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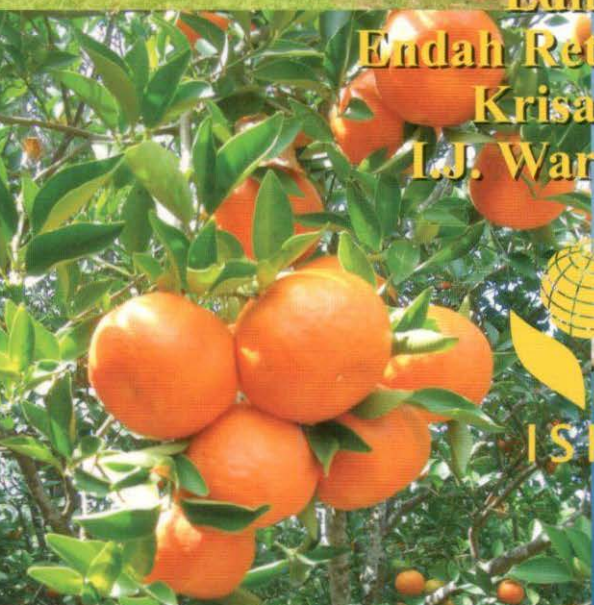

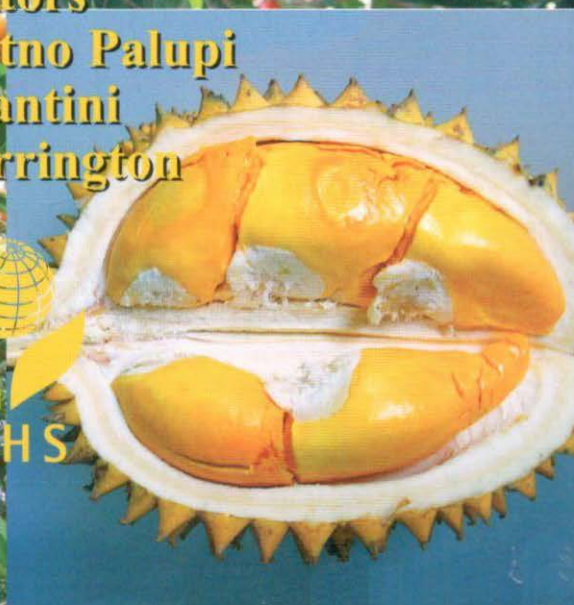

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Genetic Variability of Mangosteen, an Apomictic *Garcinia*

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Keywords: *Garcinia mangostana*, apomixis, variability, allotetraploid, genetic improvement

Abstract

Mangosteen (*Garcinia mangostana*) is native to South East Asia, including Indonesia. Due to its apomictic reproduction pattern, it is assumed that all mangosteen trees have the same genetic properties. However, field evaluation has identified variability in several morphological characters, such as tree shape, fruit shape, and petal color. Further studies using RAPD, AFLP, and ISSR markers confirmed that there is genetic variability among the mangosteen populations in Indonesia. This variation may have arisen from accumulation of natural mutations, or multi-events of natural hybridization. Observations using ISSR markers on mangosteen and close relatives indicated the possibility of *G. malaccensis* and *G. celebica* as the common ancestors of mangosteen. Crop improvement has been conducted by selection of superior trees which has resulted in four new cultivars. Genetic variation has also been successfully obtained using mutation breeding through application of gamma ray irradiation to seed. For further crop improvement, specific primers for important traits have been developed.

INTRODUCTION

The mangosteen (*Garcinia mangostana* L.) has been hailed as the "queen of tropical fruits" (Fairchild, 1915), due to its exotic visual appearance and taste appeal, and has recently been popularized for its medicinal benefits (Sakagami et al., 2005; Mahabusarakam et al., 2006). Indonesia is one of the most important mangosteen producing countries and growing areas are spread across the archipelago. This fruit grows well on lowland as well as on highland sites (up to 800 m above sea level) with diverse environmental conditions. Mangosteen is also the most important export commodity in Indonesia, constituting 46.5% of total fresh fruit exports (Ministry of Agriculture, 2009). However, most of the mangosteen fruit are derived from forests or backyards without any intensive cultural practices.

According to the Ministry of Agriculture (2009), the main production areas of mangosteen are Karo (North Sumatera), Sawahlunto and Padang Pariaman (West Sumatera), Bengkalis (Riau), Wanayasa, Tasikmalaya, and Bogor (West Java), Banjarnegara (Central Java), Blitar, Teranggalek and Banyuwangi (East Java), and Tabanan (Bali). However, despite growing market demand, mangosteen exports from Indonesia have not risen, primary due to inconsistent supply and low fruit quality, especially caused by yellow latex (gummosis), translucent flesh, and fruit scars. Less than 30% of the mangosteen produced meet export quality standards. Obstacles to quality improvement arise from on-farm practices and postharvest handling. An integrated effort is needed, therefore, to improve the quality of mangosteen produced in Indonesia, undertaken simultaneously with a program of genetic improvement.

Crop improvement of mangosteen needs special approaches due to the very long

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juvenile phase, and an apomixis reproductive mode (Horn, 1940; Richards, 1997) – i.e., mangosteen is propagated with apomictic seed, in which embryo is formed without reduction of the chromosome number and fertilization of the egg (den Nijs and van Dijk, 1993). This apomictic characteristic implies that the same genetic properties of parent spread to the progenies (Koltunow et al., 1995). Based on this assumption, mangosteen is claimed to be a fruit species with narrow genetic variability and is generally considered to be a single clone. However, our field observations identified morphological variation among mangosteen trees which, therefore, provides an opportunity for genetic improvement.

An understanding of genetic diversity and its phylogeny among cultivated plant accessions significantly influences approaches to quality enhancement and improves the management of germplasm conservation. Plant genetic improvement highly depends on available genetic resources. Wide genetic diversity will give greater opportunities in the selection processes for the best characters. Research on genetic diversity using markers could explain the phylogeny within and among populations (Fajardo et al., 2002; Hurtado et al., 2002). Genetic variability analysis can be done by using any number of markers, such as those for morphology (Talhinhas et al., 2006), isoenzymes (Ayana et al., 2001), and molecular markers (Assefa et al., 2003; Cavagnaro et al., 2006), such as AFLP markers (Vos et al., 1995). Recently, due to the rapid growth in biotechnological techniques, molecular markers have been widely used to elucidate genetic information in the molecular level.

Important steps of crop improvement in mangosteen are identifying genetic variability, and the development of DNA markers for important traits such as production consistency, fruit quality, and low gummosis incidence. Since conventional hybridization is impossible for mangosteen, we conducted irradiation experiments to enhance genetic variability, and developed DNA markers to assist important trait selection.

OBJECTIVES

The long-term objective of this research is to improve competitiveness of mangosteen production and business in Indonesia through crop improvement and improved scientific information regarding the genetics of mangosteen as a tropical fruit native to Indonesia.

Short term objectives consist of:

1. Genetic variability analysis of Indonesian mangosteen based on morphological characters.
2. Utilization of DNA markers (RAPD, AFLP and ISSR) to elucidate genetic variability among the mangosteen population.
3. Application of DNA markers to reveal phylogenetic relationships of mangosteen and several close relatives (*Garcinia* spp.).
4. Genetic variability establishment and selection method improvement.

LITERATURE REVIEW

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 occurring in Sumatra and Kalimantan (Mansyah et al., 1999). However, the production centers of mangosteen are in West Sumatra, West Java, Central Java, East Java, and Bali. Commercial production has been limited by slow tree growth and long juvenile periods (10-15 years).

Some species of *Garcinia*, including *G. mangostana*, produce fruit without pollination – a phenomenon 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 from the 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 apomictic seed, which is embryo and seed formation without reduction of the chromosome number and fertilization of the egg (den Nijs and van Dijk, 1993). Apomixis in mangosteen implies that the same genetic properties of a parent should be in its progeny (Koltunow et al., 1995).

Apomixis occurs throughout the plant kingdom, from algae to angiosperms (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., 1995). In gametophytic apomixis, the embryo sac is formed from nucelar cells or megaspore mother cells (Koltunow et al., 1995), 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 formation in mangosteen, as well as in orchids, *Citrus* and mangoes is classified as adventitious embryony (Naumova, 1992).

The mangosteen flowers arise from the tip of young shoots (terminals), mostly single to three in number (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 when they turn dry. Subsequently visual observation and potassium iodide 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 supports 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 at 8 DAI. Subsequently Stage IV is indicated by sepal primordia development at 12 DAI. At Stage V at 16 DAI, pistil and stamen primordia have already developed, and the 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 the stamens 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 the pistil; but stamens remain stunted. At Stage VIII at 34 DAI, development of the edible pulp primordia begins. Stage IX is anthesis, occurring at 40 DAI (Rai, 2004).

Microscopic observation also revealed that fruit and seed development were initiated by the 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 the pistil, it was predicted that viable seed was 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.

DISCUSSION

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

Genetic studies on apomictic plants generally are conducted through two approaches: analysis of parental plants and the variation in their progeny or by 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, and 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 types of mangosteen have been identified in terms of shape of fruit, one type producing a round shape with a semi-flat bottom end and the other type with an oblong shaped 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 a flattened fruit shape, very short peduncles, and an elliptic stigma lobe (Mansyah et al., 2010).

In our studies (Prabowo, 2002; Purwanti, 2002; Mansyah, 2002; Suhaeri, 2003), morphological characters were observed from four mangosteen populations in Java Island. They were at Leuwiliang, West Java (300 m above sea level), Wanayasa, West Java (610 m a.s.l.), Watulimo, Center Java (350 m a.s.l.) and Kaligesing, East Java (450 m a.s.l.). 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 circumference; and (2) fruit characters consisting of locule number, fruit weight, peduncle length, fruit length, fruit diameter, rind thickness, total soluble solids concentration, seed/fruit and fruit sap.

Based on field observations, variation occurred in canopy shape – either oblong or pyramidical. In both Wanayasa and Watulimo, only one tree exhibited an 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. Statistical analysis on vegetative characters showed that homogeneity of variance was found in leaf weight, individual leaf area, leaf length/width ratio and trunk circumference, but canopy diameter and chlorophyll contents were variable. These results indicate that variability in most observed variables was mainly due to variation in environment. Variability in canopy diameter suggested that it was from differences in canopy type, tree 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 intercropped with tea plants (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.

Fruit morphology variation was found in weight, length, diameter, length/diameter ratio, rind thickness and peduncle length, and also in total soluble solids concentration (TSS), and presence of fruit latex. Numbers of locules and seed per fruit did not differ significantly. Correlation analysis showed that TSS was correlated negatively with fruit diameter, fruit weight, fruit length, peduncle length, and rind thickness, whereas fruit diameter was positively correlated with fruit weight, fruit length, rind thickness, and number of seed/fruit. Analysis of variance revealed that among four populations, the fruits from Kaligesing were superior for 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 distinctive new type of mangosteen in Kalimantan (Borneo) that produces fruit with insignificant seed size (less than 1 cm in length), and has bigger fruit size without thicker rind, a more acidic taste, and a larger leaf size (twice that of common mangosteen). Variation of sepal color was also found in two populations on Java Island, with white and pale orange color of petals compared to color petal of common mangosteen.

Genetic Variability

Genetic studies on apomictic plants are generally conducted through two approaches: analysis of variation in parental plants and their progeny or by molecular analysis (Koltunow, 1993). Since mangosteen has a long juvenile phase, it is difficult to carry out progeny analysis so genetic variability analysis of mangosteen was carried out using molecular tools. Recently, rapid developments in biotechnology have 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).

Each marker system has advantages and disadvantages, so an assessment of the marker system is an important step in deciding the most suitable marker for a specific research purpose. A comparative study of some PCR-based molecular markers has been carried out, for example, by Palombi and Damiano (2002) who compared RAPD and SSR markers to detect genetic variability of kiwifruit plants, and Ferdinandez and Coulman (2002) who compared the efficiency of RAPD, SSR, and AFLP to identify plant genotypes. Saker et al. (2005) used different markers to characterize barley. Other potential markers include PCR primers based the microsatellite sequences, where repeat motifs are anchored either at the 5' or 3' end with one or a few specific nucleotides that are amplified in sequences between two microsatellite loci, referred to as inter simple sequence repeat (ISSR) markers. In addition, ISSRs can be targeted towards particular sequences, which are reported to be abundant in the genome and can overcome the technical difficulties of RFLP and RAPD (Rajesh et al., 2002; Petros et al., 2007).

1. RAPD Analysis. In order to reveal the genetic variability within the mangosteen population on 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 the genomic DNA of mangosteen. Based on the number of amplified bands, 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 bands/primer on average, and 42 bands (82.4%) were polymorphic or 8.4 bands/primer in average.

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 a similarity level of 0.73, the first of which was dominated by genetically identical trees and the second consisted of trees which showed genetic variability. This variability was higher than genetic variability in five agamospecies of *Taraxacum* which was on average 19% under isozyme analysis

(Ford and Richards, 1985). *Taraxacum* was known as an apomixis obligate, as is *G. mangostana*.

2. AFLP Analysis. AFLP analysis of 10 accessions of mangosteen using three primer combinations of ACC_CAG, ACT_CAA and ACT_CAC produced 220 polymorphic bands and the band size ranged between 50-500 bp. The number of bands that resulted from each primer combination varied between 19-94 bands or an average 73.3 bands for each primer combination. Cluster analysis results, based on AFLP markers, showed that genetic diversity among 10 accessions of mangosteen ranged from 0.59-0.85 coefficient of similarity. The value r=0.977, meaning that the dendrogram resulted in a "goodness of fit" that was very suitable for depicting the grouping (Sinaga, 2008). This result confirmed the results of genetic variability within the mangosteen population revealed by morphological and RAPD markers.

3. ISSR Analysis. In order to reveal the genetic variability within mangosteen, we employed 7 ISSR dinucleotide primer systems on 28 mangosteen accessions from four islands in Indonesia (Sumatra, Java, Kalimantan and Lombok). ISSR analysis successfully amplified 43 bands having on average 6.1 fragments for each primer system, and all these fragments were polymorphic (Sobir et al., 2011). Based on ISSR primer amplified bands, a dendogram was generated by UPGMA-link method using Nei and Li similarity (1979), suggesting a 0.78 coefficient of similarity or a 0.22 coefficient of dissimilarity.

Clustering patterns among the mangosteen accessions that were evaluated, however, did not match their origin. Mangosteen accessions from East Java (JE-1) shared the same ISSR banding pattern with accessions from West Nusa Tenggara (NW-1). Two accessions from Bengkulu-Sumatra (SB-1 and SB-2), and accessions from North Sumatra (SN-2) shared the same ISSR banding pattern with KS-1 from South Kalimantan and JE-2 from East Java. However, two accessions from same location in Wanayasa, West Java, JW-2 and JW-4 were separated at 0.22 coefficient of dissimilarity. These results are supported by previous results, using RAPD markers on 92 *Garcinia mangostana* accessions from the Indonesian Archipelago, that indicated that the clustering patterns did not represent their origin (Sinaga, 2008).

4. Source of Variability. High genetic variability was not common for mangosteen, since mangosteen is considered as an apomictic obligate plant that performs clonally seed reproduction, independent of 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. This assumption is supported by our research on several mangosteen trees with different planting times, which indicated that older mangosteen tree showed higher genetic variability among branches, as detected by Enhanced-RAPD (E-RAPD) analysis (Noorochmah, 2009).

Another possibility of genetic variability in mangosteen could be in ploidy development 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, therefore, supports the recent finding 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).

Phylogenetic Relationship of *Garcinia* spp.

Mangosteen (*Garcinia mangostana* L.) belongs to the *Guttiferae* family and the genus *Garcinia* (Verheij, 1991). *Garcinia* is a large genus that consists of about 400 species (Campbell, 1966) and, based on examination of herbarium collections and the literature, there are 64 species of *Garcinia* encountered in Indonesia. Twenty-five species were found in Kalimantan, 22 species in both Sumatra and Sulawesi, 17 species in both

Moluccas and Papua, 8 species in Java, and 5 species in Lesser Sunda Island. Six of those species are cultivated plants (*Garcinia atroviridis*, *G. beccari*, *G. dulcis*, *G. mangostana*, *G. nigrolineata* and *G. parviflora*), 58 species are wild plants, 22 species have edible fruits, and 21 species are timber plants (Uji, 2007).

In order to elucidate phylogenetic relationships in mangosteen and several close relatives (*Garcinia* spp.), morphological, anatomical and molecular ISSR (Inter Simple Sequence Repeat) markers were employed on 5 accessions of *G. mangostana* and 14 of their close relatives (*G. celebica*; *G. hombroniana-1*; *G. hombroniana-2*; *G. malaccensis-1*; *G. malaccensis-2*; *G. malaccensis-3*; *G. celebica C17*; *G. celebica C18*; *G. celebica AJ*; *G. celebica AD*; *G. porrecta*; *G. forbesii*; *G. subelliptica*; and *Calophyllum inophylum*). Analysis using 35 morphological and anatomical characters, obtained 91 loci that consisted of 86 (94.50%) polymorphic loci and 5 (5.49%) monomorphic loci. A dendogram generated by the UPGMA-link method resulted in a clustering pattern where genetic distance ranged between 0.45-1.00 coefficients of similarity. Subsequently, ISSR analysis using 11 primers, generated 134 loci that could be observed to include 129 (99.23%) polymorphic loci and 1 (0.77%) monomorphic loci, resulting in a dendogram where genetic distance ranged between 0.40-1.00 coefficient of similarity. Further analysis, using phenotypic and genetic markers, revealed that *G. malaccensis* and *G. celebica* have a close genetic distance to *G. mangostana* (Sulasih, 2010).

This result was contrary to the hypothesis of Richards (1990), based on 13 morphological characters, who suggested that mangosteen (*G. mangostana*) was an allotetraploid of *G. malaccensis* and *G. hombroniana*. We can propose that *G. malaccensis* and *G. celebica* may be the allotetraploid ancestor of *G. mangostana*, since we used more accurate tools. Moreover, anatomical analysis of epidermis cells surrounding the stomata guard cells indicated that mangosteen cell shape is intermediate between the cell shapes of *G. malaccensis* and *G. celebica* (Sulasih, 2010).

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 in cooperation with local government, has selected four potential parental trees and released them as new cultivars named 'Wanayasa' (with Purwakarta District), 'Puspahiang' (with Tasikmalaya District), 'Marel' (with Rejang Lebong District), and 'Raya' (with Bogor District). However, it appears that if new cultivars with distinctive and superior characters are to be developed then some drastic measures will have to be employed, such as treating seed or budwood with chemical mutagens or subjecting them to irradiation.

In order to increase genetic variability of mangosteen, gamma ray irradiation with doses of 0, 20, 25, 30, 35 and 40 Gy was applied to mangosteen seed. Based on morphological observation, the irradiation treatment successfully increased variability in mangosteen by 30%. The highest increase of diversity was obtained in plants with: (i) a dose of 25 Gy irradiation, (ii) with the seed cut into two equal sizes, and (iii) when cutting of the seeds was done after the gamma ray irradiation treatment. The highest increase in variability of mangosteen was obtained by irradiation treatment where seeds received a dose of 25 Gy and were then cut into two equal sizes. The density of stomata has a positive correlation with the height of plants of 90% so the density of stomata can be used as a criterium to estimate the growth of mangosteen. To get a mangosteen with greater diversity, it has been suggested that different research material should be irradiated with a dose of 25 Gy (Widiastuti et al., 2010).

Since mangosteen has a relatively long juvenile stage (more than 10 years), breeding of mangosteen is very slow and potentially has a very low success rate. In order to increase the possibility of success, marker assisted selection (MAS) should be used on seedling that result from irradiation treatment, especially for important traits such as tolerance to gummosis, green calyx, and consistent productivity.

In this regard we have established research to develop SCAR (Sequenced Characterized Amplified Region) markers that link to important traits based on DNA sequences that are already known to encode for important traits, such as CWS (cell wall strength) for tolerance to gummosis, ANM (anthocyanin biosynthesis) for green calyx, UFGTM (floral transcription) for consistent productivity, APETALA2, LPMX (lignin synthesis) for tolerance to gummosis, and UGT197 (apomixis) for consistent productivity.

Recent results indicate that a specific fragment has already been obtained for a CWS primer of 1200, 1000 and 600 bp; ANM primer of 600 bp, UFGTM primer of 700, 600, and 450 bp, FTM primer 900, 600, and 300 bp, AP2 primer of 400 bp; LPMX primer of 250 bp; and UGT197 primer of 500 bp. However, these specific fragments need more validation, and converted to pairs of primers for specific target fragments.

CONCLUSIONS

Mangosteen (*Garcinia mangostana*) is a member of the large genus of *Garcinia* that is native to South East Asia, including Indonesia. Mangosteen is reproduced from adventitious embryos, from which the seed develops without fertilization, i.e., agamospermy or apomixis. Apomictic reproduction leads to the assumption that all mangosteen trees have the same genetic properties. However, field evaluation showed variability in several morphological characters, such as tree shape, fruit shape, and petal color. Further studies using isozyme, RAPD, AFLP, and ISSR markers confirmed the existence of genetic variability among mangosteen populations in Indonesia. The use of ISSR markers on mangosteen and close relatives, indicated the possibility of *G. malaccensis* and *G. celebica* being the common ancestor of mangosteen, as suported by anatomical evidence. Crop improvement has been conducted through the selection of superior trees resulting in four new cultivars. Genetic variation has succesfully been obtained using mutation breeding through application of gamma ray irradiation to seed. For further crop improvement, specific primers for important traits have been developed.

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