Scientific Papers. Series B, Horticulture. Vol. LVII, 2013
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PHYLOGENETIC ANALISYS OF *MANGIFERA* BASE ON *RBCL* SEQUENCES, CHLOROPLAST DNA

Suparman SUPARMAN[1], Adi PANCORO[2], Topik HIDAYAT[3]

[1]Department of Biology Education, Universitas Khairun, jalan bandara Babullah Ternate, Maluku Utara, Indonesia
[2]Genetics Laboratory, School of Life Science and Technology,Institut Teknologi Bandung, Jalan Ganesha 10, Bandung, Indonesia
[3]Department of Biology Education, Universitas Pendidikan Indonesia (UPI), Jalan Setiabudi 229 Bandung, Bandung, Indonesia

Corresponding author email: suparman_bio@yahoo.com

Abstract

Genus of Mangifera has 69 species that mostly distributed around Borneo, Sumatra, Java and Malay Peninsula. Phylogenetic study of this genus is conducted in order to investigate the ancestor trait and relationships among those species. Phylogenetic tree is constructed based on nucleotide variation in rbcL gene within 16 samples of Mangifera : 13 species from Indonesia and 3 species from Thailand. Two species from the other genera are added as outgroups. Genomic DNA was extracted using CTAB protocol and amplified with rbcL primers. Sequencing result is analyzed using BLAST function on NCBI. Multiple sequence alignment from all samples of rbcL sequences is generated using Bioedit and ClustalX program. Subsequently phylogenetic is constructed by using Maximum Parsimony method in PAUP 4.0b10 software. The aligned rbcL comprised 905 characters which had 72 characters of parsimony informative with consistency index (CI) 0,889 and retention index (RI) 0,962. Phylogeny generated four main groups. Group I consist of M. cochinchinensis and M. macrocarpa (Thailand); group II : M. indica M. cesia, M. aplanat and M. altisima ; group III : M. laurina, M. longipes, M. similis, and M. gedebe ; group IV : M. laurina (Thailand), M. foetida, M. caesia, Mangifera spp, and M. odorata. Phylogenetic analysis revealed that Mangifera is monophyletic. There is a diversification between M. laurina from Indonesia and Thailand, as well as M. macrocarpa. Phylogenetic analysis also provides information which support the assumption that M. odorata is a hybrid of M. indica and M. foetida, and strongly support the assumption that M. longipes is a synonim of M. laurina.*

Key words: *Mangifera, Phylogenetic, rbcL.*

INTRODUCTION

Mangifera is a genus of Anacardiaceae. Most of its member are spread in Borneo, Sumatra, Java, Malay peninsula, and also other part of Asia (Mukherjee, 1953). Classification system of *Mangifera* has been developed. Mukherjee (1953) classified *Mangifera* in two sections with five species *incertaesedis*.

Today *Mangifera* has 69 species and classified to three subgenus they are *Mangifera*, *Limus* (Marchand), and *uncertain position* Kostermans and Bompard (1993).

Mangifera was determined from one ancestor (Mukherjee, 1953) otherwise Kostermans and Bompard (1993) contradicted that theory. They suggested that the genus original was two different ancestors.

Classification of *Mangifera* is still labile (Hidayat dkk, 2011). It is because the complexity of the vegetative and generative organ. The newer classification base on morphology is doubted (Yonemori *et al*., 2002).

It can be revealed by *uncertain position* for 11 species, beside that, there are two controversial species: *M. longipes* and *M. odorata.* In the old classification there is M. *longipes* (Mukherjee, 1953; Hou, 1978) but in the latest classification there is not (Kosterman and Bompard, 1993). They said that *M. longipes* is synonym with *M. laurina* but they showed different some morphology characters.

In other species, *Mangifera odorata* was the hybrid result between *M. indica* dengan *M. foetida* (Hou, 1978), but Kosterman and Bompard (1993) rejected the statement.

Some previous molecular phylogenetic analyses in *Mangifera* were done. A research of *internal trancribed spacer* (ITS) DNA nuclear

ribosomal to investigate 13 species of *Mangifera* (Yonemori et al., 2002); Using *amplified fragment length polymorphism* (AFLP) information by Yamanaka (Yamanaka *et al*., 2006); using *trn*L-F gene sequence to analysis four species of *Mangifera* (Fitmawati and Hartana, 2010); also phylogenetic and diversification of *Mangifera* from Indonesian and Thailand by Hidayat (Hidayat *et al.,* 2011).

All molecular phylogenetic researches of *Mangifera* were to analysis the phylogenetic and phyletic original of ancestor. So that, it is quite important to construct phylogenetic tree and analyze phylogenetic of *Mangifera* using different molecular marker, especially based on *rbcL* gene sequence as marker in plant. *rbc*L is gene for coding *ribulose-1,5-bisphospate carboxylase* (RuBisCO).

All kind of plants have this gene with moderate mutation. Mutation in *rbc*L has positive correlation with species diversification in Angiosperm (Barraclough *et al*., 1996), so it is expected will be able to give phylogenetic information closer to the real condition.

MATERIALS AND METHODS

16 samples of *Mangifera,* 13 samples of leaf are collected from Indonesia (Kebun Raya Bogor) and three samples from Thailand (Forestry Departemnt of Kasetsart University, Bangkok). Two *Outgroup,* they are *Bouea macrophylla* from Bogor and *Anacardium occidentale*. The last outgroup is taken from NCBI genebank (Aguilar and Sosa, 2004).

Three main steps of research are *rbc*L primer design, DNA genome isolation from *Mangifera* leaf and *rbc*L amplification, and the last is phylogenetic tree construction.

Template of *rbc*L gene was retrieved from *Mangiferaindicarbc*L gene in NCBI (Gadek, et al., 1996). That sequence was used for designing primer both *rbc*L-F and *rbc*L-R by *GenamicsExpression* software and confirmed with *primer blast* at NCBI.

DNA Genome was extracted from *Mangifera* leaf using CTAB method protocol (Porebski et al., 1997) with modification. Then, *rbc*L gene was amplified by PCR and sequenced in Macrogen *Inc* (Korea) with the same primer.

For constructing Phylogenetic tree, all the sequences were edited and performed alignment by *Bioedit* and *ClustalX* program (Thompson et al., 1997)

Phylogenetic tree constructed with maximum parsimony (MP) and neighbour joining (NJ) using PAUP* 40.b10 (Swofford, 2002). Appearance the phylogenetic tree use tree view win 32 software (Roderic, 2001).

Table 1. Plant material and origin

Number	NAME OF SPECIES	ORIGIN
1	*Mangifera caesia* Jack	Java, Indonesia
2	*Mangifera similis* Auet	Sumatera, Indonesia
3	*Mangiferamacrocarpa* Blume	Java, Indonesia
4	Mangifera *laurina* Blume	Java, Indonesia
5	*Mangiferagedebe* Miquel	Sumatra, Indonesia
6	*Mangifera* indica Lin	Java, Indonesia
7	*Mangifera sp*	Borneo, Indonesia
8	*Mangiferaapplanata* Kosterm	Borneo, Indonesia
9	*Mangiferacasturi* Kosterm	Borneo, Indonesia
10	*Mangiferaodorata* Griff	Java, Indonesia
11	*Mangiferafoetida* Lour	Java, Indonesia
12	*Mangiferaaltissima* Blanco	Java, Indonesia
13	*Mangiferalongipes* Griff	Java, Indonesia
14	*M. cochinchinensis* Engler	Thailand
15	*Mangiferalaurina* Blume	Thailand
16	*Mangifera macrocarpa* Blume	Thailand
17	*Bouea macrophylla* Griff *	Java, Indonesia
18	*Anacardium ocidentale* Lin**	Accession number in NCBI: AY462008.1

= outgroup

* *= outgroup which taken from* NCBI

RESULTS AND DISCUSSIONS

Phylogenetic tree result

The aligned *rbc*L comprises 905 characters. Of these, 807 characters are constant and 72 are potentially parsimony informative. From the most parsimony tree (MPTs), consistency index (CI) is 0,889 and retention index (RI) is 0,962. The values showed that all characters are important in constructing tree and RI reveal that homoplasy is very small.

Phylogenetic tree as shown in figure 1, it was constructed with maximum parsimony method and bootstrap 1000x. *Neighbourjoining* (NJ) method is also done to show difference of genetic distance and analyze similarity sequence among samples.

Phylogenetic analysis *Mangifera*

Phylogenetic analysis from the tree had revealed the important answer about the ancestor trait. It is monophyletic tree with four main groups. The first group consists of two species from Thailand which are *M. macrocarpa* (Thailand) and *M. cochincinensis*. Group II consist of *M. indica, M. caesia, M. aplanata*, and *M. altisima*. Group III consist of *M.longipes*, *M. laurina, M. similis,* and *M. macrocarpa*. Group IV consist of mix samples from Thailand and Indonesia, such as *M. laurina* (Thailand), *M.* sp*, M. kasturi, M. foetida,* and *M. odorata.*

The result reveal that two species of *Mangifera* from Thailand grouped in one but other species (*M. laurina*) join to Indonesian *Mangifera*. The group systems show some differences with classification system made by Kostermans and Bompard (1993).

Monophyletic character of *Mangifera* ancestor trait based on *rbc*L gene shows the same result with ITS (Yonemori et al., 2001) and *mat*K (Hidyat et al., 2011) with different DNA sequences. Overall results of *Mangifera* ancestor are monophyletic. The monophyletic ancestor of *Mangifera* is supported by character of stomata (Hidayat et al, 2009). Therefore, the consequence for the ancestor is agree with Mukherjee (1953), that said *Mangifera* come from one origin and divided into three species. That is *M.duperreana* as *root* of section I, *M. lagenifera* and *M.macrocarpa* as root of section II. That species are the oldest among all species of *Mangifera*.

Phylogenetic analysis also shows biogeography relationship of *Mangifera*. It can be seen from the diversification of same species, which is taken from difference land with long distance. Phylogenetic pattern among species also give information in species status and taxonomy implication in genus *Mangifera*.

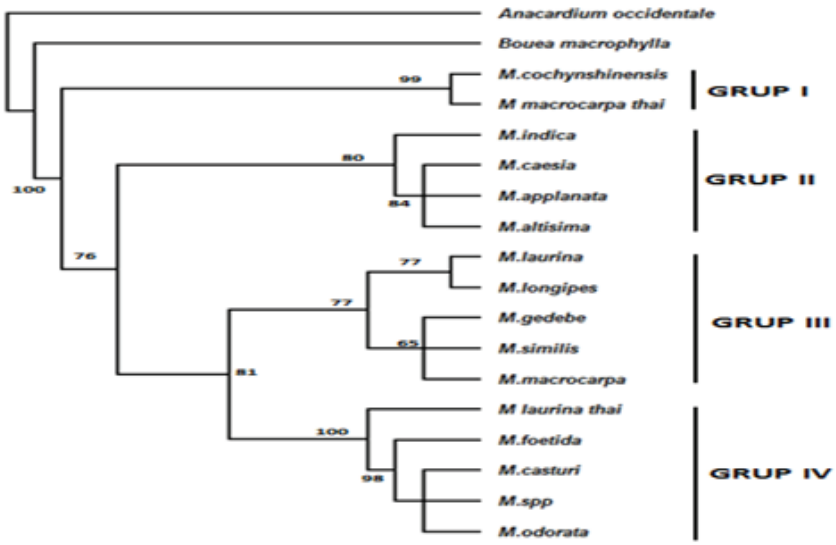

Figure 1. One of the most parsimony tree with bootstrap 1000x. The number on the node is Bootstrap value in%.

Biogeography of Genus *Mangifera*

Two species of *Mangifera* from Thailand made group I: *M. cochynsinensis* and *M. macrocarpa* (gambar III.1), meanwhile *M. laurina* joint in group III. It reveals diversification among species from Indonesian islands and Thailand especially in *M. laurina* and *M. macrocarpa* from Thailand and Indonesia.

Phylogenetic analysis based on *mat*K (Hidayat et al., 2011) in *Mangifera* also shows separation between species which come from different geography. It may be caused by different natural geographical condition since many years ago. So the sequences of DNA are changed or mutated. Another hypothesis is calculated from different variety of sample, yet this hypothesis is weak.

Phylogenetic relathionship and member status of *Mangifera*

Some closes species based on the phylogenetic tree, are *M. cochinshinensis* and *M. macrocarpa* from Thailand. These species are group I. While in group II, *M. caesia, M. aplanata*, and *M. altissima* also had *a* close relationship and *sistergroup* with *M. indica*. Relationship between *M. altissima* and *M. applanata* also close *Mangifera* phylogenetic based on *mat*K (Hidayat dkk, 2011). In group III, *M. gedebe, M. similis*, and *M. macrocarpa* closed and sister group with *M laurina* & *M. longipes*.

Group III is similar to *mat*K phylogenetic, otherwise *M. macrocarpa* in *mat*K is Thailand samples. Group IV, *M. odorata*, *M.* spp and *M. casturi* closed and *sister group* with *M. foetida,* beside *M. laurina* from Thailand is in group but outer than other.

Relationship in phylogenetic based on *rbc*L sequences also reveals status of *M. odorata* and status *M. longipes.* Species of *M. odorata* is the hybride of *M. indica* and *M. foetida* (Hou, 1978) . The conclusion does not directly agree with that opinion, but our analyses reveal it is possible. Not all the species (*M. odorata,M. indica* and *M. foetida*) are in one group. *M. odorata* and *M. foetida* are in one grup (group IV), while both of them are separated with *M. indica* (group II).

M. odorata and *M. foetida* also have close relationship based on ITS marker (Yonemori et al., 2002). AFLP analysis in showing hybrid status of *M. odorata* reveal that similarity index between *M. odorata* and *M. foetida* is higher than *M. indica* and *M. odorata* (Kiew et al., 2003; Teo et al., 2002). They indicate that *M. odorata* is hybrid result of *M indica* and *M foetida*, it was followed by *backcrossing* with *M. foetida*. So, it refers to be similar with *M. foetida* than *M. indica*.

The next research using *mat*K sequences analysis shows a different result. It shows that *M. odorata* is closer to *M. indica* than *M. foetida* (Hidayat dkk, 2011). This difference result among *rbc*L gene, ITS and *mat*K still support hybrid status of *M. odorata*). It needs more analysis using three combination of that marker to answer that controversial.

Phylogenetic tree give information of *M. longipes* status. Species *M. longipes* in newer classification of *Mangifera* is synonym with *M. laurina*. Species of *M. longipes* Griff spread in Sumatera, Malay Peninsula, Borneo, Lesser Sunda island and Philipina (Hou, 1978). however *M. laurina* Blume is endemic species in Philippines archipelago and Selayar island (Sulawesi) with local name are Mangga Aer, Mangga parih and Apale/i (local name in Palawan island). Based on the analysis, it is possible that both of them are different species.

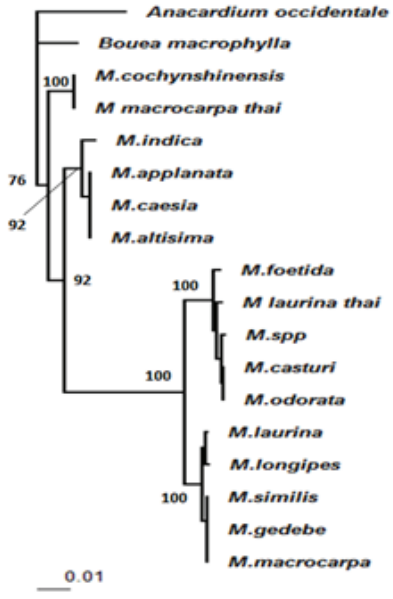

Figure 2. Phylogenetic tree using Neighbour Joining methods. Numbers on the nodes are bootstrap value in% and number bellow is genetic distance.

Phylogenetic tree using MP likely to support that *M. longipes* is synonim with *M. laurina.* It show on phylogenetic tree, *M. longipes* and *M. laurina* make one same clade at one internal nodus. In phylogenetic, it means booth of them come from one ancestor and are very close taxon. Phylogram tree (fig2) using NJ methods reveal some genetic distance between *M. laurina* and *M. longipes* but very little. Therefore, the conclusion for this controversy is strongly support that *M. laurina* is synonym with *M. longipes*.

Taxonomic implication

Phylogenetic information of Mangifera based on *rbcL* can become reference and base in *Mangifera* classification without ignore morphology and anatomy information as the first reference. Topological analysis of tree Phylogenetic uncover different pattern with newer classification of *Mangifera.* For example the closer kinship species *M. laurina, M. gedebe, M. sismilis* and *M. macrocarpa,* are different subgenus and different section. Phylogenetic based on *rbcL* gene is supported by matK gene and this difference pattern of classification also indicated by ITS marker (Yonemori et al., 2002). It means that the classification system of Mangifera today is inconsistence.

In different case, as a reference, species member of *Caragana* (Fabaceae) are reformed after molecular analysis from *tribe* of Galegeae to become different tribe of Hedysarea (Zhang et al., 2009). Based on molecular information *rbc*L, *trn*S-*trn*G and ITS, another section and group in *Caragana* are recommended to contemplate and observe

Our research was limited in samples and sequence of base so it is too early in recommending for classification reform but the result can consider in reanalysis of *Mangifera* classification. It is strongly supported by another molecular marker such as ITS and *mat*K, so it is very important for collaborating some molecular marker in making best classification system of *Mangifera*.

CONCLUSIONS

Phylogenetic analysis of 16 species of *Mangifera* using *rbc*L gene sequence in chloroplast reveal that Mangifera is a monophyletic ancestor, there are diversification between Thailand and Indonesian sample.

It result also supports that *M. odorata* is hybrid result of *M. indica* and *M.Foetida.* The analysis also support that *M. longipes* is synonym with *M. Laurina.*

The classification system is revealed quite differently with previous system.

ACKNOWLEDGEMENTS

The research is supported by AP project 2009-2010 and genetics laboratory of SITH ITB. We would like to thank Puri Arta as research assistant of Pancoro group, Asri P lestari, Husna N Praja, all ITB genetic laboratory members and Desy Apriliani (Udayana University) for discussing the grammar.

REFERENCES

Aguilar C.J., Sosa V., 2004. The evolution of toxic phenolic compounds in a group of Anacardiaceae genera. Taxon Journal 53 (2), p. 357-364.

Barrachlough T.G., Harvey P.H., Nee S., 1996. Rate of rbcL gene sequences evolution and species diversification in flowering plants (Angiospermae). The Royal Society. Proc. R. Soc. Lond. B 263, p. 589-591.

Fitmawati, Hartana A., 2010. Phylogenetic Study of Mangifera laurina and its related Species Using cpDNA trnL-F spacer Marker, HAYATI Journal Bioscience Vol. 17 No.1, p 9-14.

Gadek P.A., Fernando E.S., Quinn C.J., Hoot S.B., Terrazas T., Sheahan M.C., and Chase M.W., 1996. Sapindales: molecular delimitation and infraordinal groups. Am. J. Bot. 83 (6), p. 802-811.

Hidayat T., Pancoro A., Kusumawaty D., Eiadthong W., 2011. Molecular Diversification and Phylogeny of Mangifera (Anacardiaceae) in Indonesia and Thailand. Proceeding of the International Conference on Advanced Science, Engineering and information Technology, Putrajaya, Malaysia, p. 88-91

Hou D., 1978. Anacardiaceae (revisions). Flora Malesiana, Series I, 8 (3), p. 395-548.

Kiew R. Teo L.L, Gan Y.Y., 2003. Assesment of the hybrid status of some Malesian plants using Amplified Fragment Length Polymorphism. Telopea 10 (1), p. 225-232.

Kostermans A. J. G. H., Bompard J. M., 1993. The manggoes: Their Bothany, Nomenclature, Horti-culture and Utilization. IBPGR Academic Press. Harcourte Brace & Company. London.

Mukherjee S.K., 1953. Origin, Distribution, and Phylogenetic affinity of the species of Mangifera L. Journal of the Linnean Society, Botany. LV, p. 65-83.

Mukherjee, S.K., Litz R.E., 2009. Introduction: Botany and Importance. The mango 2nd Edition. Botany

productionand uses. Center for tropical Agriculture and Botany-CAB International.

Porebski S, Bailey L.G., Baum B.R., 1997. Modification of a CTAB DNA extraction Protocol for Plants Containing High Polysaccaride and Polyphenol Components, Plant Molecular Biology Reporter 15 (1), p. 8-15.

Roderic D.M., 2001. Tree View Win 32. http://taxonomy.zoology.gla.ac.uk/ rod/rodhtml..

Sawangchote P., Grote P.J., Dilcher D.L., 2009. Tertiary Leaf Fossils of Mangifera (Aanacardiaceae) from Li basin, Thailand as examples of the Utility of Leaf Marginal Venation Characters. American Journal of Botany 96 (11), p. 2048-2061.

Swofford D.L., 2000. PAUP*, Phylogenetic Analysis Using Parsimony (*and Other Methods). Versi 4.0b10. Sinauer Associates.

Teo L.L., Kiew R., Set O., Lee S.K. Gan Y.Y., 2002. Hybrid status of kuwini, Mangifera odorata Griff. (Anacardiaceae) verified by amplified fragmenth length polymorfism. Molecular ecology 11, Blackwell sciene Ltd., p. 1456-1469.

Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin, F., Higgins D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 25, p. 4876-4882.

Yamanaka N., Hasran M., Xu D. H., Tsunematsu H., Idris S., Ban, T., 2006. Genetic relationship and diversity of four Mangifera species revealed through AFLP analysis, Genetic Resources and Crop evolution 53, p. 949-954.

Yonemori K., Honso C., Kanzaki S., Wiadthong W., Sugiura A., 2002. Phylogentic relathionship of mangifera species revealed by ITS sequences of nuclear ribosomal DNA and a possibility of their hybrid origin. Plant Syst. Evol. 231, p. 59-75.

Zhang M., Fritsch P.W., Cruz B.C., 2009. Phylogeny of Caragana (Fabaceae) based on DNA sequence data from rbcL, trnS−trnG, and ITS. Journal Molecular Phylogenetics and Evolution 50, p. 547−559.