# ISSN 0215-4706

Floribunda 3(4): 85-112. Oktober 2007

# DAFTAR ISI

Progeny Analysis of the Tasikmalayan Mangosteens (Garcinia mangostana) with E-RAP Markers	D
Soaloon Sinaga, Sobir, Roedhy Poerwanto, Hajrial Aswidinnoor & Dedy Duryadi	35-94
Genetic Diversity of <i>Pandanus</i> and <i>Freycinetia</i> from Java Based on ISSR Marker Sri Endarti Rahayu, Alex Hartana, Tatiek Chikmawati & Kuswata Kartawinata	5-103
Anatomi Daun dan Taksonomi Syzygium zippelianum Miq., S. javanicum Miq. dan S. racemosum (Bl.) DC	
Siti Sunarti 10	4-108
Two Wild Edible <i>Russula</i> ( <i>Agaricales: Russulaceae</i> ) from East Kalimantan Atik Retnowati	9–112

Terakreditasi B berdasarkan Keputusan Dirjen Dikti Depdiknas RI No. 26/DIKTI/Kep./2005

ISSN 0215-4706



*Floribunda* merupakan organ resmi Penggalang Taksonomi Tumbuhan Indonesia, diterbitkan dua kali setahun dan menerbitkan makalah dalam bahasa Indonesia dan Inggris mengenai pelbagai gatra sistematika keanekaragaman flora Malesia pada umumnya dan Indonesia pada khususnya yang berasal dari hasil penelitian, pengamatan lapangan, pengalaman pribadi, telaahan bergagasan, dan tinjauan kritis.

### **Sidang Penyunting**

### Ketua Penyunting

Tutie Djarwaningsih (BO)

### Penyunting

Tri Mulyaningsih (UNRAM) Atik Retnowati (BO) Novita Kartika Indah (UNESA) Titien N. Praptosuwiryo (KRI) Nunik Sri Ariyanti (IPB)

### *Penyunting Pelaksana* Himmah Rustiami (BO)

Tata Letak Muhamad Ruslan (BO)

### Petunjuk kepada pengarang

### Jenis tulisan

Makalah lengkap memuat hasil penelitian floristik, revisi, atau monografi unsur-unsur flora Malesia.

Komunikasi pendek mencakup laporan kemajuan kegiatan penelitian, pengembangan dan rekayasa keanekaragaman flora Malesia yang perlu segera dikomunikasikan.

Tulisan lain meliputi obituari tokoh keanekaragaman flora, tinjauan kritis bergagasan, telaahan serta pembahasan persoalan aktual seputar kegiatan penelitian, pengembangan dan rekayasa tetumbuhan Indonesia, serta timbangan buku akan dimuat berdasarkan undangan.

### Rujukan pembakuan

Pemakaian Bahasa Indonesia sepenuhnya mengikuti Pedoman Umum Ejaan yang Disempurnakan, Pedoman Umum Pembentukan Istilah, Kamus Besar Bahasa Indonesia, serta kamus-kamus istilah yang dikeluarkan Pusat Bahasa. Bahasa Inggris yang dipakai adalah the Queen English dengan berpedoman pada The Oxford Dictionary of the English Language. Ketentuanketentuan yang dimuat dalam Pegangan Gaya Penulisan, Penyuntingan, dan Penerbitan Karya Ilmiah Indonesia, serta Scientific Style and Format: CBE Manuals for Authors, Editors, and Publishers, dan bukubuku pegangan pembakuan lain akan sangat diperhatikan. Kepatuhan penuh pada International Code of Botanical Nomenclature bersifat mutlak.

### Gaya penulisan

Penulisan naskah yang akan diajukan supaya disesuaikan dengan gaya penulisan yang terdapat dalam nomor terakhir terbitan *Floribunda*.

Abstrak informatif supaya diberikan dalam bahasa Indonesia dan Inggris yang masing-masing tidak melebihi 200 kata. Sediakan sekitar 7 kata kunci untuk keperluan pengindeksan dan pemindaian.

Bilamana diperlukan ucapan terima kasih dan bentuk persantunan lain dapat dicantumkan sesudah tubuh teks tetapi sebelum daftar pustaka.

Pengacuan pada pustaka hendaklah dilakukan dengan sistem nama-tahun. Daftar pustaka supaya disusun berdasarkan alfabet nama pengarang dengan memakai sistem Harvard.

Gambar dan tabel merupakan pendukung teks sehingga perlu disusun secara logis dalam bentuk yang mudah dimengerti. Data supaya disajikan dalam bentuk teks atau tabel atau sebagai gambar, tetapi tidak dalam bentuk ketiganya sekaligus. Siapkan gambar yang lebarnya dua kolom cetak.

### Penyumbangan naskah

Naskah dikirimkan dalam bentuk ketikan atau cetakan komputer pada kertas HVS berukuran A4 bersama-sama dengan disket komputer yang diprogram untuk serasi dengan IBM, atau melalui *e-mail*.

Naskah yang ingin diterbitkan dalam *Floribunda* akan dipertimbangkan pemuatannya *hanya* jika pengirimannya disertai pernyataan tertulis dari 2 (dua) orang mitra bestari yang dipilih sendiri oleh penulisnya (akan lebih diutamakan bila mitra bestari dipilihkan dari luar lingkungan kerja penulis), yang menyatakan bahwa secara ilmiah keorisinalan dan makna sumbangan naskah tersebut memang layak diterbitkan.

### Pengolahan naskah

Sidang penyunting bersama sekelompok mitra bestari akan mengaji ulang kesesuaian isi dan keselarasan format setiap naskah dengan *Floribunda*. Perubahan yang dilakukan akan dikomunikasikan kepada penulis dalam bentuk contoh cetak akhir sebelum diterbitkan.

### Cetak lepas

Penulis menerima 5 cetak lepas dari tulisannya secara cuma-cuma.

### Kantor penyunting

Sidang Penyunting *Floribunda* Herbarium Bogoriense, Cibinong Science Center Jalan Raya Bogor KM 46 Cibinong 16911 Telepon :(021)8765066-67 Fax :(021)8765059 E-mail : herbogor@indo.net.id

# PROGENY ANALYSIS OF THE TASIKMALAYAN MANGOSTEENS (GARCINIA MANGOSTANA) WITH E-RAPD MARKERS

Soaloon Sinaga

Department of Agronomy and Horticulture, Bogor Agricultural University (Permanent address: Kopertis Wilayah III, Jakarta. E-mail: horas97@yahoo.com) Sobir, Roedhy Poerwanto, Hajrial Aswidinnoor Department of Agronomy and Horticulture, Bogor Agricultural University Dedy Duryadi

Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agriculture University

Soaloon Sinaga, Sobir, Roedhy Poerwanto, Hajrial Aswidinnoor & Dedy Duryadi. 2007. Analisis Progeni Manggis (*Garcinia mangostana*) Tasikmalaya dengan Penanda E-RAPD. *Floribunda* 3(4): 85–94. — Apomiksis adalah reproduksi aseksual melalui biji dan merupakan strategi tanaman untuk menghasilkan biji. Pada tanaman apomiktik embrio berkembang secara otonom dan menghasilkan progeni yang identik secara genetik dengan tanaman induk. Tujuan penelitian ini adalah untuk mengungkap keanekaragaman tanaman apomiksis dan untuk mengetahui kejadian apomiksis dalam populasi manggis serta memperoleh gambaran pola-pola pewarisan genetik induk dan keturunannya. Variabilitas genetik dalam populasi adalah sebagai berikut: Salawu (5–14%), Puspahiang (9–25%), Tanjungjaya (10–25%), dan Sukaraja (22.5–29.6%), dan variabilitas di antara populasi 30–47%. Namun, 100% nilai kesamaan genetik diberikan oleh tanaman Salawu8, antara Salawu8 induk dengan Salawu8 progeni-1, dan Puspahiang progeni-2 dan progeni-3. Sementara itu, aksesi lainnya memiliki kemiripan 71.4–98%. E-RAPD berhasil diterapkan pada analisis apomiksis dalam populasi progeni di populasi manggis yang berbeda.

Kata kunci: Apomiksis, manggis, variabilitas genetik, analisis progeni, E-RAPD.

Soaloon Sinaga, Sobir, Roedhy Poerwanto, Hajrial Aswidinnoor & Dedy Duryadi. 2007. Progeny Analysis of the Tasikmalayan Mangosteens (*Garcinia mangostana*) with E-RAPD Markers. *Floribunda* 3(4): 85–94. — Apomixis is an asexual mode of plant reproduction through seeds without fertilitation. A common feature of all apomicts is the autonomous development of embryos and the generation of progenies that are the exact genetic replicas of the mother plant. The aims of apomixis study are to unlock the diversity of the apomictic plants and to know the incidence of the apomixis in the mangosteens and the description of the inheritance genetic patterns of the progenitors to their progeny. The genetic variabilities within the population are Salawu (5–14%), Puspahiang (9–25%), Tanjungjaya (10–25%), and Sukaraja (22.5–29.6%), and among the population 30–47% variability. However, 100% of genetic similarity values gave by Salawu8 progenitor with Salawu8 progeny-1, and Puspahiang progeny-2 and progeny-3. Meanwhile, the other accessions have 71.4–98% similirity. E-RAPD were successfully applied to the analysis of apomixis in the different progeny population of *mangosteens*.

Keywords: Apomixis, mangosteen, genetic variability, progeny analysis, E-RAPD.

Apomixis, asexual reproduction by seeds, is a strategy in plants to produce seeds which is genetically identical with the maternal progeny. By apomixis process, it is possible to select high quality of plants and produce seeds by large scale cloning, genotypic fixation, and to conserve heterosis characters, vigor, and high yields (Koltunow 2008).

Apomixis has a potential to revolute agriculture, if the genetic base can be determined well. Up to now, apomixis genetic control is still unclear because of the complexity of Mendelian Genetics, such as epistasis gene interaction, expressed component sporophytically and gametophytically, expression modifiers, polyploidy, aneuploidy, segregation distortion, the presence of recombination stress etc. which were accumulated during apomixis evolution.

Apomictic plant identification can be done by using some methods, namely phenotypic method, sitoembryology characterisation, genetic markers, biological examination, and their combinations to determine gene loci which control apomixis in some plant species. Those methods can be used to classify progeny deviation produced by plant crossing asexually and sexually to determine reproduction patterns of each plant. The use of these methods including sitology of embryo pouch and progeny analysis will depict characters and apomictic gene introgression use into the sexual plant species which is useful for the modern agricultural systems. The modern molecular genetic can be used as one of the ways to identify genes and physiological mechanisms or biochemistry which control certain cell regulation changes which cause the apomixis (Darrigues et al. 2008).

Within the different conditions, apomictic plants of the genus *Taraxacum* has genetic variation within the levels of ploidy and aneuploidy (Richards 1996). It also occurs in some species of *Poaceae* (Acuña et al. 2005). The newest molecular study on the mangosteen, apomixis has also showed the genetic variations (Ramage et al. 2004, Yapwattanaphun et al. 2004, Sinaga et al. 2006, Sobir & Poerwanto 2007, Sari et al. 2008 *in press*). On the other hand, the genetic variability information between the progenitors and the progeny in the apomictic mangosteens is still unknown.

The information of the genetic variability between the progenitors and the progeny suggests the phenomena of character heritability from the progenitors to the progeny. By this information, we can understand whether segregation occurred in the progeny or not, or whether the genetic patterns of the progenitors is similar to those in the progeny. The failure of the separation of spindels in chromosomes, somatic recombination caused by transposone activities and the presence of mutation was regarded as the cause of genetic variation (Richards 1996). Analysis of apomixis heredity of the progenitors to the progeny can be done by molecular markers. The use of molecular technique in the apomictic genetic study is expected to increase the understanding of the sporophytic and gametophytic apomixis plants.

The aim of this research is to know the genetic variability and genetic pattern consistency of the progenitors and progeny in the mangosteens from four locations in Tasikmalaya using E-RAPD markers. The genetic variability studied will explain whether the variability is caused by the interprogenitors and progeny or accession within location or between locations. The genetic consistency between progenitors and progeny is detected by seeing and comparing the DNA band patterns.

### **MATERIALS AND METHODS**

Samples of mangosteens were collected from four locations in Tasikmalaya Regency. E-RAPD analysis was conducted in the Molecular Laboratory of Life Science Study Center and Plant Biology, The Research Center for Biological Resources and Biotechnology IPB Bogor and the Laboratory of Study Center for Tropical Fruit IPB Bogor. This research was conducted from June 2006 to August 2007. Twelve accessions of mangosteens including one maternal progenitor with three progenies representing mangosteen populations growing in four districts in Tasikmalaya, namely: Puspahiang, Salawu, Tanjung Jaya, and Sukaraja. Sampling was collected with purposive random sampling method i.e. trunk with  $\pm$  100 cm diameter and 50 years age, with 10 plants each per location.

E-RAPD technique following William et al. (1990), with some modifications by Tanaka & Taniguchi (2002). Six random primers "*Enhanced*" used were E1 (5'-GACGCCACACT-3'), E2 (5'-GACGCCACACG-3'), E3 (5'-GACGCCACACA-3'), E4 (5'-GACGCCACACC-3'), E1E2 and E1E4 (combination). The DNA band amplified was electrophoreted together with standard DNA 1 KB DNA *ladder* (promega) on agarose gel 1.2% in buffer solution TBE 1X. Electrophoresis was run for 150 minutes on 60 volt at room temperature. The bands amplified were documented with gel and recorded in the computer.

### Data Analysis E-RAPD Technique

The bands of E-RAPD were translated as binary data (1 if presence and 0 if absence). These data were used to construct matrix of genetic similarity based on the formula Nei & Li (1979) with UPGMA (Unweighted Pair-Group Method Aritmetic) method by using NTSys (*Numerical Taxonomy and Multivariate System*) versi 2.02 (Rolf 1998).

The presence of bands was translated as the binary data. Each band represents one character and scored based on the presence and absence of bands. The polymorphic bands are bands which do not belong to the other mangosteen individual on the same size. The binary data resulted were used to arrange genetic similarity matrix based on formula of Nei & Li (1979). Cluster analysis was done based on those genetic similarity followed by production of the dendrogram using UPGMA (Unweighted Pair-Group Method Aritmetic) with the program NTSys (Numerical Taxonomy ang Multivariate System) version 2.02.

# **RESULT AND DISCUSSION**

Result

### **E-RAPD** Data Analysis

All random primers could amplify all DNA accessions (Table 1). The number of bands/samples/

primers varies between 1–11 bands with average 9 bands DNA per sample. Primers E1 and E2 had resulted in the least (7 bands), while the most bands (11 bands) were resulted by primers E1E4. The size of bands amplified by primer E-RAPD ranges from 200–1100 pb. All primers result in high polymorphism (100%). The different numbers and size of bands defining levels of genetic variability of mangosteen accession and their close relatives.

Figure 1 suggest variation patterns of DNA bands between progenitors and progeny namely the loss of progenitor DNA band in certain progenies and additional new DNA bands without loss of progenitor DNA. The range of band size produced by E-RAPD analysis was relatively shorter, less, and more dense band compared with those produced by normal RAPD primers. The number of bands produced by each primer depends on the site distribution which were homologous with the primer sequence in the genome.

Genetic relationship between Salawu3 progenitor with 3 progenies (Salawu2-P1, Salawu2-P2, and Salawu2-P3) can be determined from the genetic similarity matrices based on presence and absence of DNA bands using six random primers. The highest genetic similarity score between progeny Salawu2-P1 and Salawu2-P2 is 97.7%, while the lowest one between Salawu2-I and progeny Salawu2-P3 is 86%. Based on cluster analysis (Figure 2) Salawu2 progenitor accession and their three progenies were united on the genetic similarity 89% or 11% diversity between progeny and progenitor accession Salawu2. The cluster between progeny Salawu2 united at the similarity level of 7%. Cophenetic correlation score between accession Salawu2 was 0.857 or with the goodness of fit (Table 2). It means that there was compatibility between matrices produced by the dendrogram.

Primer	Band size (pb)	Monomorphic band	Polymorphic band	Band number
E1	200-750	0	7	7
E2	200-750	0	7	7
E3	200-625	0	9	9
E4	200-750	0	10	10
E1E2	200-750	0	9	9
E1E4	200-1100	0	11	11
E2E4	200-750	0	10	10
	Total	0	63 (100%)	63

Table 1. The number of bands amplified by seven primers with E-RAPD technique for progenitors and progeny.



Figure 1. Profile of DNA bands between progenitors and progeny with E-RAPD markers primer E1 and E1E4. Notes: 25 = T8 Progeny1, 26 = T8 Progeny3, 27 = T8 Progeny2, T8 = Tanjungjaya8 mother, 31 = SR8 Progeny1, 32 = SR8 Progeny2, 33 = SR8 Progeny3, R8 = Sukaraja8 mother, 4 = S7 Progeny1, 5 = S7 Progeny2, 6 = S7 Progeny3, S7 = Salawu7 mother, 13 = P3 Progeny1, 14 = P3 Progeny2, 15 = P3 Progeny3, P3 = Puspahiang3 Mother, a = Additional bands, b and c = band deletion.

Similarity score between accession Salawu7 progenitor and progeny was 86% or there was diversity in between progenitor and progeny accession of 14%. The accession with the highest similarity was Salawu7-P2 with Salawu7-P3 (95%) or there were diversity between progeny of 5%. Clustering patterns shown by accession Salawu2 and Salawu7 were the same, where the diversity score between progenitors and progeny was more than diversity score among progeny. Cophenetic correlation score between accession Salawu7 was 0.812 or with goodness of fit. On the other hand, the accession Salawu8, the progenitor accession formed cluster with similarity level 100% with progeny Salawu8-P1, while the other two progenies united in the level of 96% similarity. Generally, accession Salawu8 formed a cluster at the similarity 95% or there were diversity between accession of 5%. Cophenetic correlation score between accession Salawu8 was 0.927 or with very suitable goodness of fit. Three Salawu trees (Salawu2, Salawu7, Salawu8) of progenitor and progeny formed a cluster at a similarity of 70% or there were diversity within Salawu population of

Table 2. Character score and 2 of the first main component score based on E-KATD marked	Table 2. (	Character score	and 2 of the	first main	component score	based	d on E-RAPD	markers
---	------------	-----------------	--------------	------------	-----------------	-------	-------------	---------

Accession (population)	Character score	Proporsion	Cumulative	Characters	MC1	MC2	r (Cophenetic correlation)
Salawu2	2.120	0.606	0.606	E2-1	0.094	0.531	0.857
	1.113	0.318	0.924	E2-5	0.221	-0.352	
Salawu7	2.552	0.589	0.589	E4-4	0.015	-0.346	0.812
	1.307	0.302	0.890	E1E4-10	0.090	0.473	
Salawu8	1.408	0.704	0.704	E3-2	-0.464	-0.227	0.927
	0.592	0.296	1.000	E3-13	0.464	0.227	
Puspahiang2	1.531	0.593	0.593	E1E4-1	0.378	-0.171	0.946
	1.052	0.407	1.000	E1E4-8	0.119	-0.544	
Puspahiang3	3.683	0.850	0.850	E1E2-8	-0.095	0.707	0.993
	0.333	0.077	0.927	E1E4-12	-0.095	-0.707	
Puspahiang7	0.547	0.729	0.729	E1-11	0.657	0.261	0.941
	0.203	0.271	1.000	E2-3	-0.369	0.929	
Tanjungjaya2	1.625	0.453	0.453	E2-8	-0.025	-0.454	0.897
	1.153	0.322	0.775	E2-9	-0.025	-0.454	
Tanjungjaya7	4.655	0.657	0.657	E2E4-5	0.061	0.336	0.981
	1.882	0.266	0.923	E2E4-6	-0.061	-0.336	
Tanjungjaya8	1.558	0.519	0.519	E2-1	0.170	0.543	0.843
	0.932	0.311	0.830	E2E4-7	0.391	-0.249	
Sukaraja6	4.560	0.497	0.497	E2-5	0.267	0.026	0.809
	2.488	0.271	0.769	E1E2-12	-0.022	0.364	
Sukaraja8	4.497	0.650	0.650	E2E4-1	0.138	0.360	0.988
	1.321	0.191	0.841	E2E4-5	0.072	0.375	
Sukaraja9	2.763	0.436	0.436	E2-9	-0.067	-0.326	0.926
	2.112	0.333	0.770	E3-12	-0.067	-0.326	
Salawu	4.098	0.407	0.407	E1-3	0.044	-0.263	0.976
	3.355	0.333	0.740	E4-5	-0.044	0.263	
Puspahiang	4.976	0.419	0.419	E3-3	0.013	-0.247	0.968
	3.936	0.332	0.751	E1E2-1	-0.013	0.247	
Tanjungjaya	6.436	0.493	0.493	E2-13	-0.093	-0.244	0.972
	3.068	0.235	0.729	E3-1	0.093	0.244	
Sukaraja	2.956	0.269	0.269	E2E4-1	0.066	0.302	0.772
	2.366	0.216	0.485	E2E4-3	-0.001	0.312	
	1.376	0.125	0.610	E2E4-8	-0.066	-0.302	
	1.160	0.106	0.716	E2E4-11	0.064	-0.283	
Tasikmalaya	3.117	0.178	0.178	E1-5	0.214	-0.093	0.877
-	2.624	0.150	0.328	E1-6	-0.231	-0.071	
	2.240	0.128	0.456	E2-6	0.207	-0.137	
	1.378	0.079	0.535	E2-10	0.076	0.253	
	1.221	0.070	0.605	E3-9	-0.053	0.211	
	0.912	0.052	0.657	E1E2-10	0.243	0.133	
	0.810	0.046	0.703	E1E4-12	0.219	0.088	

Notes: MC1 and MC2 were the main component score 1 and 2.

30%. Cophenetic correlation between Salawu was 0.976 or with suitable goodness of fit.

Accession Puspahiang2 connected at the similarity level of 91% or there were 9% diversity among Puspahiang2. The highest similarity score was presence between Puspa2-P2 and Puspa2-P3 (100%), and the lowest score was in Puspa2-P1 (91%), while the progenitor was located in between those three progenies with similarity score 93.5%. Cophenetic correlation score between accession Puspahiang2 was 0.946 or with the suitable goodness of fit. Accession Puspahiang3 formed a cluster at similarity score of 78% or 22% diversity between progenitor and progeny. This diversity score was the highest compared to those three accessions of the other Puspahiang. While the diversity score between progeny was 98%. Cophenetic correlation score between accession Puspahiang3 was 0.993 or with very suitable goodness of fit. Accession Puspahiang7 connected at similarity level 86%, with similarity score was shown by Puspahiang7-Progenitor and Puspahiang7-progenyl of 97.5%. The range of diversity in Puspahiang7 was 2.5-16% diversity. Phenetic correlation score between accession Puspahiang2 was 0.941 or with very suitable goodness of fit. Similarity score of three accession of Puspahiang population was 60% or with diversity within population of Puspahiang either in progenitor or progeny 40%. Phenetic correlation score between accession of Puspahiang populations was 0.968 or with very suitable goodness of fit.

Accession Tanjungjaya2 formed a cluster at similarity score 90%, at this point the progenitor accession was separated from those three progenies. It means that there were variations between progenitor and progeny of 10%. The highest similarity score was present in Tanjungjaya2-P1 and Tanjungjaya2-P2 93.8%. Cophenetic correlation score between accession Tanjungjaya2 was 0.897 or with suitable goodness of fit. Accession Tanjungjaya7 formed a cluster at a similarity score of 75%. This score was the point at which Tanjungjaya7-P3 was separated between progenitor and the other progeny. The highest similarity score was given by Tanjungjaya7-I and Tanjungjaya7-P1 95.2%. Cophenetic correlation score between accession Tanjungjaya2 of 0.981 or with suitable goodness of fit. In Tanjungjaya8, a cluster was formed at a similarity score of 90% which was the similarity score between progenitor and three progenies, or with diversity between progenitor and progenies of 10%. The highest similarity score was given by Tanjungjaya8-P1 and Tanjungjaya8-P2, while

similarity score among progenies was 93.3%, or with diversity within progenies 6.66%. Cophenetic correlation score between accession Tanjungjaya8 was 0.843 or with suitable goodness of fit. Three accessions of Tanjungjaya population formed a cluster at a similarity level of 53% or with diversity within populations of 47%. Cophenetic correlation score between accession of Tanjungjaya population was 0.972 or with highly goodness of fit.

Accession Sukaraja6 formed a cluster at similarity level of 71.4% or with diversity 29.6% within accession Sukaraja6. The highest similarity score was present at Sukaraja6-I and Sukaraja6-P1, while Sukaraja6-P2 and Sukaraja6-P3 had similarity score 80%. Cophenetic correlation score among accession Sukaraja6-P3 was 0.809 or with suitable goodness of fit. Accession Sukaraja8 formed a cluster at similarity score 73% which was the point of separation between progenitor and three progenies. It means that there were diversity among progenies and progenitor of 27%. The highest similarity score was given by Sukaraja8-P2 and Sukaraja8-P3 90.4%, while the similarity score between Sukaraja8-P1 and the other progenies was 90.1%.

Cophenetic correlation score between accession Sukaraja8 of 0.928 or with highly goodness of fit. Accession Sukaraja9 formed a cluster at similarity level of 77.5% or with diversity between accession 22.5% with the highest similarity score was given by Sukaraja9-P2 and Sukaraja9-P3 86.6%, while the progenitor accession was located in between three progenies with similarity score of 82.9%. Cophenetic correlation score among the accession of Sukaraja9 was 0.926 or with very suitable goodness of fit. Three accessions of Sukaraja formed a cluster at a similarity level 64% or with diversity within Sukaraja population of 36%. Cophenetic correlation score among the accession of Sukaraja was 0.772 or with less suitable goodness of fit.

The results of genetic similarity analysis showed that the average genetic similarity within population of four populations of Tasikmalayan mangosteens, the highest was Salawu (70%), followed by Sukaraja (64%), Puspahiang (60%) and Tanjungjaya (53%). In general, the clusters formed by 48 progenitors and progenies accession were originated from four Tasikmalayan populations i.e. Salawu, Puspahiang, Tanjungjaya and Sukaraja. They formed at 47% similarity score or at diversity among populaion was 53%, which was the point of separation of population from Puspahiang with the other three populations. The highest diversity within



Figure 2. Dendrogram analysis of progenitor and progeny of Tasikmalayan mangosteens with E-RAPD markers.

population was 40% (Figure 2e), where all of the other accessions of each population gathered in a cluster of population and separated from the other populations. However, there was an accession,

namely Tanjungjaya8, which was included in accession Salawu with a similarity score of 71%. In the population, this Tajungjaya8 was separated at 47%. It was estimated that the seedling of this tree

was originated from Salawu, so that the similarity score was closer in Salawu than the original population. The populations with the closest genetic relationship were Salawu and Tanjungjaya with similarity genetic score of 55.5%, while Sukaraja population was located between Salawu and Tanjungjaya population with similarity score of 53%. Cophenetic correlation among 48 Tasikmalayan accessions was 0.877 or with suitable goodness of fit.

### Discussion

The majority of the primers employed in mapping of component 1 vs 2 was primer E2 with 11 bands, followed by E2E4 (8 bands), E3 (6 bands), E1E4 (5 bands), E1E2 (4 bands) and E4 2 (bands) (Table 2). The extraction of eigenvalue 70% or more which play a role in accession mapping in the main component of each accession only given by 2 characters, except Sukaraja population (4 characters) and Tasikmalaya 48 accession (7 characters) (Table 2). Based on E-RAPD markers, analysis of the main component has mapped all progenies clustered together with their progenitor, and the mangosteen accession had also clustered based on the area of origin except Tanjungjaya8 accession which belonged to Salawu8.

# **Apomixis Analysis of Mangosteens**

Based on criteria that progeny of the obligate apomixis plants have identical genetic patterns which identical with the progenitors, therefore a model can be made to determine whether the mangosteens apomictic or not. If one of the loci of E-RAPD of the progenitor and progeny have different allele, it will not be apomictic. If both progenitor and progeny are the same the apomictic character cannot be defined. The apomictic character of mangosteens cannot be defined because the similarity of genetic patterns of progenitor and progeny which may be caused by self fertilization or that the mangosteen is truly apomictic plant.



Figure 3. Three dimensional 48 accession of Tasikmalayan mangosteens based on E-RAPD markers. 1. Salawu2-I, 2. Salawu2P1, 3. Salawu2P2, 4. Salawu2P3, 5. Salawu7-I, 6. Salawu7P1, 7. Salawu7P2, 8. Salawu7P3, 9. Salawu8-I, 10. Salawu8P1, 11. Salawu8P2, 12. Salawu8P3, 13. Puspa2-I, 14. Puspa2-P1, 15. Puspa2-P2, 16. Puspa2-P3, 17. Puspa3-I, 18. Puspa3-P1, 19. Puspa3-P2, 20. Puspa3-P3, 21. Puspa7-I, 22. Puspa7-P1, 23. Puspa7-P2, 24. Puspa7-P3, 25. TJaya2-I, 26. TJaya2-P1, 27. TJaya2-P2, 28. TJaya2-P3, 29. TJaya7-I, 30. TJaya7-P1, 31. TJaya7-P2, 32. TJaya7-P3, 33. TJaya8-I, 34. TJaya8-P1, 35. TJaya8-P2, 36. TJaya8-P3, 37. SRaja6-I, 38. SRaja6-P1, 39. SRaja6-P2, 40. SRaja6-P3, 41. SRaja8-I, 42. SRaja8-P1, 43. SRaja8-P2, 44. SRaja8-P3, 45. SRaja9-I, 46. SRaja9-P1, 47. SRaja9-P2, 48. SRaja9-P3.

	Accession/proportion model of DNA band pattern variation (%)												
Primer	S	Salawu			Puspahiang			Tanjungjaya			Sukaraja		
	1	2	3	1	2	3	1	2	3	1	2	3	
E-1	100.0	0.0	0.0	95.8	0.0	4.2	98.6	1.4	0.0	95.8	4.2	0.0	
E-2	95.4	2.8	1.8	85.7	8.5	5.9	95.4	2.7	1.9	91.2	1.4	7.4	
E-3	89.7	5.2	5.2	89.5	2.2	8.3	91.0	4.8	4.2	81.9	5.6	12.5	
E-4	97.0	2.0	1.0	92.6	2.1	5.3	96.8	3.2	0.0	93.6	0.6	5.8	
E1E2	99.1	0.0	0.9	96.6	2.7	0.7	93.2	1.8	5.0	86.3	10.3	3.4	
E1E4	94.4	2.4	3.2	93.7	1.6	4.7	97.6	0.0	2.4	74.8	9.5	15.7	
E2E4	86.9	7.3	5.8	92.9	3.2	3.9	83.7	6.5	9.8	78.1	7.3	14.6	
Average	94.6	2.8	2.6	92.4	2.9	4.7	93.8	2.9	3.3	86.0	5.6	8.5	

Table 3. A Model of the DNA band pattern variation among 12 progenitor trees with 3 progenies and their proportion with E-RAPD markers.

Note: 1 = equals progenitors, 2 = additional new bands, 3 = lost bands

There were three patterns of band variation in the progeny analysis of Tasikmalayan mangosteens based on E-RAPD markers: (1). Bands of progeny equals progenitors; (2). New band emergence; and (3). Loss of bands in progeny. The genetic consistency resulted in the progeny analysis was relatively high with average of 91.69% (Table 3), shown by the presence of similar bands between progenitor and progeny. The occurrence of band deletion had given the highest proportion on the genetic variability between progenitor and progeny of Tasikmalayan mangosteens with average of 4.77%. While the addition of new bands with the average of 3.54%. Genetic variability proportion showed by band differences raising on each population 5.36-14.05%. The Sukaraja population showed the highest variability (14.05%), followed by Puspahiang (7.6%), Tanjungjaya (6.25%) and Salawu (5.36%). Genetic variability proportion found in this research was lower than those of the mangosteen progeny analysis in West Sumatera (41.3%) (Mansyah et al. 2004). This might be caused by the use of E-RAPD primers with relatively longer bases, so the result suggest more specific than the common RAPD primers.

Based on patterns variation, the band patterns of progeny was not identical as those in progenitor, which should occur in the obligate apomictic plants. Based on the model above, the analyzed mangosteens were not the obligate apomictic plants. It was assumed that DNA band pattern variation occurred due to the process of fertilization in flowers, although more evidence still needed. Persson-Hovmalm et al. (2004) reported that *Aronia* tetraploid population (as apomictic plants) with the use of RAPD markers, the profile between female progenitor and their descendants were identical. On the other hand, Ford & Richards (1995), reported that there were genetic variability between progenitor and progeny in three obligate apomictic plants *Taraxacum*. King & Schaal (1990) reported that in *Taraxacum officinale* there were genotypic variations among progeny. The rate of new genotype emergence in *T. officinale* was higher than the expected random mutation. Richards (1997) stated that the obligate agamosperma may result in genetic variability by somatic recombination or by autosegregation. The genetic variability was needed by apomictic plants as the basis for adaptation and evolution process (King & Schaal 1990).

Variability of obligate apomictic according to Richards (1996 and 1997), Sobir & Poerwanto (2007) could be caused by: 1) accumulation of DNA mutation change; 2) disjunction failure due to polyploidy, haploidy, oligosomy, and polisomy among the descendants; 3) chromosome translocation causing somatic recombination due to activity of transposon, and 4) chromosome mutation in the maternal genome which control apomixis. In mangosteens, genetic variation could be caused by some phenomena such as natural mutation due to domestication since thousands years ago (Ramage et al. 2004), the ploidy failure or progenitor crossing many times (Sobir & Poerwanto 2007).

Reproduction mechanism in the mangosteen was included as adventive embryony (Naumova 1993). This reproduction has started since their ancestors hybridized many times followed by somatic diploidy process in the avular tissues and apomictic sporophyte, then forming adventive embryo and embryo somatic cells (nuselus or integument) (Spillane et al. 2001). Those mechanism may have caused genetic variation between progenitor and progeny on mangosteens. According to Koltunow et al. (1995) and (Darrigues et al. 2008), generally, the apomictic plants were controlled by one dominant gene locus playing a role in explaining apomictic paths.

Genetic diversity in Salawu population (5-14%), Puspahiang (9-25%), Tanjungjaya (10-25%), and the highest was Sukaraja (22.5-29.6%), while among population ranged from 31.2-47%. The progeny was not identically similar to the progenitor. The average genetic consistency in four locations of mangosteen was 91.69%, consistency score 100% reached in Salawu8 progeny-1, and Puspahiang progeny-2 and progeny-3. while the other accession had similarity from 71.4-98%. The mangosteen regarded as plants descended from the diplospory gametophytic apomictic pathway. The best primer playing roles in clustering were E2 with 11 bands, followed by E2E4 (8 bands), E3 (6 bands), E1E4 (5 bands), E1E2 (4 bands) and E4 2 (bands). E-RAPD can be used for apomictic analysis between progenitor and progeny of different mangosteens.

# ACKNOWLEDGEMENT

This study was funded by special grant from Indonesian Ministry of Science and Technology through National Strategic Research Initiative (RUSNAS) for tropical fruit development to Centre for Tropical Fruit Studies of Bogor Agricultural University. We would like to thank to Dr. Fitmawati, Drs. Pudji Widodo, M.Sc., Sulassih, Hery Jumhair, and Pras Setyo for their kindly help.

# REFERENCES

- Acuña CA, Martínez EJ & Quarin CL. 2005. Sexual diploid and apomictic tetraploid races in *Thrasya petrosa (Gramineae). Australian Journal of Botany* 53: 479–484.
- Darrigues A, Daub J, McCord K, Rasmussen C & Rouse J. 2008. Genetic Analysis of Apomixis. Apomixis-Genetic Analysis.[terhubung berkala]. http://www.public.iastate.edu/ ~mbhattac/bhattacharyya/genetics.pdf.[9-2-2008].
- Ford H & Richard AJ. 1995. Isoenzyme variation within and between *Taraxacum* agamospecies in a single locality. *Heredity* 55: 289–291.
- King LM & Schaal BA. 1990. Evolution Genotypic variation within asexual lineages of

Taraxacum officinale. Proc. Natl. Acad. Sci. 87: 998–1002.

- Koltunow AM, Bicknell RA & Chaudhury AM. 1995. Apomixis: Molecular Strategies for the Generation of Genetically Identical Seeds without Fertilization. *Plant Physiol*.108: 1345–1352.
- Koltunow AM. 2008 Apomixis: state of the art and potential applications.[terhubung berkala]. http:///www.CSIRO.au. [7–2–2008].
- Mansyah E, Syah MJA, Usman F & Purnama T. 2004. Genetics variability between parental tree (*Garcinia mangostana* L.) and their progenies. *Jurnal Hortikultura* 14: 229–237 (in Indonesian, with English abstract).
- Naumova TN. 1993. Apomixis in Angiosperms: Nucellar and Integumentary Embryony. CRC Press, Boca Raton, FL.
- Nei M & Li WH. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* 76 (10): 5269–5273.
- Persson-Hovmalm HA, Jeppsson N & Bartish IV. 2004. RAPD analysis of diploid and tetraploid populations of Aronia points to different reproductive strategies within the genus. *Hereditas* 141: 301–312.
- Ramage CM, Sando L, Peace CP, Carroll BJ & Dew RA. 2004. Genetic diversity revealed in the apomictic fruit species Garcinia mangostana L. (mangosteen). Euphytica 136 (1): 1–10.
- Richards AJ. 1996. Genetic variability in obligate apomicts of the genus *Taraxacum*. Folia Geobotanica. *In*: AJ Richards, J Kirschner, J Stepanek & K Marhold (Eds.).Proceedings Apomixis and Taxonomy of the Symposium held in Pruhonice, Czech Republic 1995. Volume 31, Number 3/December, 1996 p 405–414.
  - . 1997. Plant Breeding Systems. 2<sup>nd</sup> Ed. Department of Agricultural and Environmental Science University of Newcastle Upon Tyne. Chapman and Hill. London. 529 p.
- RolfFJ. 1998. NTSys-pc. Numerical Taxonomy and Multivariate Analysis Syatem. Version 2.02. Exerter Software. New York.
- Sari R, Lukman R, Sinaga S & Amelia R. 2008. Evaluating genetic relationships in Indonesian Mangosteen (*Garcinia mangostana*) using AFLP profiling. *Biotropia* in press.
- Sinaga S, Sobir, Poerwato R, Aswidinnoor H & Duryadi D. 2006. Analisis variabilitas genetik

tanaman Manggis (*Garcinia mangostana*) dan kerabat dekatnya dengan penanda isoenzim dan RAPD. *Floribunda* 3(1): 1–9.

- Sobir & Poerwanto R. 2007. Mangosteen Genetics and Improvement. International Journal of Plant Breeding. *International Journal of Plant Breeding* 1(2): 105–111.
- Spillane C, Steimer A & Grossniklaus U. 2001. Apomixis in agriculture: the quest for clonal seeds. *Sex Plant Reprod.* 14: 179–187.
- Tanaka J & Taniguchi F. 2002. Emphasized-RAPD (e-RAPD): a Simple and Efficient Technique to make RAPD Band Clearer. *Breeding Science* 52: 22–229.
- Yapwattanaphun C, Subhadrapitahu S, Honsho C & Yonemori K. 2004. Phylogenetic relationship of mangosteen (Garcinia mangostana) and several wild relative. Journal of the American Society for Horticultural Science 129(3): 368-373.
- William JGK, Kubelik AR, Livak KJ, Ravalski JA, & Tingey SV. 1990. DNA polimorphisms amplified by arbitrary primer are useful as genetic markers. *Nucleic Acid Research* 18(22): 6531–6535.

 Dewan Penyunting *Floribunda* amat berterima kasih kepada Prof. Dr. Mien A. Rifai (AIPI Jakarta)
Prof. Dr. Wasmen Manalu (IPB Bogor)
Prof. Dr. Sudarsono (IPB Bogor)
Dr. Rugayah (LIPI Bogor)
Dr. Pudji Widodo (UNSOED Purwokerto)
Dr. Fitmawati (Univ. Riau Pekanbaru)

atas kesudiannya bertindak selaku mitra bestari untuk terbitan *Floribunda* 3(4)

Terbit: Oktober 2007

Tanggal terlaksana: 31 Desember 2009

\*\*\*

Penggalang Taksonomi Tumbuhan Indonesia ingin merekamkan rasa terima kasihnya pada Program Hibah Simposium Nasional Organisasi Profesi Direktorat Penelitian dan Pengabdian Kepada Masyarakat – DIKTI untuk ikut mendanai penerbitan *Floribunda* 3(4)