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Preliminary Studies on Morphological and Molecular Characterization of Wild *Garcinia* in Sijunjung, West Sumatra

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A total of 18 samples of five *Garcinia* species from two sites in Sijunjung district, West Sumatra were evaluated using morphological traits and molecular markers. The species comprised four wild species, *G. xanthocymus, G. atroviridis*, *G. nervosa* and *G. macrophyla,* and one cultivated species, *G. mangostana.* Morphological variability was observed with respect to flower and fruit position, leaf size, flower colour, fruit shape and size, the existence of stigma lobe, calyx, and the corolla in ripe fruit. Using RAPD markers, highest inter-specific diversity was observed in *G. xanthocymus* with genetic similarity coefficient ranging from 0.75 to 0.88, while the three other species exhibited similar range from 0.80 to 0.88. The genetic similarity coefficient among species was from 0.60 to 0.88. There are two types of each species based on their sites of occurrence and also their morphological and molecular characters. The information generated will be of use in devising *in situ* conservation strategy and sustainable use of *Garcinia* species.

Key Words: *In situ* conservation, Molecular markers, Morphological traits, Wild *Garcinia* species

Introduction

Indonesia is an important agrobiodiversity centre for tropical fruit species and their wild relatives. Sumatran lowland rain forests is an important center of biodiversity (Stolton *et al.,* 2006); majority of fruit species currently being cultivated in this area are indigenous and have wild relatives. Four genera of indigenous fruits are recommended to be developed in Indonesia, *i.e. Durio, Mangifera, Garcinia* and *Nephelium* (Uji, 2007). There are 77 *Garcinia* species in Indonesia of which 22 are grown in Sumatra, comprising cultivated, wild, edible and timber species. In Borneo, there are 20 *Garcinia* species native to Indonesia, four of which are cultivated while others grow wild in the forest (Uji, 2004; Uji 2007).

A study to assess the diversity of wild *Garcinia* at eight locations in West Sumatra and Jambi Provinces indicated rich diversity in Nagari Latang, and Nagari Kampung Dalam, at Sijunjung district, West Sumatra. Five *Garcinia* species occuring in the above areas, include *G. atroviridis* (asam gelugur), *G. xanthocymus (*asam kandis*), G. macrophyla* (manggis jepang) and one unidentified *Garcinia* (manggis hutan). All the above species grow in the forest interior and in the buffer zone (Winarno *et al*., 2013).

It is well known that the Sumatran lowland rain forests are under great risk, with high levels of habitat conversion (66%) and low levels of protection (4.9%). Wild and cultivated fruit species diversity in Asia is threatened by rapid genetic erosion due to habitat destruction, extension of agriculture, filling up of wetlands, conversion of biodiversity rich sites for human settlement and industrial development. Deforestation and land use conversion have put immense pressure on wild fruit tree resources leading to their loss and its relatives (FAO, 2003).

There is an urgent need to develop and upscale successful strategies to conserve both cultivated and wild relatives of tropical fruit species in Asia for economic, cultural, and ecological reasons (IPGRI, 2003). *In situ* conservation and on-farm conservation protects threatened plants in their natural habitat and takes into account social and cultural factors such as farmers' perceptions and knowledge (Maxted, 2006). Before implementing *in situ*/on-farm conservation activities, knowledge of the levels of genetic diversity is important for planning, managing and monitoring *in situ* conservation activities. Hence, the objective of the present study was to determine the level of genetic diversity in the target populations of cultivated and wild *Garcinia* species occuring at Sijunjung, West Sumatra using morphological and molecular methods.

Materials and Methods

The experimental material consisted of 18 trees belonging to five, wild and cultivated *Garcinia* species, collected

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Table 1. Details of *Garcinia* species used in the present study

Samples	Code	Vernacular name	Botanical name	Habitat	Source of sample/Origin
1	X1	Asam kandis	*Garcinia xanthocymus*	Forest	Mother tree/Latang
2	X2	Asam kandis	*Garcinia xanthocymus*	Forest	Mother tree/Latang
3	X3	Asam kandis	*Garcinia xanthocymus*	Forest	Mother tree/Latang
4	X4	Asam kandis	*Garcinia xanthocymus*	Forest	Mother tree/Latang
5	X5	Asam kandis	*Garcinia xanthocymus*	Forest	Seedling/Latang
6	X6	Asam kandis	*Garcinia xanthocymus*	Forest	Seedling/Latang
7	X7	Asam kandis	*Garcinia xanthocymus*	Forest	Seedling/Latang
8	A1	Asam gelugur	*Garcinia atroviridis*	Forest	Mother tree/Latang
9	A2	Asam gelugur	*Garcinia atroviridis*	Forest	Mother tree/Latang
10	A3	Asam gelugur	*Garcinia atroviridis*	Forest	Seedling/Latang
11	A4	Asam gelugur	*Garcinia atroviridis*	Forest	Seedling/Latang
12	A5	Asam gelugur	*Garcinia atroviridis*	Forest	Seedling/Latang
13	G1	Manggis Jepang	*Garcinia macrophyla*	Forest	Seedling /Kampung Dalam
14	G2	Manggis Jepang	*Garcinia macrophyla*	Forest	Seedling/Kampung Dalam
15	G3	Manggis Jepang	*Garcinia macrophyla*	Forest	Mother tree/Latang
16	H1	Manggis Hutan	*Garcinia nervosa*	Forest	Mother tree/Latang
17	H2	Manggis Hutan	*Garcinia nervosa*	Forest	Seedling/Latang
18	M	Manggis	*Garcinia mangostana*	Home Garden/ Orchard	Mother tree/Latang

from Nagari Latang and Kampung Dalam, Lubuk Tarok subdistrict, Sijunjung district, West Sumatra. The location was selected based on previous survey of *Garcinia* species diversity found in this region (Winarno *et al.*, 2013). The five species comprised four wild species (*G. xanthocymus*, *G. atroviridis*, *G. macrophyla* and *G. nervosa*, and one cultivated species (*G. mangostana*) (Table 1).

Morphological Evaluation

All the 18 samples were evaluated for 25 morphological traits based on descriptors developed by IPGRI (2003) (Table 3). The main traits studied were the branching system, leaves, flowers and fruits position. The flower characters included the colour of inner and outer calyx and corolla, and the existence of calyx and corolla in the ripe fruits. Fruit characters included shape, size, pedicel length, colour, fruit surfaces, number of segments, the existence of stigma lobe, seed number and latex colour (Table 3).

Molecular Evaluation

DNA Extraction

About 0.1 mg fresh leaflets of 18 *Garcinia* species (Table 1) were ground for DNA extraction. Total DNA was extracted according to the modified CTAB protocol (Doyle and Doyle, 1990) using 1% PVPP (polyvinyl polypyrrolidone). DNA concentration was determined with electrophoresis in agarose gel, ethidium bromide staining solution and visualization on UV transilluminator.

DNA Amplification

DNA was PCR-amplified by using eight RAPD primers (Table 2) in a 96-well Applied Biosystems 2720 thermal cycler. Reactions were carried out in a total volume of 25 µl consisting of 2 µl (20 ng) of template DNA, 12.5 µl Go Taq Green Master Mix (Promega M7122), 1 µl primer (20 µM), and 9.5 µl free nuclease water. Amplification was performed under the following conditions: 4 min at 94°C for 1 cycle, followed by 0.5 min at 94°C, 0.5 min at annealing temperature (depending on the primer used), and 1 min at 72°C for 35 cycles, and 5 min at 72°C for final extension. Forty five cycles were repeated with initial denaturation at 94°C for 30 sec. Primer annealing temperature was 37°C for 45 sec and extension at 72°C for 2 min. The final extension step was carried out at 72°C for 7 min. The amplification products were electrophoresed on 2% agarose gel in 1X TAE buffer.

Data Analysis

The DNA banding pattern was observed under UV light after staining with ethidium bromide (0.1µg/ml of gel solution) and photographed in Gel-Doc (Biometra). PCR

Table 2. Primers used for the molecular characterization of *Garcinia* species

Primer	Sequence (5'----------3')
OPH13	CACGCCACAC
OPH18	GAATCGGCCA
P1	GGTGCGGGAA
P2	GTTTCGCTCC
P3	GTAGACCCGT
P4	AAGAGCCCGT
P5	AACGCGCAAC
P6	CCCGTCAGCA

Table 3. Morphological variability for different traits in *Garcinia* species from Sijunjung, West Sumatra

Characters	*G. xanthocymus*		*G. atroviridis*		*G. macrophyla*		*G. nervosa*		*G. mangostana*
	Type 1 (site 1 and 2)	Type 2 (site 1 and 2)	Type 1 (site 1)* Type 2	Type 2 (site 2)** Type 2	Type (site 1) Type 2	Type 2 (site 2) Type 2	Type 1 (site 1) Type 2	Type 2 (site 2) Type 2	
Tree									
Branch position	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite
Leaf									
Leaf position	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite	Opposite
Leaf length (cm)	7-10	4-5	25-30	25-30	30-35	30-35	50-60	50-60	25-30
Leaf width (cm)	4-5	2-3	10-16	10-16	15-20	15-20	20-25	20-25	15-20
Flower									
Flower/fruit position	Terminal	Terminal	Terminal	Terminal	Axillary	Axillary	Axillary	Axillary	Terminal
Colour of outer calyx	Absent	Absent	Green	Green	Green	Green	Green	Green	Green
Colour of inner calyx	Absent	Absent	Red	Red	Red	Red	Yellow	Yellow	Red
Corolla	Light Yellow	Light Yellow	Red, remains attached until ripe	Red, remains attached until ripe	Red	Red	Light Yellow	Light Yellow	Red margin, yellowish in the centre, fall after full bloom
Fruit									
Outer calyx	Absent	Absent	Green	Green	Absent	Absent	Absent	Absent	Green
Colour of inner calyx	Absent	Absent	Green	Green	Absent	Absent	Absent	Absent	Green
Corolla	Absent	Absent	Red	Red	Absent	Absent	Absent	Absent	Absent
Stigma lobe	Not prominent	Not prominent	Prominent	Prominent	Prominent	Prominent	Not prominent	Not prominent	Prominent
Stigma lobe diameter (cm)	–	–	2-2.5	2-2.5	0.3-0.5	0.3-0.5	–	–	1.5-2
Fruit weight (gram)	10-20 (Big)	5-10 (Small)	200-600	200-400	55-100	55-100 gram	250-300	250-300	50-300
Calyx	Absent	Absent	Present	Present	Absent	Absent	Absent	Absent	Present
Fruit shape	Round	Oblong	Flattened	Flattened	Flattened	Flattened	Round	Oblong	Round and Flattened
Fruit surface	Smooth	Smooth	Corrugated	Corrugated	Stripes	Smooth	Smooth	Smooth	Smooth
Mature fruit colour	Orange	Orange	Orange	Orange	Yellow	Yellow	Yellow	Yellow	Deep Purple
Pedicel length (cm)	None	None	None	None	None	None	1-2	3-4	1.5-3.0
Pericarp thickness (cm)	2-3	2-3	4-5	4-5	3-4	3-4	1-1.5	1-1.5	0.5-1.0
Flesh colour	Orange	Orange	Light Yellow	Light Yellow	Light Yellow	Light Yellow	Yellow	Yellow	White
Taste	Sour	Sour	Sour	Sour	Sour	Sour	Sour, Astringent	Sour, Astringent	Sweet-sour
Number of fruit segment	7-8	7-8	8-13	13 or more	9-11	9-11	–	–	4-8
Number of seed	2-4	2-4	2-6	2-6	1-8	1-8	3-5	3-5	1-2
Latex colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow

* Nagari Latang, Sijunjung district, West Sumatra

** Nagari Kampung Dalam, Sijunjung district, West Sumatra

products were then scored as present (1) or absent (0) for the 18 samples analysed in the present study. Genetic diversity and the relationship between samples were calculated through Jaccard's coefficient and a sequential, agglomerative, hierarchical and nested (SAHN) cluster analysis was carried out. This analysis was performed using the un-weighted pair group method with arithmetic means (UPGMA) algorithm computed by NTSYS-pc *(*Numerical Taxonomy and Multivariate Analysis) software version 2.1 (Rohlf, 2000).

Results and Discussion

Morphological Evaluation

Garcinia species have a characteristic feature of opposite position of the branches and leaves. Amongst species there was profound variability in flower and fruit position, leaf size, flower colour, fruit shape and size, the existence of stigma lobe, calyx and the corolla in the ripe fruit (Table 3). The flower and fruit of *G. mangostana* and *G. atroviridis* are terminal buds, while in the three others, these are borne as axillary buds. Two species, *G. xanthocymus* and *G. nervosa* have no stigma lobe, while three others have the stigma lobes but of different sizes. The stigma lobe diameter of *G. atroviridis, G. mangostana* and *G. macrophyla* are 2-2.5 cm, 1.5-2.0 cm and 0.3-0.5 cm, respectively. The species *G. xanthocymus* has the smallest leaves and fruits with leaf length varying from 5-7 cm and fruit weight ranging from 10-20 g. The species *G. nervosa* has the largest leaves, about 50-60 cm in length, whereas *G. atroviridis* and *G. mangostana* have medium leaves, 25-30 cm in length.The calyx and corolla are present in the ripe fruit of *G. atroviridis*, whereas in *G. mangostana* there is only a calyx as the corolla falls when the flowers are in full bloom. Three other species have no calyx and corolla in the ripe fruits. The general morphological traits of the five species are the leaves and branching system which are born in pairs and have yellow latex in all parts of the plant.

Based on these morphological characters each of the wild species could be divided into two types which were separated into different sites. It was observed that the *G. atroviridis, G. macrophyla* and *G. nervosa* from Nagari Latang (site1) were different from those of Nagari Kampung Dalam (site 2), while the two types of *G. xanthocymus* occuring at each site differed with respect to leaf and fruit shape. The first type has bigger leaves, (12 cm long and 3.5 cm wide) with round fruits. The leaves of the second type are about 8 cm long and 2.5 cm wide, with round fruits.

G. atroviridis showed variability in the number of fruit segments. The first type found at site 1 has 8 to 13 segments, and the second type present at site 2 has more than 13 segments. The key character which differentiates the two types of *G. macrophyla*, is the type of stripes on the fruit surface. The fruit skin surface of *G. macrophyla* from site 1 has longitudinal stripes while that from site 2 has a smooth skin with no stripes on pericarp. The two types of *G. nervosa* differ in fruit shape and pedicel length. The first type has round fruits with a short pedicel and the second type has an oblong fruit with a long pedicel. Comparative morphological variation within species can be observed from data presented in Table 1 and from Figure 1.

Molecular Characterization

Polymorphism of RAPD Markers

It is clear from Table 4 that there is polymorphism amongst 18 trees of five *Garcinia* species. Eight RAPD primers yielded a total of 152 bands, with a maximum of 26 for P3 (AAGAGCCCGT) and a minimum of 15 for P2 (GTAGACCCGT) and P4 (AACGCGCAAC). The

Table 4. The amplification products by eight RAPD primers in 18 *Garcinia* plants

Primer code	Primers sequence (5'----------3')	Total scorable bands	Polymorphic bands	Monomorphic bands
OPH13	GAATCGGCCA	22	22	0
OPH18	GGTGCGGGAA	24	24	0
P1	GTTTCGCTCC	16	16	0
P2	GTAGACCCGT	15	15	0
P3	AAGAGCCCGT	26	26	0
P4	AACGCGCAAC	15	15	0
P5	CCCGTCAGCA	18	18	0
P6	CACGCCACAC	16	15	1
	Total	152	151 (99.003%)	1(0.007%)
	Average	19	18,875	0,11

Fig. 1. The two types of *G. macrophyla*: (a) fruit surface with stripes in pericarp, (b) smooth surface with no stripes in pericarp, (c) two types of *G. nervosa*: short pedicel and round fruits, (d) long pedicel and oblong fruits

Table 5. Morphological variations within *Garcinia* species from Sijunjung, West Sumatra

Species	Main morphological characters	Locations
G. xanthocymus		
Type 1	Large leaves (10 to 12 cm in length and 3 to 3.5 cm in width), oblong fruits	Site 1 and 2
Type 2	Small leaves (8 to 9 cm in length and 2-2.5 cm in width), round fruits	Site 1 and 2
G. atroviridis		
Type 1	Fruit segments 8 to 13	Site 1
Type 2	Fruit segment more than 13	Site 2
G. macrophyla		
Type 1	fruit surface with stripes in pericarp	Site 1
Type 2	Smooth surface with no stripes in pericarp	Site 2
G. nervosa		
Type 1	Short pedicel, round fruit	Site 1
Type 2	Long pedicel, oblong fruit	Site 2

152 markers consisted of 151 (99.003%) polymorphic bands and 1 (0.007 %) monomorphic band, and these ranged in size from 250 to 2500 bp. This result indicates the wide genetic variation among the species.

Amplification products of eight RAPD primers produced several DNA fragments and DNA banding patterns that were unique to certain samples. Part of PCR products of *Garcinia* species by primer OPH 13, OPH 18, P1 and P5 are presented in Figure 2. The arrows indicate the unique DNA fragments and DNA banding patterns. *G. xanthocymus* and *G. atroviridis* exhibited considerable variability in molecular data. Among the seven samples of *G. xanthocymus* the tree number 4 (X4) exhibited very different and unique banding pattern for each primer used. Morphologically, the X4 tree belongs to type 2 (small leaves) while the other trees belong to type 1 (large leaves).

Similar to *G. xanthocymus*, the DNA banding pattern of *G. atroviridis* samples were variable indicating the wide genetic variability in this species. The RAPD analysis showed that the offsprings differ in genetic diversity from the mother plant. This condition is related to the cross-pollinated reproduction system of *G. atroviridis*. The *G. atroviridis* is gynodioecoius plant in which female and hermaphrodite individuals co-occur (Pangsuban *et al*., 2007).

The aditional bands, OPH 13/1600 bp, OPH 18/ 250 bp, P1/250 bp and 1000 bp, were present in *G. macrophyla* collected from site 1 (G3) and these were absent in two others samples from site 2 (G1 and G2). The bands indicated by the arrow in Figure 2. The G3 plants are morphologically unique due to presence of stripes in fruit skin. The unique morphology (Figs. 1a, 1b) of this sample correlates with the unique banding patterns (Fig. 2).

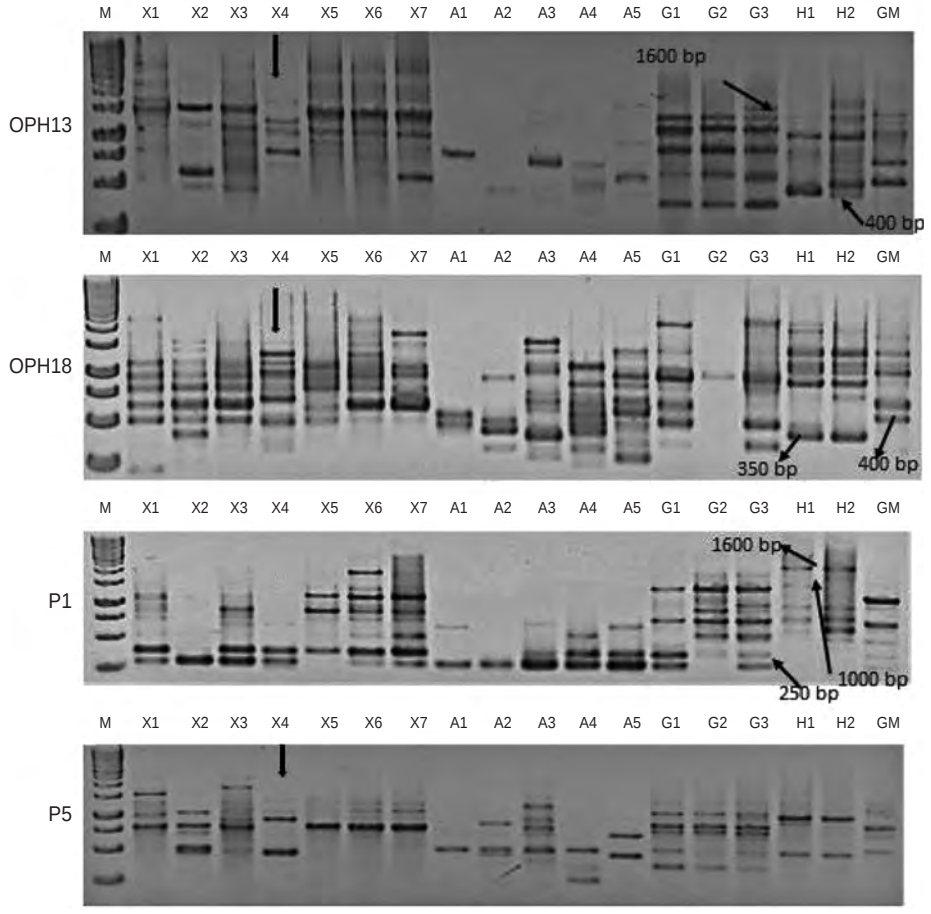

Fig. 2. Part of PCR products of *Garcinia* species by primer OPH 13, OPH 18, RAPD1 and RAPD 5

It is clear from the amplification pattern in *G. nervosa* that the two samples, mother tree (H1) and the offspring (H2) exhibit genetic differences. The PCR products of *G. nevosa* also displayed a number of unique bands. The bands produced by primer OPH13 at 400 bp are present in H2 and absent in H1 whereas OPH-18/400 bp and P1/1600 bp are present in H1 and absent in H2.

From these analyses, all the samples could be differentiated using the eight RAPD markers. This indicates that RAPD markers can be used to distinguish between and within species of *Garcinia*. Random Amplified Polymorphic DNA (RAPD) technique is a simple and quick method to determine the level of genetic diversity. This technique requires less DNA 10-25 ng, does not require DNA sequence information, not radioactive, its implementation is relatively easy, and produce higher estimates for interspecific similarity (Powell *et al.*, 1996; Gupta *et al*., 1996). Nevertheless, RAPD technique has some limitations, among others, cannot distinguish homozygous and heterozygous individuals for being as dominant markers (Williams *et al.*, 1990). Small changes in the reaction conditions can significantly alter the amount and intensity of the amplification products so that reproducibility is difficult to be maintained. (Hallden *et al*., 1996). Further research is needed using more samples of *Garcinia* and better molecular methods such as microsatellite or Simple Sequence Repeat (SSR)

Genetic Relationships among Samples

Genetic similarity coefficient among the 18 *Garcinia* trees based on RAPD analysis varied from 0.60 to 0.88 (Fig. 3.). These results are similar to genetic diversity among 11 *Garcinia* spp. using ISSR (Inter Simple Sequence Repeat) markers which was 0.61 to 1.0 in similarity coefficient (Sobir *et al*., 2011). Isozyme analysis of *G. mangostana* and 10 related species showed genetic similarity coefficient of 0.14 to 0.97 (Sinaga *et al*., 2010). According to Ramage *et al.* (2004) regarding genetic diversity among nine *Garcinia* spp. using Randomly

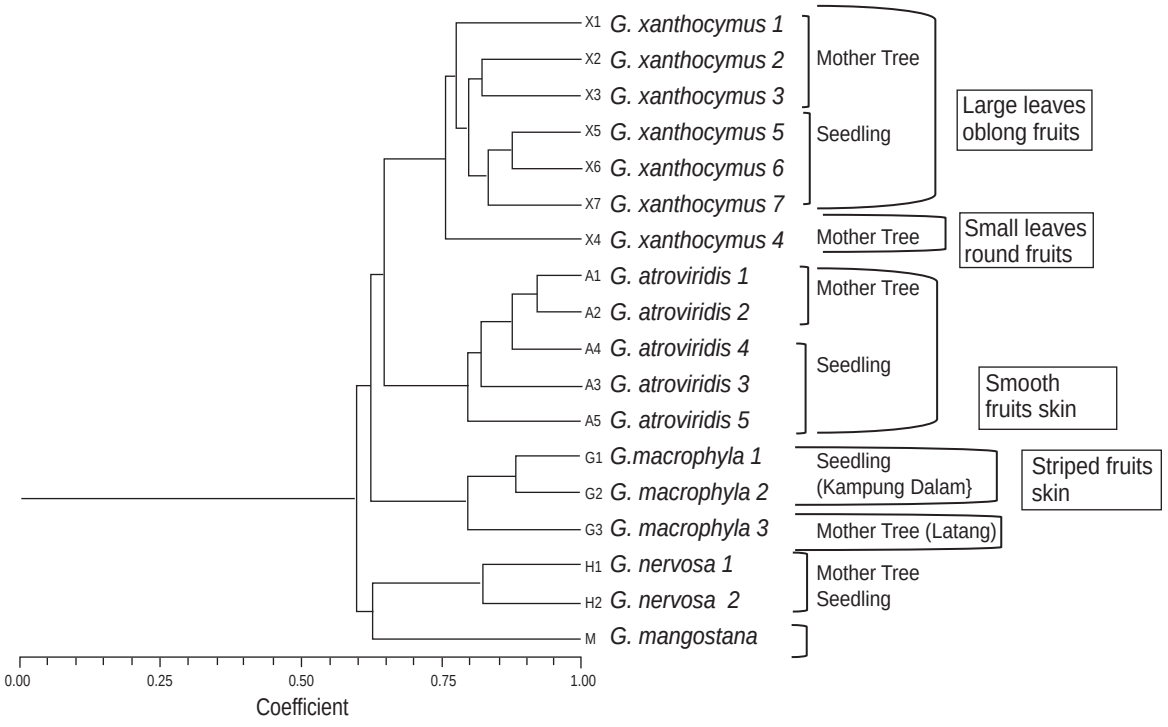

Fig. 3. Dendrogram of 18 *Garcinia* samples based on eight RAPD primers along with morphological correlates

Amplified DNA Fingerprinting (RAF), there was wider diversity with similarity index from 0.20-0.88. The variations in the genetic similarity coefficient were caused by different markers and the samples used.

The 18 *Garcinia* trees were clustered into four distinct groups which represented different species. *G. xanthocymus* displayed the greatest genetic variation with the genetic similarity coefficient varying from 0.75 to 0.88. The four other species exhibited similar range from 0.80 to 0.88. The sequence of the species groups on dendogram (Fig. 3) based on similarity coefficient value from the largest to smallest are *G. xanthocymus*, *G. atroviridis*, *G. macrophyla* and *G. nervosa*, and *G. mangostana*.

The first group contained seven *G. xanthocymus* samples, divided into two sub-clusters which were different in morphology as well as in the banding patterns. The first sub-cluster consisted of the trees with large leaves and oblong fruits (X1, X2 and X3), and three seedlings (X5, X6 and X7); the second sub-cluster contained one *G. xanthocymus* tree with small leaves and round fruits. The second group, *G. atroviridis* species, consisted of five trees which could be separated into two sub-clusters. The two mother trees were in the same sub-cluster and the three seedlings within the other sub-cluster.

The third group consisted of three *G. macrophyla* samples. This group is divided clearly into two sub-clusters *i.e*., *G. macrophyla* 1 and 2 (G1 and G2) from Nagari Latang and *G. macrophyla* 3 (G3) from Nagari Kampung Dalam, having 0.80 in genetic similarity. The two sub-clusters were different in their origin and morphology, and were separated by the unique DNA fragments OPH13/1600 bp, OPH18/350bp, P1/1600bp and P5/250 bp. Two samples of *G. nervosa* and one of *G. mangostana* were clustered within the group four. The cluster analysis showed that *G. nervosa* is very close to *G. mangostana*. The mother tree of *G. nervosa* (H1) was also genetically different from its offsprings (H2)**.**

It is clear from the present study that there is high genetic diversity among and within the five *Garcinia* species. The diversity was exhibited at both morphological and molecular levels. The variations occur among species, within species and between the seedlings and their mother tree. Based on these results, it is suggested to propagate the *Garcinia* vegetatively by grafting. Such different forms or genotypes need to be domesticated and conserved on-farm and in orchards to reduce human pressure to the natural and community forest. These results can be used as a basis for *in situ* conservation

activities of *Garcinia* in Sijunjung, West Sumatra and for educating the community for better management plans. Since the species come from the forest, it is necessary to domesticate and conserve them at home gardens or orchards to maintain their existence together with their utilization.

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