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Mangosteen Genetics and Improvement

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ABSTRACT

Mangosteen (*Garcinia mangostana* L.) originated from natural hybridization of *G. malaccensis* and *G. hombrioniana*. Mangosteen is reproduced from adventitious embryos, from which the seed develops without fertilization, i.e. agamospermy or apomixis. Studies on mangosteen flowers revealed that the stamens and pistils developed at an early stage of flower development, however subsequently staminate growth is stunted and aborted. Apomictic reproduction leads to the assumption that mangosteen trees have same genetic properties. However, field evaluation showed variability in several morphological characters, such as tree shape, fruit shape, and petal color. Further studies using DNA markers confirmed genetic variability among the mangosteen population. The variation may have arisen from accumulation of natural mutations. Another hypothesis is that the mangosteen population may have developed from more than a single hybridization of its two wild progenitor species. Crop improvement has been conducted using mutation breeding through application of gamma ray irradiation on seed callus, as well as on cell culture. Early results indicated genetic variability increased three-fold compare to that of natural levels as detected by RAPD analysis.

Keywords: apomixis, *Garcinia mangostana*, mutation breeding, variability

CONTENTS

INTRODUCTION

The mangosteen (*Garcinia mangostana* L.) has been hailed as the "queen of tropical fruits" due to its instant visual and taste appeal (Cruz 2001), and has recently been popularized for its medicinal benefits (Iinuma *et al*. 1996; Sakagami *et al*. 2005; Mahabusarakam *et al*. 2006). The binomial *G. mangostana* was established by Carolus Linnaeus, derived from the first collector and the local name of the fruit. *Garcinia* was first described by *Linnaeus* based on a specimen he received from Laurentius Garcin (1683-1571), a naturalist and correspondent of Linnaeus who worked periodically as a ship doctor in the Nederland Indies (Indonesia), and the specimen was from Moluccas and called mangostan by the locals (Whitmore 1973).

Mangosteen belongs to the *Guttiferae* family, 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 (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, long juvenile periods (10-15 years). Seed has low viability and short life, and must be planted within a few days (Purseglove 1968).

Some species of *Garcinia*, including *G. mangostana* produce fruit without pollination, the phenomenon is referred to as agamospermy, which is the production of seed without fusion of gametes (Thomas 1997). The process of embryo formation in *G. mangostana* was first studied by Treub (1911) who reported that the early development of woodiness in the endocarp soon after anthesis made observation of embryo development difficult. However, Lan (1989) provided a detailed account of mangosteen embryology and reported that the embryo of *G. mangostana* is derived from tissue of integument instead of from the egg. Based on its reproductive mode, mangosteen has been classified as an apomictic plant (Horn 1940; Richards 1997). Such plants propagate through apomixis seed, which is embryo and seed formation without reduction of the chromo-

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some number and fertilization of the egg (den Nijs and van Dijk 1993). Apomixis in mangosteen implies that the same genetic properties of parent should be in its progenies (Koltunow *et al*. 1995).

Apomixis occurs throughout the plant kingdom, from algae to angiosperm (Asker and Jerling 1992). Apomictic processes occur in the ovule, resulting in progeny that are exact copies of the female plant. The apomictic embryo is formed via two fundamentally different pathways, gametophytic or sporophytic (Asker and Jerling 1992; Koltunow *et al*. 1993). In gametophytic apomixis, embryo sac is formed from nucelar cells or megaspore mother cells (Koltunow *et al*. 1993), and in sporophytic apomixis, the embryo arises directly from the nucellus or the integument of the ovule in a process generally called adventitious embryony. Apomictic seed in mangosteen, as well as in orchids, *Citrus* and mangoes are classified as adventitious embryony (Naumova 1992).

FLOWER DEVELOPMENT

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 iod treatment revealed that mangosteen anthers have no viable pollen (Mansyah 2002). This was reported earlier by Horn (1940). The failure of mangosteen flowers to produce fertile pollen support the theory of apomictic reproduction (Horn 1940; Richard 1990).

It takes 30 to 35 days for mangosteen flowers to develop to anthesis (Mansyah 2002). Rai (2004) through microscopic observation reported that from flower induction to anthesis required 40 days. Flower initiation (Stage I) is indicated by enlargement of the shoot base 40 days prior to anthesis. In Stage II, four days after initiation (DAI), flower primordia emerged as a compact mass of 0.2 mm diameter. Stage III is denoted by flower primordia and calyx development on 8 DAI. Subsequently Stage IV is indicated by sepal primordia development on 12 DAI. At Stage V on 16 DAI, pistil and stamen primordia have developed already, and flower stalk has been extended. Stage II to V are classified as flower differentiation (Bernier *et al*. 1985) which started with initiation of flower primordia, followed by sepal and petal primordia, and development of stamen and pistil. Stage VI at 22 DAI is indicated by pistil and stamen development, followed by Stage VII at 28 DAI, denoted by enlargement of pistil; but stamens remain stunted. At Stage VIII on 34 DAI, development of edible pulp primordial is begins. Stage IX is anthesis, occurred at 40 DAI (Rai 2004).

Microscopic observation also revealed that fruit and seed development was initiated by development of edible pulp at Stage VIII of flower development, and seed primordia were developed prior to anthesis (Rai 2004). Since staminodes fail to reach pistil, it was predicted that the viable seed produced without fertilization of the egg. These observations confirmed previous finding that mangosteen seed is apomictic (Asker and Jerling 1992). Bicknell and Koltunow (2004) summarized that apomixis has been described in more than 400 flowering plant taxa, including representatives of more than 40 families.

GENETIC VARIABILITY

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 carrying the same genetic properties, even in obligate apomixis (Asker and Jerling 1992). Variability in progeny of obligate apomixis plant has been reported in genus *Taraxacum* (Ford and Richards 1985).

Genetic studies on apomictic plants generally are 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. Genetic variability analysis of mangosteen was carried out through evaluation of morphological characters of several mangosteen populations, studies on seedling characters of seedlings 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 shape of fruit, one type producing a round shape with semi-flat bottom end and the other type with oblong shape fruit 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 show wide variability in leaf length, fruit weight and rind thickness. Mangosteen found in Tembilahan, Sumatera Island, exhibit flattened fruit shape, very short peduncle, elliptic stigma lobe (Mansyah *et al*. 2005)

In our studies (Mansyah 2002; Prabowo 2002; Purwanti 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, 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 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**).

In fruit morphology the variation was found in weight, length, diameter, length/diameter ratio, rind thickness and peduncle length, and also Total soluble solids (TSS), and presence of fruit latex. Numbers of locules and seed per fruit did not significantly. Correlation analysis showed that TSS was correlated negatively with fruit diameter, fruit

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
Compilation, with permission from Prabowo 2002, Purwanti 2002, Mansyah 2002, Suhaeri 2003.

Table 2 Analysis of variance (Bartlett test) for seedling performance of three populations of mangosteen.

Characters	Origin of parent tree			χ^2
	Wanayasa	Watulimo	Gunung Salak	
Germination rate (%)	91.76	90.75	100.00	16.05*
Flush rate of second leaf (days)	45.81	36.44	42.85	2.40ns
Leaf Area Index (cm^2)	14.02	12.45	11.7	1.19ns
Leaf Area (cm^2)	20.932	18.671	18.807	0.89ns
Plant Height 7 WAP (cm)	3.16	2.62	3.32	1.73ns
Plant Height 27 WAP (cm)	5.48	4.32	4.97	1.54ns
Leave Numbers 7 WAP	2.8	2.4	2.8	1.53ns
Leave Numbers 27 WAP	6.2	5.2	5.5	2.25ns

ns, * : non-significant and significant at p=0.05, respectively by Bartlett test
Compiled, with permission from Anggraeni 2003.

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

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 larger fruit size and seed number/fruit, and the fruits from Watulimo for superior sweetness and lower yellow latex occurrence.

In recent exploration we found a new distinctive type of mangosteen in Kalimantan (Borneo) that produces fruit with insignificant size of seed (less than 1 cm in length), and have bigger fruit size, out with 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 (**Fig. 1**), with white and pale orange color of petals compared to color petal of common mangosteen.

Seedling analysis

The apomictic character in mangosteen resulted the seed are not really true seed but advetitious embryos since sexual fertilization is absent. The seed is described as tubercolous hypocotyl with underdeveloped embryo and seeds develop from cells of the inner carpel walls (Horn 1940). Mangosteen seed clasified as polyembryoni seed, our examination on transversal and longitudinal cutting of seed up to four sections, resulted development of shoot after 3-6 weeks, however smaller section generate shoots later (Harahap 2005).

Phenotypic variability is arises from genetic variability, environment variability and interaction between genetics and environment (Allard 1960). Hence, genetic variability can be elucidated through phenotypic variability if environmental variability is reduced to a low level. In order to reveal genetic variability pattern of mangosteen populations from three-production centers in Java, Wanayasa and Gunung Walat in West Java and Watulimo in East Java seedlings of were grown under the same environmental conditions (Wulan 2002; Anggraeni 2003). Ten parent trees were randomly chosen for each population, and from each tree ten fruits were picked randomly. The data were analyzed by homogeneity of variance test (Bartlett test) and followed by Tukey's Studentized Range test. Seeds were randomly obtained from fruits from each parent tree, grown in polybag containers under arrangement of Completely Randomized Design consisting of ten replications for populations from Gunung Walat and Wanayasa, and six replications for the population from Watulimo. The data of seedling performance were analyzed using Bartlett test, followed by Analysis of Variance and Tukey's Studentized Range Test.

Results showed that, except for germination rates, the variance was homogenous in flush rate, leaf area, plant height, and the number of leaves. Analysis of variance and Tukey's Studentized Range test also confirmed that no differences occurred among three seedling population on flush rate, leaf area, plant height, and leaf number (**Table 2**). Variability in germination rate predictably arises from differences in length of storage of seed prior to germination,

Wanayasa seed was stored for 25 days, Gunung Walat seed for 16 days and Watulimo seed was stored for 7 days, before germination attempts.

Genetic variability

Genetic variability among populations can be detected visual by protein and by DNA markers (Paterson *et al*. 1990). Visual markers are based on phenological and morphological characters of the plant. These markers are often employed because they are easy, less demanding technique as well as more applicable, but they vary by development stages are influenced by environment (Bai *et al*. 2000). Protein markers, also called isoenzymes are based on protein polymorphisms detected by electrophoresis. In order to characterize and analyze variation of gene number, isoenzyme markers are limited to the number of available loci and variability in electric charge of the protein (Murphy *et al*. 1996).

Recently, burgeoning in the biotechnological field 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). Random amplified polymorphic DNA (RAPD) analysis (Williams *et al*. 1990) has been used for diversity analysis in a vast array of crops, in the determination of genotypes (Hasizume *et al*. 1993), gene mapping (Ohmori *et al*. 1995) and QTL analysis (Grandillo and Tanskley 1996). This approach is based on the polymerase chain reaction (PCR) (Saiki *et al*. 1988) amplification of template DNA genome using short, synthetic deoxyribonucleotides of random sequence as primers. Each primer can direct the amplification of several unrelated regions of the genome (Sondur *et al*. 1996). The resolving power of RAPD technique is several folds higher than visual and protein markers and is much simpler and technically less demanding than RFLP and other similar techniques (Williams *et al*. 1990).

In order to reveal genetic variability of mangosteen population in Java Island, we utilized RAPD analysis (Prabowo 2002; Mansyah 2002) on genomic DNA extracted from leaves of 21 trees: 10 from Wanayasa, 5 from Leuwiliang, 4 from Kaligesing and 2 from Watulimo. Primer screening was done on 40 decamers primers and resulted in 39 primers that were successful in amplifying bands from genomic DNA of mangosteen. Based on the number of amplified band, five primers were chosen for further RAPD analysis. They were SB13, SB19, OPH12, OPH13 and OPH18. RAPD analysis revealed that five primers produced 51 bands or 5.1 band/primer on average, and 42 bands (82.4%) were polymorphic or 8.4 band/primer in average (**Table 3**).

A dendogram based on the UPGMA-link method using Nei and Li similarity (1979) was generated to separate and examine the relationships among the trees by using a computer program NTSYS-pc, version 1.80 (Exeter software, New York). The mangosteen trees were separated into two main clusters at dissimilarity level of 27%, the first of which was dominated by genetically identical trees and the second consisted of trees which showed genetic variability (**Fig. 2**). These results were higher than genetics variability of five agamospecies *Taraxacum* on average of 19% under isozymes analysis (Ford and Richards 1985). *Taraxacum* was known as apomixis obligate, as *G. mangostana*.

At 27% of dissimilarity level, mangosteen trees were divided into two main groups. The first group consisted of two sub groups at 7% of dissimilarity level. The first subgroup shared the same genetic properties in all trees from Leuwiliang and Watulimo, and two trees from Kaligesing; the second subgroup comprised two trees from Wanayasa. The second group consisted of eight trees from Wanayasa and two trees from Kaligesing, which exhibit higher genetic variability compared to those in the first group. Higher genetic variability in trees from Wanayasa predictably resulted from parental variation of these trees. In Wanayasa trees varied in age, since several of them were planted with seedling from other regions.

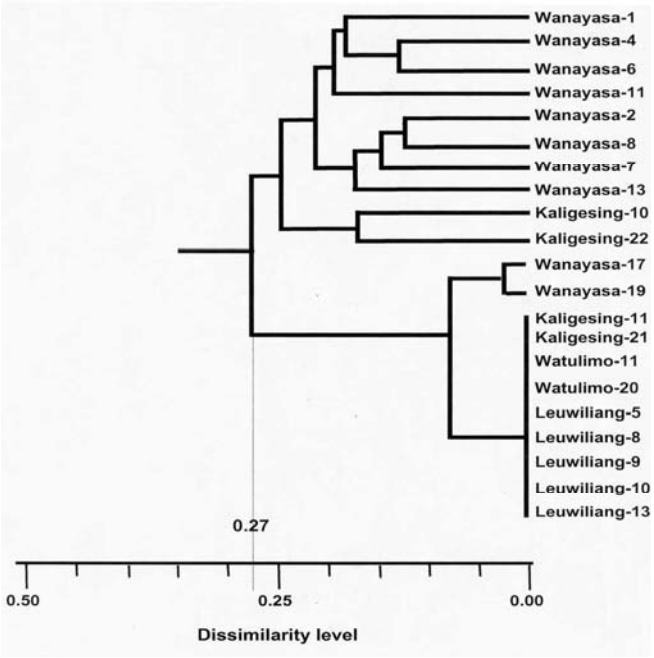

Fig. 2 Dendogram constructed using UPGMA and based on Nei and Li dissimilarity values (1-F) from pair wise comparison of RAPD among 23 mangosteen (*G. mangostana* L.) trees from four locations in Java Islands.

Table 3 RAPD analysis result of 21 genomics DNA of mangosteen leaves from four populations of mangosteen in Java island.

Primer	Sequences	Total bands	Polymorphic bands
SB 13	AGTCAGCCAC	8	5 (62.5%)
SB 19	CAGCACCCAC	14	13 (92.7%)
OPH 12	ACGCGCATGT	7	6 (85.7%)
OPH 13	GACGCCACAC	12	12 (100.0%)
OPH 18	GAATCGGCCA	10	6 (60.0%)
Total		51	42 (82.4%)

Source, with permission Mansyah 2002.

Ramage *et al*. (2004) surveyed the genetic relationship between 37 accessions of mangosteen and among 11 accessions of eight other *Garcinia* species by molecular markers of Randomly Amplified DNA Fingerprinting (RAF). The result revealed genetic diversity within *G. mangostan*a and among *Garcinia* species. For 26 (70%) of the accessions, no marker variation was detected in over 530 loci, eight (22%) accessions exhibited very low variation (0.2-1%), and the other three (8%) showed extensive variation (22-31%) compared with the majority of accessions. Compared to other *Garcinia* species the three groups of mangosteen differed at 63-70% of dissimilarity level. Our recent analysis using isozyme, Enhanced RAPD (ERAPD), and AFLP markers also confirmed variability among mangosteen accessions from 20 provinces in Indonesia (unpublished data).

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).

Carman (2001) suggests that apomicts result from wide hybridization of ancestral sexual parents having distinct phenotypic traits related to reproduction. 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, as South East Asia, including Indonesia, is a diversity center of *Garcinia*. Thomas (1977) reported genetic variability in both *G. hom-*

brioniana and *G. malaccensis*. Our recent analysis using isozymes, RAPD and AFLP markers revealed genetics variability among accessions of *G. malaccensis*. The possibility that development of the ancestral mangosteen was not established from a single hybridization, would lead to variation among mangosteen populations separately generated.

Another possibility of genetic variability in mangosteen could be in ploidy developmental processes. Our research on three groups of parents and progenies of mangosteen indicated genetic variability among the progenies, where their genetic similarity to parent trees ranged from 0.59 to 1.0. This result can, therefore, support the recent findings concerning the existence of genetic variation in apomictic mangosteen (Mansyah *et al*. 2007). In a previous study (Mansyah *et al*. 2004), genetic variation occurred between mangosteen mother plants and their offspring. Many forms of genetic variation may have arisen after hybridization of sexual ancestors with divergent reproductive traits (Spillane *et al*. 2001).

GENETIC IMPROVEMENT

Since the diversity in mangosteen is limited, selection of trees with oustanding characteristic is also limited. However, based on their morphological characteristic and confirmed by RAPD analysis, The Center for Tropical Fruit Studies of Bogor Agricultural University has chosen two potential parent trees and released them as new varieties namely Wanayasa and Puspahiang. However, its apear that to produce new varieties with distinct and supperior characters, some drastic measures have to be employed, such as treating seed or budwood with chemical mutagens or subjecting them to irradiation.

Irradiation of seed

In order to generate genetic variation of mangosteen, our first attempt was irradiating mangosteen seeds by gamma rays at four different doses i.e. 0 Gy, 10 Gy, 20 Gy, and 30 Gy, using Gamma Chamber 4000A, at the rates of 2.39 kGy, with ^{60}Co as a source of radiation. Subsequently, the seedlings were grown in 5 kg polybag containers, and observation was conducted in 18th month after planting including morphological, and anatomical parameters, as well as molecular changes by means of RAPD analysis (Chasanah 2005).

Analysis of variance results revealed that Gamma Ray Irradiation affected seedling growth and their rooting system, and several leaf anatomy parameters (upper cuticle thickness, spongy mesophyll thickness, and leaf thickness). Higher gamma ray doses seemed to inhibit seedling growth and their rooting systems, but the effect on leaf anatomy parameters was not affected by gamma ray dose (**Table 4**).

RAPD analysis on several irradiated seedlings by using five random primers (OPH 12, OPH 13, OPH 18, SB 13, and SB 19) resulted in 24 polymorphic bands, and separation among mutants using computer program NTSYS-pc, version 2.02 (Exeter software, New York) showed that genetic distance among irradiated seedlings based on dissimilarity level of Dice was 0.62, which was higher than genetic variability of mangosteen accessions in Java Island of 0.27. Furthermore, a dendogram based on un-weighted pair group method of arithmetic average (UPGMA) function showed that clustering among irradiated seedlings was not associated with gamma ray dose, indicated the effects of gamma ray irradiation was random.

In another experiment gamma ray irradiation was conducted on mangosteen seed cultured on ½ N Murashige and Skoog (MS ½ N) medium added with 5 ppm of benzyl aminopurine (BAP). Mangosteen seeds were divided into four sections and subjected to 11 levels of dose of gamma ray irradiation (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 Gy) using Gamma Chamber 4000A, at the rates of 2.39 kGy, with ^{60}Co as a source of radiation (Harahap 2005). Morphological observation revealed that doses of gamma ray irradiation affected plant regeneration indicated by change of shoot and leaf morphology, and anatomy parameters.

In order to elucidate the effect of gamma ray irradiation on enzymes properties, isoenzyme analysis was conducted on leaves of 73 plantlets by using six enzyme systems of aspartate amino transferase (AAT), acid phospatase (ACP), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), esterase (EST), and peroxidase (PER). Since AAT enzyme system produced a monomorphic band, it was excluded in further analysis. Five other enzyme systems generated 38 bands and 37 bands (97.4%) were polymorphic. Further analysis using computer program NTSYS-pc, version 2.02 (Exeter software, New York), revealed that gamma ray irradiation resulted in separation among plantlets seedlings at 0.70 based on dissimilarity level of Dice.

RAPD analysis on 80 plantlets was conducted by using ten random primers (OPH 12, OPH 13, OPH 18, SB 12, SB 13, SB 16, SB 19, OPN 4, OPN 12, and OPN16) and resulting 98 bands with 86 polymorphic bands (87.75%). Further analysis revealed that gamma ray irradiation caused loss of RAPD bands in certain samples and addition of RAPD bands in other samples; however, these results were not associated with gamma ray doses. Separation among mutants using computer program NTSYS-pc, version 2.02 (Exeter software, New York) showed that genetic distance among irradiated seedlings based on dissimilarity level of Dice was 0.38 (**Fig. 3**), this was higher than genetic variability of mangosteen accessions in Java Island of 0.27, indicating the gamma ray irradiation attempt had successfully increased genetic variability of mangosteen.

Table 4 The effects of gamma irradiation on seed to mangosteen growth, leaf anatomical structure, and root system of the seedling.

Parameters	Gamma Ray Dose			
	0 Gy	10 Gy	20 Gy	30 Gy
Plant height (cm)	27.61 a	13.31 b	13.58 b	11.93 b
Number of leaves	22.86 a	18.6 ab	17.0 b	17.71 b
Stomata length (µm)	32.63	31.38	31.85	30.45
Stomata width (µm)	15.48	15.63	16.18	15.88
Number of stomata/cm^2	17.56	20.92	17.04	19.00
Stomata density	109.75	130.75	106.50	118.75
Upper cuticle thickness (µm)	4.93 a	4.10 b	4.98 a	4.30 ab
Upper epidermis thickness (µm)	11.43	11.63	12.18	11.03
Palisade thickness (µm)	77.48	62.88	76.45	70.98
Spongy mesophyll thickness (µm)	256.8 ab	201.9 c	272.2 a	236.5 b
Lower cuticle thickness (µm)	11.15	10.79	11.53	10.63
Lower epidermis thickness (µm)	4.50	3.89	4.43	4.15
Leaf thickness (µm)	364.8 a	299.2 b	381.1 a	340.5 b
Root length (cm)	22.57	21.57	23.71	22.07
Number of secondary root	21.3 a	17. 3 ab	16.4 ab	12.0 b
Density of tertiary root	3.0 a	1.7 b	2.0 b	1.3 b

Values within row followed by the same letter indicating not significantly different under Duncan Multiple Range test at p = 0.05
Source, with permission, Harahap 2005.

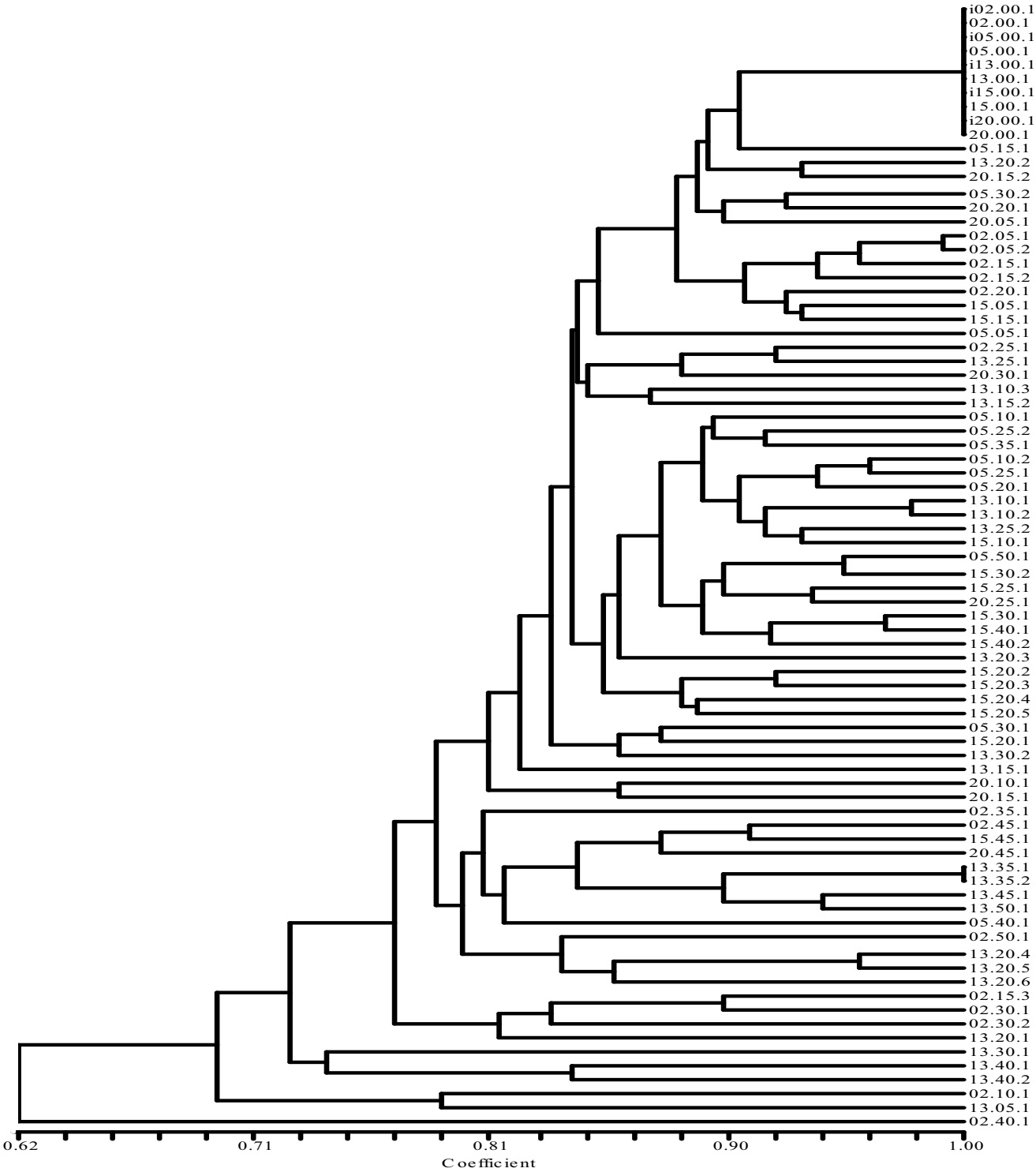

Fig. 3 Dendogram constructed using Un-weighted Pair Group Method of Arithmetic average (UPGMA) and based on Nei and Li dissimilarity values (1-F) from pair wise comparison of RAPD among 80 mangosteen plantlets and 10 primer pairs.

Irradiation of nodular callus

In order to obtain solid mutants, we conducted gamma ray irradiation or nodular callus, with 9 levels of gamma ray irradiation of 0, 5, 10, 15, 20, 25, 30, 35, and 40 Gy (Qosim 2006). Gamma rays irradiation affected the regeneration capacity of nodular callus. Regeneration capacity of nodular callus decreased linearly with increasing level of irradiation for variable percentage of nodular callus forming shoot and variable of number of shoot per callus nodular, while variable of time of formed shoot increased linearly with increasing level of irradiation.

Gamma irradiation affected leaf anatomy of regenerants. Several putative mutant regenerants were variable in stomata area, stomata index and stomata density, the thickness of palisade parenchyma, spongy mesophyll, and cuticle. Several mutant regenerants had higher stomata density and stomata index compare to control regenerants, however, several other mutant regenerants had thinner adaxial cuticle layer than control regenerants, while some had thicker spongy mesophyll and thicker lamina than that of control regenerant. Generally, the thickness of spongy mesophyll and the number of vasculars of the mutant regenerants were greater than those of control regenerants.

RAPD analysis on 22 putative mutants revealed that gamma irradiation changed the banding pattern of DNA as they were amplified with five random primers. Separation among mutant using computer program NTSYS-pc, version

2.02 (Exeter software, New York) showed that genetic distance among regenerants was 0.40 based on dissimilarity level of Dice was 0.38 (**Fig. 3**), which was higher than genetic variability generated by irradiation of seed, indicating that gamma ray irradiation of nodular callus was more effective than on seed.

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REFERENCES

Alexander DM (1984) Guttiferae. In: Page PE (Ed) *Tropical Tree Fruits for Australia*, Queensland Department of Primary Industries, Brisbane, pp 66-69

Allard RW (1960) *Principles of Plant Breeding*, John Willey and Sons. Inc., New York, 485 pp

Almeyda N, Martin FM (1976) Cultivation of neglected tropical fruits with promise. Part I. The mangosteen. Agricultural Research Service. USDA, 18 pp

Anggraeni TDA (2003) Study on variability pattern of morphological traits of *Garcinia mangostana* L. population from four locations in Java Island. BSc Thesis, Bogor Agricultural University, 26 pp

Asker SE, Jerling L (1992) *Apomixis in Plants*, CRC Press, London, 297 pp

Bai G, Ayele M, Tafera H, Nguyen HT (2000) Genetic diversity in tef (*Eragrostis tef* (Zucc) Trotter) and its relatives as revealed by Random Amplified Polymorphic DNAs. *Euphytica* **112**, 15-22

Bernier GB, Kinet JM, Sachs RM (1985) The initiation of flowering. In: Haley AH (Ed) *The Physiology of Flowering* (Vol I), CRC Press, Florida, pp 3-116

Bicknell RA, Koltunow AM (2004) Understanding apomixis: Recent advances and remaining conundrums. *Plant Cell* **16**, S228-S245

Campbell CW (1966) Growing mangosteen in Southern Florida. *Proceedings of the Florida State of Horticulture Society* **79**, 399-401

Carman JG (2001) The gene effect: Genome collision and apomixis. In: Savidan Y, Carman JG, Dresselhaus T (Eds) *The Flowering of Apomixis: From Mechanisms to Genetic Engineering*, CIMMYT, IRD, European Commission DG, Mexico, DF, pp 95-110

Chasanah DC (2005) Studies on morphological, anatomical and RAPD analysis irradiated mangosteen (*Garcinia mangostana* L.) by gamma ray (Scientific Project). Dept. Agron. Hort., Bogor Agricultural University, 52 pp

dela Cruz FS (2001) Status report on genetic resources of mangosteen (*Garcinia mangostana* L.) in Southeast Asia. National Agriculture Science Center (NASC). New Delhi, 30 pp

den Nijs APM, van Dijk GE (1993) Apomixis. In: Hayward MD, Bosemark NO, Romagosa I (Eds) *Plant Breeding: Principles and Prospects*, Chapman and Hall, London, pp 229-245

Ford H, Richards AJ (1985) Isozyme variation within and between *Taraxacum agamospecies* in a single locality. *Heredity* **55**, 289-291

Grandillo S, Tanskley SD (1996) QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*. *Theoretical and Applied Genetics* **92**, 935-951

Harahap F (2005) Genetic variability induction of mangosteen (*Garcinia mangostana* L.) by gamma ray irradiation. PhD Dissertation, Graduate School, Bogor Agricultural University, 143 pp

Hasizume T, Sato T, Hirai M (1993) Determination of genetic purity of hybrid seed in watermelon (*Citrullus lanatus*) and tomato (*Lycopersicon esculentum*) Using Random Amplified Polymorphic DNA (RAPD). *Japanese Journal of Breeding* **43**, 367-375

Horn CL (1940) Existence of only one variety of cultivated mangosteen explained by asexually formed "seed". *Science* **92**, 237-238

Iinuma M, Tosa H, Tanaka T, Asai F, Kobayashi Y, Shimano R, Miyauchi K (1996) Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. *The Journal of Pharmacy and Pharmacolology* **48**, 861-865

Kindiger B, Dewald C (1996) A system for genetic change in apomictic eastern gamagrass. *Crop Science* **36**, 250-255

Koltunow AM (1993) Apomixis: Embryo sacs and embryos formed without meiosis or fertilization in ovules. *Plant Cell* **5**, 1425-1437

Koltunow AM, Bicknell RA, Chaudhury AM (1995) Apomixis: Molecular strategies for the generation of genetically identical seeds without fertilization. *Plant Physiology* **108**, 1345-1352

Lan LA (1989) The embryology of Garcinia mangostana L. (*Clusiaceae*). *Garden Bulletin Singapore* **37**, 93-103

Mahabusarakam W, Kuaha K, Wilairat P, Taylor WC (2006) Prenylated xanthones as potential antiplasmodial substances. *Planta Medica* **72**, 912-916

Mansyah E, Anwarudinsyah MJ, Sadwiyanti L, Susiloadi A (1999) Genetics variability of mangosteen base on isozymes analysis and its relationship to phenotypic variability. *Zuriat* **10**, 1-10

Mansyah E (2002) Genetics variability analysis of mangosteen population in Java and Sumatra island trough their phenotypic performance and RAPD technique (Thesis). Graduate School, Padjadjaran University, 108 pp

Mansyah E, Anwarudin Syah MJ, 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)

Mansyah E, Santoso PJ, M Jawal AS (2005b) Genetic variation of mangosteen (*Garcinia mangostana* L.) progenies derived from polyembryonic seed based on RAPD markers. *Proceedings of 6th National Congress on Genetics*, 12-14 May, 2005, Kuala Lumpur, Malaysia, pp 87-90

Mansyah E, Santoso PJ, Sobir (2007) Evaluation of genetic diversity among and within mangosteen (*Garcinia mangostana* L.) trees. *Journal of Applied Horticulture* in press

Masruroh N (2006) Vegetative growth and morphological characterization of mangosteen seedling cv. Kiarapedes and Wanayasa. BSc Thesis, Bogor Agricultural University, 51 pp

Morton J (1987) Mangosteen. In: Morton JF (Ed) *Fruits of Warm Climates*, Miami, FL, pp 301-304

Murphy RW, Sites JW, Buth DG, Haufler CH (1996) Proteins: Isozyme electrophoresis. In: Hillis DM, Moritz C, Mable BK (Eds) *Molecular Systematics*, Sinauer Associates Inc. Publ. Sunderland, Massachusetts, pp 45-126

Naumova TN (1992) *Apomixis in Angiosperms: Nucellar and Integumentary Embryony*, CRC Press, Boca Raton, FL, 160 pp

Nei M, Li WH (1979) Mathematical model for studying genetics variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences USA* **74**, 5269-5273

Ohmori T, Murata M, Motoyoshi F (1995) Identification of RAPD markers linked to the *Tm-2* locus in tomato. *Theoretical and Applied Genetics* **90**, 307-311

Oostrum HV, Sterk AA, Wijsman HJW (1985) Genetic variation in agamospermous microspecies of *Taraxacum sect. Erythrosperma* and sect *obliqua*. *Heredity* **55**, 223-228

Paterson AH, Tanksley SD, Sorrells ME (1990) DNA markers in plant improvement. *Advances in Agronomy* **8**, 39-89

Prabowo LP (2002) Morphological variability studies on mangosteen (*Garcinia mangostana* L.) population at Trenggalek, Purworejo, Purwakarta and Leuwiliang. BSc Thesis, Bogor Agricultural University, 28 pp

Purwanti A (2002) Genetics variability studies on mangosteen (*Garcinia mangostana* L.) using RAPD analysis. BSc Thesis, Bogor Agricultural University, 25 pp

Qosim WA (2006) Studies on gamma ray irradiation of mangosteen nodular callus for improvement of genetical and morphological variability of the renegenerants. PhD Dissertation, Graduate School, Bogor Agricultural University, 156 pp

Rai IN (2004) Physiology of growth and flowering of seedling and grafted mangosteen (*Garcinia mangostana* L.). PhD Dissertation, Graduate School, Bogor Agricultural University, 163 pp

Ramage CM, Sando L, Peace CP, Carol BJ, Drew RA (2004) Genetics diversity revealed in the apomictic fruit species *Garcinia mangostana* L. (mangosteen). *Euphytica* **136**, 1-10

Richards AJ (1990) Studies in *Garcinia*, dioecious tropical forest trees: the origin of the mangosteen. *Botanical Journal of the Linnean Society* **103**, 301-308

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487-491

Sakagami Y, Iinuma M, Piyasena KG, Dharmaratne HR (2005) Antibacterial activity of alpha-mangostin against vancomycin resistant Enterococci (VRE) and synergism with antibiotics. *Phytomedicine* **12**, 203-208

Sondur SN, Manshardt RM, Stiles JI (1996) A genetic linkage map of papaya based on randomly amplified polymorphic DNA markers. *Theoretical and Applied Genetics* **93**, 547-553

Spillane C, Vielle-Calzada JP, Grossniklaus U (2001) APO2001: A sexy apomixer *in como*. *Plant Cell* **13**, 1480-1491

Suhaeri M (2003) Studies on fruits morphology variability of mangosteen (*Garcinia mangostana* L.) in Java Island (Scientific Project). Dept. Agron., Bogor, 23 pp

Thomas SC (1997) Geographic parthenogenesis in a tropical forest tree. *American Journal of Botany* **84**, 1012-1015

Tixier P (1955) Contribution a' l' etudes des Garcinia. *Fruits* **10**, 209-212

Treub M (1911) Le sac embryonnaire et l'embryon dans les angiosperms. Nouvelle serie de recherches. *Annales du Jarding Botanique de Buitenzorg* **24**, 1-17

van Steenis CGGJ (1981) *Flora*, Paradnya Paramitha. Jakarta, 495 pp

Verheij EWM (1991) *Garcinia mangostana* L. In: Verheij EWM (Ed) *Plant Resources of South East Asia, Edible Fruit and Nuts*. *Bogor a Selection*, PUDOC, Wageningen, pp 177-181

Whitmore TC (1973) Guttiferae. In: Whitmore TC (Ed) *Tree Flora of Malaya*, Forest Research Institute, Longman, London, pp 162-236

Williams JGK, Kubelik AR, Livak KJ, Raflski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* **18**, 6531-6535

Wulan MK (2002) Studies on variability of morphological characters and seedling performance of mangosteen (*Garcinia mangostana* L.) from three locations in Java Island. BSc Thesis, Bogor Agricultural University, 29 pp

Yaacob O, Tindall HD (1995) Mangosteen Cultivation. FAO Plant Protection Paper 129. Food and Agriculture Organization of the United Nations, Rome, 100 pp