HAYATI Journal of Biosciences March 2010
Vol. 17 No. 1, p 9-14
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.17.1.9

Phylogenetic Study of *Mangifera laurina* and its Related Species Using cpDNA *trnL-F* Spacer Markers

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Received January 30, 2009/Accepted February 25, 2010

Phylogenetic study of cpDNA intergenic spacer *trn*L-F of *Mangifera laurina* and their related species within the genus *Mangifera* in Indonesia was conducted using Rutaceae as the outgroup. This study was to reconstruct phylogenetic relationships and to understand infraspecific relationships within *Mangifera* based on cpDNA *trn*L-F intergenic spacer sequences. The results showed that *Mangifera* sp. Hiku (*mangga hiku*) as the basic cultivar in the clade, and it supported the monophyletic group in *Mangifera*. And phylogenetic construction indicated that *Mangifera* sp. Hiku was the progenitor of *M. laurina* and their related species.

Key words: *Mangifera laurina* var. Hiku, phylogentic, cpDNA, *trnL*-F intergenic spacer, progenitor

INTRODUCTION

Mangoes (*Mangifera*) have a high number of species. They are distributed in tropical and subtropical areas. One of diversity centers of mangoes in the world is Indonesia. The latest revision of mangoes was described 68 species of mangoes (Kostermans & Bompard 1993). However, the delimination of *M. laurina* and its related species i.e. *M. aplanata, M. indica,* and *M. rubropetala* was still unclear (Kochumen 1996). Mangoes have allotetraploid chromosomes (2n=4x=40) and polyembrionic seed (Litz 2004). And interspecific hybridization was commonly occurred among *M. laurina* and its related species. Therefore, their morphological plasticity was high and their species deliminations were various. Fruit morphology of *Mangifera* sp. Hiku (*mangga hiku*) is similar to *M. indica* but the characters were very sour, roughly fibrous, and the carpel is whitish yellow. The morphology characters of *mangga hiku* were more primitive than that of other Indonesian mangoes.

Mango classification based on molecular markers was scarcely studied and there was limited information available on molecular phylogeny of mangoes. However, the molecular phylogeny is important for classifying their taxonomic status, maintaining and conserving their genetic diversity.

Some phylogeny studies of plants based on cp DNA 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 the phylogeny among families of flowering plants (Kajita 1998). The sequences of *trn*L-F region of cpDNA are frequently used on the phylogenetic studies at generic and specific levels (Alejanro *et al*. 2005; Barfuss *et al*. 2005; Shaw *et al*. 2005). The cpDNA markers are commonly used in the phylogetic studies because they are easily isolated, purified, characterized and cloned. The *trn*L-F region of cpDNA is conservative with low rate evolution (Bayer *et al*. 2000).

The *trn*L-F intergenic spacer of cpDNA is non coding characters, and this region is more variable than the coding regions. Some studies on non coding region of cpDNA showed higher variations and more often mutation than that of coding regions (Baldwin 1995). Accordingly, the *trn*L-F intergenic spacer is a suitable parameter to investigate evolution relationship on the lower taxa (Bayer *et al*. 2000). Therefore this study was analyzed and reconstructed molecular phylogeny of *M. laurina* and its related species based on cpDNA *trn*L-F transgenic spacer sequences.

MATERIALS AND METHODS

Leaf samples of six mangoes were collected from six areas in Indonesia namely *M. laurina* Betul (*mangga betul*) and *M. aplanata* (*mangga depeh*) from West Kalimantan, *M. indica* (*mangga golek*), and *M. indica* (*mangga kiyal*) from East Java, *M. laurina* (*mangga dodol ternate)* from Maluku, and *Mangifera* sp. Hiku (*mangga hiku*) from Sulawesi. And three species of Rutaceae were chosed as the outgroup.

DNA Isolation. DNA was extracted using CTAB method (Doyle & Doyle 1987) with modifications by soaking in water bath at 65 °C over night. *trn*L-F intergenic spacer was amplified using E primer (GGTTCAAGTCCCTC TATCCC) and F primer (ATTTGAACTGGTGACACGAG) (Small *et al*. 2005). Amplification of *trn*L-F intergenic DNA spacer region used PCR machine (GeneAmp PCR system 2400 Perkin Elmer) with 35 cycles. The pre PCR was set up

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at 95 °C for 4 minutes. Denaturation was set up at 94 °C for 30 seconds, annealing was at 52 °C 30 seconds, and extension was at 72 °C for 1 minute. And it was followed by post PCR at 72 °C for 7 minutes.

DNA Sequencing and Phylogenetic Analysis. PCR products were sequenced using ABI 377 automated DNA sequencer (Applied Biosystems) at First BASE Laboratories, Malaysia. The sequences were edited using BioEdit 7.0.0.1 program, and then compared using BLASTN with DNA sequences of *Guarea glabra, Citrus sinensis, Citrus medica* from GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Analysis of maximum parsimony and likelihood was done using PAUP version 4.0b8 with 1000 times bootstrap(Swofford 2000).

RESULTS

The aligned length of *trn*L-F DNA spacer of six *Mangifera* species and three outgroup species was 433 nucleotides. The sequence was consist of a total of 357 bp constant characters, 7 bp non informative parsimony character, and 69 bp parsimony informative character. The average ratio of mango *trn*L-F DNA spacer nucleotides of Adenine (A), Thymine (T), Cytosine (C) and Guanine (G) was 0.3014, 0.3316, 0.206, and 0.1608 respectively; and the G+C content was 0.3668 indicating that the region was AT rich (Tabel 1).

The sequence region of cpDNA *trn*L-F *M. laurina* and its related species was descended from the sequence of Anacardiaceae, it had 91 and 94% similarity with *Rhus caryophila* and *Pistacio weinmaniifolia* respectively. Phylogenetic tree construction based on *trn*L-F region had consistency Index Value (CI) of 0.9625 and resistance index (RI) of 0.99 (Figure 1), while homoplasy index (HI) was 0.0375. This value showed that homoplasy was occurred only 3.75%. Grouping confirmation of *M. laurina,* and their related species of 11 members of Rutaceae based on the size of *trn*L-F region of mangoes showed similarity

Tabel 1. Alignment of *trn*L-F DNA spacer sequences of six mangoes and their Outgroup

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CLUSTAL W(1.60) multiple sequence alignment

Mbetul          -TTTAACTCCCTAACGATTTATCCTA-------------------TGTTAGCGGTTCCAA
Depeh           -TTTAACTCCCTAACGATTTATCCTA-------------------TGTTAGCGGTTCCAA
Golek           -TTTAACTCCCTAACGATTTATCCTA-------------------TGTTAGCGGTTCCAA
Hiku            --TTTACTCCCTAACGATTTATCCTA-------------------TGTTAGCGGTTCC-A
Dternate        -TTTAACTCCCTAACGATTTATCCTA-------------------TGTTAGCGGTTCCAA
Kiyal           -TTTAACTCCCTAACGATTTATCCTA-------------------TGTTAGCGGTTCCAA
C_macrop        ATTTGACTCTCTAACCATTTCTCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
G_macrop        ATTTGACTCTCTAACCATTTCTCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
C_diverg        ATTTGACTCTCTAACCATTTCTCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
C_toment        ATTTGACTCTCTAACCATTTCTCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
C_aurant        ----------------------------------------TTTTTTTGTTAGTGGTTCAAA
C_limon         -----ACTCCCTAACCATTTCTCCTACCTTCTC-----CTTTTTTTGTTAGTGGTTCAAA
G_guidon        ATTTGACTCTCTAACCATTTCTCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
G_glabra        ATTTGACTCTCTAACCATTTATCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
C_sinens        ---------------------------------------TTTTTTTGTTAGTGGTTCAAA
C_macrop        ATTTGACTCTCTAACCATTTCTCCTACCCTCTCTTTTTCTCTTTTTGTTAATGGTTCAAA
M_panicu        -----ACTCCCTAACCATTTCTCCTAACCT-TC---TCCTTTTTTTGTTAGTGGTTCAAA
C_medica        ----------------------------------------TTTTTTTGTTAGTGGTTCAAA


Mbetul          TTTCGTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAA-
Depeh           TTTCGTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAA-
Golek           TTTCGTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAA-
Hiku            TTTCGTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAA-
Dternate        TTTCGTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAA-
Kiyal           TTTCGTTATGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCCGAGCAGAA-
C_macrop        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
G_macrop        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
C_diverg        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
C_toment        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
C_aurant        AGTCGGTAGGTTTCTCATCTATCCTACTCTTTTCCATTTCCAAAAGGATATGGGCAGAAT
C_limon         AGTCGGTAGGTTTCTCATCTATCCTACTCTTTTCCATTTCCAAAAGGATATGGGCAGAAT
G_guidon        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
G_glabra        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
C_sinens        AGTCGGTAGGTTTCTCATCTATCCTACTCTTTTCCATTTCCAAAAGGATATGGGCAGAAT
C_macrop        ATTCGTTAGGTTTCTCATTCATCCTACTCTTTTCCATTTACAAACGTATCTGAGCAGAAT
M_panicu        AGTCGGTAGATTTCTCATCTATCCTACTCTTTTCCATTTCCAAAGGGATATGGGCAGAAT
C_medica        AGTCGGTAGGTTTCTCATCTATCCTACTCTTTTCCATTTCCAAAAGGATATGGGCAGAAT


Mbetul          TTTTTTCTCTTATCATATACAAGTCGTGTGGTATATAGGATACACGTAGAAATGAACACT
Depeh           TTTTTTCTCTTATCATATACAAGTCGTGTGGTATATAGGATACACGTAGAAATGAACACT
Golek           TTTTTTCTCTTATCATATACAAGTCGTGTGGTATATAGGATACACGTAGAAATGAACACT
Hiku            TTTTTTCTCTTATCATATACAAGTCGTGTGGTATATAGGATACACGTAGAAATGAACACT
Dternate        TTTTTTCTCTTATCATATACAAGTCGTGTGGTATATAGGATACACGTAGAAATGAACACT
Kiyal           TTTTTTCTCTTATCATATACAAGTCGTGTGGTATATAGGATACACGTAGAAATGAACACT
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Tabel 1. Continue

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C_macrop        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
G_macrop        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
C_diverg        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
C_toment        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
C_aurant        TTTTTTCTCTTATC----ACAAGCCGTATGGTCTATACGATATATGTAGAAATGAACACC
C_limon         TTTTTTCTCTTATC----ACAAGCCGTATGGTCTATACGATATATGTAGAAATGAACACC
G_guidon        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
G_glabra        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
C_sinens        TTTTTTCTCTTATC----ACAAGCCGTATGGTCTATACGATATATGTAGAAATGAACACC
C_macrop        TTTTTTCTCTTATC----ACCAGTCGTGTGTTATATATGATAGACGTACAAATGAACACC
M_panicu        TTTTTTCTCTTATC----ACAAGCCGTATGGTCTATACGATATATGTAGAAATGAACACC
C_medica        TTTTTTCTCTTATC----ACAAGCCGTATGGTCTATACGATATATGTAGAAATGAACACC

Mbetul          TTGGAGCAGGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAAC
Depeh           TTGGAGCAGGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAAC
Golek           TTGGAGCAGGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAAC
Hiku            TTGGAGCAAGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAAC
Dternate        TTGGAGCAGGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAAC
Kiyal           TTGGAGCAGGGAATCTCCATGTGAATGATTCACAATCCATCTCATTGCTCATACTGAAAC
C_macrop        CTTGAGCAAGGAATCCCTATTTGAATGATTCACAATTCATATCATTGCTCATACTGAAAC
G_macrop        CTTGAGCAAGGAATCCCCATTTGAATGATTCACAATCCATATCATTGCTCATACTGAAAC
C_diverg        CTTGAGCAAGGAATCCCTATTTGAATGATTCACAATTCATATCATTGCTCATACTGAAAC
C_toment        CTTGAGCAAGGAATCCCTATTTGAATGATTCACAATTCATATCATTGCTCATACTGAAAC
C_aurant        TTTGAGCAAGGAATCCCCGTTTTAATGATTCCCAATCCATATTATTGCTCATACTGAAAC
C_limon         TTTGAGCAAGGAATCCCCGTTTTAATGATTCCCAATCCATATTATTGCTCATACTGAAAC
G_guidon        CTTGAGCAAGGAATCCCCATTTGAATGATTCACAATCCATATCATTGCTCATACTGAAAC
G_glabra        CTTGAGCAAGGAATCCCCATTTGAATGATTCACAATCCATATCATTGCTCATACTGAAAC
C_sinens        TTTGAGCAAGGAATCCCCGTTTTAATGATTCCCAATCCATATTATTGCTCATACTGAAAC
C_macrop        CTTGAGCAAGGAATCCCTATTTGAATGATTCACAATTCATATCATTGCTCATACTGAAAC
M_panicu        TTTGAGCAAGGAATCCCCGTTTTAATGATTCCCAATCCATATTATTGCTCATACTGAAAC
C_medica        TTTGAGCAAGGAATCCCCGTTTTAATGATTCCCAATCCATATTATTGCTCATACTGAAAC

Mbetul          TTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGTCCAAGACTTTTAATA
Depeh           TTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGTCCAAGACTTTTAATA
Golek           TTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGTCCAAGACTTTTAATA
Hiku            TTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGTCCAAGACTTTTAATA
Dternate        TTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGTCCAAGACTTTTAATA
Kiyal           TTACAAAGTCTTCTTTTTGAATATTCAAGAAATGCAATTTCCCGTCCAAGACTTTTAATA
C_macrop        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAAGACTTTGAATA
G_macrop        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAAGACTTTGAATA
C_diverg        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAACACTTTGAATA
C_toment        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAACACTTTGAATA
C_aurant        TTACAAAGTCTTCTTTTTGATGATTCAAGAAATGAAATTT--------------------
C_limon         TTACAAAGTCTTCTTTTTGATGATTCAAGAAATGAAATTCCCCTCCCAAGACTTTTAATC
G_guidon        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAAGACTTTGAATA
G_glabra        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAAGACTTTGAATA
C_sinens        TTACAAAGTCTTCTTTTTGATGATTCAAGAAATGAAATTT--------------------
C_macrop        TTACAAAGTCTTCTTTTTGAAGATTCAAGAAATGAAATTCCCCCTGCAAGACTTTGAATA
M_panicu        TTACAAAGTCTTCTTTTTGATGATTCAAGAAATGAAATTCCCCTCCCAAGACTTTTAATC
C_medica        TTACAAAGTCTTCTTTTTAATGATTCAAGAAATGAAATTT--------------------

Mbetul          CTGAATTGCGTCTTTTTTAATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATG
Depeh           CTGAATTGCGTCTTTTTTAATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATG
Golek           CTGAATTGCGTCTTTTTTAATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATG
Hiku            CTGAATTGCGTCTTTTTTAATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATG
Dternate        CTGAATTGCGTCTTTTTTAATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATG
Kiyal           CTGAATTGCGTCTTTTTTAATTGACATCGACCCAACCCATCTAGTAAAATGAAAATGATG
C_macrop        CTTTTTTT-GTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG
G_macrop        CTTTTTTT-GTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG
C_diverg        CTTTTTTT-GTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG
C_toment        CTTTTTTT-GTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG

C_aurant        ------------------------------------------------------------
C_limon         CCTGTTTA----TTTTTTAATTGACATAGACCCAAGTCATCTAGTAAGATGAGAACGGTG
G_guidon        CTTTTTTT-GTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG
G_glabra        CTTTTTTTTGTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG
C_sinens        ------------------------------------------------------------
C_macrop        CTTTTTTT-GTCTTTTTTAATTGACATAGACCCAAGTCATCTAGTAAAATGAGGATGGTG
M_panicu        CC----------TTTTTTAATTGACATAGACCCAAGCCATCTAGTAAGATGAGAACGGTG
C_medica        ------------------------------------------------------------
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Tabel 1. Continue

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Mbetul          CGTCGGTAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCA
Depeh           CGTCGGTAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCA
Golek           CGTCGGTAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCA
Hiku            CGTCGGTAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCA
Dternate        CGTCGGTAATGGTCGGGATAGCTCAGCTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCA
Kiyal           CGTCGGTAATGGTCGGGATAGCTCAGCTGGTARAGCARARGACTGAAAATCCTCGTGTCA
C_macrop        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
G_macrop        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
C_diverg        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
C_toment        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
C_aurant        ------------------------------------------------------------
C_limon         TGTCGGAAATGGTCGGGATAGCTCAGCT--------------------------------
G_guidon        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
G_glabra        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
C_sinens        ------------------------------------------------------------
C_macrop        TGTTGGGAATGGTCGGGATAGCTCAGCTGGTAGAGCAG----------------------
M_panicu        TGTCGGAAATGGTCGGGATAGCTCAGCT--------------------------------
C_medica        ------------------------------------------------------------

Mbetul          CCAGTTCAAAAAA
Depeh           GCAGTTCAAATAA
Golek           CCAGTTCAAATAA
Hiku            CCAGTTCAAATAA
Dternate        CCARTTCAAAAAA
Kiyal           WCAGTTMAAAAAA
C_macrop        -------------
G_macrop        -------------
C_diverg        -------------
C_toment        -------------
C_aurant        -------------
C_limon         -------------
G_guidon        -------------
G_glabra        -------------
C_sinens        -------------
C_macrop        -------------
M_panicu        -------------
C_medica        -------------
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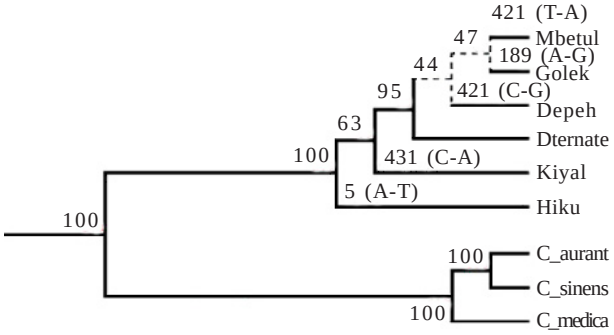

Figure 1. Cladogram of six accession of mangoes and outgroup based on *trnL-F* markers. Mbetul: *Mangifera laurina* cv. Betul; *M. indica* cv. Golek, Depeh: *M. aplanata*, Dternate: *M. laurina* cv. dodol ternate, *M. indica* cv. Kiyal; and *M.laurina* cv. Hiku.

with Rutaceae. *Mangga dodol* ternate (*M. laurina*) from Ternate and *mangga kiyal* from East Java were located at the position of between *mangga hiku* and *mangga betul*. The second clade was occupied by *Chisocheton* and *Guerea*, while the third group by *Citrus*, with bootstrap values 100%.

The phylogenetic tree performed three branches of *M. laurina* and their related species (Figure 2). *Mangifera* group was monophyletic or descended from the same

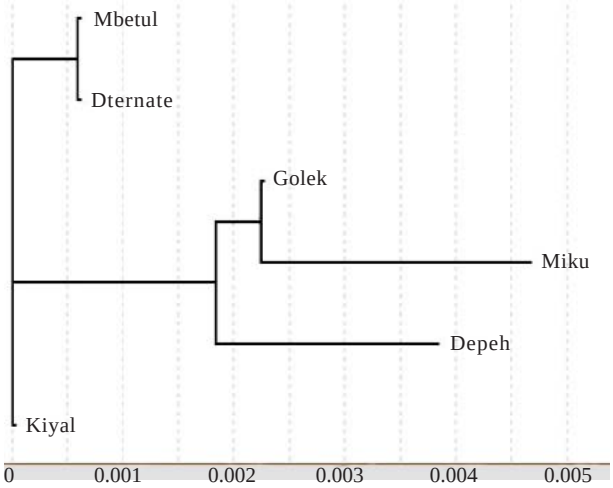

Figure 2. Cladogram of six accession of mangoes based on *trn*L-F markers with Neighbor Joining method. Mbetul: *Mangifera laurina* cv. Betul, *M. indica* cv. Golek, Depeh: *M. aplanata*, Dternate: *M. laurina* cv. dodol ternate, *M. indica* cv. Kiyal, and *M.laurina* cv. Hiku.

common ancestor. *Mangga hiku* (*Mangifera* sp.) from South East Sulawesi was located at the lowest of the clade and separated from the other species.

While *mangga dodol* ternate (*M. laurina*) from Ternate and *mangga kiyal* from East Java were located at the position of between *mangga hiku* and *mangga betul*. The second clade was occupied by *Chisocheton* and *Guerea*, while the third group by *Citrus*, with 100% bootstrap values.

DISCUSSION

Gaps within cpDNA occurred due to insertion and deletion process. Deletion of nucleotide on position number of 2 and 59, and insertion of nucleotide on nucleotide number 5 and 431 (a→t) occurred in *mangga hiku* as an accession. While in *mangga depeh* (*M. aplanata*) accession, insertion occurred on nucleotide of 421 (c→g) and 431 (a→t) and in *mangga golek* (*M. indica*) accession on nucleotide of 431 (a→t). Mutation rate of

cpDNA sequence among and within species of *M. laurina* is very low (<1%). Mutation in nucleotide levels can be used to reconstruct a phylogenetic tree. Insertion and deletion phenomena in *mangga hiku* (*M. laurina*) support formation of phylogenic branching within the ingroup species.

It was found that cpDNA nucleotide of *mangga hiku* was changed from Adenine to Thymine. While the other five species of *Mangifera* sp. were separated each other because of their base was changed i.e. in *mangga betul* (*M. laurina*), nucleotide 421 of cpDNA, Thymine, was altered by Adenine, in *mangga golek* (*M. indica*) nucleotide 189 of cpDNA, Adenine, was altered by Guanine, and in *mangga depeh* (*M. aplanata*) nucleotide 412 of cpDNA, Adenine, was altered by Thymine.

Sequence tracing of cpDNA *trn*L-F nucleotides of mango accessions showed a high homology (99%). This value was higher that the homology of 14 species of Anacardiaceae family (75%) in the ITS-1 regions of the nuclear genome (Hidayat & Pancoro 2001). The homology of *trn*L-F intergenic spacer sequence in *M. laurina* and their related species was more conservative than that of nuclear DNA and uniparentally inherited. However, nucleotide change could be used to estimate homology patterns of the cpDNA fragment (Raubeson & Jansen 2005). This could also be used to analyze the relationships among progenies and patterns of gene mutation process in chloroplast genomes. Even though, cpDNA was commonly conservative, the diversity of cpDNA was reported occurred in different plant species such as *Fagopyrum cymosum*, *Astragalus* sp., Conifers and different species of Dipterocarpaceae (Tsumura *et al.* 1996; Yamane *et al.* 2003; Liston 2008). Variations in cpDNA sequence was usually caused by mutation of single nucleotide in long period of time. Mutation rate of cpDNA loci was between 3.2×10^{-5} and 7.9×10^{-5} (Provan *et al.* 1999). Although nucleotide change in cpDNA was very little than that of in nuclear genome, it was highly valuable to provide some information to explain the process of evolution.

Based on Neighbor Joining Analysis (Saitou & Nei 1987) (Figure 2), *mangga hiku* (*Mangifera* sp.) had the longest branch and occurred earlier than the others, so that *mangga hiku* could be presumed as a common progenitor of *M. laurina* and each related species. The length of line indicated that the change of cpDNA sequence. Therefore, *mangga hiku*, which is still wild in South East Sulawesi was more primitive than other Indonesian cultivated mangoes.

The clustering pattern of *M. laurina* and their related species based on E-RAPD markers of nuclear DNA (Fitmawati 2006) showed similar pattern with the clustering using *trn*L-F markers, except for *mangga depeh* (*M. aplanata*) which was separated from their related species. On the other hand, the clustering *trn*L-F intergenic spacer differed from that the clustering based on morphological markers, i.e. *mangga Hiku* was located together with *M. laurina*. The diversity pattern of chloroplast markers can be different from that of the morphological markers. Chloroplast was inherited only by the female parent, while the morphology was descended from both parents and affected by the environment. Therefore clustering Analysis using cpDNA *trn*L-F of six accession i.e. mangga betul (*M. laurina*), 'Depeh' (*M. aplanata*), 'Golek' (*M. indica*), 'Hiku' (*Mangifera* sp.), 'Dodol Ternate' (*M. laurina*), and 'Kiyal' (*M. indica*) did not agree to the clusters of morphological markers reported by Kostermans and Bompard (1993).

The markers of cpDNA *trn*L-F could be used to investigate the phylogenetic relationships of *M. laurina* and their related species. Phylogenetic cluster of cpDNA *trn*L-F markers may be different from those of morphological markers. *M. laurina* and their related species were clustered separately from the outgroup. *Mangga hiku* was presumed as the common progenitor of *M. laurina* and their related species.

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