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Molecular Biodiversity of Selected Mango Cultivars Based on DNA Sequences of Internal Transcribed Spacer Region

[1]Topik Hidayat, [2]Shahkila Mohd. Arif and [2]Azman Abd. Samad
[1]Department of Biology Education, Universitas Pendidikan Indonesia (UPI), Bandung, Indonesia
[2]Department of Biological Sciences, Universiti Teknologi Malaysia (UTM), Johor Bahru, Malaysia

Abstract: The mango (*Mangifer indica* L.) is an important species of the family Anacardiaceae and is one of the most important crops cultivated commercially in many parts of the world. Hence, a better understanding of the phylogeny in this species is crucial as it is the basis knowledge of improving its genetic resources which is beneficial for breeding programs. Phylogenetic relationships among 13 mango cultivars from Indonesia, Malaysia and Taiwan were carried out by comparing DNA sequence data sets derived from the Internal Transcribed Spacer (ITS) region pf nuclear ribosomal DNA (nrDNA). Analysis using parsimony method showed that the cultivars were classified into three major groups. The first group composed almost Malaysian cultivars although with low bootstrap value, the second group consisted of mainly Taiwan cultivars and the last group included mostly Indonesia one. The results indicated that some cultivars have a close relationships with each other even it is originated from different countries. With regards to the relationship among these cultivars, this gives better insight for generating new cultivar.

Key words: ITS region, mango, new cultivar, parsimony, phylogenetic analysis

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most prominent members of the family Anacardiaceae that is grown commercially in many parts of the world, particularly tropical and subtropical countries (Rajwana *et al*., 2008, 2011). This is due to several characteristics possess by this climacteric fruit such as stupendous quality (attractive appearance, great taste and adorable flavour) and excellent nutritional composition (diverse amount of fibre, minerals, vitamins and various antioxidant compounds).

Its genotypes are classified into two categories, namely monoembryonic which is mostly from subtropical regions (Indian types) and polyembryonic which is from tropical regions (Southeast Asian types) (Luo *et al*., 2011). Over 4000 years ago, mango is originated from India and Burma and has been spread to Eastern Asia, Eastern Africa and Malaysia (Luo *et al*., 2011). Malaysia is among countries that cultivates mango for commercial production in Southeast Asia. There are several cultivars of mango grown in Malaysia, including Chokanan (MA 224), Harumanis (MA 128), Maha 65 (MA 165) and Nam Doc Mai (223).

In terms of production, it is ranked as the fifth major fruit grown throughout the world after apples, bananas, grapes and oranges and the second most vital tropical fruit (Bally *et al*., 2009). Generally, there are numbers of mango cultivars that arise from naturally occurred open-pollinated seedlings (Iyer and Degani, 1997). As for commercially grown cultivars, seedling selections with respect to characteristics like colour, flavour, size and taste has been done and subsequently, these cultivars are propagated and cultivated in a huge area (Ravishankar *et al*., 2004).

According to Ravishankar *et al*. (2004), information with regards to genetic diversity is one of the most crucial parts in designing breeding programmes in order to maintain mango production. Furthermore, another factor that contributes to the effective production of hybrids is the well-defined phylogenetic relationship between the parents (Nishiyama *et al*., 2006). However, the phylogenetic relationship among numbers of mango cultivars is poorly studied and remains unclear as there are numbers of mango cultivars and sometimes different or synonym names are used referring to a single cultivar. Additionally, the morphological based approach has led to confusion on cultivars identification. Thus, creating a conflict in classification of mango cultivars.

Rapid advances of molecular techniques such as Polymerase Chain Reaction (PCR) bring an impact for the use of DNA sequences in molecular phylogenetic studies

Corresponding Author: Topik Hidayat, Department of Biology Education, Universitas Pendidikan Indonesia (UPI), Bandung, Indonesia

(Topik *et al*., 2005). DNA sequencing-based method is very reliable due to the reproducibility of the results and the scope of application. Duneman (1994) has mentioned that DNA-based markers are very advantageous in characterizing and studying genetics similarities among cultivars, land races and varieties.

Now-a-days, various DNA markers namely Restriction Fragment Length Polymorphism (RFLP) (Ravishankar *et al*., 2004), Random Amplified Polymorphic DNA (RAPD) (Karihaloo *et al*., 2003; Ravishankar *et al*., 2004), Amplified Fragment Length Polymorphism (AFLP) (Yamanaka *et al*., 2006) and Simple Sequence Repeats (SSRs) (Viruel *et al*., 2005; Schnell *et al*., 2006) have been utilized to determine taxonomic identity (Schnell *et al*., 2006), estimate genetic diversity (Viruel *et al*., 2005) and infer evolutionary histories of mango (Yamanaka *et al*., 2006).

In this study, phylogenetic relationship of selected mango cultivars was inferred by using DNA sequences of Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA), a widely used DNA marker in order to resolve phylogenetic relationship at various taxa (Karehed *et al*., 2008). This DNA marker is very useful due to several features such as high copy number, small in size, possess highly conserved flanks, rapid concerted evolution and universality of primers (Baldwin *et al*., 1995).

MATERIALS AND METHODS

In this study, a total of five mango cultivars namely Chokanan (MA 224), Harumanis (MA 128), Maha 65 (MA 165), Mangga Epal (MA 194) and Nam Doc Mai (MA 223) were chosen. The sampling of young and fresh mango leaves were carried out in Institute Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI), Serdang, Selangor with the help of Mr. Ahmad Najib, research officer at MARDI. The collected samples were labeled and kept in a proper storage bag and stored in -20°C freezer to maintain its freshness. Furthermore, the DNA sequences of ITS region of mango cultivars from Indonesia and Taiwan were retrieved from Topik (Unpublished data).

The genomic DNA was extracted from young, flushing mango leaves by using Qiagen DNeasy Plant Mini Kit with a slight modification. The ITS region was amplified by using Polymerase Chain Reaction (PCR) with a set of primers namely AB101 as the forward primer and AB102 as the reverse primer (Table 1).

About 20 μL of amplicon for each sample was inserted to 1.5 mL Eppendorf tube and sealed using parafilm. The amplicons were sent for sequencing at First BASE Laboratories, Seri Kembangan, Selangor.

Table 1: Primer sequence used in this study

Primer	Sequence*
AB101	5-ACG AAT TCA TGG TCC GGT GAA GTG TTC G-3
AB102	5-GAA TTC CCC GGT TCG CTC GCC GTT AC-3

*Topik *et al*. (2005)

The DNA sequences obtained were combined with the DNA sequence of ITS region for Indonesian cultivars, Taiwanese cultivars and the outgroup, *Mangifera oblongifolia* (Topik, Unpublished data) and saved in FASTA format. After that, multiple sequence alignment was performed using ClustalX and the sequences were adjusted manually. Subsequently, phylogeny reconstruction analysis based on the maximum parsimony method was performed to the aligned DNA sequences using PAUP version 5.10 (MEGA 5.1 Beta 3). The bootstrap test with 1000 replicates was conducted to assess the degree of support for each branch with the consensus tree option of retaining groups with frequency >50%.

RESULTS AND DISCUSSION

In phylogenetic study, the construction of phylogenetic tree is very important, as it function as a tool that helps in inferring and elucidating evolutionary relationships among organism. As for this study, relationships among selected mango cultivars were analyzed based on the DNA sequences obtained.

The phylogenetic analysis revealed that the 13 cultivars were classified into three major groups (Fig. 1). The first group consisted of Harumanis (MA 128), Chokanan (MA 224), Mangga Epal (MA 194) and Nam Doc Mai (MA 223) with 54% BS value. The second group included Irwin, Li'ar, Haden and Saigon with moderate BS (72%). Remain cultivars are grouped in group 3.

As shown in the consensus tree (Fig. 1), it is suggested that Mangga Epal (MA 194) is a sister cultivar to Nam Doc Mai (MA 223). A similar pattern of relationship among cultivars such as Harumanis (MA 128) and Chokanan (MA 224), both Mangga Epal (MA 194) and Nam Doc Mai (MA 223) are supported by fruit morphology and characteristics such as tender in texture, yellowish orange colour of flesh, aromatic and sweet in flavour. However, there are few differences between these cultivars such as the shape of the fruit, Mangga Epal (MA 194) is round in shape whereas Nam Doc Mai (MA 223) has oblong shape, the peel of Mangga Epal (MA 194) is yellowish green and sometimes a bit red while Nam Doc Mai (MA 223) has yellowish peel colour (Rajwana *et al*., 2008).

Group 2 is predominant by Taiwanese cultivars. Member of this group shares reddish peel colour. Saigon

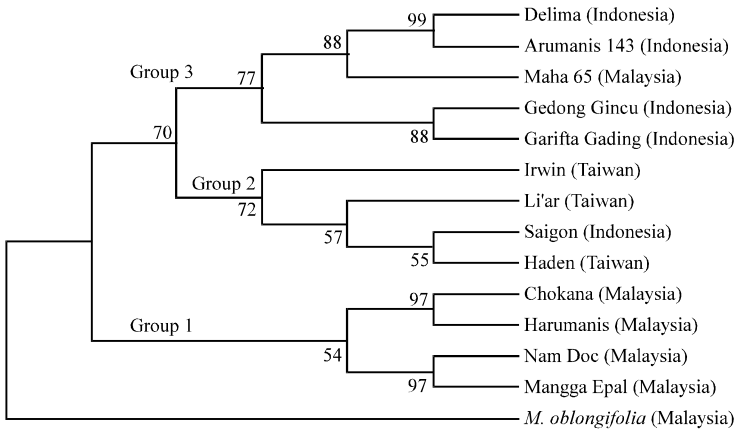

Fig. 1: Strict consensus tree from the parsimony analysis of ITS region (2 most parsimonious tree, Tree length: 471 steps, CI: 0.883, RI: 0.556). The value in the branch represents the bootstrap support (BS)

has yellowish red peel colour, Haden and Irwin has bright yellow with crimson or red blush peel with numerous large yellowish and whitish glands (dots) and Li'ar has reddish peel colour. In addition, Saigon, Haden and Li'ar shared similar flesh texture, which is moderate. On contrary, Irwin possesses soft and tender flesh texture (Rajwana *et al*., 2008; Bally *et al*., 2009).

Group 3 comprises of mostly Indonesian cultivars, with one Malaysian cultivar (Maha 65). They are related since they share similarities in the fruit morphology and characteristics such as oval to oblong in shape and sweet in flavour (Rajwana *et al*., 2008; Bally *et al*., 2009).

From the phylogenetic tree constructed, the evolutionary pattern of the cultivars can be determined. The tree suggested that both group 2 dan 3 has evolved earlier as compared to the group 1 that consisted of cultivars from Malaysia only based on the length of the nodes that branch out from the outgroup. This finding was in line with the previous study that most of Malaysian cultivars were originated from India, Indonesia and Thailand (Yamanaka *et al*., 2006). However, Maha 65 (MA 165) is Malaysian local cultivar which is derived from Kelantan and evolved earlier than Delima, Arumanis 143, Gedong Gincu of Indonesia. This is supported by Bally *et al*. (2009) which indicated that some of the *Mangifera* species are originated from Malaya.

Generally, some cultivars are formed by hybridization between two cultivars in order to improve its genetic resources. For instance, hybridization of Arumanis 143 with Delima has increased the attractiveness and quality of Arumanis 143 (Bally *et al*., 2009). It is suggested that hybridization is possible among different cultivars from the same group rather than between two different groups. This is because hybridization is the result of combination of two or more attributes from different cultivars. Therefore, the cultivars in the same group share similar genetic pattern and tends to possess the same evolutionary pathway (Baldwin *et al*., 1995).

There are several recommendations that can be applied for the betterment of this study in the future. Firstly, it is recommended to employ molecular marker which derived from different genome of the plant such as *rbcL* and *matK* that is derived from chloroplast (cpDNA) and protein-coding genes of mitochondria (mtDNA) in order to determine the most appropriate genetic marker to reconstruct phylogenetic relationship among mango cultivars. Moreover, it is suggested to increase number of mango cultivars samples which enables confirmation of good tree topology and increase the robustness of the tree, thus the relationship among cultivars is predicted accurately.

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