RESEARCH ARTICLE

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PHYLOGENETIC ANALYSIS OF MANGOSTEEN (*Garcinia mangostana L.*) AND ITS RELATIVES BASED ON MORPHOLOGICAL AND INTER SIMPLE SEQUENCE REPEAT (ISSR) MARKERS

SULASSIH[1], SOBIR[2*] and SANTOSA E[2]

[1]Center for Tropical Horticulture Studies, Bogor Agricultural University, Jl Pajajaran Baranangsiang, Bogor, Indonesia
[2]Department of Agronomy and Horticulture, Faculty of Agriculture at Bogor Agricultural University and Center for Tropical Horticulture Studies, Bogor Agricultural University, Jl Pajajaran Baranangsiang, Bogor, Indonesia
*Corresponding author's email: sobir@ipb.ac.id

SUMMARY

Mangosteen and its relatives within the genus *Garcinia* L. belong to the family Guttiferae that contains about 35 genera and up to 800 species. *Guttiferae* diversity is found across the Indonesian archipelago. In order to elucidate the genetic diversity of mangosteen and its relatives, morphological and molecular analyses were conducted. The objectives for this study were: (1) to determine the relationships between mangosteen and its relatives; and (2) to confirm the true diversity of allotetraploid mangosteen relatives *G. mangostana*. Analysis was conducted using morphological and inter simple sequence repeat (ISSR) between 19 accessions of *G. mangostana* and their close relatives revealed. Diversity analysis was based on 212 polymorphic characters and 3 groups were formed. Group A consisted of *Garcinia mangostana*, *Garcinia malaccensis*, *Garcinia celebica*, *Garcinia hombroniana* and *Garcinia porrecta*; group B comprised *Garcinia forbesii* and *Garcinia subellptica*; and group C solely with *Calophyllum inophyllum*... The genetic similarity of *Garcinia mangostana*, *Garcinia malaccensis* and *Garcinia celebica* were 0.78 and 0.63. The epidermis cell observations around stomata cells on the lower surface of leaves revealed that *Garcinia mangostana* has the intermediate shape between *Garcinia celebica* and *Garcinia malaccensis*. It shows that there is a close relationship among *Garcinia celebica, Garcinia malaccensis* and *Garcinia mangostana*. It was determined that *Garcinia malaccensis* and *Garcinia celebica* were ancestors based on morphological and ISSR markers.

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Keywords: Garcinia mangostana, molecular markers, morphological markers, phylogenetic analysis

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INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) known as the "queen of tropical fruits" (Fairchild, 1915), belongs to family *Guttiferae* and genus *Garcinia* (Verheij, 1991). Almeyda and Martin (1976) stated that mangosteen is a native of Indonesia where it is distributed throughout the archipelago, with the main populations in Sumatra and Kalimantan (Mansyah *et al*., 1999).*Garcinia* is a large genus that consists of about 400 species. Based on the examination of herbarium collections and a literature review, there are 77 species of *Garcinia* in Indonesia. The 25 species are found in Kalimantan, 22 species in Sumatera and Sulawesi, 17 species in

Moluccas and Papua, 8 species in Java, and 5 species in Lesser Sunda Island of Indonesia. Six species of these are cultivated (*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).

Richard (1990) stated that mangosteen originated from Southeast Asia and is an allotetraploid derivate of *Garcinia hombroniana* (2n = 48) and *Garcinia malaccensis* (2n = 42) based on 13 morphological markers. Yapwattanaphun and Subhadrabandhu (2004) stated *G. mangostana* has similarity with *G. atroviridis, G. cowa, G. dulcis, G. malaccensis, G. mangostana, G. rostrata and G. vilersiana* using internal transcribed spacer regions in the ribosomal DNA (rDNA). It shows that the other species than *G. malaccensis* and *G. hombroniana* could be candidates as ancestors of mangosteen.

It is important to determine the genetic diversity and relationship between *G. mangostana* and several close relatives. Genetic diversity can be determined using a morphological and molecular analysis such as ISSR. The advantages of ISSR markers include: (1) they are not being influenced by season and environment; (2) require 5-50 ng template of DNA per reaction; (3) represent loci throughout the genome; (4) can generate higher polymorphism higher than RAPD (Gao *et al*., 2006); (5) produce inter species polymorphisms (Zietkiewicz *et al*., 1994; Soltis *et al*., 1998; Kumar *et al*., 2009). Therefore, the objectives of this study were to: (1) determine the relationships between mangosteen and its relatives; and (2) to confirm mangosteen relatives that were suspected to be parents of allotetraploid *G. mangostana* using the morphological and molecular markers.

MATERIALS AND METHODS

Plant materials

The plant materials included 21 from the Guttiferae family, 7 from the genus *Garcinia* and 1 from the genus *Calophyllum*. They were collected from 4 locations in Bogor and West Java, Indonesia (Table 1). The elevation was measured using GPS Garmin type eTrex 30. Material was planted several years ago at Bogor Botanical Garden Indonesian Institute of Sciences ($06^0$35'S, $106^0$47'E): *Garcinia hombroniana* was planted in 2005, *Garcinia hombroniana* in 2006, *Garcinia porrecta* (P1 and P2) unknown and *G. celebica* unknown but had the first flower in April 1965. Other material was planted at Mekarsari Fruit Garden ($06^0$25'S, $106^0$59'E) (*Garcinia malaccensis* in 1997 and *Garcinia celebica* in 1995). *Garcinia subelliptica* and *Chalophylum inophyllum* was planted in 1993 and *Garcinia celebica, Garcinia porrecta* and *Garcinia forbesii* were planted in 1999 at Tajur station of Center for Tropical Horticulture Studies ($06^0$38'S, $106^0$49'E). *Garcinia mangostana* was planted in 1999 at Leuwiliang mangosteen farm Bogor ($06^0$36'S, $106^0$37'E).

Morphological analysis

Observations were taken for 29 morphological characters consisting of 25 characters including flower, fruit, leave, latex color, 2 stomata characters and 2 epidermis cell character (Table 2). Morphological characters of flowers, leaves and fruits were observed referring to IPGRI (2003). Documentation was done using a digital camera (Canon Powershot A480). Colors were measured according to standard color chart of Royal Horticultural Society (5th Edition).

Stomata characters and epidermis cell based on Sass (1958) technique. Epidermis cell walls divided into sinuous and flat type (Musa *et al*., 1989). Rugayah (2007) classified the epidermis based on cell walls into deeply sinuous and slighty sinuous. Epidermis cell size consists of short cells (approximately 18-35 μm) and long cell (around 17-92 x 8-12 μm to 50-192 x 6-14 μm) (Tabrani G *et al*., 1989). Each of the morphological characters are divided into several subcharacters, depend on the number of sub-character variations found.

Molecular analysis

The DNA was isolated from 0.1 g young leaf tissue (3-week old plant) at the terminal position

using a modified CTAB method (Doyle and Doyle, 1987), by adding 1% polyvinyl pyrolidone (PVP) and 1% 2-mercaptoethanol to the isolation buffer to inhibit phenolic compounds. DNA concentration was determined by comparing with 1 µl λ DNA (Promega catalog number D150A). PCR reactions were carried out in a total volume of 13 µl containing reaction mixture 20 ng of genomic DNA 1 µl approximately, 1 µl primer, 6 µl Go Taq master mix (catalog number M712B) and 5 µl pure water. Amplification was performed in an Applied Biosystem 2720 thermal cycler, with 35 cycles after pre PCR for 5 minutes at 94^0C. Each cycle was for 1 minute at 94^0C for denaturation, 1 minute at 48-54^0C for primer annealing, 1 minute at 72^0C for DNA fragment elongation and ended with post PCR for 5 minutes at 72^0C. Amplified products were electrophoresed on 1.2% agarose gel (Promega catalog number V3121) at 50 volt for one hour in

Table 1. List of mangosteen accessions and its close relatives used in the analysis.

No.	Accession	Code	Location	Elevation (m above sea level)	Origin
1.	*G. celebica*	Ce2	Bogor Botanical Garden Indonesian Institute of Sciences	272 m	Sulawesi
2.	*G. hombroniana1*	H1	Bogor Botanical Garden Indonesian Institute of Sciences	274 m	Bangka Belitung
3.	*G. hombroniana2*	H2	Bogor Botanical Garden Indonesian Institute of Sciences	260 m	Bangka Belitung
4.	*G. malaccensis no1*	M1	Mekarsari Fruit Garden Bogor	102 m	Jambi
5.	*G. malaccensis no2*	M2	Mekarsari Fruit Garden Bogor	125 m	North Sumatera
6.	*G. malaccensis no3*	M3	Mekarsari Fruit Garden Bogor	104 m	North Sumatera
7.	*G. celebica C17*	C17	Mekarsari Fruit Garden Bogor	103 m	South Sumatera
8.	*G. celebica C18*	C18	Mekarsari Fruit Garden Bogor	102 m	South Sumatera
9.	*G. celebica AJ*	AJ	Tajur, Center for Tropical Horticulture Studies Bogor	351 m	Mekarsari Fruit Garden Bogor
10.	*G. celebica AD*	AD	Tajur, Center for Tropical Horticulture Studies Bogor	350 m	Mekarsari Fruit Garden Bogor
11.	*G. porrecta*	AB	Tajur, Center for Tropical Horticulture Studies Bogor	356 m	Mekarsari Fruit Garden Bogor
12.	*G. porrecta*	P1	Bogor Botanical Garden Indonesian Institute of Sciences	290 m	Ambon, Maluku
13.	*G. porrecta*	P2	Bogor Botanical Garden Indonesian Institute of Sciences	292 m	Ambon, Maluku
14.	*G. Forbesii*	For	Tajur, Center for Tropical Horticulture Studies Bogor	356 m	Mekarsari Fruit Garden Bogor
15.	*Calophyllum inophyllum*	Ny	Tajur, Center for Tropical Horticulture Studies Bogor	351 m	Cilacap, Center Java
16	*G. subelliptica*	Fu	Tajur, Center for Tropical Horticulture Studies Bogor	353 m	Okinawa, Japan
17.	*G. mangostana*	L1	Leuwiliang mangosteen farm Bogor	406 m	Leuwiliang Bogor
18.	*G. mangostana*	L2	Leuwiliang mangosteen farm Bogor	410 m	Leuwiliang Bogor
19.	*G. mangostana*	L3	Leuwiliang mangosteen farm Bogor	412 m	Leuwiliang Bogor
20.	*G. mangostana*	L7	Leuwiliang mangosteen farm Bogor	382 m	Leuwiliang Bogor
21.	*G. mangostana*	L10	Leuwiliang mangosteen farm Bogor	399 m	Leuwiliang Bogor

Table 2. Morphological markers used in this study.

No	Morphological markers	Sub-characters
1.	Color of young leaf	Light green, light green with brownies, red brown
2.	Color of mature leaf	Green, dark green
3.	Leaf blade shape	Ovate, obovate, elliptic, oblong, lanceolate
4.	Leaf apex shape	Acute, acuminate, retuse, obtuse,
5.	Flower clustering habit	One, two, more than three flower per cluster
6.	Number of petal	Four, five
7.	Petal color	Yellow, yellow green, yellow with pink margin, white, greenish yellow, pink
8.	Sepal color	Green, white
9.	Color of peduncle	Green, white
10.	Flower size	Small, medium, large
11.	Position of flower	Axillary, terminal, both
12.	Number of sepal	Four, five
13.	Fruit clustering habit	One, two, three, more than three
14.	Position of fruit	Axillary, terminal, both
15.	Fruit shape	spherical/round, flattened, ovoid, oblong
16.	Color of stigma lobe	Brown, dark brown, red
17.	Epicarp thickness	Thin, medium, thick
18.	Mature fruit color	Purple, red, green, yellow, deep red
19.	Petal of fruit	Absent, present
20.	Fruit tip	Flat, long tip
21.	Fruit segment color	Snowy white, yellow, creamy white, no aril
22.	Number of fruit segments	Four, six, seven, eight, no aril/segment
23.	Seed shape	Reniform, rounded
24.	Seed coat color	Light brown, brown, dark brown, black
25.	Color of latex	Yellow, white
26.	Width and length of stomata cell ratio	Large, medium, small
27.	Shape of epidermis cell wall at abaxial/upper surface leaf	Sinuous sharply, sinuous deeply, flat
28.	Shape of epidermis cell wall at adaxial/lower surface leaf	Sinuous sharply, sinuous deeply, flat
29.	Stomata cell at abaxial and adaxial of leaf	Absent, present

Table 3. The 11 ISSR primers used in this study.

No.	Primer	Sequence	Annealing temperature	No	Primer	Sequence	Annealing temperature
1	PKBT 2	(AC)8 TT	53 ^{0}C	7	ISSRED 20	(TCC) 5A	48 ^{0}C
2	PKBT 3	(AG)8 T	53 ^{0}C	8	ISSRED 23	(CT) 8T	48 ^{0}C
3	PKBT 4	(AG)8 AA	53 ^{0}C	9	ISSRED 17	(GAC) 5	48 ^{0}C
4	PKBT 8	(GA)9 C	54 ^{0}C	10	ISSRED 12	(AGAC) 4	36 ^{0}C
5	PKBT 9	(GA)9T	54 ^{0}C	11	ISSRED 18	(GGAT) 4	48 ^{0}C
6	PKBT 11	(GT)9 C	54 ^{0}C				

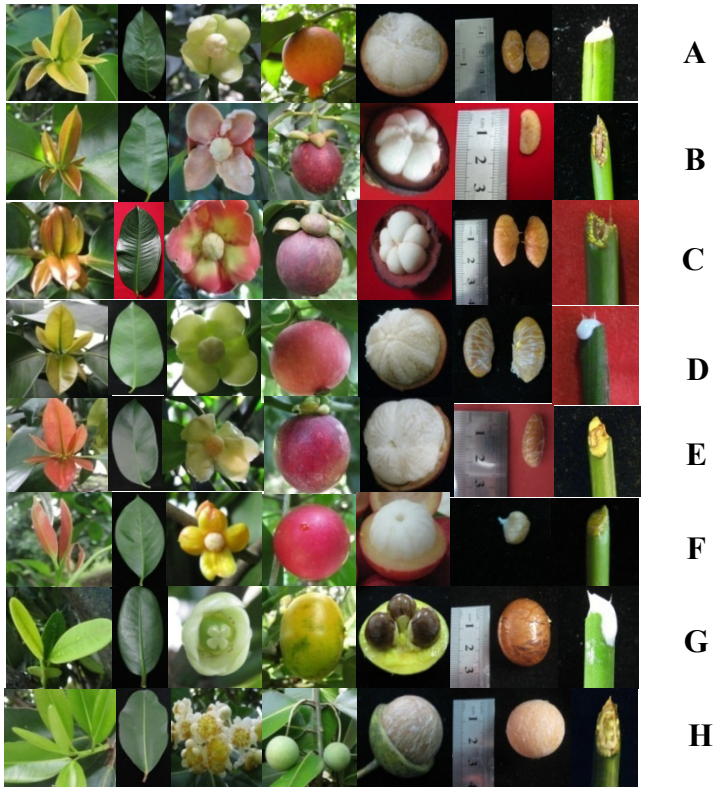

Figure 1. Morphological marker observed color of young leaf, mature leaf, flower, fruit, segment/aril, seed and latex for (A) *G. hombroniana,* (B) *G. malaccensis,* (C) *G. mangostana,* (D) *G. celebica,* (E) *G. porrecta,* (F) *G. forbesii,* (G) *G. subelliptica* (G) and (H) *C. inophyllum*.

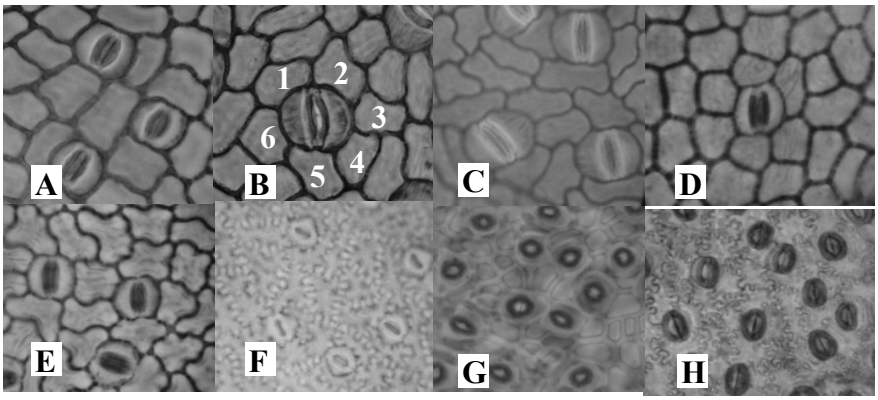

Figure 2. Epidermis cell and stomata type at lower leaf surface for (A) *G. hombroniana,* (B) *G. malaccensis,* (C) *G. mangostana,* (D) *G. porrecta,* (E) *G. celebica,* (F) *G. forbesii,* (G) *G. subelliptica,* and (H) *C. inophyllum* was observed using a microscope (400x zoom and Canon Powershot A480 digital camera at 3.3x optical zoom). Cells around the stomata are indicated by number 1-6.

Table 4. Number of amplified marker alleles in *G. mangostana* and its relatives using ISSR primers.

Primer	Total number of bands	Number of polymorphic bands	Number of monomorphic bands
PKBT2	13	12	1
PKBT3	9	9	0
PKBT4	11	11	0
PKBT8	10	10	0
PKBT9	12	12	0
PKBT11	10	10	0
ISSRED12	15	15	0
ISSRED17	14	14	0
ISSRED18	13	13	0
ISSRED20	9	9	0
ISSRED23	14	14	0
Total	130	129 (99.23%)	1 (0.77%)

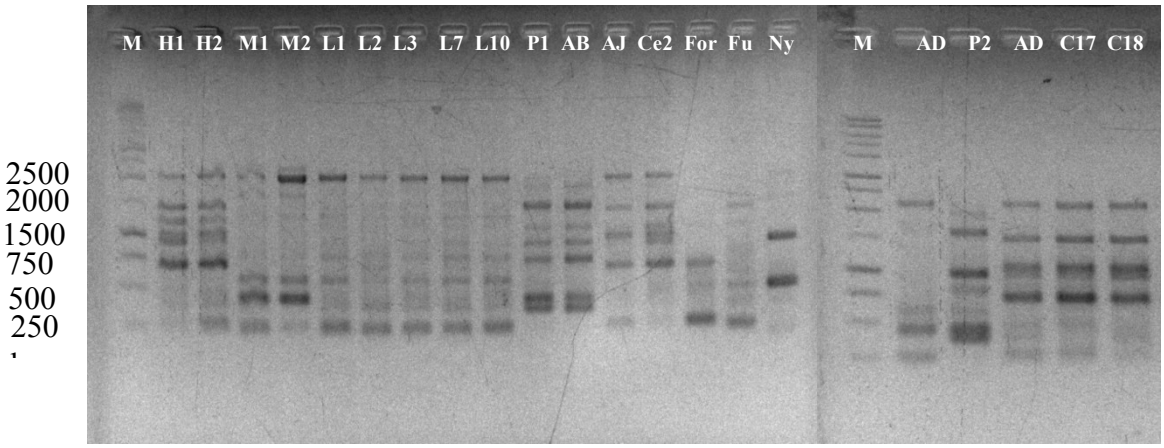

Figure 3. ISSR fingerprint pattern of mangosteen and its close relatives generated using PKBT8 primer. Lanes: *G. hombroniana* (H1, H2), *Garcinia malaccensis* (M1, M2, M3), *G. celebica* Tajur Center for Horticulture Tropical Studies (AD, AJ), *G. celebica* Botanical Garden (Ce2), *G. celebica* Mekarsari Garden (C17, C18), *G. porrecta* Tajur Center for Horticulture Tropical Studies (AB), *G. porrecta* Botanical Garden (P1, P2), *G. mangostana* (L1, L2, L3, L7, L10), *G. subeliptic* (Fu), *G. forbesii* (For) and *C. inophyllum* (Ny) , respectively. M: DNA size marker.

Table 5. The combination of morphological markers and ISSR markers.

No.	Characters/Primers	Number of amplified characters or marker	Number of polymorphic characters or markers
1.	Color of young leaf	3	3
2.	Color of mature leaf	2	2
3.	Leaf blade shape	2	2
4.	Leaf apex shape	4	4
5.	Flower clustering habit	3	3
6.	Number of petal	2	2
7.	Petal color	6	6
8.	Sepal color	2	2
9.	Flower of stalk	2	2
10.	Flower size	2	2
11.	Position of flower	3	3
12.	Number of sepal	2	2
13.	Fruit clustering habit	3	3
14.	Position of fruit	2	2
15.	Fruit shape	3	3
16.	Color of stigma lobe	3	3
17.	Fruit skin thickness	3	3
18.	Mature fruit color	5	5
19.	Petal of fruit	2	2
20.	long shape at the tip of fruit	2	2
21.	Segment color	4	4
22.	Number of segment per fruit	5	5
23.	Seed shape	2	2
24.	Seed coat color	3	3
25.	Color of latex	2	2
26.	Width and length of stomata cell ratio	3	3
27.	Shape of epidermis cell at upper surface leaf	3	3
28.	Shape of epidermis cell lower surface leaf	3	3
29.	Stomata cell at upper and lower of leaf	2	2
30.	PKBT-2(AC)8 TT	13	12
31.	PKBT-3 (AG)8T	9	9
32.	PKBT-4 (AG)8TT	11	11
33.	PKBT-8 (GA)9C	10	10
34.	PKBT-9 (GA)9T	12	12
35.	PKBT-11 (GT)9C	10	10
36.	ISSRED-12 (AGAC)4	15	15
37.	ISSRED-17 (GAC)5	14	14
38.	ISSRED-18 (GGAT)4	13	13
39.	ISSRED-20 (TCC)5A	9	9
40.	ISSRED-23 (CT)8T	14	14
	Total	213	212 (99.53%)

1X TAE buffer (Promega catalog number V4271) and stained with ethidium bromide (Sigma catalog number E8751). DNA bands were visualized under UV light and photographed using digital camera. The ISSR primers used in this study target the dimer repeat as forward and reverse primers (Table 3).

Data Analysis

The genetic relationships were analyzed using 29 morphological characters and 11 different ISSR primers. Morphological characters that were evaluated are described in Table 2. Characters were scored as 1 (present) and 0 (absent).

Since ISSRs were dominant, a locus was considered to be polymorphic if the band was present in one lane and absent in the other. Polymorphic DNA bands were scored as 1 for present band or 0 for absent. The binary data were used to calculate a genetic similarity matrix based on the formula of Nei and Li (1979). Based on the genetic similarity values, cluster analysis and a phylogenetic tree dendrogram constructed using the method of UPGMA (Unweighted Pair-Cluster Method Arithmetic) with NTSys (Numerical Taxonomy and Multivariate System) version 2.01 (Rohlf, 1998)

RESULTS

Morphological variation

Morphological marker data indicated that *Garcinia mangostana* and the other *Garcinia* species have morphological differences that can be seen in their phenotype characteristics, such as the flower color, young leaves, fruit pericarp, latex and fruit shape (Figure 1) and stomata shape and epidermis cell type (Figure 3). Young leaf color consisted of light green with brown for *G. hombroniana, G. malaccensis, G. mangostana,* and *G. celebica,* red-brown for *G. porrecta* and *G. forbesii* and light green for *G. subelliptica* and *G. inophyllum*. Petal color categories were: (1) *G. celebica, G. porrecta* and *G. hombroniana,* which have yellow green color; (2) *G. malaccensis* had pink color; (3) *G. mangostana* had a yellow color with a pink margin; (4) *G. forbesii* with yellow; (5) *G. subelliptica* had greenish yellow; and (6) *C. inophyllum* looked white.

Latex color was classified into yellow and white. *G. malaccensis, G. mangostana, G. porrecta, G. forbesii, C. inophyllum* all have yellow latex and *G. hombroniana, G. celebica, G. subelliptica* have white latex. Pericarp color for mature fruit included purple for *G. malaccensis, G. mangostana* and *G. porrecta*; (2) deep red for *G. celebica* and *G. hombroniana*; (3) red for *G. forbesii*; (4) green for C. *inophyllum*; and (5) yellow *G. subelliptica.* Fruit segment color was divided into snowy white *G. mangostana, G. malaccensis* and *G. forbesii*, yellow *G. subelliptica*, creamy white *G. celebica, G. porrecta*, *C. inophyllum.* The character of *G. hombroniana* had a long tip whereas the others have flat tip.

Stomata cells for 19 accessions were the anomocitic type which has 4-7 cells around the guard cell. This cell was not really different than epidermis cell on shape and size (Cutler *et al.* 2008). Almost all of the accessions had stomata at the lower leaf surface, except *G. forbesii* which had stomata at the upper and lower leaf surface. Epidermis cell walls at the lower surface were divided into flat, deeply and slightly sinuous categories (Rugayah, 2002): (1) flat for *G. hombroniana, G. porrecta* and *G. subelliptica*; (2) deeply sinuous for *G. malaccensis, G. mangostana, G. celebica*; and (3) slighty sinuous for *G. forbesii and C. inophyllum*. Epidermis cell walls on *G. mangostana* looked like a combination shape between *G. Malaccensis* and *G. celebica* shape (Figure 2). Stomata size ratios based on width to length consist of: (1) large size (1:2-3) for *G. hombroniana* (1:2)*, G. malaccensis* (1:2)*, G. celebica* (1:2), *G. porrecta* (1:2); (2) medium size (ratio 1:2) for *C. inophyllum* (1:1.6); and (3) small size (ratio 1:1) for *G. subelliptica* (1:0.98).

Molecular variation

G. mangostana and its relatives amplified 130 markers using 11 primers ISSR of which only 1 was monomorphic (Table 4). The most informative primer was ISSRED12 which produced 15 markers and the primers PKBT3

and ISSRED20 produced the lowest numbers of polymorphic markers.

G. mangostana has genetic variability based on DNA markers although the reproduction is clonal. The variability within *G. mangostana* was revealed using ISSRED18 and ISSRED23 primers. ISSR detected genetic variation but morphological characters did not.

G. malaccensis has similarity with *G. mangostana* using markers generated by PKBT9 primer. The ISSR marker profile for *G. mangostana, G. malaccensis* and *G. celebica* based on PKBT8 primer is shown in Figure 3.

Combination of morphological and molecular markers

The morphological and ISSR markers were used to clarify the relationship between the proximity of the character *G. mangostana* and its relatives. Both markers were successfully amplified, in which out of 213 bands there were 212 polymorphic bands (99.53%) and 1 monomorphic band (0.47%) as shown in Table 5.

The cluster analysis divided into 3 groups at a level of 52% similarity. The first group (A) consisted of 5 species: *G. malaccensis*, *G. mangostana, G. hombroniana*, *G. celebica* and *G. porrecta.* The second group (B) includes *G. forbesii* and *G. subelliptica*, and the third group (C) contained only species *C. inophyllum* (Figure 4). The correlation matrix (r) was 0.98997, which means that grouping was separated within sub genus Garcinia, sub *Brindonia/Xanthocymus* and the out-group. Sari (2000) classified the genus Garcinia into 14 sub-genus/sections. Group A was classified into sub-genus Garcinia and sub-genus Brindonia/Xanthocymus. *G. subelliptica* is classified into sub-genus Brindonia or Xanthocymus. The out-group of the sub-genus consisted of *C. inophyllum.*

The dendrogram generated by UPGMA using Nei and Li similarity coefficient (1979) suggested that the genetic diversity in 8 genera of *Garcinia* ranged between 40% to 100% similarity (Figure 4). This is consistent with previous results based on isozymes analysis which indicated 48% dissimilarity, and AFLP analysis that detected 79% dissimilarity (Sobir and Poerwanto, 2007; Sinaga, 2008) as well as the result by Randomly Amplified DNA Fingerprinting (RAF) markers revealed 63-70% dissimilarity among *Garcinia* spp. (Ramage et al, 2004).

The dendrogram also indicated that the close relatives of *G. mangostana* are *G. malaccensis*, *G. hombroniana*, *G. celebica*, and *G. porrecta*. They belong to group A, while *G. forbesii* and *G. subelliptica* (Group B) and *C. inophyllum* (group C) are more distant to *G. mangostana.* It shows *G. mangostana* is similar to *G. malaccensis* (0.73-0.78) (Table 6). *G. mangostana* and *G. celebica* were similar (0.60-0.63). *G. hombroniana* was similar with *G. mangostana* (0.59-0.61). Based on similarity coefficient, *G. celebica* was closest to *G. mangostana* rather than *G. hombroniana.* Results indicated that *G. malaccensis* and *G. celebica* were probable ancestors.

DISCUSSION

Results of morphological and molecular ISSR analysis confirmed the previous studies using molecular tools (Mansyah 2003; Ramage *et a*l., 2004; Sobir and Poerwanto, 2007; Sinaga, 2008). Mangosteen is considered as an apomixis obligate plant that performs clonal seed reproduction, (i.e. independent from fertilization; Koltunow *et al*. 1995). Genetic variation was detected on molecular markers but variation was not detected using morphological marker.

Carman (2001) suggested that the apomicts result from wide hybridization of ancestral sexual parents had distinct phenotypic traits related to reproduction. It is possible that *G. mangostana* was a hybrid from ancestral sexually reproducing parents. Richards (1990) hypothesized that *G. mangostana* is a hybrid of dioecious plants with *G. hombroniana* as the female ancestor and *G. malaccensis* as the male ancestor. However, this hypothesis is not supported by our results based on the clustering pattern of mangosteen accessions in present study. Our analysis using the morphological and ISSR markers revealed genetic diversity among accessions. The highest similarity coefficient between *G. mangostana* and *G. malaccensis* was

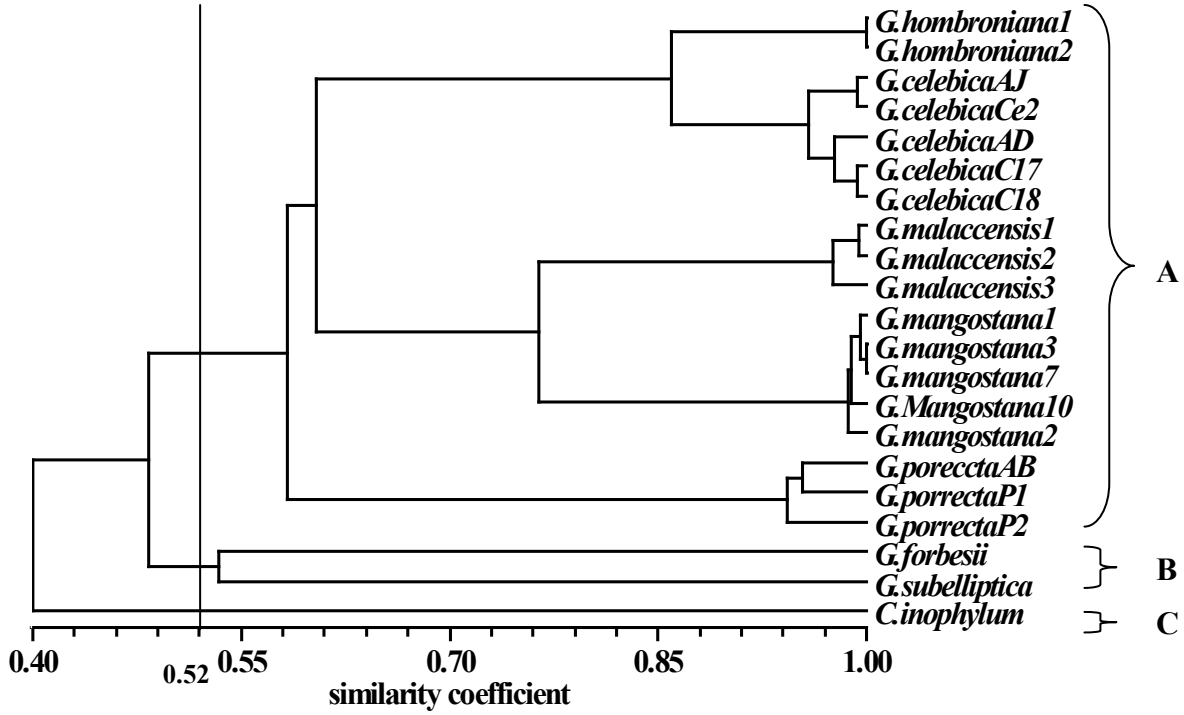

Figure 4. A dendrogram based on UPGMA generated from 29 morphological and 11 ISSR primers of mangosteen and its close relatives.

Table 6. Similarity coefficient *G. mangostana* and its relatives based on a combination of morphological and ISSR markers.

Accession	H1	H2	M1	M2	M3	L1	L2	L3	L7	L10	AB	P1	P2	AJ	Ce2	AD	C17	C18	For	Fu	Ny
H1	1.00																				
H2	1.00	1.00																			
M1	0.56	0.56	1.00																		
M2	0.56	0.56	0.99	1.00																	
M3	0.55	0.55	0.98	0.97	1.00																
L1	0.61	0.60	0.77	0.77	0.74	1.00															
L2	0.59	0.59	0.76	0.76	0.73	0.98	1.00														
L3	0.61	0.61	0.77	0.77	0.75	0.99	0.99	1.00													
L7	0.61	0.61	0.77	0.77	0.75	0.99	0.99	1.00	1.00												
L10	0.60	0.60	0.77	0.78	0.75	0.98	0.98	0.99	0.99	1.00											
AB	0.58	0.58	0.57	0.58	0.55	0.59	0.60	0.59	0.59	0.59	1.00										
P1	0.54	0.54	0.56	0.56	0.53	0.55	0.56	0.55	0.55	0.56	0.95	1.00									
P2	0.58	0.58	0.58	0.58	0.55	0.57	0.57	0.57	0.57	0.58	0.93	0.94	1.00								
AJ	0.87	0.87	0.58	0.58	0.57	0.61	0.61	0.62	0.62	0.61	0.61	0.59	0.63	1.00							
Ce2	0.86	0.86	0.58	0.58	0.57	0.62	0.62	0.62	0.62	0.61	0.60	0.59	0.62	0.99	1.00						
AD	0.84	0.83	0.59	0.60	0.59	0.61	0.60	0.61	0.61	0.60	0.59	0.58	0.62	0.95	0.95	1.00					
C17	0.86	0.86	0.60	0.60	0.59	0.62	0.62	0.62	0.62	0.62	0.61	0.58	0.63	0.95	0.96	0.97	1.00				
C18	0.85	0.85	0.60	0.60	0.60	0.62	0.62	0.63	0.63	0.62	0.60	0.59	0.63	0.96	0.96	0.97	0.99	1.00			
For	0.46	0.46	0.51	0.51	0.51	0.49	0.50	0.49	0.49	0.49	0.36	0.37	0.37	0.50	0.49	0.48	0.47	0.48	1.00		
Fu	0.51	0.51	0.50	0.50	0.49	0.50	0.50	0.51	0.51	0.50	0.44	0.43	0.44	0.52	0.51	0.51	0.50	0.50	0.53	1.00	
Ny	0.41	0.41	0.39	0.38	0.39	0.38	0.39	0.38	0.38	0.38	0.39	0.37	0.39	0.41	0.40	0.40	0.40	0.40	0.45	0.46	1.00

Note : H1 = *G. hombroniana1* Botanical Garden, H2 = *G. hombroniana2* Botanical Garden, M1 = *G. malaccensis1* Mekarsari Fruit Garden, M2 = *G. malaccensis2* Mekarsari Fruit Garden, M3 = *G. malaccensis3* Mekarsari Fruit Garden, L1 = *G. mangostana* Leuwiliang, L2 = *G. mangostana2* Leuwiliang, L3 = *G. mangostana3* Leuwiliang, L7 = *G. mangostana7* Leuwiliang, L10 = *G. mangostana10* Leuwiliang, AB = *G. porrecta Tajur,* P1 = *G. porrecta* Botanical Garden, P2 = *G. porrecta* Botanical Garden, AJ = *G. celebica* Tajur, Ce2 = *G. celebica* Botanical Garden, AD = *G. celebica* Tajur, C17 = *G. celebica* Mekarsari Fruit Garden, C18 = *G. celebica* Mekarsari Fruit Garden, For = *G. forbesii* Tajur, Fu = *G. Subelliptica* Tajur, Ny = *Calophyllum inophyllum* Tajur

0.78. Between *G. mangostana* and *G. celebica* similarity was 0.63. Based on this analysis, it can be assumed that *G. mangostana* is closely related with *G. celebica* and *G. malaccensis*. Our results strengthened the findings of Sobir and Poerwanto (2007) and Sinaga (2008) that *G. celebica* is similar to *G. mangostana* (based on AFLP markers). Matra (2010) used SSR alleles through IGMB001 (Ibaraki/IPB *Garcinia mangostana* Bogor 001) indicated that *G. mangostana* has an allele of equal size to *G. malaccensis* (233 bp) and *G. Mangostana* has the same allele at 252 base pairs as *G. celebica*. Yapwattanaphun (2004) stated that *G. hombroniana* did not group with *G. malaccensis* and *G.mangostana* based on internal transcribed spacer. Tirtawinata (2003) reported in grafting compatibility between *G. mangostana* and *G. celebica* on vegetative propagation.

CONCLUSION

Diversity analysis was based on 212 polymorphic characters revealed relationship *Garcinia mangostana* to other species of Garcinia, and successfully grouped *Garcinia* genus separated from *Calophyllum inophylluminophyllum.* The relationship of *G. mangostana*, *G. malaccensis* and *G. celebica* is at the similarity coefficient level of 0.78 and 0.63. The epidermis cell observations around the stomata cell on the lower leaf surface revealed that *G. mangostana* has an intermediate shape between *G. celebica* and *G. malaccensis*. It shows that there is close relationship between *G. celebica, G. malaccensis* and *G. mangostana*. It was determined that *G. malaccensis* and *G. celebica* were ancestors based on morphological and ISSR markers.

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