Jurnal Natur Indonesia 13(1), Oktober 2010: 53-58
ISSN 1410-9379, Keputusan Akreditasi No 65a/DIKTI/Kep./2008

Genetic Diversity and The Relationship Between The Indonesian Mangosteen (*Garcinia Mangostana*) and The Related Species Using Isozyme Markers

Soaloon Sinaga[1*)], Sobir[2)], Roedhy Poerwanto[2)], Hajrial Aswidinnoor[2)], dan Dedy Duryadi[3)]

[1)] Kopertis Region III, Jakarta, Indonesia
[2)] Department of Agronomy and Horticulture, Bogor Agricultural University
[3)] Department of Biology, Bogor Agricultural University

Diterima 09-12-2009 Disetujui 04-05-2010

ABSTRACT

Indonesia was known to have high diversity of mangosteens (*Garcinia mangostana*) and the related species. In order to elucidate the genetics variability of the diversity, thirty three accessions were examined by using isozyme analysis. The genetic diversity and relationships among several mangosteens and other *Garcinia* sp were established by using four isozymes. The level of polymorphism as revealed by isoenzyme was 88%. Although mangosteen is believed to reproduce exclusively through apomixis, our result show that considerable genetic diversity exists within *G. mangostana* and between other *Garcinia* species. Based on 27 bands there were 5-42% dissimilarity level among mangosteen accessions, while the other species has 75% dissimilarity. The dendrogram is built based on isozyme marker analysis to separate clusters of mangosteen and other *Garcinia sp*. The data showed that *G. mangostana* is a close relative of *G. malaccensis*, *G. porrecta*, *G. celebica,* and *G. hombroniana*. The concurrence analysis on isozyme analysis result showed the very good fit of Rolf correlation value (0.914). This result indicated that isozymes could group *G.mangostana* and the related species.

Keywords: genetic diversity, isozymes, mangosteens, related species

INTRODUCTION

The mangoesteen (*Garcinia mangostana* L) or known as *Queen of Tropical Fruits* is one of the best exported commodity from Indonesia. To increase the economic values, the production needs to be increased. This can be reached by the improvement of cultivation techniques and the use of the best seedlings as a result of the correct and strategic breeding.

Genetic improvement of the mangoesteen depends on the genetic diversity sources. Indonesia is centre of diversity of mangoesteen in the South East Asia. Exploration, identification, and characterization were needed to find out the information about the new genetic diversity resources to improve the genetic characters and production. This study was aimed to trace the presence of variation in Indonesia mangoesteens (Drew, 1997).

Mangoesteen is obligate apomictic that is the seeds were not the product of fertilization and the genetic diversity is narrow, so that it was estimated that there is only one species of mangoesteen on nature. In facts, there are some variations which may be caused by the environment or genetic factors due to natural mutation following the history thousands of years ago (Ramage *et al*., 2004), high yields, various color of the seedlings and isozyme band analysis (Supriyanto *et al*., 1999), and various in morphology (Mansyah *et al*., 1994).

Based on isozyme *glucose phosphateisome-rase* (GPI) analysis of West Sumateran mangoesteens with 14 samples examined showed similar band patterns although the phenotypes were variable. On other words, the genetic variability was narrow, but the phenotypes were wide (Mansyah *et al*., 1999). Genetic analysis with contemporary techniques for wider species could be used to identify the paternal progenitors for breeding with the mangoesteen as the maternal progenitors (Osman & Abdul, 2006).

The mangoesteen has a long life cycle, so that a genetic study is difficult to conduct. Thus estimation of genotype variability by using morphological markers and isozymes was important. Isozyme analyses had been done on plants like *Aristolochia manshuriensis* (Nakonechnaya *et al.,* 2007), *Terminalia paniculata* (Thangaraja & Ganesan, 2007).

*Telp: +6281385065093
Email: horas97@yahoo.com

The use of isozyme has given important contributon for plant breeders to handle the apomixis (Ramage *et al*., 2004). Variability analysis in the mangoesteen and genotyping the other *Garcinia* were important to find the best progenitors for breeding. The aims of this research were to find out genetic variability and phylogentics among mangoesteens and related species, as well to find out the progenitors.

MATERIALS AND METHODS

Plant Material. Thirty four samples of mangoesteen were collected from some areas in Indonesia and from Bogor Botanic Garden and Mekarsari Fruit Park (Table 1).

Thirty four samples of fresh leaves were used for isozyme analysis. Before analysis, all leaves were cleaned, dried, and sprayed with alcohol 70%. The rest of alcohol was stored in the freezer (-20^{0}C). This analysis was done following Soltis and Soltis (1989). The enzymes analyzed were peroxidase (PER), phosphatase acid (ACP), Malic Dehydrogenase (MDH), and Esterase (EST). The isozyme bands were separated by using electrophoresis with 10% strach gel for 4 hours and 100 volt.

The bands were translated as binary data. These data were used to arrange matrices of genetic diversity based on formula of Nei and Li (1979) with method of UPGMA (*Unweighted Pair-Group Method Aritmetic*) using NTSYS (*Numerical Taxonomy and Multivariate System*) version 2.02 (Rolf 1998). The coefficient of Nei dan Li (Nei and Li 1979) calculated genetic similarity (GS) between two samples, i and j, with formula $GS(i,j) = 2Nij / (2Nij + Ni + Nj)$, where *Nij* is the number of bands presence between i and j, Ni number of bands present in i and absent in j, Nj is the number of bands present in j and absent ini.

RESULTS AND DISCUSSION

Fragment of 34 accessions of mangoesteens and related species with four isozymes results in 28 bands

Table 1. Accession of mangoesteen and related allies analyzed using isozyme markers

N0.	Accession	Provice/country of origin	N0.	Accession	Provice/country of origin
1	Lampung	Lampung	18	IPBPAU	West Java
2	Kalteng	Central Kalimantan	19	IPBREK	West Java
3	Hatalas	Maluku	20	Pontianak1	West Kalimantan
4	Kaligesing	Central Java	21	Pontianak2	West Kalimantan
5	Kusu-Kusu	Maluku	22	Pontianak3	West Kalimantan
6	Semarang	Central Java	23	Pontianak4	West Kalimantan
7	Ponorogo	East Java	24	*G.malaccensis*	Jambi
8	Cicurug	West Java	25	*G.bancana*	Bangka
9	Banten	Banten	26	*G.hombroniana*	Malay Peninsula
10	Jayanti	West Java	27	*G.celebica*	Sulawesi
11	Wanayasa	West Java	28	*G.benthami*	Vietnam
12	Kaliagung	West Java	29	*G.xanthochymus*	Malay Peninsula
13	Tasikmalaya	West Java	30	*G.livingstone*	Afrika Tropik
14	Sukabumi	West Java	31	*G.cowa*	Jora Sumatera
15	Kaliangger	West Java	32	*G.latiflora*	Java/Lesser Sunda
16	CengalLW	West Java	33	*G.forbesii*	West Java
17	Soya	Maluku	34	*G.sizygiifolia*	Sarawak

Isozyme Analysis

Table 2. The number of bands and levels of polymorphism of 4 isoenzymes in 13 accession of mangoesteens and closely related species

NO	Isoenzymes	No bands	Polymorphic bands	Monomorphic bands
1	Esterase 1	4	4 (100%)	0
2	Esterase 2	3	3 (100%)	0
3	Esterase 3	3	3 (100%)	0
4	Peroxydase 1	3	3 (100%)	0
5	Peroxydase 2	3	3 (100%)	0
6	Peroxydase 3	1	0 (0%)	1
7	Acid phosphatase 1	1	1 (100%)	0
8	Acid phosphatase 2	4	2 (50%)	1
9	Malate dehydrogenase 1	1	0 (0%)	1
10	Malate dehydrogenase 2	5	5 (100%)	0
		28	24 (85.7%)	3

(Table 2). This could be used to determine diversity of the 34 accessions with high polymorphism (85.7%). The cluster analysis results in a dendrogram which separate the mangoesteen from the other rewlated species with dissimilarity index 43%, except *G. malaccensis* which grouped with the mangoesteen (Figure 1).

The cluster of mangoesteens and related species was formed with similarity coefficient of 14 - 97%. The mangoesteen group had similarity level higher (43 - 97%) than the related species (14-80%). At the similarity 73%, the mangoesteen formed five groups. Group I consists of 2 subgroups, in which group I consists of *G. malaccensis* which came from Jambi grouped together woth those from Lampung and Central Kalimantan with similarity 80%.

Subgroup 2 consisted of mangoesteens from West Java (Kaliagung, Tasikmalaya, Sukabumi, IPB Rektorat, Cengal, and Kaliangger) and Maluku (Soya). Group 2 consists of those from West Java (Jayanti, Cicurug, and Wanayasa), Central Java (Semarang and Kaligesing), Banten, and East Java (Ponorogo). Group 3 and 4 consists of one accession each namely Hatalas and IPB PAU respectively. Group 5 comprises five mangoesteens from Pontianak (West Kalimantan). The close relatives of *G. hombroniana* which was regarded as the mangoesteen maternal progenitor was located with 39 similarity (Figure 1). The cophenetic correlation score of dendrogram with function MxComp r = 0.911, which means that the dendrogram was with goodness of fit very suitable with isozymes to depict those grouping (Rolf, 1998).

Component analysis based on eigenvalue showed precentage accumulation of seven first main components representing 72.647% diversity (Table 2) of total 100% of 28 bands (characters). It means there were seven main characters which significantly play roles in the clustering of the mangoesteen and its related species. Those seven characters were selected from seven absolute score PC1 and PC2 with the highest score (Table 2 and 3). There were 21 characters which did not significantly influence the plotting of the 34 accessions.

Based on PC1 and PC2, spread diagram was made to map the accession of mangoesteen and related species. It was different from mapping with dendrogram, there were some accessions from Pontianak which was

Table 3. Cgaracter scores, proportion, cummulatives, and character number contributing in grouping

Eigen value	Proporsion	Cummulative	Character number playing roles
1.143	0.243	0.243	7
0.804	0.171	0.414	5
0.437	0.093	0.507	3
0.347	0.074	0.581	2
0.336	0.072	0.653	2
0.265	0.056	**0.709**	2
		Total	21

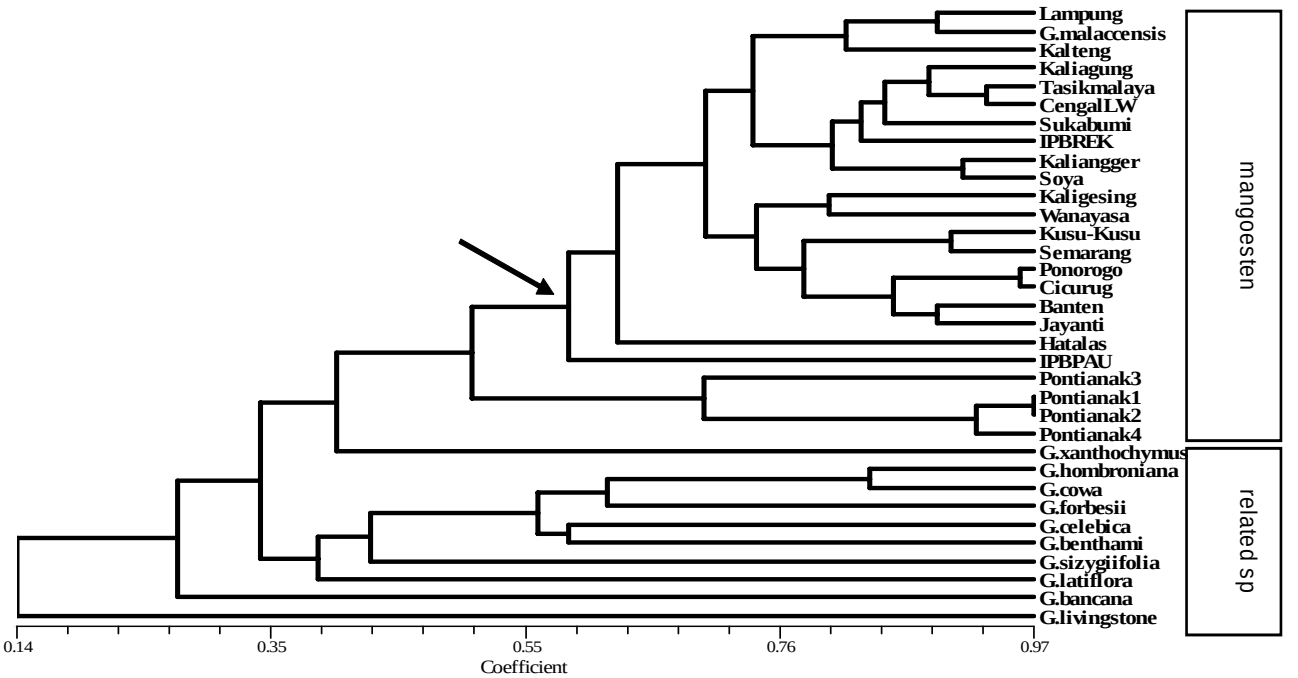

Figure 1. Dendrogram of 34 accession of mangoesteen and its closely related species based on isozyme markers

relatively far from the other mangoesteen groups. However, the mangoesteen accession still could be mapped separately from the other closely related species except *G. malaccensis* 1 with 2 (Figure 2).

Two dimension mapping by using component 1 and 3 (Figure 3) tend to be more similar with those produced by the dendrogram. This result showed that the characters playing the most important roles in constructing the dendrigram came from component 1 and 3. While mapping based on component 2 and 3 did (Figure 4) was not similar as those based on morphology.

The analysis results of the principle component on 28 isozyme bands showed that 21 bands had the highest the main component absolute score (Table 4).

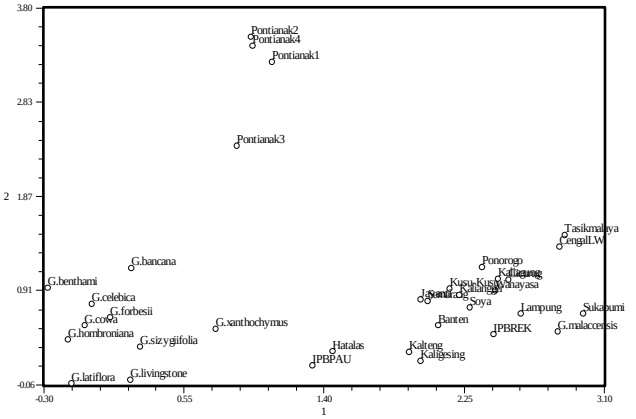

Figure 2. Spread diagram of two dimensionof component 1 and 2 of mangoesteen and relatives 1 and 2

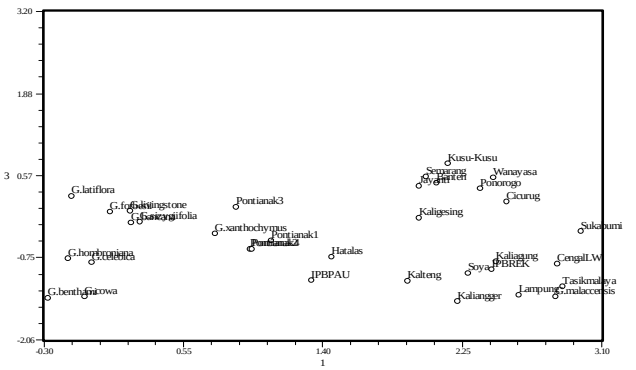

Figure 3. Spread diagram of two dimensionof component 1 and 3 of mangoesteen and relatives 1 and 3

Determination of the 21 bands was obtained from the accummulation diversity proportion of each PC i.e. 70% with details contributions as follows: PC1 with diversity proportion 21.81%, PC2 with diversity proportion 15.83%, PC3 13.39%, PC4 9.01%, PC5 8.91% and PC6 9.23%. There were 6 repeated bands within 2 PC which caused total number of bands became 14 not 21. Esterase system enzymes (EST) contributed the majority bands to cluster namely 6 bands, Malate dehydrogenase (MDH) and Peroksidase (PER) with 3 and Acid phosphatase (ACP) with 2 bands.

Mapping with three dimension showed clusters matching those based on dendrogram with a tendency of mangoesteen separated from relatives (Figure 5). Therefore the use of three components continually parallel with grouping with dendrogram using all characters.

Correlation analysis between bands of each enzyme system can be used to select enzyme system prioritized in isozyme analysis in mangoesteens and allies (Table 5). The presence of EST7 bands significantly correlated with 3 bands on 3 other enzyme systems namely PER6, MDH4 and ACP5. Band EST10 significantly correlated with MDH3, MDH5 and ACP4. This suggests that Esterase enzyme systems was more effective in grouping and could represent bands of the other enzyme systems.

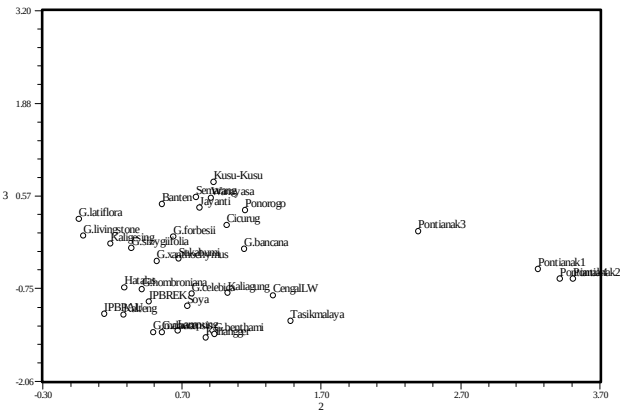

Figure 4. Distribution diagram of two dimension of component 2 and 3 of mangoesteen and relatives

Table 4. Characters contributing clustering in mangoesteen and related species

C	PC1	C	PC2	C	PC3	C	PC4	C	PC5	C	PC6
EST10	0.424	EST1	0.358	PER4	0.594	ACP2	0.571	PER2	0.484	EST8	0.603
MDH5	0.370	PER6	0.336	PER5	0.470	EST5	0.330	EST5	0.407	PER4	0.320
EST3	0.317	MDH4	0.322	ACP2	0.275						
EST2	0.309	EST2	0.287								
MDH3	0.283	PER2	0.280								
ACP3	0.248										
PER5	0.230										

Notes: C = isozyme band characters

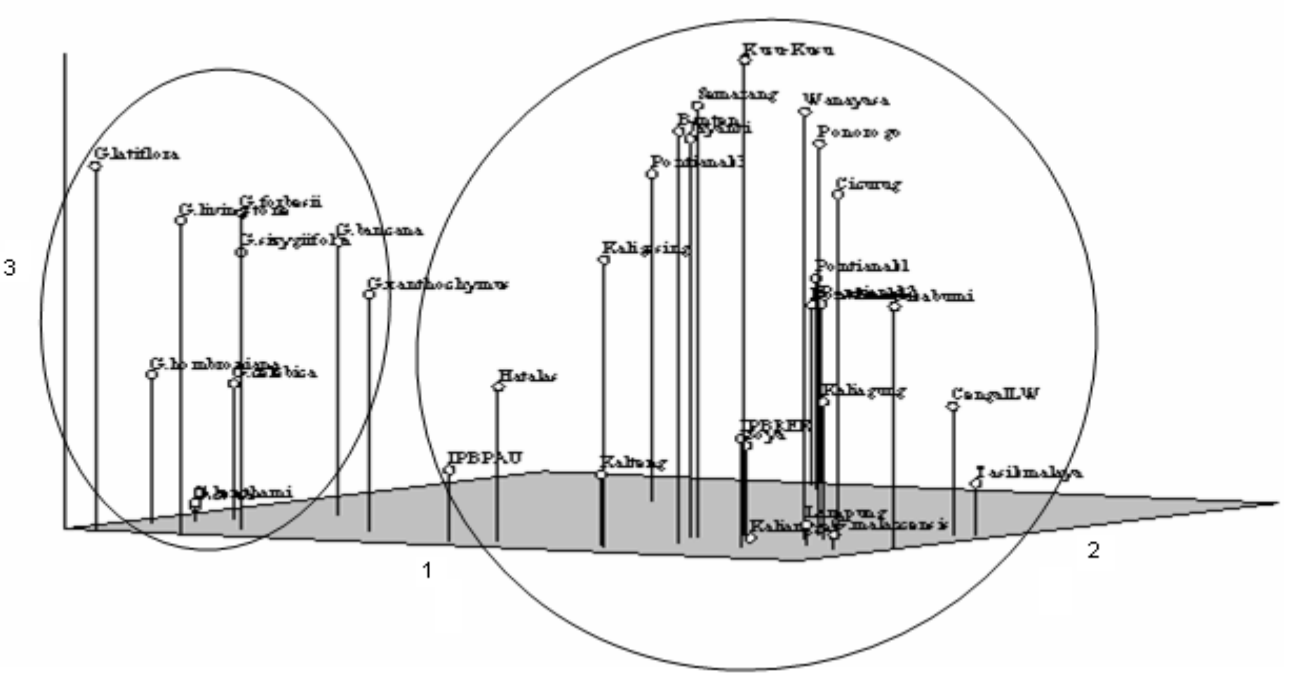

Figure 5. Analysis of similarity main conponents of mangoesteen and related species mapped in three main axis based on isozyme markers

Table 5. Significant correlation among isozyme bands

Characters	EST4	EST7	PER3	EST10	PER6	MDH1	MDH3	MDH4	MDH5
PER6	¤	0.602	0.610	¤	¤	¤	¤	¤	¤
MDH2	-0.685	¤	¤	¤	¤	¤	¤	¤	¤
MDH3	¤	¤	¤	-0.609	¤	¤	¤	¤	¤
MDH4	¤	0.602	¤	¤	0.766	¤	¤	¤	¤
MDH5	¤	¤	¤	0.699	¤	0.600	-0.736	¤	¤
ACP3	¤	¤	¤	¤	¤	¤	-0.640	¤	0.736
ACP4	¤	¤	¤	-0.640	¤	¤	¤	0.643	¤
ACP5	¤	0.804	¤	¤	0.749	¤	¤	0.749	¤

Notes: ¤ = non significant, PER = Peroxydase , MDH = Malate Dehidrogenase, ACP = Acid phosphatase, and EST = Esterase.

Table 6. Characteristics of flowers and fruits of *G. mangostana, G. malaccensis,* and *G. hombroniana*

Karakter	*G. Mangostana*	*G. malaccensis*	*G. hombroniana*
Flowering time(month)	3-4; 7-9	4-7	1-3(-6)
Fruit color	violet	maroon	red
Fruit taste	sweet/acidic	sweet/neutral	kelat
Petal ♂	-	red	yellow
Stigma	4-8 lobes	8 deep lobed	shallowly lobed

Source: Richards (1990).

Progenitor Analysis if Mangoesteens Based in Isozyme. Distribution patterns and isozyme band number of accession of mengoesteen and allies from all isozyme systems can be used to trace the progenitors based on band contribution to the progenitors. Band proportion contributed by two progenitors were 13.5% (*G. hombroniana*) and 44.2% (*G. malaccensis*) respectively, while bands owned by mangoesteen only 42.3% (Figure 6). Morphological characters such as flower colors, fruit colors, fruit taste, and stigma number (Table 6) showed that mangoesteens was located among twi progenitor characters. Morphologically, the mangoesteen tend to be similar as *G. malaccensis* rather than *G. hombroniana.*

Therefore, hypothesis stating that mangoesteens were hybrid of *G. malaccensis* and *G. hombroniana* is acceptable. Genetic diversity of mangoesteen and

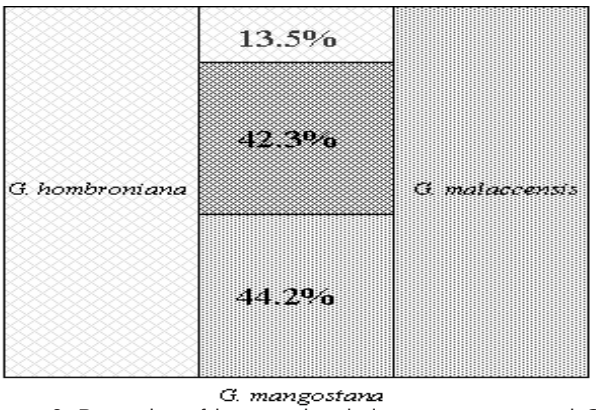

Figure 6. Proportion of isozyme bands in mangoesteen and *G. hombroniana* and *G. malaccensis*

related species based on isozyme (83%) was high for obligate apomixis than *Taraxacum* (19%) (Ford & Richards, 1985). Variation presence in apomixis accurred more rapidly than mutation (Hughes & Richards, 1985). High polymorphic percentage was incommon in the mangoesteen as an obligate apomixsis. Possibly, this was caused by the fact that *G. mangostana* was not monohybrid. Repeated hybridization among progenitors had caused the presence of wider genetic variation among the progenitors.

The high genetic variation among mangoesteens was very potential for priducing high quality plants. This can be done by mass selection in some plants to produce new varieties.

Based on isozyme markers, *G. celebica* and *G. hombroniana* which were estimated as the progenitors were located at genetic similarity if 43%, while *G. malaccensis* was within the mangoesteens (90%). Richards (1990) stated that the mangoesteen is allotetraploid (2n=90) descended from *G. malaccensis* (2n = 42) and *G. hombroniana* (2n = 48), with intermediate morphology of both diploid species.

CONCLUSIONS

Genetic diversity of mangoesteen and closely related species can be traced with four isozymes with 85,7% polymorphism. Genetic variability of mangoesteen and related species was wide (3-86%). *G. mangostana* as an obligate apomictic plant have wide genetic variation. They can be grouped into 5 groups, while their closely related species had formed three groups. The hypothesis stating that *G. mangostana* was the hybrid of *G. hombroniana* and *G. malaccensis* based on isozyme markers is acceptable.

ACKNOWLEDGEMENT

The authors would like to tanhk to the ministry of research and tehnology trhrough the National strategis commodity research project (Rusnas) in 2003-2007, and to center for tropical fruit (PKBT) IPB for the availability of representative laboratory.

REFERENCES

Drew, R.A. 1997. The Application of Biotechnology to the Conservation and Improvement of Tropical and Subtropical Fruit Species. Seed and Plant Genetic Resources Service. Rome: Food and Agriculture Organization of the United Nations.

Ford, H. & Richards, A.J. 1985. Isozyme variation within and between *Taraxacum* agamospesies in a single locality. *Heredity* **55**: 289-291.

Hughes, J. & Richards, A.J. 1985. Isozyme in heritance in diploid *Taraxacum* hybrid. *Heredity* **54**: 245-249.

Mansyah, E., Edison, H.S. & Winarno, M. 1994. Eksplorasi dan Studi Keragaman Manggis (*Garcinia mangostana* L.) di Sumatera Selatan. *Laporan Hasil Penelitian Balai Penelitian Hortikultura Solok.*

Mansyah, E., Jawal, M., Lukitariati, A. & Susiloadi, A. 1999. Variabilitas genetik tanaman manggis melalui analisis isoenzim dan kaitannya dengan variabilitas fenotipik. *Zuriat* **10(1)**: 1-9.

Nakonechnaya, O.V., Koren, O.G. & Zhuravlev, Yu. N. 2007. Allozyme variation of the relict plant *Aristolochia manshuriensis* Kom. (Aristolochiaceae). *Russian Journal of Genetics* **43(2)**: 156-164.

Nei, M. & Li, W.H . 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl acad Sci USA* **76(10)**: 5269-5273.

Osman, M. & Abdul, R.M. 2006. Mangosteen *Garcinia mangostana* L. Southampton Center for Underutilised Crops, University of Southampton, UK. 169p.

Ramage, C.M., Sando, L., Peace, C.P., Carroll, B.J. & Dew, R.A. 2004. Genetic diversity revealed in the apomictic fruit species *Garcinia mangostana* L. (mangosteen). *Euphytica* **136(1)**: 1-10.

Richards, A.J. 1990. Studies in Garcinia, dioecious tropical forest trees: the origin of the mangosteen (*G. mangostana*). *Botanical Journal of The Linn.Soc* **103**:103-308.

Rolf, F.J. 1998. NTSys-pc. Numerical Taxonomy and Multivariate Analysis Syatem. Version 2.02. Exerter Software. New York.

Soltis, D.E. & Soltis, P.S. 1989. Isoenzimes in Plant Biology. *Portland, Oregon:* Discorides Press.

Supriyanto, A., Muharam A. & Hariyanto, B. 1999. Evaluasi keragaman pohon manggis pada sentra produksi di Jawa dan Lombok dengan analisis isoenzim. *Bul. Plasma Nutfah* **5(1)**: 6-10.

Thangaraja, A. & Ganesan V. 2007. Allozyme diversity in South Western Ghats populations of *Terminalia paniculata* Roth. (Combretaceae). *African Journal of Plant Science.* **1(1)**: 7-13.