Phylogenetic Study of *Mangifera* Central Sumatra Based on *rbcl* Sequences

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Abstract: *Mangifera* from Central Sumatra has a unique ability to adapt to the high rainfall region. Therefore this genus is a potential germplasm source in Sumatera. However the diversity of *Mangifera* was decreasing due to many factors such as deforestation and habitat changes. Those factors may cause loss information of *Mangifera* whereas its diversity and taxonomical status were not yet clear. The aim of this study was to reconstruct the relationship of *Mangifera* based on the *rbc*L sequences. DNA was isolated from leaves using CTAB method. DNA sequencing was done in First Laboratories, Malaysia. The phylogenetic reconstruction was conducted using PAUP* Version 4.0b10 with Maximum Parsimony (MP) and Neighbor Joining (NJ) analysis. Cladogram using MP showed monophyletic tree and was divided into two main clades. The first clad only consist of *M. casturi* and the second clad consist of *M. indic*a, *M. laurina*, *M. sumatrana*, *M. quadrifida*, *M. odorata*, *M. kemanga*, *Mamgifera* sp. and *M. torquenda*. The NJ cladogram supported MP analysis and showed that *Mangifera* sp. has the highest genetic distance to other species.

Keywords: Central Sumatera; *Mangifera*, Molecular Marker; Phylogenetic Analyses; *rbc*L

1. Introduction

Sumatera Island has a high diversity of flora among Central Sumatera region. Mango (*Mangifera*) is member of Anacardiaceae [1] which are naturally dispersed widely in this region, has a unique ability to adapt to the high rainfall region. These characters are different from the mango that grows in dry areas, which makes the summer and drought as a trigger flowering and fruiting. Therefore this Sumatra mango has a potential germplasm source *Mangifera* typical wet areas [2]. Fitmawati *et al.* [2] reported the results of exploration and identification of *Mangifera* in Central Sumatra consisting of Riau Province, Jambi and West Sumatra. *Mangifera* stated that diversity is decreasing due to many factors such as deforestation, habitat changes, industrialization, the expansion of oil palm plantations and others. Those factors may cause loss information of *Mangifera* whereas its diversity and taxonomical status were not yet clear.

The morphology of the genus *Mangifera* quite difficult to distinguish, especially in species closely related. This is because the high morphological plasticity due to high cross conformity between the types *Mangifera*, so morphologies are very common in this genus. Therefore, in addition to characterization using morphological markers, we also needed the support of the data in the form of DNA barcoding to justify the position of the kinds *Mangifera* which firmer and more comprehensive. This approach can be used to study the phylogenetic status and relationship analysis based on evolutionary lineages [3]. In this study, the marker which was used is a coding gene sequences *rbc*L ribulose-1,5-bisphospate carboxylase (RuBisCO) located in the chloroplast genome. This marker set by Barcode of Life Database (BOLD) as a DNA barcode sequences to analyze the genetic diversity of species and is universal in all plant genes[4]. *rbc*L is a marker of a conservative and has a pretty good variety for use in distinguishing between species [5]. Research on the study of molecular-based phylogenetic marker of *Mangifera* Indonesia with *rbc*L already been done by [6]. But for mango Sumatera,

especially in Central Sumatera has not been done. The purpose of this study was to analyze and reconstruct the phylogenetic relationship of *Mangifera* species in Central Sumatera through phylogenetic studies based on *rbc*L sequences that are in the chloroplast genome.

2. Material & Methodology

Plant Material

9 samples of *Mangifera* consisted of *Mangifera indica, M. odorata, M. sumatrana, M. laurina, M. quadrifida, M. torquenda, M. kemanga, M. casturi* and *Mangifera* sp. and *Bouea macrophylla* as outgroup.

DNA Extraction

DNA extraction using CTAB method of Doyle and Doyle [7] with modification. DNAs were then suspended in TE buffer.

Amplification and Sequencing

The genomic DNA was amplified by using universal primer *rbc*L F(CTTGGCATTCCGAGTA) and *rbc*L R(TCACAAGCAGCCAGTTC) [6]. Thirty five cycles of PCR were conducted using Thermal Cycle under following profiles: 95^{o}C for 4 m, 94^{o}C for 30 s, 53^{o}C for 30 s, 72^{o}C for 2 m, 72^{o}C for 10 m. PCR products were sealed by using parafilm before sending them to First Base Laboratories, Malaysia.

Phylogenetic Analysis *Mangifera*

DNA sequences of *rbc*L were first alligned by ClustalW Multiple Allignment in Bioedit [8] both *Mangifera* species and outgroup taxa. The aligned sequence data matrix was analyzed by PAUP 4.0 program [9] for parsimony and neighbor joining method with bootstrap replicate method.

3. Results and Discussion

*rbc*L Sequence Analysis

*rbc*L nucleotide sequencing results with long bases on *Mangifera* species ranged between 1083-1267 bp. DNA base composition is expressed as G+C content, where the content of G+C on all species with the average of 32.2% (Table 1). The results of the long alignment of the nucleotide bases on sequences *Mangifera* used was 1083 bp by the number of indels in a sequence of 227 bases (Table 1). Another sequence which have many indels are *Mangifera* sp.

Phylogenetic Analysis of *Mangifera* Species in Central Sumatra

The results of parsimony analysis based on the sequence data of *rbc*L are summarized in Table 1. The aligned *rbc*L comprises 1083 characters. Of these, 974 characters are constant and 14 characters are parsimony-informative. Based on parsimony criteria obtained cladogram with CI value 0.95 dan RI value 0.83. Evolution tree from 9 *Mangifera* species formed two clade with bootstrap value 100%.

Cladogram as shown in figure 1, it was constructed with maximum parsimony (MP) method and figure 2, was to reconstruct the relationship of *Mangifera* based on neighbor joining (NJ) method. The results of maximum parsimony and neighbor joining analysis reconstructed two clades. The first clad consisted of *Mangifera casturi* and the second clad consisted of *M. indic*a, *M. laurina*, *M. sumatrana, M. quadrifida*, *M. odorata, M. kemanga*, *Mamgifera* sp. and *M. torquenda.*

Table 1 The characteristic features of The *rbc*L among *Mangifera* species and combination with outgroup

	Kisaran Panjang (nt)	Rata-rata Panjang (nt)	Panjang Setelah Allignment (nt)	Kandungan G+C (%)	Rerata G+C (%)	Jumlah Indels	Jumlah Situs Informatif	Panjang Pohon	CI	RI	RC
Mangifera spp.											
Keseluruhan Seluas	1083-1299	1191	1083	21,4-43	32,2	227	12	114	0,98	0,82	0,80
Mangifera spp. + 1 *outgroup*											
Keseluruhan Seluas	1083-1299	1191	1083	21,4-43	32,2	227	14	121	0,95	0,83	0,79

Phylogenetic Relationship Among *Mangifera* Species in Central Sumatra Based on *rbc*L Sequence

Cladogram with maximum parsimony method is divided into two main clades *Mangifera*. Clad I was separating *Mangifera casturi* with other species. This species is assumed as endemic plants and introduction plant in the island of Borneo[10], brought by the tribal people to the Tembilahan Banjar area, Riau and cultivated. This is supported by [11] which shows the separation between species from different geographical conditions since many years ago can cause changes in the DNA sequence.

Clad II consists of two subclad separating sister group of *M. indica* and *M. Laurina* (IIA) with ingroup with bootstrap value of 78%. This is supported by the opinion of [12] using the ITS sequences of the 14 kinds of *Mangifera* in Thailand which grouped seven cultivar of *M. indica* that forms a clad with *M. Laurina*. According to [1], based on morphological characters *M. indica* and *M. Laurina* has floral disks that cushion-like (like pillows) by the number of fertile stamen 1. The distinguishing features of this species is *M. indica* has glomerulate interest, there is a hair on a flowering branch while *M. Laurina have* not glomerulate interest.

Subclad (IIb) consists of two groups with 97% bootstrap values. The first group consisted of *M. quadrifida* and *M. sumatrana* separately with other ingroup with ≤50% bootstrap value. According Fitmawati *et al.* (2013), the texture of the leaves on the mango is divided into two chartaceous on *M. quadrifida* and *M. sumatrana* and coriaceous on *Mangifera* types such as *M. odorata*, *M. kemanga*, *Mangifera* sp. and *M. torquenda*. Based on the differences in character, *M. quadrifida* have the flower color of white and red flowers on *M. sumatrana.*

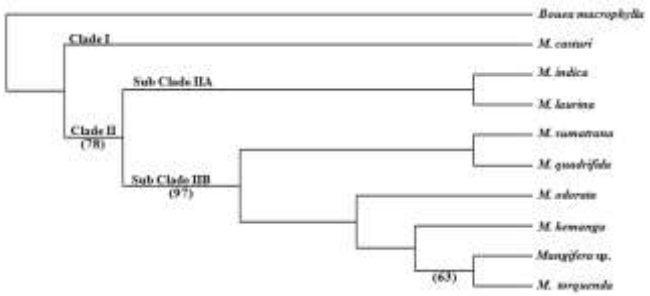

Figure 3 The phylogenetic tree based on the ITS sequences generated from maximum parsimony analysis with bootstrap value below the branch

The second group consists of *M. odorata*, *M. kemanga*, *Mangifera* sp. and *M. torquenda*. [1] grouping *Mangifera* based on morphological characters disk on the flowers into two subgenus, namely subgenus *Limus* and *Mangifera*. *M. odorata* and *M. kemanga* classified in subgenus *Limus* (more primitive), but both of these species belong to a different section. *M. kemanga* in Deciduae section which has bractea covering young leaves while *M. odorata* was not have bractea and included in section Perennes. Based on (Figure 2), *Mangifera* sp. has a close relationship with *M. torquenda* with 63% bootstrap value and formed a group with *M. kemanga*. Based on morphological characters of flowers [2], *Mangifera* sp. and *M. torquenda* have jewelry white flowers and flower (multiple of 4) differs from *M. kemanga* which has a purplish pink flowers and flower (multiples of 5), and the characters are classifying this species is the white flesh [1].

Cladogram with MP method to form a monophyletic clad is derived from a common ancestor [13]. This is supported by the statement [6] who studied 16 species of *Mangifera* from Indonesia and Thailand based on the sequences *rbc*L, [12] and analysis 19 *Mangifera* from Indonesia and Thailand based on the sequence matK [14]. The overall results of supported monophyletic character with character anomocytic stomate on *Mangifera* [14].

Neighbor Joining analysis to reconstruct the phylogenetic tree based on the distance or proximity of relationship between species on the phylogenetic tree was described from a different nucleotide changes. Different genetic distance showed the rate of evolution of each species. Based on the phylogenetic tree *Mangifera* sp. currently on the longest branch of the tree compared to other species. This suggests that this species has a long evolutionary history and assumed as a primitive of *Mangifera* group in Central Sumatra (Figure 2). *Mangifera* sp. assumed to be a new species because it has the distinction based on morphological characters with other types of *Mangifera* Sumatra. Based on exploration conducted [2] derived morphological characters at a different young shoots covered by bract (deciduous), the thickest leaf texture (coriaceous)

among species *Mangifera*, leaf edge wavy, leaf blade opens only 900, petals and white crown four strands, disk 5 large cup-shaped lobes, the head of a black stamens, fruit obovate, soft-textured white pulp and fiber quantity is low. Obovate leaf shape, stem color and white wreath [2]. When viewed from the record [1] possible characters *Mangifera* sp. This has similarities with *Mangifera magnifica* Kochummen distributed Borneo and ecology in the area of Riau, Sumatera. However [1] grouping *M. magnifica* into the subgenus *Mangifera* who do not have a cover bractea or young leaves (deciduous). Furthermore, based on the sequences *rbc*L Phylogenetic analysis showed *Mangifera* sp. closely related to *M. torquenda*. This is supported by the opinion of [1], that *M. torquenda* and *M. magnifica* subgenus *Mangifera* included in the group Tetramerous with morphological characters sepals and petals amounted to 4, so the presence of *Mangifera* sp. is still an opinion that needs to be studied further.

Figure 4. The phylogenetic tree of the genus *Mangifera* based Neighbor Joining method. Figures in brackets under the branches indicate bootstrap values 100x

The nucleotide sequences in each species vary in *Mangifera.* This shows the evolutionary process, one of which is caused by mutations such as deletions, insertions or changes in the composition of bases. Furthermore, mutations may lead to differences in phenotypic characters are encoded by genes. Variations of nucleotide bases obtained by gene sequences coding cpDNA is *rbc*L as ribulose-1,5-bisphospate carboxylase (RuBisCO) obtained quite high, so the sequence can be used in the analysis of phylogenetic relationships at the level of genus, species and infraspesies.

4. Conclusion

Phylogenetic studies of nine species *Mangifera* Central Sumatra based on *rbc*L sequences can be concluded that cladogram analysis results maximum parsimony (MP) formed a monophyletic clad with two main clad *Mangifera*. The first clad consists of *M. casturi* first and the second clad composed of *M. indica, M. Laurina, M. sumatrana, M. quadrifida, M. odorata, M. kemanga, Mangifera* sp. and *M. torquenda*. Cladogram by Neighbor Joining method (NJ) was supported MP analysis and showed *Mangifera* sp. has the longest genetic distance compared to other species. Species that have a close genetic relationship is with *M. Laurina M. indica, M. quadrifida* with *M. sumatrana*, and *Mangifera* sp. by *M. torquenda.*

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