which was equal to 64.22%, while in GC was 35.78% (Table 2). These results are according to Li (1997), which stated that the most composition of nucleotides in a non-coding area of chloroplast DNA is adenine and thymine. The variation occurs between different species within the same or different genera. Sequences variation found in cpDNA sequences was generally caused by a mutation in a single nucleotide which represents mutation that has happened for a very long period of time (Fitmawati and Hartana 2010).

*Trn*L-F intergenic spacer region as non-coding sequences (intron) has more variation and higher mutation rate than the coding region. It is also clear that non-coding region (intron) plays a role in the regulation of gene expression that can be affected by the environment or habitat niches, which expressed in phenotype characters.

Phylogenetic analysis of *Mangifera*

From the result of Parsimony analysis based on the sequences data of *trn*-LF intergenic spacer region, it was obtained a cladogram (Figure 1) with CI valued 0.9928 and RI valued 0.97, while homoplasy index (HI) was 0.0072. This value showed that homoplasy index was occurred at only 0.72%. Based on the Parsimony analysis, *M. kemanga* became the ancestor to the species of *Mangifera* examined. This finding was supported by nucleotide base changes in specific sites which are separating *M. kemanga* with the other five *Mangifera* species (Table 1). In this study, *Mangifera kemanga* has a very high nucleotide variation, this is due to mutation events.

Table 3. Variation in AT and GC contents on 313 bp *trn*L-F alligned sequences on *Mangifera*

Species	T (U)	C	A	G	GC (%)	AT (%)
B. macrophylla	30.1	19.5	31.8	18.5	36.74	63.26
M. foetida	30.5	19.2	31.8	18.5	36.42	63.58
M. indica	30.5	19.2	31.5	18.9	36.74	63.26
M. kemanga	29.9	20.3	31.6	18.3	37.06	62.94
M. laurina	26.3	16.7	27.7	29.3	44.09	55.91
Mangifera sp.	33.9	18.4	31.1	16.6	31.63	68.37
M. odorata	39.5	11.8	28.4	20.3	27.79	72.21
Average	31.4	18.0	30.6	20.1	35.78	64.22

Table 2. Alignment of *trn*L-F DNA spacer sequences of six *Mangifera* spp. and outgroup

```
B.mac       AGCAGAATTT--TTTCTCTTATCATACACAAGTCGTGTGGTATATAGGA-TACACGTAGAAATGAACA
M.foe       AGCAGAATTT--TTTCTCTTATCATATACAAGTCGTGTGGTATATAGGA-TACACGTAGAAATGAACA
M.ind       AGCAGAATTT--TTTCTCTTATCATATACAAGTCGTGTGGTATATAGGA-TACACGTAGAAATGAACA
M.kem       AGCAGAATTT--TTTCTCTTATCATACACAAGTCGTGTGGTATATAGGA-TACACGTAGAAATGAACA
M.lau       AGCATTCCTTGAATATTTAAAAAGAGTAGTTGGTAAGTTCCGAAATGAGCTATGAGATGGATTGTGAA
M.sp        AGCAGAATTT--TTTCTCTTATCATATACAAGTCGTGTGGTATATAGGA-TACACGTAGAAATGAACA
M.odo       AGCAATATTAA-TTTCTTGCACAGGGTCGGTATTGCTCCGT-TATTTAG-TAGTTTTTTATTTACATA

B.mac       CTTTGGAGCAAGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAACTTACAA
M.foe       CTTTGGAGCAAGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAACTTACAA
M.ind       CTTTGGAGCAGGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAACTTACAA
M.kem       CTTTGGAGCAAGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAACTTACAA
M.lau       CTATCACACTCTGCATTCCCTGTCCTCAAAGGTTCATTTCTCCGCGTATCCTCTTTTCCAC-------
M.sp        CTTTGGAGCAAGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAACTTACAA
M.odo       CGTTTCGTTTGGTTGTTTGTTTTTCAACAAAACAAAATT--TTCTGCTTCTTGTTNTGTA--------

B.mac       AGTCTTCTTTTTGAATATTCA-AGAAATGCAATTTCCCGTCCAAGACTTTTAATACTGAATTGCGTC-
M.foe       AGTCTTCTTTTTGAATATTCA-AGAAATGCAATTTCCCGTCCAAGACTTTTAATACTGAATTGCGTC-
M.ind       AGTCTTCTTTTTGAATATTCA-AGAAATGCAATTTCCCGTCCAAGACTTTTAATACTGAATTGCGTC-
M.kem       AGTCTTCTTTTTTAATATTCA-CGAAATGCAATTTCCCGTCCAAGACTTTTAATACTGAATTGCGTC-
M.lau       --ACCCCATTGGATATGGTAAGAGCAAAAAAATTTTTCGGAAACTACTGTGGATGGGGAAAAATAGGA
M.sp        AGTCTTCTTTTTGAATATTCA-AGAAATGCAATTTCCCGTCCAAGACTTTTAATACTGAATTGCGTC-
M.odo       --TCTTCATTTCGAG-ATT----GATAAGTAAGGTACCATAAAAAAAGGAGAATGATCAAAAATATG-

B.mac       -TTTTTT--AATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATGCGTCGGTAATGGTCGG-
M.foe       -TTTTTT--AATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATGCGTCGGTAATGGTCGG-
M.ind       -TTTTTT--AATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATGCGTCGGTAATGGTCGG-
M.kem       -TTTTTT--AATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGA-GCGTCGGCAATGGTCGG-
M.lau       GGCAGACGCAAACACTACCGAAAAAGGAATTTTTTTTTTTTGGAAAGGG----CGGGGGGGGGTTAAA
M.sp        -TTTTTT—AATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATGCGTCGGTTATTTTTTTT
M.odo       -GTAGA---AATTGTAATCCTTGTATTTTTTGTAATTTTTTAAGAGGGG-----CGGATGTAGCCAA-

B.mac       GATAGCTCAGCTGGT--AGAGCAGAGGACTGAAAATCCTCG
M.foe       GATAGCTCAGCTGGT--AGAGCAGAGGACTGAAAATCCTCG
M.ind       GATAGCTCAGCTGGT--AGAGCAGAGGACTGAAAATCCTCG
M.kem       GATAGCTCAGCTGGT--AGAGCAGAGGACTGAAAATCCTCG
M.lau       GGGGGGCCCTTTGGGGGGGGGGAATGGGGTGGAGGGGGTCC
M.sp        GATAGCTCAGCTGGT--AGAG--------------------
M.odo       -GTGGATC---------AAGGCAGTGGATTGTGAATCCTTT
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Consistency index (CI) was used to measure the relative amount of homoplasy on the phylogenetic tree. Homoplasy happens when the characters are similar but not derived from a common ancestor. CI = 1 means the consistent character with the high parsimonious rate. Meanwhile, retention index (RI) was used to measure the proportion of synapomorphy on phylogenetic tree that is intended to reflect the proportion of similarity in the phylogenetic tree. RI value = 1 means the code is consistent with the complete DNA phylogeny.

Evolution tree from six *Mangifera* species formed two clades. Clade I consisted of *M. kemanga*, while Clade II consisted of two subclades as following: subclades IIA consisted of *M. foetida* and *Mangifera* sp.; while subclade IIB consisted of *M. indica*, *M. odorata* and *M. laurina*. Bootstrap value for clade II showed more than 50%. *Mangifera kemanga* was connected to the branch with 3 changes in base nucleotides: the change of A-C in the character number of 159, G-T in the character number of 149, and T-C in the character number of 262. The branch connected to clade II was different from *M. kemanga* with one character where the change C-T was in character number of 27.

The close relationship between *M. odorata* and *M. laurina* was supported by 100% bootstrap value (Figure 1). This finding was similiar to Hou (1978) which stated that *M. laurina* was the synonym of *M. indica*. However, Fitmawati and Hartana (2010), also used *trn*L-F intergenic spacer for *Mangifera* from Sulawesi, did not agree with that statement and stated that *M. laurina* was different species from *M. Indica,* also, *M. laurina* was the ancestor for *M. indica* which is the cultivated form of *M. laurina.* This result also contrasts with the previous study using ITS sequence (Fitmawati et al. 2016) which stated that *M. odorata* has a close relationship with *M. foetida.*

According to Kostermans and Bompard (1993), *M. odorata* was placed in a section of *Limus*, while *M. laurina* was of *Mangifera*. There was a contradiction between the morphology and molecular characters in this study. The delimitation of species on the separation of the section is

based on a floral intrastaminal disk (Kostermans and Bompard 1993), but in this study, it used molecular marker from cpDNA which is more reliable than morphological characters. It is because the morphological characters are inherited biparentally, while cpDNA inherited maternally. Therefore, for looking up the evolutionary traces, molecular markers are consistent to be used.

The evolutionary model does not explicitly describe the mechanism of mutation and natural selection, but this model describes the relative rate of changes in the different nucleotides. For example, biased mutation that supports the conservative changes may be associated with the transition, substitution, and mutation rate which are relatively higher than transversion during DNA sequences which have been evolved, but HKY85 Model is just trying to capture the effects of four parameters which are consisted of transition, substitution, mutation rate, and transversion that reflect the relative speed of transition and transversion. The evolutionary model was used in the calculation of the p-distance index to the nucleotide sequence of *trn*L-F intergenic. Evolution tree from HKY85 model evolution that allows the base frequency is relatively varied and differentiated between the pace of transition and transversion substitution by using four free parameters (Hasegawa et al. 1985).

Neighbor Joining (NJ) analysis was different from Parsimony analysis. From the results of NJ analysis, it was obtained a phylogram (Figure 2) with a total of branch length of 1.77604 with a minimum evolution score of 1.80615. There were no significant differences in NJ tree for the separation of clade, except for the clade which is consisted of *M. odorata* and *M. laurina*. The species of *M. laurina* had the longest horizontal branch (0.74762) and it also showed the longest genetic distance with other species of *Mangifera* (Table 3). This supported the statement of Fitmawati and Hartana (2010) that *M. laurina* was the common ancestor of *Mangifera* in Indonesia. *Mangifera kemanga*, *M. foetida* and *Mangifera* sp. did not form a clade because there were not many strong characters found in order to separate them from the basis of the clade. *Mangifera indica*, however, formed a clade with *M. odorata* and *M. laurina*.

The variation of nucleotide bases obtained by the gene sequences of *trn*L-F intergenic spacer of cpDNA was quite high. This means that the gene sequences of cpDNA *trn*L-F is very proper to be used in distinguishing the phylogenetic relationship at the level of species and infraspecies. The pattern that emerges from cpDNA markers was not always associated with the pattern created by morphological markers and vice versa. Chloroplast was inherited uniparentally, or passed down from the female parent. While the morphology characters were inherited from two parents through the recombination process and influenced by the environment. Therefore, this was a reason there were differences in morphology and cpDNA grouping.

Differences in nucleotide sequence variations indicate the evolutionary processes caused by mutations (changes in the nucleotide sequence). Mutations cause differences in phenotypic characters encoded by genes as a form of adaptation to different environments.

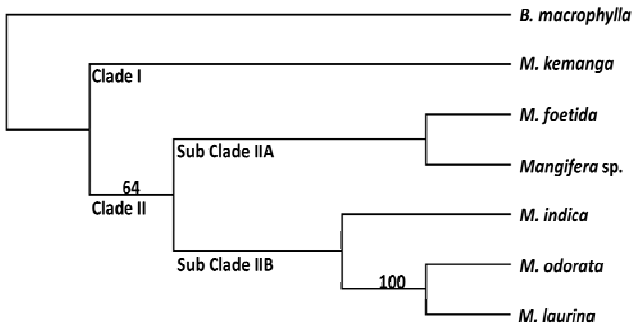

Figure 1. Cladogram of six species of mangoes based on *trn*L-F markers with Maximum Parsimony method with a bootstrap value above the branch. Branches with no number have bootstrap values under 50

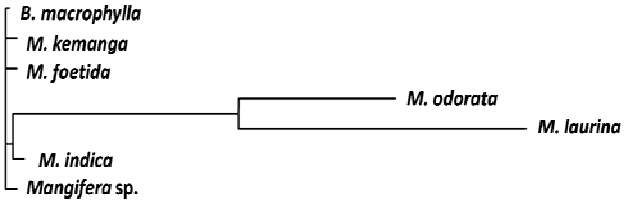

Figure 2. Cladogram of six species of mangoes based on *trn*L-F marker with the Neighbor Joining method with bootstrap values 1000x

The line on the phylogenetic tree by the Neighbor-Joining method showed the much nearby evolutionary process that occurs between ingroup species studied. The longer the line of branching, the greater the distance its evolution and the shorter the line as well as the closer the distance evolution.

Diversity indicated by cpDNA markers diversity was relatively different from indicated by morphological markers (Fitmawati et al. 2013). The pattern that emerges from cpDNA markers was not always associated with the resulting pattern of morphological markers and vice versa. This was possible because the expression at the level of morphology was a result of the recombination of two parents and the environmental factors. Additionally, the gene sequences located on the DNA chloroplast experienced a lower rate of evolution than on DNA core (Taberlet et al. 1991). A non-coding area had a high mutation rate, so it had more variations and more informative than the coding area (Taberlet et al. 1991; Hamilton 1999).

The differences in genetic distance describe the rate of evolution of each type of *Mangifera*, so the greater the genetic distance, the higher the genetic adaptation strategies developed by a species, in order to survive in its environment. The genetic distance also showed the greater the variation within a species that lead to species with a variety of properties that can survive be going to leave nature to the next generation. The rate of evolution can take place faster/slower depending on the mechanism of adaptation and the environmental circumstance habitat.

Table 4. Genetic distance matrix by using HKY85 evolution model

		1	2	3	4	5	6	7
1	*B. macrophylla*	-						
2	*M. kemanga*	0.01004	-					
3	*M. foetida*	0.00332	0.01344	-				
4	*M. odorata*	1.00191	1.01861	0.98474	-			
5	*Mangifera* sp.	0.02597	0.03738	0.02222	1.09495	-		
6	*M. indica*	0.00667	0.01684	0.00332	0.96450	0.02593	-	
7	*M. laurina*	1.36745	1.37963	1.34405	1.14397	1.38653	1.34344	-

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