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Genetic variability in apomictic mangosteen (*Garcinia mangostana*) and its close relatives (*Garcinia* spp.) based on ISSR markers

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ABSTRACT

*Sobir, Poerwanto R, Santosa E, Sinaga S, Mansyah E (2011) Genetic variability in apomictic mangosteen (*Garcinia mangostana*) and its close relatives (*Garcinia *spp.) based on ISSR markers. Biodiversitas 12: 59-63.* In order to reveal phylogenetic relationship of mangosteen and several close relatives (*Garcinia* spp.), we employed seven ISSR dinucleotide primer systems on eleven close relatives of mangosteen and 28 mangosteen accessions from four islands in Indonesia (Sumatra, Java, Kalimantan and Lombok). ISSR analysis successfully amplified 43 bands on average 6.1 fragments for each primer system, and these all fragments were polymorphic. Seven close relatives of mangosteen were separated with mangosteen accessions at 0.22 level of dissimilarity, while other four including *G. malaccensis*, were clustered with mangosteen accessions, this results supported proposal that *G. malaccensis* was allopolyploid derivative of mangosteen. Clustering pattern among mangosteen accessions, however, not represented their origin, indicated that distribution of the accessions was not linked to their genetic properties.

Key words: *Garcinia* spp., ISSR analysis, genetic diversity.

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) belongs to the *Guttiferae* family and the genus *Garcinia* (Verheij 1991). *Garcinia* is a large genus that consists of about 400 species (Campbell 1966), and based on examination of herbarium collections and literature study, there are 64 species of *Garcinia* encountered in Indonesia. Twenty-five species were found in Kalimantan, 22 species in Sumatra and Sulawesi respectively, 17 species in Moluccas and Papua respectively, 8 species in Java, and 5 species in Lesser Sunda Island. Six species of those are as cultivation plants (*Garcinia atroviridis, G. beccari, G. dulcis, G. mangostana, G. nigrolineata* and *G. parviflora*), 58 species as the wild plants, 22 species as edible fruits, and 21 species as timber plants (Uji 2007).

The mangosteen has been hailed as the *queen of tropical fruits* (Fairchild 1915), due to its exotic visual appearance and taste appeals, and has recently been popularized for its medicinal benefits (Sakagami et al. 2005; Mahabusarakam et al. 2006). Based on morphological and cytological studies, Richard (1990) proposed that mangosteen originated from South East Asia, and is an allotetraploid derivate of *Garcinia hombroniana* (2n= 48) and *Garcinia malaccensis* (2n = 42). Almeyda and Martin (1976) proposed that mangosteen is a native of Indonesia. In Indonesia mangosteen is distributed almost throughout the archipelago, with the main populations in Sumatra and Kalimantan (Mansyah et al. 1999). However the production centers of mangosteen are in West Sumatra, West Java, Central Java, East Java, and Bali. Commercial production has been limited by slow tree growth, and long juvenile periods (10-15 years).

Base on its reproductive mode mangosteen has been classified as an apomictic plant (Horn 1940; Richards 1997). This plant propagates trough apomixis seed, which is embryo in seed formed without reduction of the chromosome number and fertilization of the egg (den Nijs and van Dijk 1993). Apomixis in mangosteen implies that same genetics properties of parent spread to its progenies (Koltunow et al. 1995); Apomictic processes occur in the ovule without fertilization, resulting in progeny that are genetically exact copies of the female plant (Koltunow et. al. 1995). Based on this assumption mangosteen Horn (1940) stated that existence of only one variety of cultivated mangosteen.

Due to its reproductive manner, mangosteen trees are essentially clonal. However, some distinct variations in morphological characters have been reported. Two type of mangosteen have been identified in terms of shape of fruit, one type producing a round shape with semi-flat bottom end and the other type with oblong shape fruit which cannot stand on its distal end (van Steenis 1981). A wild type containing only four carpels with fully developed seed was also found in north Borneo (Morton 1987). Mansyah et al. (1999) found that mangosteen in West Sumatra show wide variability in leaf length, fruit weight and rind thickness. Mangosteen tree that found in Tembilahan, Sumatra, exhibit flattened fruit shape, verry short peduncle, and elliptic stigma lobe (Mansyah et al. 2005). In recent exploration; we found a new distinctive type of mangosteen in Kalimantan that produces fruit with insignificant size of

seed (less than 1 cm in length), and have bigger fruit size, out with thicker rind, more acidic taste, and larger leaf size (two fold to those of common mangosteen). Variation of sepal color was also found in our collection, with yellow, white and pale orange color of petals compared to red color petal of common mangosteen (Sobir and Poerwanto 2007).

Genetic studies on apomictic plants generally are conducted, through two approaches, parental plants and their progeny variation analysis or molecular analysis (Koltunow 1995). Since mangosteen has a long juvenile phase, it is difficult to carry out progeny analysis, genetic variability analysis of mangosteen was carried out by utilization of molecular tools, such as isozymes (Sinaga 2008), RAPDs (Mansyah et al. 2003; Ramage et al. 2004), and AFLPs (Sinaga 2008). Review on the use of molecular marker has been published by Sobir and Poerwanto (2007). Other potential markers is PCR primers based the microsatellite sequences, where repeat motifs are anchored either at 5' or 3' end with one or few specific nucleotides and amplify the sequences between the two microsatellite loci referred to as inter simple sequence repeat (ISSR) markers. In addition, ISSRs can be targeted towards particular sequences, which are reported to be abundant in the genome and can overcome the technical difficulties of RFLP and RAPD (Rajesh et al. 2002; Petros et al. 2007). Application ISSR has been successfully to reveal genetic variability of mangosteen grown from different Sumatra region (Mansyah et al. 2010)

MATERIALS AND METHODS

Plant material and DNA isolation

Eleven close relatives of mangosteen and 28 mangosteen accessions from four islands in Indonesia (Sumatra, Java, Kalimantan and Lombok) were used in this study (Table 1).

The DNA's were isolated from approximately 1 g leaf by employed modified CTAB method (Doyle and Doyle 1987), by adding 1% polyvinyl pyrolidone (PVP) and 1% 2-mercaptoethanol to the isolation buffer to inhibit phenolic compound interruption, and extracted DNA was purified with RNase.

ISSR Analysis

Purified DNA samples were subjected to ISSR analysis by following protocols. PCR reactions were carried out in a 25 μL reaction mixture containing approximately 25-50 ng templates DNA, 1X solution buffer (50mM KCL, 10 mM Tris-HCL pH 9, 0.01% Tripton X-100), 2.5 mM $MgCl_2$, 200 uM dNTP, 0.4 mM primer, and 1 unit Taq polymerase DNA. Amplification was performed in GeneAmp PCR system 2400 Perkin Elmer, with 40 cycles after pre PCR for 5 minutes at 94ºC. Each cycle consisted of 1 minute 94ºC for denaturation, 1 minute 55ºC for primer annealing 1 minute 72ºC for DNA fragment elongation and finalized with post PCR for 5 minutes 72ºC. Amplification products were electrophoresed in 1.2% Agarose gel at 60 volt for 1 hour. Primers system used in this study represented dimer repeat, used as single primer (PKBT-2, PKBT-4, PKBT-5, PKBT-10) and pair of primer (PKBT-2 + PKBT-4; PKBT-2 + PKBT-6; PKBT-3 + PKBT-6).

Table 1. List of mangosteen accessions and its close relatives subjected analysis.

Name	Accession code	Origin
G. malaccensis	GM-1	Mekarsari Fruit Garden
G. xanthochymus	GX-1	Bogor Botanical Garden
G. celebica-1	GC-1	Mekarsari Fruit Garden
G. porrecta	Gpor	Bogor Botanical Garden
G. sizygiifolia	GS-1	Bogor Botanical Garden
G. picrorhiza	Gpic	Bogor Botanical Garden
G. bancana	GB-1	Bogor Botanical Garden
G. livingstonei	GL-1	Bogor Botanical Garden
G. dulcis	GD-1	Bogor Botanical Garden
G. celebica-2	GC-2	Bogor Botanical Garden
G. hombroniana	GH-1	Bogor Botanical Garden
Tasik-BPSB	JW-1	West Java
Wanayasa-BPSB	JW-2	West Java
Jayanti	JW-3	West Java
Kaliagung-Wanayasa	JW-4	West Java
Cicurug	JW-5	West Java
Cidahu	JW-6	West Java
TWM	JW-7	West Java
Kaliangger-Wanayasa	JW-8	West Java
BungaPutih	CT-1	CETROFS
Bunga3	CT-2	CETROFS
Bunga4	CT-3	CETROFS
Kali-Tajur	CT-4	CETROFS
Kaligesing	JC-1	Central Java
Semarang	JC-2	Central Java
Trenggalek	JE-1	East Java
Ponorogo	JE-2	East Java
Tarutung	SN-1	North Sumatra
Sibolga	SN-2	North Sumatra
Ratu Kamang	SR-1	Riau
Kampar	SR-2	Riau
RejangLebong-1	SB-1	Bengkulu
RejangLebong-2	SB-2	Bengkulu
Lampung	SL-1	Lampung
Pontianak	KW-1	West Kalimantan
Kalteng	KC-1	Central Kalimantan
Malinau	KE-1	East Kalimantan
Kal-Sel	KS-1	South Kalimantan
Lingsar	NW-1	West Nusa Tenggara

Data analysis

The genetic relationships between 13 were studied by means of scorable bands using 5 different ISSR primers. Since ISSRs are dominant, a locus was considered to be polymorphic if the band was present in one lane and absent in the other. The presence or absence of bands was scored as binary code (1 and 0). The binary data were used to arrange the matrix of genetic similarity based on the formula of Nei and Li (1979). Based on the genetic similarity values, a cluster analysis and phylogenetic tree dendogram were constructed using the method of UPGMA (Unweighted Pair-Cluster Method Arithmetic) with NTSYS (Numerical Taxonomy and Multivariate System) version 2.01.

RESULTS AND DISCUSSION

ISSR amplification

The seven ISSR primer systems used in this study successfully amplified 43 bands. The fragment number of each primer ranging from 5 to 8, on average 6.1 fragments per primer, and these entire fragments showed as polymorphic band (Table 2). DNA fragment amplified by ISSR dimer primers in *Garcinia* spp. genomic DNA were slightly lower to the those of amplification in *Cicer arietinum* that average produced 6.7 band for each primer system (Rajesh et al. 2002). Visualization of ISSR finger-print pattern from primer PKBT-2 presented in Figure 1.

Table 2. Sequence of ISSR primers and number of fragment amplification products.

Primer	Sequence	Ampli-fied band	Poly-morphic band
PKBT-2	ACACACACACACACACTT	5	5
PKBT-4	AGAGAGAGAGAGAGAGAA	6	6
PKBT-5	AGAGAGAGAGAGAGAGTA	8	8
PKBT-10	GTGTGTGTGTGTGTGTGTA	5	5
PKBT-2 + PKBT-4	ACACACACACACACACTT AGAGAGAGAGAGAGAGAA	7	7
PKBT-2 + PKBT-6	ACACACACACACACACTT AGAGAGAGAGAGAGAGTT	6	6
PKBT-3 + PKBT-6	AGAGAGAGAGAGAGAGT AGAGAGAGAGAGAGAGTT	6	6
Total		43	43

Clustering pattern of *Garcinia* spp.

Based on ISSR primers amplified bands, a dendogram was generated by UPGMA-link method using Nei and Li similarity (1979), suggesting that genetic diversity among genus *Garcinia* was 0.61 coefficient similarity or 39% dissimilarity (Figure 2). This results indicated that variability of twelve *Garcinia* species revealed by ISSR analysis in this study was lower to our previous study using isozyme analysis that showed 62% dissimilarity, and AFLP analysis that reached 79% dissimilarity (Sobir and Poerwarnto 2007; Sinaga 2008), also as detected by Randomly Amplified DNA Fingerprinting (RAF) marker that observed 63-70% dissimilarity among *Garcinia* spp. (Ramage et al. 2004).

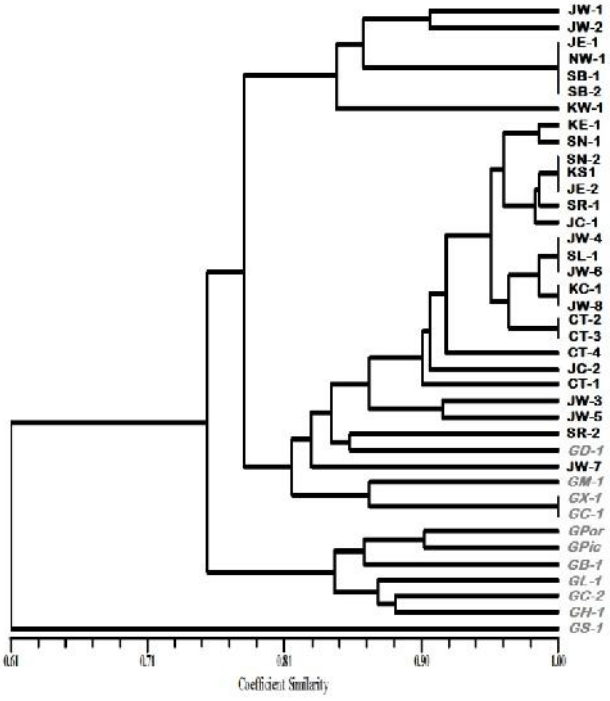

Figure 2. A dendogram based on UPGMA-link method generated from ISSR analysis data using seven ISSR primers system on 28 accessions of mangosteen and 11 its close relatives.

The dendogram also indicated that seven close relatives of mangosteen (*G. sizygiifolia, G. hombroniana, G.celebica-2, G. livingstonei, G. bancana, G. picrorhiza, G.porrecta*) were separated from *G. mangostana* accessions, while other four of *G. xanthochymus, G.malaccensis, G. celebica-1,* and *G. dulcis,* were grouping in same clustered with mangosteen accessions at 22% dissimilarity level. This results resemble to our previous studies using isozyme and AFLP markers (Sobir and Poerwarnto 2007; Sinaga 2008). However the dendogram also indicated that *G. malaccensis* flanked *G. mangostana* accessions with equal genetic distance with other six close relatives (*G. hombroniana, G. celebica-2, G. livingstonei, G. bancana, G. picrorhiza, G. porrecta*), raised question

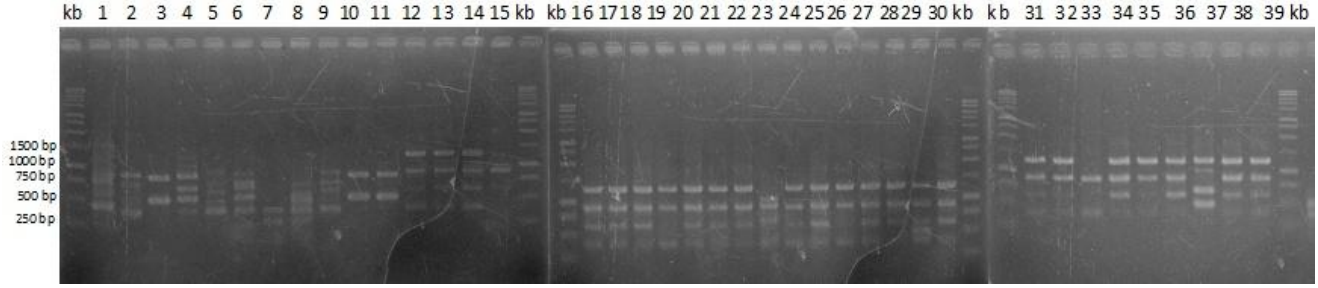

Figure 1. ISSR fingerprint pattern 28 accessions of mangosteen and 11 its close relatives generated using PKBT-2 primer. Lanes 1-39: *Garcinia malaccensis , G. xanthochymus, G. celebica* TWM, *G. porrecta, G. sizigyfolia, G. picrorhiza, G. bancana, G. livingstonei, G. dulcis, G. celebica* KRB, *G. hombroniana*, Tasik BPSB, Wanayasa BPSB, Jayanti, Kaliagung Wanayasa, Cicurug, Cidahu, TWM, Kalianger Wanayasa, Bunga Putih, Bunga3, Bunga4, Kali-Tajur, Kaligesing, Semarang, Trenggalek, Ponorogo, Tarutung, Sibolga, Kampar, Rejang Lebong1, Rejang Lebong2, Lampung, Pontianak, Kalteng, Malinau, and Kalsel, respectively. Kb: DNA size marker.

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regarding the ancestor of the mangosteen. Our analysis using isozyme and AFLP markers suggested that *G. porrecta* has higher share of genetic properties compare to *G. hombroniana* (Sobir and Poerwarnto 2007; Sinaga 2008), and supported by higher similarity in fruit morphology of *G. mangostana* to *G. porrecta* than to *G. hombroniana*. Based on obtained molecular data, the proposal of Richard (1990) that mangosteen is an allotetraploid derivate of *G. hombroniana* and *G. malaccensis* should be reviewed.

Genetic variability of mangosteen

A dendogram based on UPGMA-link method generated from ISSR analysis data using seven ISSR primers system on 28 accessions of mangosteen and 11 its close relatives, revealed that all mangosteen accessions clustered at 0.78 coefficient of similarity or 0.22 coefficient of dissimilarity (Figure 2). This results indicated that variability revealed by ISSR analysis was lower to our previous study using RAPD analysis 0.33 (Mansyah 2003), isozyme analysis that showed 0.58 coefficient of dissimilarity, and AFLP analysis that reached 0.58 coefficient of dissimilarity (Sobir and Poerwarnto 2007; Sinaga 2008). However, this result higher as detected by Randomly Amplified DNA Fingerprinting (RAF) that observed only 0.2-1% dissimilarity among *G. mangostana* accessions (Ramage et al. 2004).

Clustering pattern among mangosteen evaluated accessions, however, not following their origin. Mangosteen accessions from East Java (JE-1) shared same ISSR banding pattern with accession from West Nusa Tenggara (NW-1) and two accessions from Bengkulu, Sumatra (SB-1 and SB-2), subsequently accessions from North Sumatra (SN-2) shared same ISSR banding pattern with KS-1 from South Kalimantan and JE-2 from East Java. However, two accessions from same location Wanayasa West Java, JW-2 and JW-4 were separately at 0.22 coefficient of dissimilarity. This results supported by previous observation results using RAPD markers on 92 *G.mangostana* accessions from Indonesian Archipelago indicated that the clustering pattern not represented their origin (Sinaga 2008).

Discussions

The results of ISSR analysis in this study confirmed previous study using molecular tools (Mansyah 2003; Ramage et al. 2004; Sobir and Poerwanto 2007; Sinaga 2008), however a question arise what is the source of the variation, since mangosteen is considered as apomixis obligate plant that performs clonally seed reproduction, independent from fertilization (Koltunow et al. 1995). The variation may have arisen from accumulation of natural mutations. Spontaneous somatic mutations have played an essential role in the speciation and domestication of vegetativelly propagated crops such as banana and plantain.

Carman (2001) suggests that apomicts result from wide hybridization of ancestral sexual parents having distinct phenotypic traits related to reproduction. It was possible that *G. mangostana* did not originate from a single hybridization of its ancestral sexual parents, as Southeast Asia, including Indonesia, is a diversity center of *Garcinia*. Our recent analysis using isozymes, RAPD and AFLP markers revealed a genetic variability among accessions of *G. malaccensis* that collected from Jambi, Sumatra (Sinaga 2008). The possibility that development of the ancestral mangosteen was not established from a single hybridization, would lead to variation among mangosteen populations. However, this assumption not supported by our results, since clustering pattern of mangosteen accessions in this study was not following the accessions origin.

Another possibility of genetic variability in mangosteen could be in ploidy developmental processes. Our research on three groups of parents and progenies of mangosteen indicated genetic variability among the progenies, where their genetic similarity to parent trees ranged from 0.59 to 1.0. (Sinaga 2008). In a previous study (Mansyah et al. 2004), genetic variation occurred between mangosteen mother plants and their offspring. Many forms of genetic variation may have arisen after hybridization of sexual ancestors with divergent reproductive traits (Spillane et al. 2001).

CONCLUSION

ISSR analysis on 28 accessions of mangosteen and 11 its close relatives using seven primer system have successfully amplified 43 bands on average 6.1 fragments for each primer system, and these all fragments were polymorphic. Seven close relatives of mangosteen were separated with mangosteen accessions at 0.22 level of dissimilarity, while other four including *G. malaccensis*, were clustered with mangosteen accessions, this results supported proposal that *G. malaccensis* was allopolyploid derivative of mangosteen. Clustering pattern among mangosteen accessions, however, not represented their origin, indicated that distribution of the accessions was not linked to their genetic properties

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