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Toward Harmonization between Development and Environmental Conservation in Biological Production

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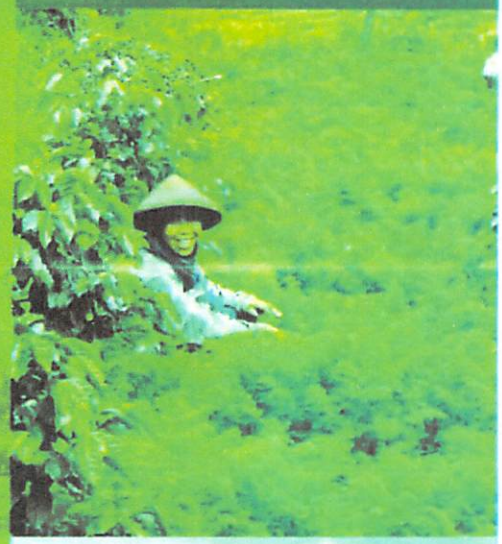

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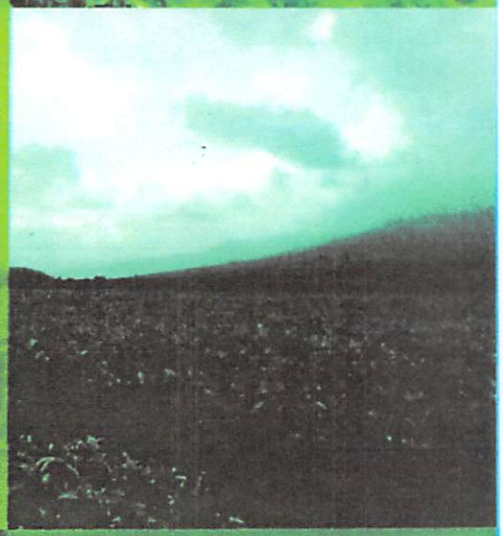

Venue:
Ichijo Hall of Yayoi Auditorium
Graduate School of Agricultural and Life Sciences
The University of Tokyo, Japan

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The 21st Century COE Program in the University of Tokyo
on "Biodiversity & Ecosystem Restoration"

CONTENTS

2. Poster presentations

Genetic Variability in Apomictic Mangosteen

Sobir*, Roedhy Poerwanto, Soaloon Sinaga, Elina Mansyah

Center for Tropical Fruit Studies, Bogor Agricultural University,
Kampus IPB Baranangsiang, Jl Pajajaran, Bogor 16144, Indonesia
Corresponding author: * sobir@ipb.ac.id

ABSTRACT

Mangosteen (*Garcinia mangostana* L.) originated from natural hybridization of *G. malaccensis* and *G. hombroniana*. Mangosteen is reproduced from adventitious embryo, from which the seed develops without fertilization, i.e. agamospermy or apomixis. Apomictic reproduction mode leads to the idea that the mangosteen population is genetically uniform. However, field evaluation showed variability in both of vegetative and reproductive characters. Further studies using isoenzyme and RAPD markers confirmed the genetic variability among the mangosteen population. Isoenzyme marker also elucidates high variability among *Garcinia* genus, and did not support the idea of the single origin of *G. mangostana*. Furthermore, ERAPD and RAPD analysis on parental tree and its progeny shows genetic variability, and deep analysis on polyembriony seedlings from the same seed also show variability on RAPD banding pattern.

INTRODUCTION

Based on its reproductive mode, mangosteen has been classified as an apomictic plant (Horn, 1940; Richards, 1997). This plant is propagated through apomictic seed, of which embryo is formed without reduction of the chromosome number and fertilization of the egg (den Nijs and van Dijk, 1993). Apomixis in mangosteen implies that same genetic 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 is claimed as a species with narrow of genetic variabilities and noticed as a single clone.

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; Richards, 1990). Based on morphological and cytological studies, Yaacob and Tindal (1995) proposed that mangosteen originated from South East Asia, and is an allotetraploid derivate of *Garcinia hombrioniana* (2n= 48) and *Garcinia malaccensis* (2n = 42). This suggestion has been confirmed on our recent finding using isozymes and Amplified Fragment Length Polymorphism (AFLP) markers (Sinaga et. al. 2007, unpublished data). 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).

The mangosteen flowers arise from the tip of young shoots (terminals), mostly single to three (van Steenis, 1981). However, several trees produce flowers in clusters of up to 12 (Rai, 2004). The flower size is 4-6 cm in diameter (Morton, 1999) and fleshy. Richard (1990) reported that mangosteen trees produce perfect flowers that are functionally female due to infertile staminodes. Observation indicated that mangosteen produces 14-18 stamens, 5 - 6 mm in length; however, they do not bear fertile pollen. Anthers consist of four (Mansyah, 2002) to eight compartments (van Steenis, 1981), and anther color changes to brown after anthesis and they turned dry. Subsequently visual observation and potassium iodine treatment revealed that a mangosteen anther has no viable pollen (Mansyah 2002). This was reported earlier by Horn (1940). The failure of mangosteen flowers to produce fertile pollen supports the theory of apomictic reproduction (Horn, 1940; Richard, 1990).

Due to its reproductive manner, mangosteen trees are essentially clonal. While this species is almost exclusively propagated by seed, the resulting trees are little variable because the seed is not zygotic but vegetative, being maternal in origin. Variation of mangosteen in the field is predicted due to differences of environmental conditions. However, several studies revealed that population from apomictic reproduction does not always carry the same genetic properties, even in obligate apomixis (Asker and Jerling, 1992). Variability in progeny of obligate apomictic plant has been reported in genus *Taraxacum* (Ford and Richards, 1985).

Genetic studies on apomictic plants are generally conducted through two approaches, parental plants and their progeny variation analysis or molecular analysis (Koltunow, 1993). Since mangosteen has a long juvenile phase, it is difficult to carry out progeny analysis. Therefore, genetic variability analysis of mangosteen was carried out through evaluation of morphological characters of several mangosteen populations grown in the same location to eliminate environmental influence, as well as by utilization of molecular tools.

MORPHOLOGICAL VARIABILITY

Some distinct variations in morphological characters have been reported. Two type of mangosteen have been identified in terms of fruit shape, one type producing a round shape with semi-flat bottom end and the other type with oblong shape which cannot stand on its distal end (van Steenis, 1981). A wild form containing only four carpels with fully developed seed was also found in north Borneo (Morton 1987). In Yan Bukit Pinang, Malaysia, a tree bearing seedless fruits was reported (Thomas, 1997). Mansyah et al. (1999) found that mangosteen in West Sumatra shows wide variability in leaf length, fruit weight and rind thickness. Mangosteen found in Tembilahan, Sumatera Island, exhibits flattened fruit shape, very short peduncle and elliptic stigma lobe (Mansyah et al., 2005)

In our studies (Prabowo, 2002; Purwanti, 2002; Mansyah, 2002; Suhaeri, 2003), morphological characters were observed from four mangosteen populations in Java Island. They were Leuwiliang, West Java (300 m above sea level), Wanayasa, West Java (610 m asl), Watulimo, Center Java (350 m asl) and Kaligesing, East Java (450 m asl). In each population, 20 plant samples were chosen randomly for further morphological studies. Observation was conducted on two groups of parameters, (1) vegetative characters consisting of canopy diameter, leaf weight, individual leaf area, leaf length, leaf width and trunk ring; and (2) fruit characters consisting of locule number, fruit weight, peduncle length, fruit length, fruit diameter, rind thickness, total soluble solids, seed/fruit and fruit sap.

Based on field observations, variation occurred in canopy shape, either oblong or pyramidical. In Wanayasa and Watulimo only one tree exhibited oblong canopy out of 20 trees, in Leuwiliang five trees had an oblong canopy, but in Kaligesing 11 trees out of 20 trees had an oblong canopy.

For vegetative characters, homogeneity of variance was found in leaf weight, individual leaf area, leaf length/width ratio and trunk ring, but canopy diameter and chlorophyll contents were variable (Table 1). These results indicate that variability in most observed variables were mainly due to variation in environment. Variability in canopy diameter suggested that it was from differences in canopy type, trees age, and plant spacing. The observed trees ranged from 25 years to more than 50 years old, and grew in a very dense population (Leuwiliang) in mixed-culture with other trees (Kaligesing, Watulimo) or intercropping with tea plant (Wanayasa). Tukey's Studentized Range Test on vegetative characters revealed that trees observed from Watulimo showed better vegetative performance than trees of populations from Leuwiliang, Wanayasa and Kaligesing (Table 1).

Table 1. Homogeneity of variance analysis (*Bartlett test*) for morphological characters of 4 populations of mangosteen

Characters	Location				χ^2
	Leuwi-liang	Wana-yasa	Kali-gesing	Watu-limo	
Canopy diameter (m)	12.85	14.58	12.55	17.06	13.28**
Leaf weight (g)	5.61	6.21	5.42	7.48	4.82ns
Leaf area (cm^2)	141.48	104.78	78.83	156.97	6.01 ns
Leaf length/length ratio	2.14	1.98	2.22	2.14	3.64 ns
Trunk ring (cm)	51.55	60.30	61.88	82.20	2.18 ns
Locule number	6.08	5.92	6.20	6.19	6.304ns
Fruit weight (g)	93.62	123.73	125.25	85.23	50.36**
Fruit length (cm)	5.07	5.44	5.49	4.86	33.61**
Fruit diameter (cm)	5.82	6.31	6.28	5.58	32.06**
Rind thickness (cm)	0.86	0.90	0.83	0.66	30.06**
Total soluble solid (%)	18.66	17.75	17.13	17.68	12.94*
Number of seed/fruit	1.66	1.70	1.88	1.52	5.60ns
Fruit latex	2.34	1.81	1.66	2.42	32.58**

ns, *, **: non-significant, significant at p=0.05 and significant at p=0.01, respectively by Bartlett test.

In fruit morphology, the variation was found in weight, length, diameter, length/diameter ratio, rind thickness, peduncle length as well as total soluble solids (TSS) and presence of fruit latex. Numbers of locules and seed per fruit did not differ significantly. Correlation analysis showed that TSS was correlated negatively with fruit diameter, fruit weight, fruit length, peduncle length, and rind thickness, whereas fruit diameter was positively correlated with fruit weight, fruit length, rind thickness, and number of seed/fruit. Analysis of variance revealed that among four populations, the fruits from Kaligesing were superior for fruit size and seed number/fruit.

In recent exploration, we found a new distinctive type of mangosteen in Kalimantan (Borneo) that produces fruit with insignificant size of seed (less than 1cm in length) and has bigger fruit size, thicker rind, more acidic taste, and larger leaf size (two fold to those of common mangosteen). Variation of sepal color was also found in two populations in Java Island (Figure 1), with white and pale orange color of petals compared to petal color of common mangosteen.

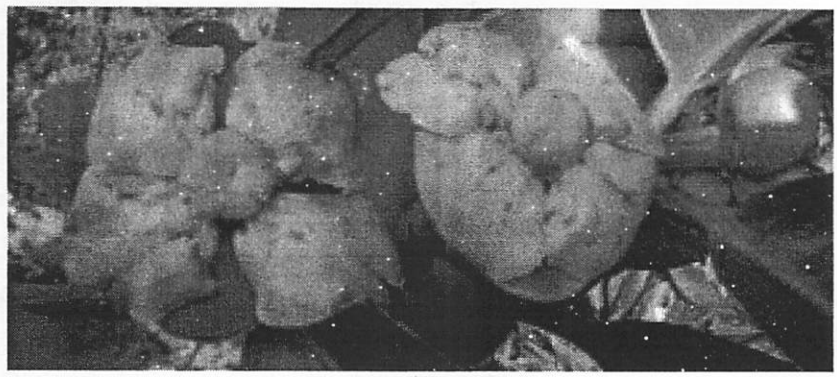

Figure 1. Variation of sepal color of mangosteen, obtained from population in Wanayasa, West Java.

GENETIC VARIABILITY

Recently, significant progress in the biotechnological techniques has made it possible to elaborate genetic variability at the DNA level. DNA markers provide a quick and reliable method for estimating genetic relationships among genotypes of any organism (Thormann *et al.*, 1994). In order to confirm previous studies on genetic variability of apomictic mangosteen by using RAPD markers (Mansyah et al., 2002) and RAF markers (Ramage et al., 2004), we were conducting genetic variability analysis by utilized isozymes analysis on 19 accessions of mangosteen and 14 its wild relatives, and RAPD analysis on 11 accessions of mangosteen and 7 its wild relatives.

Isozymes analysis were conducted by utilized four enzyme systems of *Esterase (EST), Peroxidase (PER), Acid Phosphatase (ACP), and Malate Dehidrogenase (MDH)* by using electrophoreses method of Soltis and Soltis (1989). The four enzyme system produces 27 bands, i.e., 10 bands by Esterase (EST), 7 bands by Peroxidase (PER), 5 bands by Acid Phosphatase (ACP) and 5 bands by Malate Dehidrogenase (MDH). However, only three bands (11.1 %) among them were monomorphic, the rest of 24 bands (88.9%) were polymorphic. These results indicated that genetic variability as DNA that revealed previously, also confirmed with variability in enzyme system.

Nei and Li similarity (1979) was calculated using binary data of 24 isozyme bands and a dendogram based on UPGMA-link method was generated (Figure 2). It revealed that genetics diversity among *Garcinia* was 85%, and the mangosteen accesions were clusterd in one group at genetics variability level of 42%. The dendogram also succesfully confirms the statement that *G. mangostana* is an autotetraploid from *G. malaccencis* and *G. hombroniana* (Richards, 1990), since the *G. malaccencis* and *G. hombroniana* were flanked the cluster of *G. mangostana*.

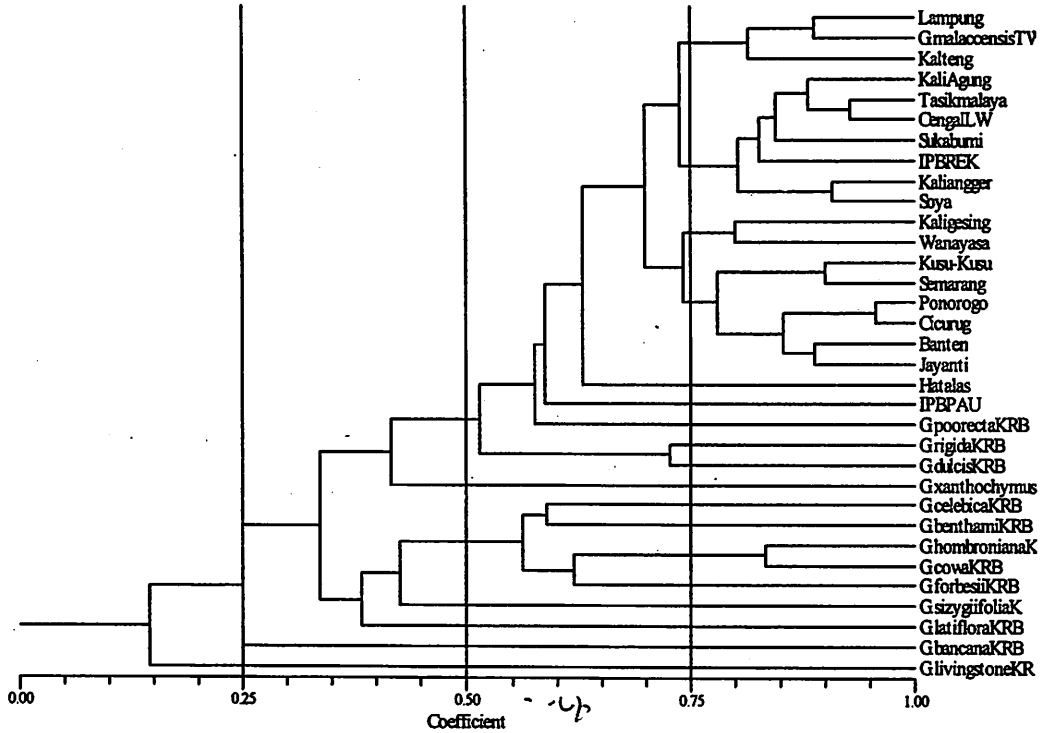

Figure 2. A dendogram based on UPGMA-link method generated from isozyme bands of four enzyme systems on 19 accessions of mangosteen and 14 its wild relatives.

RAPD analysis was applied by using seven random primers of SBH12 (5'-ACGCGCATGT-3'), SBH13 (5'-GACGCCACAC-3'), SBH19 (5'-CTGACCAGCC-3'), OPA14 (5'-TCTGTGCTGG-3'), OPA16 (5'-AGCCAGCGAA-3'), OPA17 (5'-GACCGCTTGT-3'), and OPA18 (5'-AGGTGACCGT-3') by using method of Williams et al. (1990) with slight modifications. Those primers were produced 69 bands with average of 9.9 bands for each primer and all of the bands were polymorphic.

Based on RAPD bands, a dendogram was generated by UPGMA-link method using Nei and Li similarity (1979), suggesting that genetic diversity among *Garcinia* genus (80%) is slight lower than detected by isozyme analysis. Those result presumably is due to the smaller number of accessions and species analyzed on RAPD (7 *Garcinia* and 11 *G. mangostana*) than those of on isozyme analysis (14 *Garcinia* and 19 *G. mangostana*). However, the mangosteen accessions were not clustered in one group, instead they mixed in sveral groups with its wild relatives, and the clustering cast doubt on the idea of *G. mangosstana* origin of development (Figure. 2). Further analysis indicated that RAPD analysis results were not concurrent with isozyme analysis results. However, other research using AFLP analysis suported the idea that *G. mangostana* is an autotetraploid from *G. malaccencis* and *G. hombroniana.*

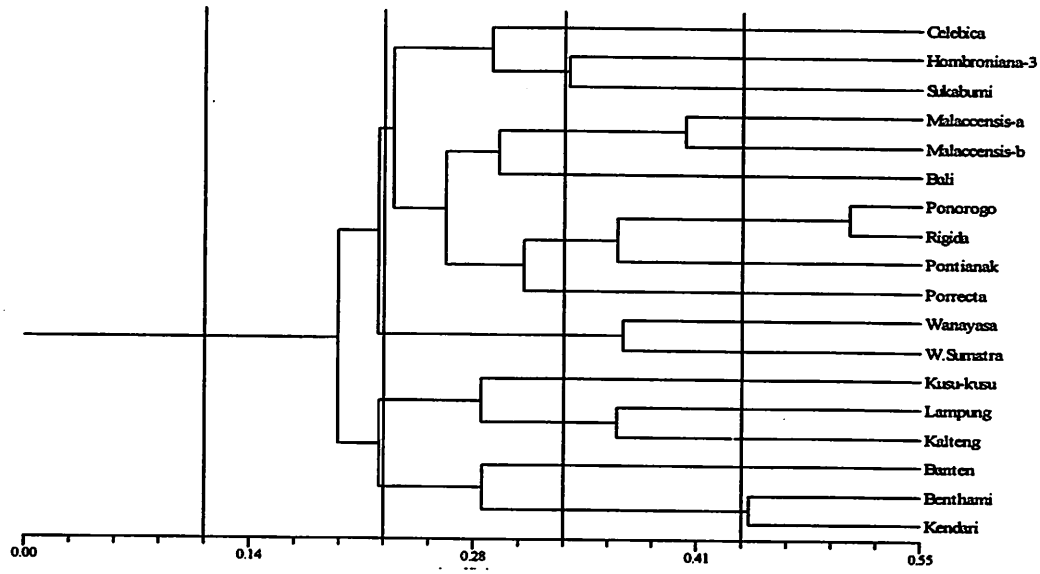

Figure 3. A dendogram based on UPGMA-link method generated from RAPD analysis data using seven random primers on 11 accessions of mangosteen and 7 its wild relatives.

Such high genetic variability was not common for mangosteen, 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 vegetatively propagated crops such as banana and plantain (Buddenhagen, 1987).

GENETIC STABILITY AMONG PARENTAL TO PROGENY

In order to elucidate genetic stability among parental trees to progenies, we were employed enhanced RAPD markers, with adding one base to 3 end random marker SBH13 (5'-GACGCCACAC-3'), to become ESBH-131 (5'-GACGCCACACT-3'); ESBH-132 (5'-GACGCCACACG-3)'; ESBH-133 (5'-GACGCCACACA-3'); and ESBH-134 (5'-GACGCCACACC-3'). These four individual ERAPD primers and three combinations of ESBH-131+ ESBH-132; ESBH-131+ ESBH-134; and ESBH-132+ ESBH-134 were used to amplify 40 genomic DNA of parental trees and their progeny seedlings originated from seed of the parental fruits. PCR reactions were carried out in Perkin Elmer thermal cycler programmed for 45 cycles of each of the following: 95 ^{0}C for 1 min, 38 ^{0}C for 1 min, and 72 ^{0}C for 2 min. A final elongation step of 5 min at 72 ^{0}C was included.

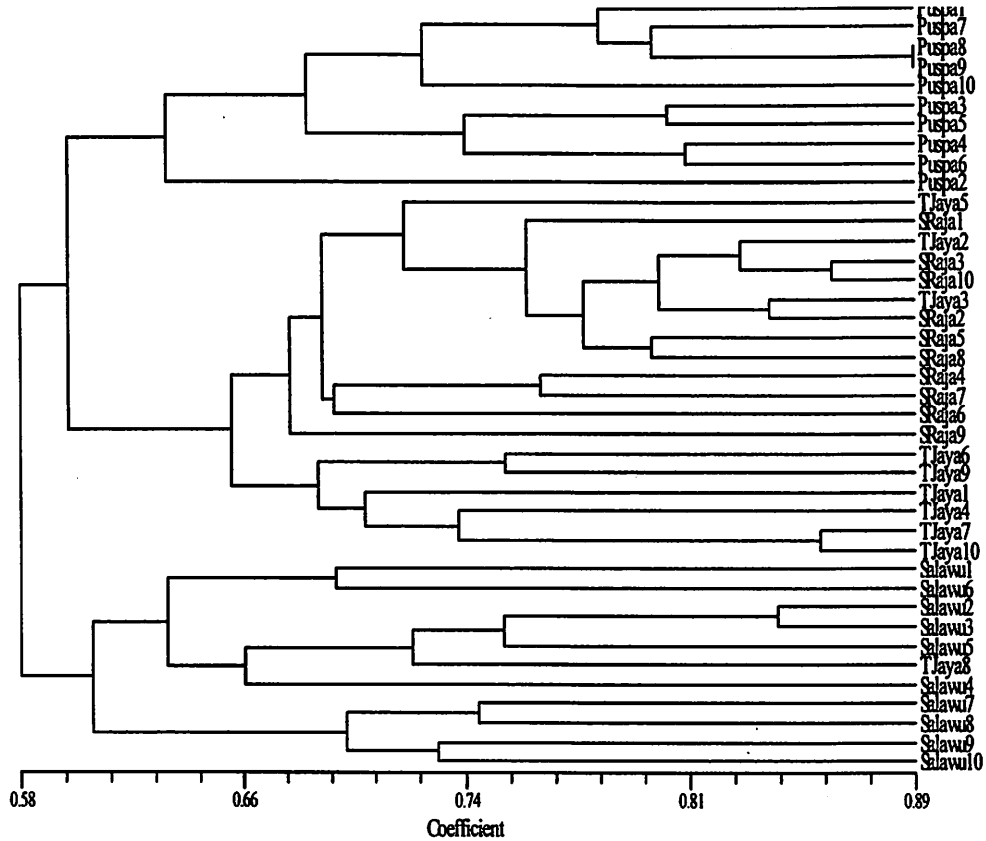

Figure 4. A dendogram based on UPGMA-link method generated from seven ERAPD markers data on 40 parental trees in Tasikmalaya regency, West Java.

Based on ERAPD data, a dendogram was generated by UPGMA-link method using Nei and Li similarity (1979). It revealed that genetic diversity among mangosteen four populations in Tasikmalaya regency was 42 %, similar to those detected by isozyme markers. This results indicated that the size of genetic variability of mangosteen in Tasikmalaya was reflected variability in boarder population in Indonesia. However, the dendogram also showed that mangosteen trees from same area tends to clustered in the same group (Figure 4).

Utilization the same ERAPD primers and their combination to those of used in their parental resulted in same banding pattern, subsequently cluster analysis based on UPGMA-link method, revealed the cluster pattern of progenies similar to the parental banding pattern. However, genetics variability level among progenies was 48%, slightly higher from their parentals.

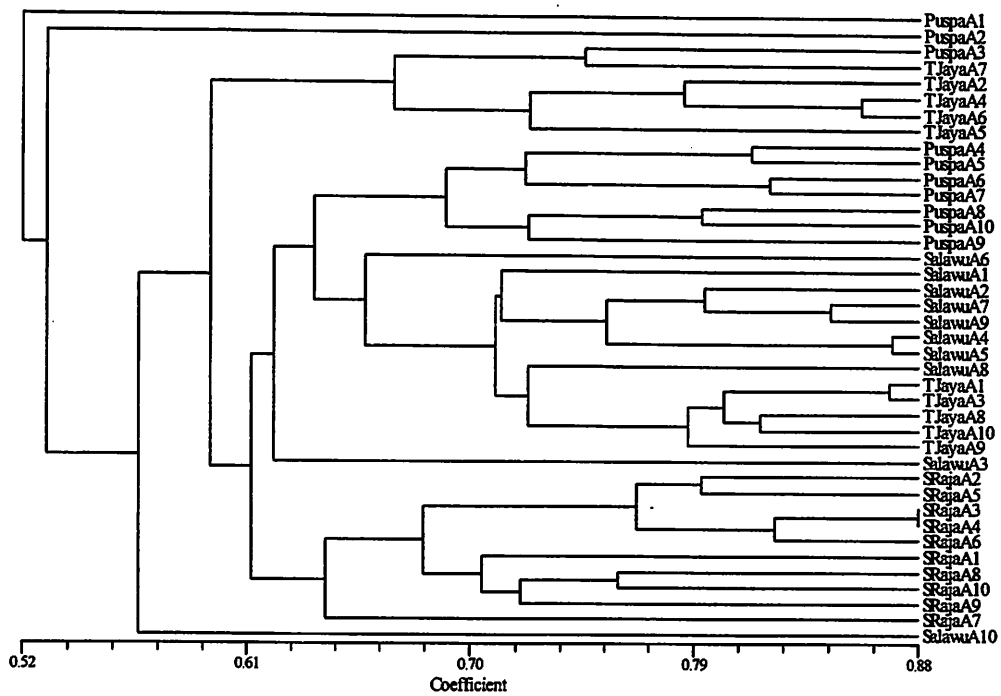

Figure 5. A dendogram based on UPGMA-link method generated from seven ERAPD markers data on 40 progenies trees in Tasikmalaya regency, West Java.

To confirm the source of elevation of genetics variability in progenies, we choosed 12 parental trees, and germinating seed from three different fruits for each parental tree. The result revealed that among them 47% of progenies trees showed identical ERAPD banding pattern, but different banding patterns from their parental trees, and the rest of 53% progenie groups were show variability banding pattern among three of the progenies and its parental tree.

Subsequentlly, in order to elucidate the variability elevation from parental to progenies, we conducted RAPD analysis with three random primers of OPH-12 (5-ACGCGCATG T-3); OPH-18 (5-GAATCGGCC A-3), and OPA-20 (5-GTTGCGATC C-3) on monoembryonic seedling and polyembrionic seedlings. Primer OPH-12 showed that two progenies from different polyembryonic seeds were identical in DNA banding patterns, but they were different from their mother plant. Primer OPH-18 produced four non maternal banding patterns in progenies from different polyembryonic seeds and two individuals from monoembryonic seeds. OPA-20 revealed the variations among the 14 progenies, of which nine progenies from polyembryonic seeds and five from monoembryonic seeds were identified as non maternal genotypes (Figure 5).

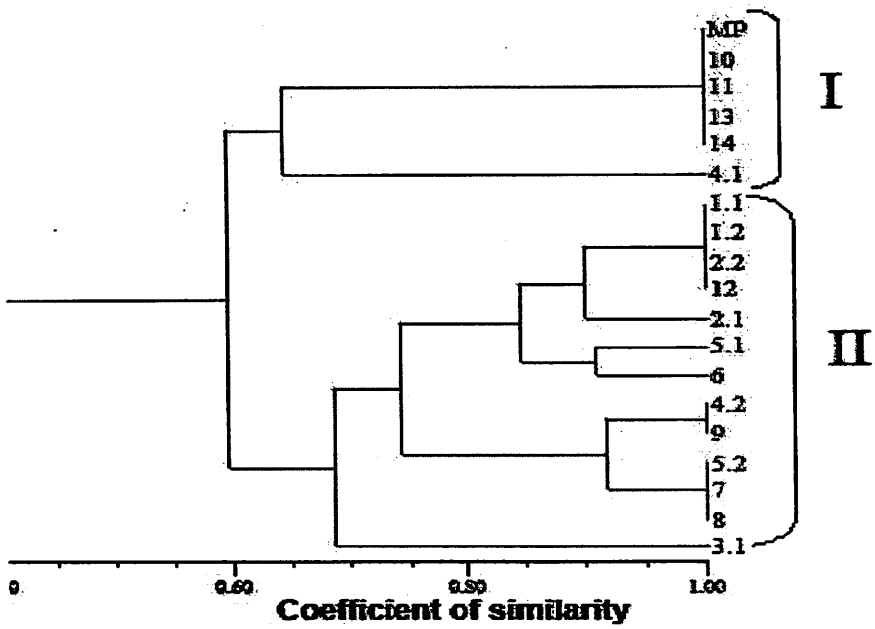

Figure 5. Dendogram of 18 mangosteen progenies and their mother plant revealed by UPGMA cluster analyses of RAPD data. MP = Mother Plant, 1.1 to 5.2 were progenies derived from polyembryonic seeds and 6 to 14 progenies from monoembryonic seeds.

The result is in accordance with the previous study reported by Mansyah *et al.* (2004), in which genetic variations were found between mangosteen mother plant and its progeny's, and a study by Oostrum *et al.* (1985), in which genetic variations were found among progenies on obligate apomicts in *Taraxacum.* Further observation in genetic change of tetraploid apomictic eastern gamagrass (*Tripsacum dactyloides* var *dactyloides* L.) has found 4 % genetic variant in its progeny. These findings indicate that it is unlikely that obligate or facultative apomictic species are unexpectedly incapable or weakly capable of inducing genetic changes, as stated by Kindiger and Dewald (1996).

DISCUSSION

Genetic variation in apomictic plants is assumed as a result of the somaclonal variation, auto segregation, and somatic-crossing over, amplification or loss of DNA material, chromosome rearrangements and further activity of gene regulating changes in transposable genetic elements (Walbot and Cullis, 1985). In the obligate apomicts, variation may occur through the mechanisms such as DNA mutational accumulation, cytology disjunctional accidents, and somatic recombination from chromosomal translocations. In addition, mutational or chromosomal changes in the maternal genome may be concerned with agamospermous behavior (Richards 1997).

Carman (2001) suggests that apomicts result from wide hybridization of ancestral sexual parents having distinct reproductive phenotypic traits. According to Yaacob and Tindal (1995) mangosteen (*G. mangostana*) is a hybrid of *G. hombrioniana* and *G. malaccensis*, and it was possible that *G. mangostana* did not originate from a single hybridization of its ancestral sexual parents. Thomas (1977) reported genetic variability in both *G.*

hombrioniana and *G. malaccensis*. Our recent analysis using RAPD markers revealed genetics variability among accessions of *G. malaccensis*. Therefore, it is possible that development of the ancestral mangosteen was not established from a single hybridization, leading to variation among mangosteen populations.

ACKNOWLEDGEMENT

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