Jurnal Riau Biologia 1(2) : 130-134
Periode Juli-September 2016

JURNAL RIAU BIOLOGIA
ISSN ONLINE : 2527-6409

Evolutionary Relationship of Common Mangoes: Insight from ITS

IBNA HAYATI[1], FITMAWATI[2], NERY SOFIYANTI[3]

[123]Jurusan Biologi, FMIPA, Universitas Riau
[1]email: nana.ibna@gmail.com

ABSTRACT

Mango (*Mangifera*) is one the most important fruit from Asia. The center of origin and diversity of this genus is in Southeast Asia. This region has great economic development in recent years. Due to the deforestation and habitat change, the occurance of *Mangifera* species in their natural habitat is threathened. This research aimed to analyze evolutionary relationship among common mangoes from Central Sumatra. This research was conducted from November 2015 until April 2016. DNA was isolated using CTAB method. The isolated DNA were then sequenced in First Base Laboratories, Malaysia. Phylogram was generated using Neighbor Joining Method, while T92+G evolution model was used for Distance Analysis. NJ analysis revealed monophyletic group of common mangoes. *M. laurina* was considered as the most primitive species in common mangoes clade due to the longest branch length. Relationship among *M. kemanga* and *M. laurina, M. indica* and *M. zeylanica* was a new finding. It would be highlighted to determine newer classification, cultivation and conservation strategies of mango.

Key words : Central Sumatra, Common Mangoes, Evolutionary Relationship, ITS, Molecular Marker

ABSTRAK

Mangga merupakan salah satu buah terpenting di Asia. Pusat asal dan keanekaragaman marga *Mangifera* saat ini berada di Asia Tenggara. Asia Tenggara adalah daerah dengan sejarah perkembangan ekonomi terbesar dalam beberapa tahu terakhir. Akibat deforestasi dan alih fungsi lahan, jenis *Mangifera* terancam punah di habitat alaminya. Penelitian ini bertujuan untuk menganalisis hubungan evolusi di antara jenis mangga umum di Sumatera Tengah. Penelitian ini dilakukan dari November 2015 sampai April 2016. DNA diisolasi menggunakan metode CTAB. Sekuensing DNA dilakukan di First Base Laboratories, Malaysia. Filogram diperoleh menggunakan analisis *Neighbor Joining*. Analisis jarak kekerabatan menggunakan model evolusi T92+G. Pohon NJ mengungkapkan kelompok monofiletik dari jenis mangga umum di Sumatera Tengah. *M. laurina* merupakan jenis paling primitif dalan kelompok mangga umum akibat jarak genetik terpanjang yang dimilikinya. Hubungan di antara *M. kemanga, M. laurina, M. indica* dan *M. zeylanica* merupakan penemuan baru yang perlu disoroti dalam menentukan klasifikasi terbaru, kultivasi dan konservasi mangga.

Kata kunci : Hubungan Evolusi, ITS, Mangga Umum, Penanda Molekuler, Sumatera Tengah

INTRODUCTION

Sumatra island is located in western part of Indonesia. Its geographical location and physiographic condition is very unique. The mountain ranges face the monsoon winds could affect rainfall pattern was varied in Sumatra (Oldeman *et al.* 1979). Consequently, plants in Sumatra have adaptation strategies, tolerate to high rainfall and wet climate that may cause variation among plant species. Exploration of *Mangifera g*ermplasm in Central Sumatra had been conducted during 2012-2013 (Fitmawati *et al.* 2013). The common mangoes were found in Central Sumatra have high economic value and have been cultivated for a long time. Improvement of mango breeding depend on the use of genetic variability of *M. indica* which highlighted as the most common mango around the world. The primitive species which has remarkable characters can support the improvement of mango production.

JURNAL RIAU BIOLOGIA
ISSN ONLINE : 2527-6409

Southeast Asia is the centre of origin and diversity of the genus *Mangifera*, this region has high economic development in recent years (Litz 2009). The deforestation for expanding agriculture or removal of tropical hardwoods for export causes the high genetic erosion and loss of species in natural habitat, including *Mangifera* species.

Morphological characters had been use for a long time in phylogenetic analysis. However morphological plasticity whitin species became a major threat to determine whether the rank of taxon is cultivar or species. Therefore, we try to reconstruct phylogenetic tree of common mangoes in Central Sumatra based on Internal Transcibed Spacer (ITS) sequence. ITS of nrDNA has been used for molecular markers at specific level of Angiospermae (Baldwin *et al.* 1995; Yonemori *et al.* 2002). Sequences of ITS is also useful because it has conserve region, short size (±700 bp), high evolution rate, informative and universality (Baldwin *et al.* 1995). Molecular study of specific *Mangifera* in Central Sumatra based on ITS sequences has never been reported. Molecular approach is important to find the best phylogenetic tree model which useful in conservation and cultivation strategies. Therefore, this research is important to support the classification, cultivation and conservation of mango.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Plant samples of five *Mangifera* species, (*M. foetida* Lour. (macang), *M. indica* L. (mangga), *M. laurina* Bl. (mempelam), *M. zeylanica* (BI.) Hooker f. (apel), *M. kemanga* Bl. (kemang) were obtained from the collection of Fitmawati *et al.* (2013) from Central Sumatra. Fresh leaves were dried in silica gel at the time of collection. Two genera from Anacardiaceae family used as outgroup were obtained from Genebank Data (NCBI) by Yonemori *et al.* (2002). DNA was isolated using CTAB method of Doyle and Doyle (1987) with modification. DNAs were then suspended in TE buffer.

Tabel 1. List of five species of *Mangifera* from Central Sumatra and two outgroups taxa with location of their collection

Species name	Sub genus	Collected location	Acc. code for ITS sequence	Reference
M. kemanga Bl.	*Limus*	Central Sumatra, Indonesia	KX347955	Fitmawati *et al.* (2013) and this study
M. foetida Lour.	*Limus*		KX347956	
M. indica L.	*Mangifera*		KX347960	
M. zeylanica (BI.) Hooker f.	*Mangifera*		KX347962	
M. laurina Bl.	*Mangifera*		KX347963	
Anacardium occidentale		Thailand	AB071690	Yonemori *et al.* (2002)
Bouea macrophylla			AB071691	

Amplification and Sequencing

The genomic DNA was amplified using universal primer ITS4 and ITS5 (White *et al.* 1990) for the entire ITS regions. Reaction mixture (50 µL) contained 5 µL 10X DreamTaq Buffer; 5 µL 0,2 mM dNTP Mix; 2,5 µL 0,25 µM primer ITS5F; 2,5 µL 0,25 µM primer ITS4R; 0,25 µL 5 U/µL DreamTaq DNA Polymerase; 1 µL 40-80 ng/µL template DNA and 33,75 µL dH_2O (water, nuclease-free). Thirty five cycles of PCR were conducted using Thermal Cycle under following profiles: 94^{o}C for 5 m, 94^{o}C for 1 m, 47.4^{o}C for 30 s, 72^{o}C for 1 m 30 s, 72^{o}C for 7 m. PCR products were sealed by using parafilm before being sent First Base Laboratories, Malaysia.

Sequence Alignment and Phylogenetic Analysis

DNA sequences obtained from ITS regions were first aligned using ClustalW (Thompson *et al.* 1997) and then adjusted manually. The boundaries of ITS1 and ITS2 were determined by comparing the aligned sequence with previous published sequences (Yonemori *et al.* 2002). Disparity index test of substitution pattern homogeneity analysis with 500 replicates of Monte Carlo was used to determine the utility of gamma distribution in phylogenetic tree reconstruction. Phylogeny reconstruction was analyzed using Neighbor Joining (NJ) method. The evaluation of internal support of clades was conducted using bootstrap analysis (100 replicates). Model Selection (ML) analysis was used to determine substitution models selected as best-fit and gamma parameter for site rates. The best-fit model evolution and gamma

JURNAL RIAU BIOLOGIA
ISSN ONLINE : 2527-6409

distribution (shape parameter) were also used to generate NJ phylogram. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.* 2013).

RESULTS AND DISCUSSION

ITS Sequence of *Mangifera*

Amplifications of ITS regions were successful for the five species. The length of ITS sequence ranged from 652 to 656 bp. A 652-bp sequence was observed in *M. foetida* which we include in this study as comparison and most of other species had a 656-bp ITS sequence. The length of ITS1 was 265 bp and did not show the variation, while the length of ITS2 was 226 to 229 bp. The length of conserved region 5.8S gene was in the ranged of 162 to 163 bp. Alignment of the entire sequences among *Mangifera* and outgroup obtained 666 bp with 63 indels (42 in ITS1, 4 in 5.8S, 17 in ITS2), 187 variable sites and 41 parsimony-informative sites. We found remarkable conserved sites especially near the 5.8S gene which encoded for ribosomal RNA.

The probability of rejecting the null hypothesis that sequences have evolved with the same pattern of substitution was judged from the extent of differences in base composition biases between sequences (Disparity Index test) (Kumar and Gadagkar 2001). Monte Carlo test (500 replicates) was used to estimate the *P*-values which are shown below the diagonal. *P*-values smaller than 0.05 are considered significant (marked with yellow highlight). The estimates of the disparity index per site are shown for each sequence pair above the diagonal (Table 1). There are rate variation among sites, especially between *M. laurina* with *M. kemanga* and *M. laurina* with *M. indica*. This results are important to determine utility if gamma distribution for phylogenetic tree reconstruction using NJ analysis.

Model Selection (ML) analyses was obtained T92+G (Tamura 3-parameter) as a best-fit substitution model selected by MEGA6 (Tamura 2013) from 24 different nucleotide substitution models. This models has the lowest BIC scores (Bayesian Information Criterion) which were considered to describe the substitution pattern the best. BIC value of T92+G model was 4009.52 and maximum likelihood value (InL) was -1945,81. Rates of base substitutions for each nucleotide pair had variation among sites therefore we modeled with a gamma distribution (shape parameter=0.73). The nucleotide frequencies are A=18.63%, T/U=18.63%, C=31.37% and G=31.37% and the detailed substitution rate can be seen in Table 2.

Tabel 1. Test of the Homogeneity of Substitution Patterns Between Sequences. Green marks are Disparity Index and yellow marks are probability computed. P<0.05 for hypothesis rejection at 5% level

	1	2	3	4	5	6	7
1. *A. occidentale*		0.262	1.741	1.951	1.801	1.622	1.823
2. *B. macrophylla*	0.048		1.034	0.778	1.099	0.543	1.068
3. *M. kemanga*	0.000	0.000		0.000	0.000	0.111	0.000
4. *M. foetida*	0.000	0.000	1.000		0.000	0.000	0.000
5. *M. zeylanica*	0.000	0.000	1.000	1.000		0.078	0.000
6. *M. laurina*	0.000	0.004	0.042	1.000	0.108		0.113
7. *M. indica*	0.000	0.000	1.000	1.000	1.000	0.042	

Tabel 2. The best-fit substitution models and its parameter

T92 + G parameter for ITS sequence				
Substitution Rate				
From/To	A	T	C	G
A	-	0.03	0.06	0.20
T	0.03	-	0.20	0.06
C	0.03	0.12	-	0.06
G	0.12	0.03	0.06	-

JURNAL RIAU BIOLOGIA
ISSN ONLINE : 2527-6409

Phylogenetic Relationship among Common Mango

The evolutionary history was inferred using the Neighbor Joining method (Saitou and Nei 1987). The total of branch length was 0.451. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura 1992). The rate variation among sites was modeled with gamma distribution (shape parameter=0.73). NJ phylogram is presented in Figure 1.

The tree is drawn to scale, with branch lengths correspond the evolutionary distance used to infer phylogram. The longest branch obtained from *M. laurina* with 0.07 length (Fig. 1). This indicate *M. laurina* as the most primitive species among common mangoes. This finding was agree to Fitmawati and Hartana (2010) who revealed mangga hiku (*M. laurina* from Sulawesi) as the common progenitor of Indonesian mango.

NJ analysis was supported monophyletic group (descended from the same common ancestor) of common mangoes with 100% bootstrap value. This clade separated *M. indica* with the other species. *M. kemanga* was related to *M. laurina*. Kostermans and Bompard (1993) classified *M. laurina, M. indica* and *M. zeylanica* in to sub genus *Mangifera* while *M. kemanga* was put in to sub genus *Limus* together with *M. foetida* (we used *M. foetida* as comparison). However, ITS sequence did not form a clade based on this floral disk character and the place of *M. kemanga* was still remain unclear.

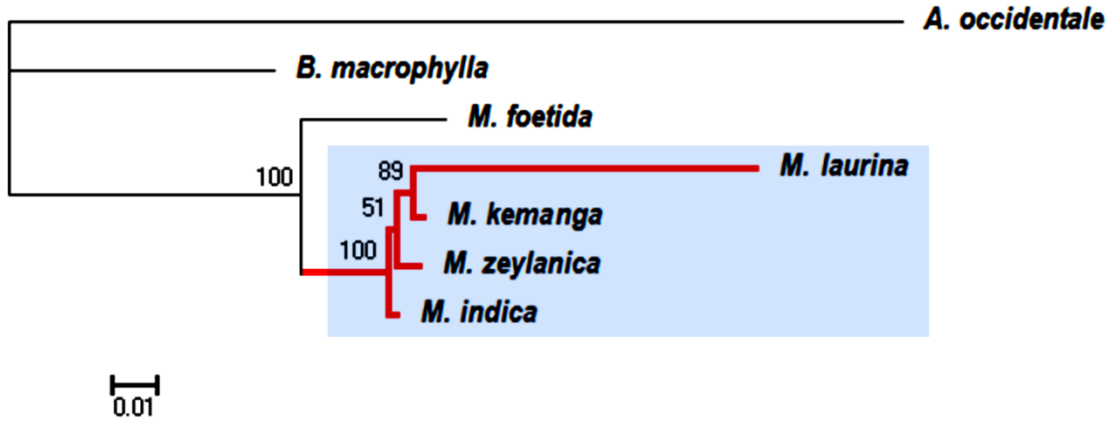

Figure 1. Phylogenetic tree generated from the neighbor joining analysis of the ITS region. Numbers above branches indicate the support level (for branches with >50%) determined from 100 bootstrap replication.

Leaf morphological character of *M. kemanga* (obovate) was very distinct compared with *M. laurina, M. indica* and *M. zeylanica* (oblong-lanceolate and ovate-oblong) (Fitmawati *et al.* 2013). The previous study based on morphological characters did not inform the relationship of *M. kemanga* with this three species. Recently, molecular study of *M. caesia* (close relative of *M. kemanga*) was revealed *M. caesia* origin from the same common ancestor of *M. indica* and *M. laurina* based on matK gene (Hidayat *et al.* 2012). Another study based on rbcL sequence (Suparman *et al.* 2013) was revealed the relationship between *M. indica* and *M. caesia.* This result corresponded with this study. Therefore this finding will be highlighted to support revision on *Mangifera* classification based on morphological characters by Kostermans and Bompard (1993) with support of molecular data. Phylogenetic study based on morphological characters and ITS sequences has similar pattern, were inherited biparentally. Hoewever, this research could be use as strong principal to develop classification system. It is also due to the fact DNA is functional unit which encoded organism (Hillis *et al.* 1996).

CONCLUSION

Phylogenetic study of common mangoes in Central Sumatra based on ITS sequence was obtained monophyletic group. *M. laurina* was assumed as the most primitive species among common mangoes

JURNAL RIAU BIOLOGIA
ISSN ONLINE : 2527-6409

based on the longest branch length. Relationship among *M. kemanga* and *M. laurina*, *M. indica* and *M. zeylanica* was a remarkable finding.

ACKNOWLEDGEMENT

This research was supported by HIBAH KOMPETENSI, DIKTI 2015 led by the first co-author. Thanks to all parties involved in this study.

REFERENCES

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ. 1995. The ITS region of nuclear ribosomal DNA: A valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82(2): 247-277.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11-15.

Fitmawati, Hartana A. 2010. Phylogenetic Study of *Mangifera laurina* and its related species using cpDNA *trnL-F* spacer markers. *HAYATI Journal of Biosciences* 17(1): 9-14

Fitmawati, Swita A, Sofiyanti N, Herman. 2013. Analisis Kekerabatan Morfologi *Mangifera* dari Sumatera Tengah. *Floribunda* 4(7): 169-179

Hidayat T, Pancoro A, Kusumawaty D, Eiadthong W. 2012. Development *matK* Gene as DNA Barcode to Assess Evolutionary Relationship of important tropical forest tree genus *Mangifera* (Anacardiaceae) in Indonesia and Thailand. *Jurnal Teknologi (Science & Engineering)* 59: 17-20.

Hillis DM, Moritz C, Mable BK. 1996. Molecular Systematics, Second Edition. Sinauer Associates. Massachusetts.

Kostermans AJGH, Bompard JM. 1993. The Mangoes: Their Botany, Nomenclature, Horticulture and Utilization. Academic Press. London.

Litz RE. 2009. The Mango: Botany, Production and Uses, *Second Edition*. CAB International. Massachussets.

Oldeman LR, Las I, Darwis SN. 1979. An Agromatic Map of Sumatra. *Contributions from the Central Research· Institute for Agriculture Bogor* No 52.

Saitou N, Nei M. 1987. The Neighbour-Joining Methods: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution* 4(4):406-425.

Suparman, Pancoro A, Hidayat T. 2013. Phylogenetic Analysis of *Mangifera* based on *rbcL* Sequences, chloroplast DNA. *Scientific Papers. Series B, Horticulture* LVII: 235-240.

Tamura K. 1992. Estimation of the Number of Nucleotide Substitutions When There re Strong Transition-Transversion and G + C-Content Biases. *Molecular Biology and Evolution* 9:678-687.

Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*30: 2725-2729.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876-4882.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes For Phylogenetics. Di dalam: Innis M, Gelfand D, Sninsky J, White T., (eds). *PCR Protocols: A Guide To Methods And Apllications*. Academic Press. San Diego, California, USA. hlm. 315-322.

Yonemori K, Honsho C, Kanzaki S, Eiadthong W, Sugiura A. 2002. Phylogenetic relationships of *Mangifera* species revealed by ITS sequences of nuclear ribosomal DNA and a possibility of their hybrid origin. *Plant Systematics and Evolution* 231: 59-75.