

## Diversity analysis of mangosteen (*Garcinia mangostana*) irradiated by gamma-ray based on morphological and anatomical characteristics

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Manuscript received: 13 December 2009. Revision accepted: 2 March 2010.

**Abstract.** Widiastuti A, Sobir, Suhartanto MR. 2010. Diversity analysis of mangosteen (*Garcinia mangostana* L.) irradiated by gamma-ray based on morphological and anatomical characteristics. *Nusantara Bioscience* 2: 23-33. The aim of this research was to increase genetic variability of mangosteen (*Garcinia mangostana* L.) irradiated by gamma rays dosage of 0 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy, and 40 Gy. Plant materials used were seeds collected from Cengal Sub-village, Karancak Village, Leuwiliang Sub-district, Bogor District, West Java. Data was generated from morphological and anatomical characteristics. The result indicated that increasing gamma-ray dosage had inhibited ability of seed to grow, which needed longer time and decreased seed viability. Morphologically, it also decreased plant height, stem diameter, leaf seizure, and amount of leaf. Anatomically, stomatal density had positive correlation with plant height by correlation was 90% and 74%. Gamma rays irradiation successfully increase morphological variability until 30%. Seed cleavage after irradiation increased variability and survival rate of mangosteen.

**Keywords:** *Garcinia mangostana*, gamma ray, genetic variability.

**Abstrak.** Widiastuti A, Sobir, Suhartanto MR. 2010. Analisis keragaman manggis (*Garcinia mangostana*) diiradiasi dengan sinar gamma berdasarkan karakteristik morfologi dan anatomi. *Nusantara Bioscience* 2: 23-33. Tujuan penelitian ini adalah meningkatkan keragaman genetik manggis (*Garcinia mangostana* L.) yang diiradiasi dengan sinar gamma dosis 0 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy dan 40 Gy. Bahan tanaman yang digunakan adalah biji yang dikumpulkan dari Kampung Cengal, Desa Karancak, Kecamatan Leuwiliang, Kabupaten Bogor, Jawa Barat. Data dihasilkan dari karakteristik morfologi dan anatomi. Hasil penelitian menunjukkan bahwa peningkatan dosis sinar gamma dapat menghambat pertumbuhan benih, sehingga membutuhkan waktu lebih lama untuk tumbuh dan menurunkan viabilitas benih. Secara morfologi, hal itu juga menurunkan tinggi tanaman, diameter batang, ukuran daun, dan jumlah daun. Secara anatomi, kepadatan stomata berkorelasi positif dengan tinggi tanaman dengan nilai korelasi adalah 90% dan 74%. Iradiasi sinar gamma dapat meningkatkan keragaman morfologi hingga 30%. Pemotongan benih setelah iradiasi dapat meningkatkan keragaman dan tingkat kelangsungan hidup manggis.

**Kata kunci:** *Garcinia mangostana*, sinar gamma, keragaman genetik.

### INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is Indonesian original fruit commodities which have very good prospects for further development. Mangosteen is a tropical fruit that is very well known and known as the Queen of Fruit because of its delicious taste and its a lot of fans (Test 2007). In addition, the mangosteen has long been used as medicine among them are as anti-inflammatory (Chen et al. 2008), antibacterial (Chomnawang et al. 2009), and for treatment of infections and wounds. Improvement on varieties of mangosteen aims to obtain high yielding varieties that are directed to accelerate the growth of mangosteen through improved root system, rapid production (early maturing), high productivity and good fruit quality. Mangosteen plant breeding to improve those characteristics is constrained because the mangosteen plant has low genetic variability and no possibility of increasing

genetic variability through crossbreeding because of the male flowers are rudimental (Morton 1987).

Mangosteen is a type of plant with very long juvenile period, where the slow growth which is caused by poor root system, the slow absorption of nutrients and water, low photosynthetic rate and low cutting rate of cells in the apical meristems (Ramlan et al. 1992; Wible et al. 1992; Poerwanto 2000). The mangosteen seeds shape themselves apomictically and develop from adventive's embryos asexually (Sobir and Poerwanto 2007). The asexual regeneration of mangosteen leads to its low genetic variability (Richard 1990b) and is genetically inherited female elders' characteristics (Koltunow and Grossniklaus 2003). According to Ramage et al. (2004), based on the study *Randomly Amplified DNA Fingerprinting (RAF)* of 37 accessions of mangosteen, 70% showed no variation. Mansyah's research et al. (1999) on 30 plants of West Sumatra's mangosteens it can be concluded that the

variability is narrow, although a few characters show wide phenotypic variability.

Efforts to improve the quality of mangosteen by increasing genetic diversity need to be done. With the wide variability, the selection process can be done effectively because it will give more opportunity to gain the desired characteristics or quality. One of the alternatives to increase variability in apomictic plant is through artificial mutation (Sobir and Poerwanto 2007). The use of radiation to cause mutations or changes in genetic makeup has a lot of positive impacts with an increase in the number of new plant varieties. This technique contributes to the increase genetic diversity and from the gained mutants there are some which have superior characteristics. Fauza et al. (2005) state that gamma-ray irradiation on the mangosteen seeds shows an increase in phenotypic variability in several characters such as plant height, leaf number per plant, stem diameter, and leaf width. In rice plants, radiation with gamma rays at specific doses is known to be able to induce chlorophyll mutations and increase the genetic resistance to blast disease (Mugiono 1996). *Institute of Radiation Breeding* in Japan has been using mutation induction since 1969 to gain potential mutants. Some new varieties of crops of apples, sugarcane, barley, and ornamental plants have been released until 1998 (IRB 2001).

Radiation is enlightening process using radioactive rays that can cause mutations. High energy radiation is usually the form that releases energy in large quantities and is sometimes called ionization radiation because the ions are generated in the material penetrated by the energy (Crowder 1997). Mutations with radiation can increase genetic variation. Cells that can survive well after irradiation will undergo several changes in physiological or genetic. These changes can produce better-looking plants (plants superior) than before (Harahap 2005). Mutations have resulted from all types of material changes derived. DNA, which is a major component of genes as carriers of genetic information from generation to generation, is the main target of radiation delivery. DNA changes that occur as a result of mutation, will lead to new genetic variations that will be deployed on its derivatives. The success of mutation can be observed through changes in morphology, anatomy, and also at the DNA level. Mutants that show morphological characteristics better than previous elders and show the existence of a genetic difference is expected to be developed into new varieties which are superior.

## MATERIALS AND METHODS

### Time and place

This research was conducted from January to August 2009 in the greenhouse and laboratory Research Center of Tropical Fruits (PKBT), Bogor Agricultural University, Laboratory of Microtechniques, Department of Biology, Bogor Agricultural University, and Center for Research and Development of Isotopes and Radiation Technology (IP3TIR), National Agency of Nuclear Energy (BATAN), Jakarta.

### Plant material

Plant material used is the mangosteen seed harvested in Kampong Cengal, Karancak Village, Leuwiliang Subdistrict, Bogor District, West Java.

### Experimental design

This study consisted of two experiments, namely: (i). Mangosteen seeds cut after gamma-ray irradiation treatment, and (ii). Mangosteen seeds cut before gamma irradiation treatment. Seeds were selected based on the weight of > 1 g. The dose Gamma-ray radiation used is according to Harahap (2005) who states that 50% lethal dose (LD 50%) derived from the mangosteen seeds were 32.09 Gy, so the doses used in this study was 0 Gy (I0) as a control were 20 Gy (I1), 25 Gy (I2), 30 Gy (I3), 35 Gy (I4), and 40 Gy (I5). The tool used for irradiation is a Gamma Chamber 4000A with radiation source is a Co-60 radiation dose rate of 96.481 krad / hr (0.96481 kGy / hr). Treatment on cutting off the mangosteen seeds consists of three levels, i.e., the seeds are left intact (B0), seeds are cut into two equal parts (B1), and the seed is cut into three equal parts (B2). Each experiment consisted of 18 experimental units so that the total of the two experiments were 36 experimental units. Each treatment consisted of 10 replications so that the total population in this study was 360. Coefficient of variation (KK) is calculated based on each level of treatment by using a completely randomized design (CRD).

### Implementation of experiments

Mangosteen seeds that had been extracted and cleaned were broken to be divided into two groups. Group 1 (experiment 1) the seeds were cut after irradiation treatment and group 2 (experiment 2) the seeds were cut prior to irradiation treatment. Each group was divided according to standard treatment combination of gamma irradiation (0 Gy, 20 Gy, 25 Gy, 30 Gy, 35 Gy, and 40 Gy) and the standard of cutting seeds (whole, cut two, cut three). Next, the seeds were inserted into a different paper pocket and separated for each treatment combination. One container is one experimental unit. seeds that have been irradiated were planted in polybags in accordance with their respective treatment.

### Morphological observation

Observations were done on the seeds that formed buds on each treatment combination. Morphological observations were made by observing the number, the length, the width and shape of the leaves, and the diameter and height of the stems. Observations were made since the seeds germinated until they reached the age of 6 months.

### Anatomical observations

Anatomical observations on the mangosteen's leaves were made on both the transferal and paradermal slices. The paradermal slice was made using intact preparations (*whole mount*) while transversal incision was made by following the paraffin method. The leaf was slashed using a rotary microtome with a thickness of 10 µm, and then it was colored.

### Data analysis

From the morphological data, the coefficient of variation was measured using SAS ver. 9.1.3 (SAS 2004). The data was also scored in binary measurement and was analyzed using SAHN, after that its similar matrix were calculated using the program NTSYS ver. 2.01 and displayed in a dendrogram (Rohlf 2002). The grouping showed similar relationship between each individual in the form of morphological characteristics. Genetic distance is the difference in value of the percentage of similarity to the value of 100%. From the dendrogram, it can be seen how far the changes in crop radiation results when compared with controls. The same was not reported for transverse slices leaf anatomical parameters due to the data which was so various, so it did not show a specific pattern. Relationships and morphological parameters of the stomata were gained by doing a correlation analysis using SAS program ver. 9.1.3 (SAS 2004).

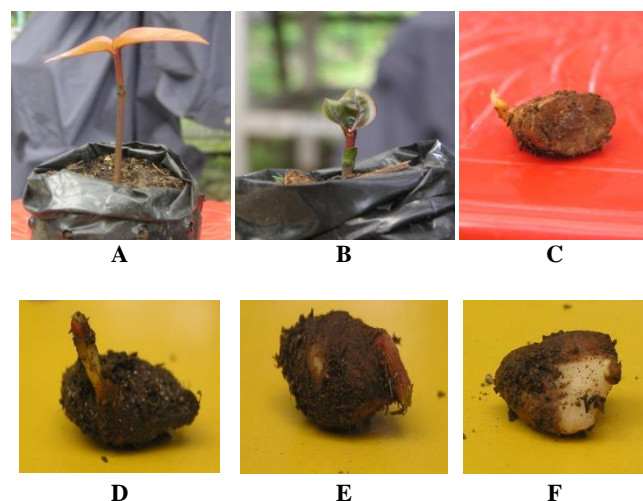
## RESULTS AND DISCUSSION

At the beginning of the study, the number of seed planted were 360 seeds and each seed was planted in a poly bag. Not all seeds were grown to form buds, total number of seeds capable of forming buds both in the control treatment results and gamma irradiation were 57 seeds. Data shown in this study is one related to the morphological observation and the anatomy of the seed treated by being cut and enlightened by gamma-ray irradiation. Morphological observations were carried out on all the mangosteen seeds that formed buds, while the anatomical observations were carried out on seedlings that survived after 30 weeks of planting and showed normal growth, which was not stunted.

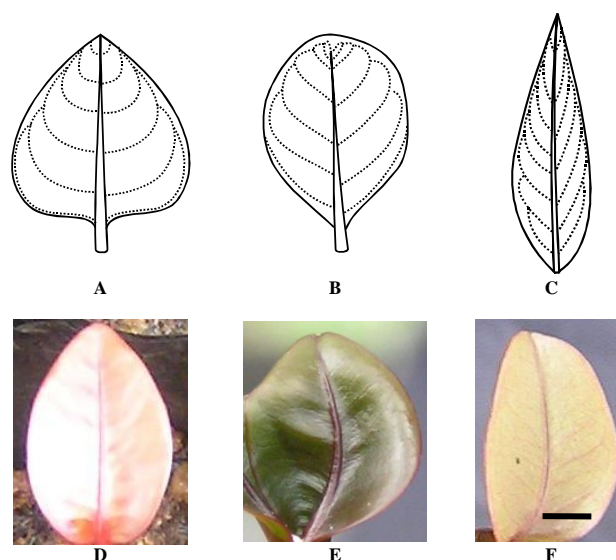
### Morphological observations

Morphological observations showed that the number and time when the seeds to form buds, plant height, stem diameter, leaf number, length and width of leaves of some plants that underwent irradiation, experienced abnormalities. Of 360 units of the experiment, only 57 seeds that formed buds, 14 of which belong control group. This number decreased in the subsequent week of observation because some plants died that was preceded by experiencing necrosis and the loss of leaves. The largest number of buds was shown of the period 20 weeks after planting, and in some control plants there grew more than one sprout from one single seed. Control plants showed normal growth in which plants did not experience any delays in forming buds and the growth was not inhibited. Harahap (2005) stated that the mangosteen seeds which did not undergo gamma rays treatment, the buds generally appear after 2 weeks of planting. Seeds which received irradiation treatment showed a delay emergence of buds. The formation and growth of buds on the control seeds and the results of irradiation treatment are presented in Figure 1.

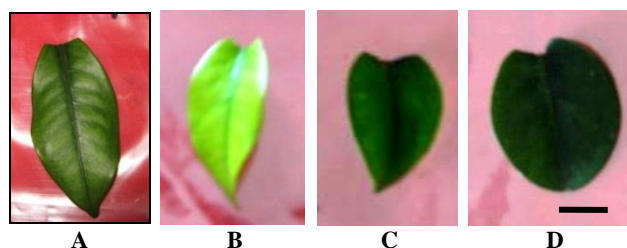
The morphological shape of mangosteen leaves both in plants that received both treatment and controls were generally ovate, obovate, and a small portion of them were



**Figure 1.** The growth of buds on the mangosteen seeds untreated with gamma-ray irradiation (A) and (B), and seeds that received gamma irradiation treatment (C), (D), (E), and (F).



**Figure 2.** Mangosteen leaf's morphology after 30 weeks of planting: A. ovate, B. obovate, and C. lanceolate. The color of young leaves of mangosteen: D. control of red brick, E. brownish green irradiated, and E. reddish brown control (f). Bar = 1 cm.



**Figure 3.** The color of the leaves after 30 weeks of planting: A. control, B and C. 20 Gy irradiation treatment, D. 25 Gy irradiation treatment (d). Bar = 1 cm.

lanceolate. From this observation, no specific pattern distinguished the shape of leaves in the control plants from the irradiated ones. *Flush* that appeared on the buds that did not receive irradiation treatment was brownish red, while the flush that appeared on the irradiated buds was usually light green with very slow growth. The morphological shape of the leaves and the color of young leaves of mangosteen which appeared in this study are presented in Figure 2.

Most of the young leaves of mangosteen were red brick and reddish brown, but in some plants that received irradiation treatment emerged young leaves that were green with brownish color on the edges. Plant response to gamma irradiation treatment is individual one, but there is a general description of several variables of the outcomes. Leaves emerged from the results of irradiation plants are generally smaller, darker green in color, and thicker in texture.

The response of each mangosteen plant to the stress of gamma-ray irradiation was different. At the dose of 20 Gy appeared the light green leaves and looked transparent, but at higher doses, i.e. 25 Gy and 30 Gy appeared leaves that have a smaller size, dark green and thicker (Figure 3). Abnormality was a response to disruption of physiological processes due to stress caused by gamma-ray radiation. According to Soeranto (2003), abnormalities in the irradiated populations showed the occurrence of major changes in the level of genomes, chromosomes, and DNA, so that physiological processes within cells that are genetically controlled became not normal. Meanwhile, according to Harahap (2005), changes in the leaf due to irradiation are thought to occur because of the increased amount of chlorophyll due to gamma-ray irradiation stress.

#### Time and number of seeds that formed buds

Mangosteen seeds that are not treated with gamma-ray irradiation and the average deduction showed the emergence of buds after 3 weeks of planting (Table 1), while seeds which received gamma irradiation treatment alone (without cutting seed) the emergence of buds varied between 3 and 16 weeks after planting. Buds of which formation took the longest time were the individuals with 40 Gy irradiation treatments, but these plants grow very slowly and not able to survive at the end of observation (25 weeks after planting). From this observation, it is seen that the higher dose of gamma irradiation treatment is, the less and the longer time required for the seeds for the emergence of buds. At the doses high enough that above 30 Gy, most of seeds did not die or decay despite being 20 weeks of sowing, but they also showed no signs of emerging buds/sprouts. Then at the end of the study the seeds which did not germinate eventually died and decomposed. At a dose of 35 Gy, none of the seeds that showed the emergence of buds, while at 40 Gy irradiation treatment, there was one seed that emerged buds that took a very long period which was 16 weeks after planting.

The number of plants that germinated in the control treatment continued to increase until 15 weeks of planting and reduced in on the 16<sup>th</sup>, 20<sup>th</sup>, and 25<sup>th</sup> week after planting, that was from 33 plants on the 16<sup>th</sup> week getting

reduced to 30 plants on 20<sup>th</sup> week and 25 plants on 25<sup>th</sup> week after planting. In the treatment at 20 Gy and 25 Gy, the number of surviving plants reduced after 20 weeks of planting, respectively from 15 to 14 and 9 to 7 (Table 1).

**Table 1.** Number of mangosteen seeds that form buds.

Treatment	Level of irradiation (Gy)	Number of buds on the WAP										
		3	4	8	9	10	11	13	14	15	16	20
Seeds irradiated before cut												
Whole seed	0	5	7	9	8	8	8	10	10	10	10	6*
	20	0	3	3	3	3	3	3	3	3	3	4*
	25	0	2	2	2	2	1	3	4	4	6	5
	30	0	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0
Seed cut two	0	4	5	5	5	5	6	6	6	6	6	3*
	20	0	2	2	3	3	3	3	3	3	3	3
	25	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0
Seed cut three	0	3	3	3	3	3	3	4	4	5	4	3*
	20	0	0	0	0	1	1	1	1	1	1	1
	25	0	0	0	0	1	1	2	2	2	2	1*
	30	0	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0
Seeds irradiated after cut												
Whole seed	0	5	8	8	8	7	7	8	10	10	8	9
	20	2	2	4	5	4	4	4	4	5	5	4*
	25	0	0	0	0	0	1	1	1	1	1	1
	30	0	0	0	1	1	1	1	1	1	1	1
	35	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	1	1
Seed cut two	0	0	2	6	3	3	2	2	2	2	2	4
	20	0	0	0	0	0	1	1	1	1	2	1
	25	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0
Seed cut three	0	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	1	1	1	1	1	1	1	1	1
	25	0	0	0	0	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0	0	0	0	0
	35	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0

Note: \* Some plants were dead so that in on the 25<sup>th</sup> week the number of plants got reduced. WAP = weeks after planting.

This was due to some individual plants have sprouted and emerged dying leaves, started with the drying of leaf tips, followed by the entire leaf and eventually the fall of the leaves. The death of those individual plants is due to the irradiation stress. According to van Harten (1998), gamma irradiation is destructive towards the path network it goes through. In addition, because its penetrability is very deep, the damage that it can cause can reach a few centimeters. Ahnstroem (1977) and Datta (2001), state that both the abnormality and the death of the irradiated plants are caused by the formation of free radicals such as Ho, that was a very unstable ion and caused a lot of collisions in different directions that will create mutations in DNA, as well as caused changes at the level of cellular and network. It can even cause death in plants. In the control group, the death of plants was suspected to be caused by



physiologically immature seeds. In the treatment of 30 Gy only one seed that formed buds which survived until the age of 20 weeks after planting, while at the 40 Gy treatment, the buds that appeared was dead after 25 weeks of planting.

Cutting the mangosteen seeds actually has effects on the number of buds that emerged (Table 1). On the irradiation level of 25 Gy, the truncated seeds after gamma irradiation showed the higher number of seeds that formed buds that were three seeds for the seeds that were cut into two, and two seeds for the ones cut into three, compared to seeds that were cut first before irradiation gamma rays, which were the two seeds in the treatment group that was two seeds for the seeds cut into two cut and no buds appeared on the seeds cut into three after 16 weeks of planting. Seeds treated with irradiation before being cut showed the ability to form buds faster and higher than the ones cut prior to irradiation. This is because on the seeds cut prior to irradiation; the tissues damaged due to radiation are greater than the seeds cut after irradiation.

#### Plants height, stem diameter, and number, length, and width of leaves

The growth of the mangosteen plants can be seen by performing detection on the morphological characteristics. In this study, the measurement of morphological characters was conducted on the height of the plants, the diameter of stems, the number, the length and width of the leaves. The height of the plants was measured from the neck of the root up to the point where the plants grow, while the trunk diameter was measured at a height of 1 cm above the root's neck. The length and width of the leaves were measured on the leaves that emerged secondly.

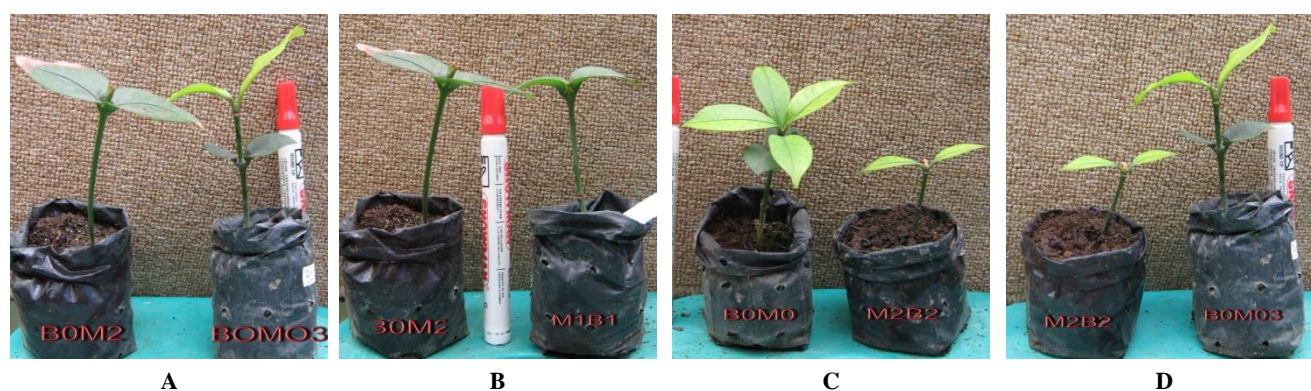
The height of the plants, the stems' diameter, the number, length and width of the leaves get decreased as the doses of gamma irradiation increases (Table 2). This happens because cellular damage happens to the plant's meristem. According to Handayati et al. (2001), the damage leads to the degradation of *indole acetic acid* (IAA) because *indole acetaldehyde dehydrogenase* enzyme is inhibited (Moore 1979).

The decreasing number of leaves creates patterns in the control treatment at the level of 20 Gy and 25 Gy, where the higher the dose of the treatment using gamma-ray irradiation means the less number of the leaves. The effect is due to physiological damage caused by gamma irradiation. The length and width of the leaves also showed a decrease in the size of some irradiated crops. According to Ashri (1970), the reduced size of the leaves can occur due to irradiation and chemical mutagen treatment.

**Table 2.** Average high of mangosteen, leaf number, stem diameter, leaf length and width on the 25<sup>th</sup> week after planting.

Characteristics	0 Gy	20 Gy	25 Gy
Plant height	5.85 ± 2.58	5.00 ± 1.77	4.40 ± 2.74
Stem diameter	0.19 ± 0.09	0.15 ± 0.04	0.15 ± 0.06
Leaf number	1.65 ± 0.47	1.33 ± 0.47	1.33 ± 0.47
Leaf length	3.79 ± 1.67	3.36 ± 1.40	2.7 ± 1.30
Leaf width	2.32 ± 0.68	2.23 ± 0.48	1.45 ± 0.91

Gamma-ray irradiation has effects on the morphological characters of mangosteen. These changes appear to be individual, although they were irradiated at the same dose. The comparison between control plants and plants produced with gamma-ray irradiation is presented in Figure 4. Gamma-ray irradiation can affect the growth and morphology of mangosteen. In this research it is gained that at higher doses than 25 Gy, the mangosteen seeds require just 9 weeks to form buds, while seeds without irradiation only take 3 weeks. The height of the plants, the number, length, and width of the leaves also shows the response to irradiation dose of gamma rays, where the higher dose of irradiation, the less the characteristics in value. This suggests that high doses cause stunted growth and even cause the seeds not able to grow. Barriers to growth are in the form of physiological damage due to gamma-ray irradiation. The length and width of the leaves also showed a decrease in the size of some irradiated crops. Qosim (2006) stated that nodular callus of mangosteen with



**Figure 4.** Comparison of control plants and the results of irradiation on the 25<sup>th</sup> week: A. Results of irradiation at the level of 25 Gy and control. B. Results of irradiation at the level 25 Gy and 20 Gy, the combined seed is cut into two equal after irradiation. C. Control and irradiated 25 Gy combinations of seeds cut into three equal after irradiation. D. Results of irradiation 25 Gy combined seeds cut into three equal after irradiation.

**Table 3.** The coefficient of variation (%) of each morphological character in (i) the extent of irradiation control (I0), 20 Gy (I1), and 25 Gy (I2), (ii) cutting seed: whole, cut in half, and cut into three (iii) treatment of seeds irradiated before being cut, and seeds irradiated after the cut.

Morphological character		Coefficient of variance (%)							
		level of gamma irradiation			Cutting seed			Seed cutting time	
		0 Gy	20 Gy	25 Gy	Whole	Cut two	Cut tree	After irradiation	Before irradiation
Number of buds	16 WAP	67.16	188.91	267.46	137.82	243.01	306.81	112.38	161.34
	20 WAP	78.53	170.44	222.51	131.76	236.79	342.26	114.28	178.37
	25 WAP	84.219	170.44	257.64	162.01	218.60	367.15	122.90	143.11
Plant height (cm)	16 WAP	35.023	36.780	80.07	42.93	40.78	30.53	29.47	41.13
	20 WAP	37.26	31.28	80.07	43.65	38.50	48.01	34.26	34.22
	25 WAP	36.190	39.6	76.08	41.99	44.79	17.06	42.94	22.26
Number of leaves	16 WAP	34.10	27.3	35	35.49	0	37.50	36.36	17.42
	20 WAP	27.08	35.18	33.126	30.37	30.73	0	41.14	14.67
	25 WAP	27.91	39.95	41.92	31.38	43.47	31.25	52.01	22.98
Diametar stem (cm)	16 WAP	30.82	24.84	25.064	25.47	33.14	102	29.33	32.97
	20 WAP	25.49	19.84	27.59	24.60	20.39	91.20	36.52	30.39
	25 WAP	35.79	23.47	34.65	27.37	26.51	101.03	0	0
Leaf length (cm)	16 WAP	35.51	45.38	52.906	39.20	45.53	39.21	35.13	34.50
	20 WAP	38.41	40.85	54.16	41.06	46.81	16.15	0	34.77
	25 WAP	43.15	43.94	55.50	42.99	64.29	11.78	0	33.94
Leaf width (cm)	16 WAP	32.59	49.17	31.4	36.38	37.99	19.10	0	31.95
	20 WAP	26.85	42.23	45.08	34.10	31.56	8.97	0	23.94
	25 WAP	21.15	23.81	72.53	31.04	23.78	23.10	0	24.77

Note: WAP = weeks after planting.

gamma ray irradiation at the level above 25 Gy took 129 weeks to form buds. Irradiation gamma rays can also cause changes in the anatomy of mangosteen's leaves. The number of seeds capable of forming buds also decreases as the dose of irradiation increases. At the doses greater than 25 Gy, only one seed that is able to form buds, i.e. at a dose of 30 Gy on the 10th week of planting, and one seed at 40 Gy on the 16th week of planting. Decreased ability to form buds or seed germination occurring with increasing dose of irradiation was also observed in wheat (Gou et al. 2007), peanut (Baddiganavar and Murty 2007), and soy seed (Manjaya and Nandawar 2007).

### Diversity test towards the mangosteen phenotypic

#### Effect of gamma irradiation

According to Baihaki (1999), to determine the variation of a population, the following rules of measurement and analysis that are in accordance with statistical way need to be done. The various populations will have specific characteristics that can be seen from the coefficient value describing diversity in a single treatment. Gamma-ray irradiation is a physical mutagen which can cause an increase in the diversity of initial population.

In this study, the dose of gamma irradiation which provides the highest variability based on coefficient of variation is 25 Gy, while the dose that gives the lowest diversity is 0 Gy (control) (Table 3). Almost all the characters showed increased coefficients of variability as the levels of irradiation increased, except for the stem's diameter, which is at the dose of 20 Gy the coefficients of variability on the 16th, 20th, and 25 weeks of planting, are lower than those in the control group. At the dose of 25 Gy, the coefficient of variation for the stem's diameter that is greater than that of the control group is only found in the one on the 20th week of planting (Table 3).

Increasing dose for irradiation led to the less ability of seeds to form buds, which is marked by the declining number of seeds that form the buds compared with the control (Table 1). At doses where the seeds are still able to form buds and grown into plants, it is known that 25 Gy is the dose that most suppressing dose towards the growth of mangosteen, which is characterized by small average value for each observation of morphological characters compared to those belonging to control group and at lower doses (Table 2).

#### Effect of seed cutting level

Mangosteen seeds are polyembryonic ones which can grow more than one bud. Coefficient of variation on a level of cutting seed treatment with the highest number of buds is obtained from the seed treatment which is cut crosswise into three equal sizes. For the height of the plant, the highest coefficient of variation on 16th and 20th week of planting is found in the treatment of intact seeds, while on the 25th week of planting the highest coefficient diversity is found from the seed treatment which is cut into two equal sizes. The highest coefficient of the number of leaves from the ones on the 16th week of planting is found in the seed treatment which is cut into three equal sizes, while from the ones on the 20th and 25th week of planting is found in the treatment of seed which is cut into two equal sizes. For the stem's diameter, the highest coefficient of diversity is found on the seed treatment which is cut into three equal sizes, while for the length of leaves the highest coefficient is obtained in the treatment of the seed which is into two equal sizes. The highest coefficient of diversity for the width of the leaves found in the plants on 16th and 20th week of planting is obtained on the seed treatment which is cut into two equal sizes, meanwhile the highest coefficient of

diversity for the plants on the 25<sup>th</sup> week of planting is found on the seed treatment which is left intact (Table 3).

Coefficient of variation which is higher on the morphological characters derived from the cutting of mangosteen seeds is related to the nature of polyembryonic of the mangosteen seeds. A single of mangosteen seed can grow more than one bud, where each bud emerges from different sections, and allegedly carries different genetic constitutions as well. Mansyah et al. (2008) stated that of the nine seeds of polyembryonic mangosteen it could be seen the differences in the DNA bands on the buds that grow from the seeds of the same mangosteen.

#### *Effect of seed cutting time*

The time for cutting seeds can be divided into after and before irradiation. The treatment for the seeds cut before irradiation has a higher coefficient of variation than the ones cut after irradiation (Table 3). This is because in the treatment for the seeds cut before irradiation, the exposure of irradiation directly hits the surface of the seed that experiences injury from the cutting so that the effect becomes larger, while in the seeds that are cut after irradiation, the exposure to gamma rays only hit the surface of the seeds so that the radiation effect is smaller.

#### **Increasing of morphological diversity due to gamma-ray irradiation**

Mangosteen is included in obligate apomixes plants, whose seeds are not derived from the results of fertilization but developed from adventive's embryos asexually (Sobir and Poerwanto 2007), thus might have low genetic diversity (Richards 1990b; Verheij 1992; Cox 1996). Apomixes on mangosteen plants cause the same genetic trait in the progeny the same as with that in the parents (Koltunow and Grossniklaus 2003). Induction of irradiation with gamma rays is one alternative to increase genetic diversity in plants in which the occurrence of cross-fertilization is not possible.

In this study, the dendrogram drawn from the morphological observation of mangosteen plants showed that gamma irradiation treatments could increase the diversity compared to ones belonging to the control group. The similarity on values in the plants that do not have gamma-ray irradiation treatment ranged from 13-83% (Figure 5A), while the ones the plants are treated with gamma-ray irradiation ranged from 0-100% (Figure 5B). Diversity increased by 30% after the induction of gamma-ray irradiation. Gamma rays include mutagens that produce ions and free radicals in the form of hydroxyl (OH<sup>-</sup>). If hydroxyl radicals are attached to the chain of nucleotides in DNA, the single strand of DNA will be broken and undergo some genomic changes (Mohr and Schopfer 1995). Visually the diversity in maize growth due to the influence of gamma irradiation becomes larger.

In the mangosteen plants that do not get gamma-ray irradiation treatments, the greatest similarity is found in plants without the cutting seed treatment which is IOB0 and BOIO, respectively by 83%. The research results are divided into two that are plants derived from intact seeds and plants whose seeds are cut into two or three equal sizes.

MXComp cophenetic value generated from the control plants is ( $r = 0.967$ ) with very appropriate *goodness fit*, while the value of the irradiated ones is ( $r = 0.956$ ). Clustering analysis on the results of gamma irradiation plants does not provide specific grouping between control plants and plants produced with gamma-ray irradiation. This happens because the nature of mutations caused by irradiation of gamma rays is random. One mutant plant derived from irradiation of 25 Gy (BOI2) is in the group of plants without irradiation treatment that is on the similarity of 100%. This shows that at the morphological level, these plants do not differ from the ones untreated with gamma-ray irradiation.

#### **Increasing morphological diversity by cutting seed process**

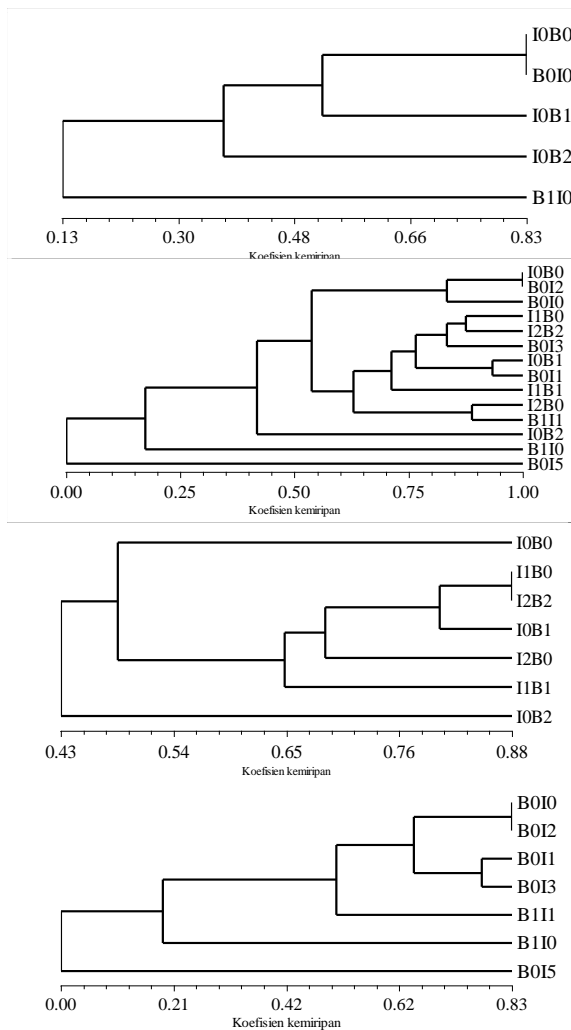
The time of cutting the mangosteen seeds, whether before or after irradiation, makes difference in improving the diversity based on morphological observations. Mangosteen seeds irradiated prior to getting cut gives a smaller similarity of 43-88% (Figure 5C), while the mangosteen seeds receiving irradiation after the cut has a similarity of 0-83% (Figure 5D). The pattern in cutting the seeds is also seen to lead to diversity, both in the irradiation treatment before and after cutting. In Figure 5C, based on the cutting pattern of mangosteen seeds, the dendrogram is divided into two groups on the similarity of 43%, i.e., the first group only consisted of plants from the intact seeds only (IOB0) and group two consisted of plants from the seeds of the mangosteen which are cut into two equal (IOB1) or three equal (IOB2) sizes. At intervals of 60% similarity, the individual plants IOB2 and IOB1 are in different groups, indicating the existence of diversity between both of them.

Mangosteen seeds are polyembryonic ones, meaning that one seed can grow more than one bud. Each bud has a different genetic constitution because they come from different embryos. Mansyah et al. (2008) stated that four out of nine seeds of polyembryonic mangosteen seeds it can be seen the different DNA bands in the buds that grow from the seeds of the same mangosteen.

#### **Anatomical observations**

##### *Transversal section*

The structure of mangosteen leaves on the transferal slice consists of the layers of cuticle, upper epidermis, palisade parenchyma, spongy parenchyma, and lower epidermis. The epidermal tissue is covered by cuticles which are spread throughout the upper and lower leaf surfaces. The structure of mangosteen's leaves belong to the dorsiventral as a palisade parenchyma tissue is between the upper epidermis and spongy tissue. The results showed that the cuticle is on the upper and lower surfaces. The palisade parenchyma of Mangosteen's leaf consists of two layers which are under the upper epidermis, while the sponge layer is under the parenchyma palisade (Figure 7). The observation on the mangosteen leaves transversally sliced was conducted towards 16 plants that visually show good growth.



**Figure 5.** Mangosteen dendrogram based on morphology: A. Control. B. Gamma-ray irradiation treatment. C. Cutting the seed after irradiation. D. Cutting the seed prior to irradiation.

Based on Table 4 it can be gained that the range of values in a thick cuticle, upper epidermis, lower epidermis, palisade parenchyma, spongy parenchyma and the thickness of the mangosteen's leaves vary greatly. There is no particular pattern between the thickness of cuticle of the individual belonging to control plants and that of the irradiated ones.

**A**

Table 4 shows that the thickness of upper epidermis and lower epidermis for most crops is almost the same. Epidermal tissue is the tissue that serves to protect the underlying tissue and serves as a coating for gas exchange to and from outside the body through the hole plant stomata. Changes in the thickness of the epidermis can be caused by the ionizing nature of gamma rays which can penetrate the epidermal layer and cause the changes. Other factors affecting the changes in leaf anatomical characters beside the *regeneration* are the environmental factors such as the availability of water, light intensity, the concentration of CO<sub>2</sub>, and the temperature which can affect the density of stomata (Willmer 1983).

**B**

**C**

**D**

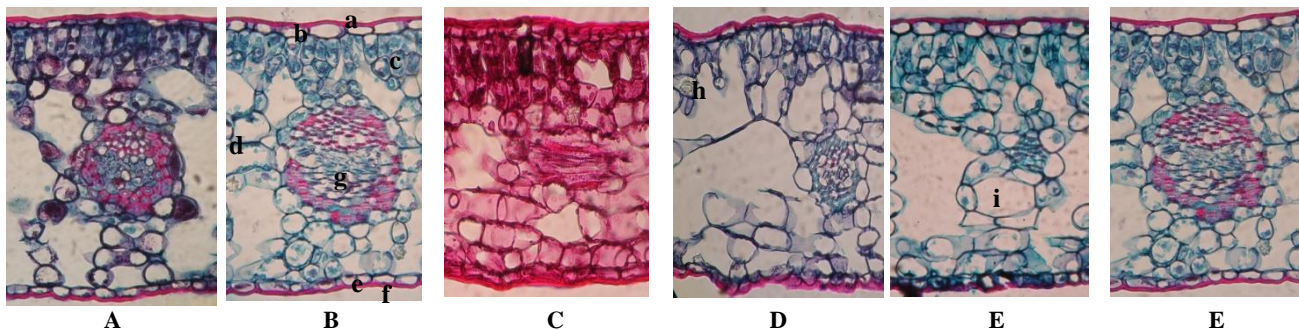
Some plants which are the result of irradiation treatment showed substantial palisade thickness values, namely BOI3, I1B01, and IOB02. The thickness of sponges and the highest thickness of leaves are obtained from the control plants (BOI03), while the lowest obtained from the plants produced at the irradiation level of 25 Gy. The thickness of the sponge tissue is associated with the thickness of space between cells, where the thicker the sponge tissue, the greater the spaces between cells that are useful for storing water and CO<sub>2</sub>. According to Fahn (1991), the important factors that can increase the efficiency of photosynthesis is the space between cells which is very well located in the mesophyll, thus facilitating gas exchange quickly.

**Table 4.** Observations of leaf transversal section in some individuals of mangosteen (μm).

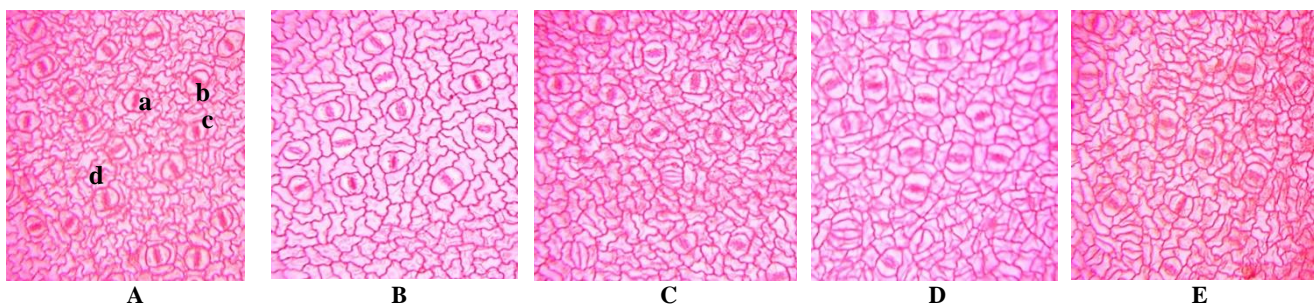
Individual plants	Average thickness (μm)					
	Cuticle	Upper epidermis	Under epidermis	Palisade	Sponges	Leaf
<b>Seeds irradiated before cut</b>						
IOB01	3.88 ± 1.24	10.00 ± 2.04	7.75 ± 1.53	42.50 ± 6.87	184.51 ± 25.02	64.37 ± 7.85
IOB02a	2.88 ± 0.60	14.00 ± 1.29	10.25 ± 1.42	48.00 ± 6.21	180.75 ± 5.01	75.38 ± 5.76
IOB03	4.00 ± 1.29	7.75 ± 1.84	9.25 ± 2.64	44.51 ± 11.59	202.25 ± 31.27	65.77 ± 13.81
I1B02b	2.50 ± 0	10.13 ± 2.53	11 ± 3.37	46.53 ± 5.29	176.51 ± 6.89	70.37 ± 7.56
I1B01	3.75 ± 1.17	12.00 ± 2.58	9.25 ± 1.20	51.52 ± 8.26	178.75 ± 24.24	76.75 ± 6.61
I1B1	2.63 ± 0.39	10.52 ± 2.29	9 ± 1.29	42.52 ± 6.97	188.25 ± 13.64	64.88 ± 7.45
I2B0	3.38 ± 1.19	7.13 ± 1.87	6.25 ± 1.77	42.03 ± 8.32	151.03 ± 8.99	58.96 ± 10.08
I2B2	3.13 ± 1.06	9.13 ± 2.04	7.5 ± 1.67	43.25 ± 6.13	140.75 ± 20.01	63.20 ± 6.06
<b>Seeds irradiated after cut</b>						
BOI02	3.52 ± 1.49	10.25 ± 1.84	9.25 ± 2.059	48.25 ± 6.46	195.25 ± 27.11	71.51 ± 7.06
BOI03	3.75 ± 1.18	10.50 ± 3.07	10.00 ± 2.63	55.00 ± 8.97	203.25 ± 19.04	79.53 ± 9.95
BOI04	4.54 ± 0.87	9.75 ± 2.75	9.75 ± 2.19	55.00 ± 10.99	167.50 ± 19.01	79.24 ± 12.52
BOI01	4.50 ± 1.05	9.87 ± 2.8	8.50 ± 1.74	53.50 ± 5.02	168.50 ± 12.08	76.62 ± 5.08
BOI2	4.01 ± 1.15	9.25 ± 2.37	8.88 ± 1.71	51.25 ± 7.38	185.5 ± 11.71	73.63 ± 6.16
BOI3	5.37 ± 2.13	13.12 ± 3.19	8.63 ± 2.91	57.25 ± 11.27	176.51 ± 24.58	84.63 ± 13.59
B1I02	3.13 ± 0.88	10.52 ± 1.97	8.50 ± 2.11	52.75 ± 9.75	173.01 ± 10.85	75.12 ± 8.77

Note: IO = 0 Gy irradiation, I1 = 20 Gy irradiation, I2 = 25 Gy irradiation, I3 = 30 Gy irradiation, B0 = seeds intact, B1 = cut two seeds, B2 = cut three seeds





**Figure 6.** The comparison of the anatomical structure of mangosteen leaf transversely sliced (magnification 400x). A. Control, B. Irradiation of 20 Gy the whole seeds, C. Irradiation of 25 Gy seeds cut in half, D. Irradiation of 30 Gy whole seeds, E. Irradiation of 20 Gy seeds cut in half, F. Seeds cut into two without irradiation. Description: Cuticle of (a), upper epidermis (b), palisade parenchyma (c), sponges (d), below the epidermis (e), lower cuticle (f), beam vessel (g), idioblast (h), gland secretion (i).



**Figure 7.** Comparison of leaf anatomical structure paradermal slices. (100x). Description: A. Control, B. Seed is cut in two, C. 20 Gy, seed cut in two, D. 25 Gy, seed cut three, E. Irradiation of 25 Gy whole seeds. Description: opening of stomata (a), guard cells (b), the neighboring cell (c), and epidermal cells (d).

Almost all plants produced by gamma-ray irradiation show a thick palisade and leaves except for I2B2 plants that instead showed the smallest number in thickness. According to Dickison (2000), the plants' response to gamma-ray radiation that has the nature of ionization can cause changes in the leaf's anatomy. Leaves may experience changes such as tissue necrosis, distortion of leaves' bones, changes in the composition and size of palisade tissue and the enlargement of spongy tissue. The differences of the sectional of paradermal slices on the mangosteen's leaves both the control plants and the ones treated with gamma irradiation are presented in Figure 6.

#### Paradermal section

This observation shows the stomata of mangosteen leaf is found on the upper part of the leaf. Paradermal slices show that in the epidermal layer of mangosteens leaves there are stomata, guard cells, neighboring cells, and epidermis cells. Observations on the paradermal slice on the mangosteen's leaves show the characteristics of the observed variations. The highest number of stomata on the plants is found in the plants without irradiation treatment and the plants irradiated with 25 Gy combined with the cutting of the seeds into three equal sizes, while the lowest is obtained in the plants irradiated with gamma-ray irradiation at the level of 25 Gy, with an intact form of the seeds. The highest number for epidermis is obtained in the plants with 25 Gy irradiation with seeds cut into 3 (I2B2), while the lowest number is obtained from the plants without irradiation treatment (I0B03). The index for the

stomata ranges from 5.223 to 9.78 (Table 5). The highest index and density of stomata is obtained in the plants without the treatment of gamma irradiation (I0B03), while the lowest ones are obtained from the plants with irradiation of 25 Gy (Figure 7).

The mangosteens which are the results of gamma irradiation which survive experience anatomical changes on the leaves. The same thing is also reported by Harahap (2005) and Qosim (2006) of mangosteen leaves that are grown in vitro. Dickison (2000) states that gamma-ray radiation that has ionization in nature can cause changes in the leaf's anatomical structure. Gamma-ray irradiation is known to increase the thickness of the cuticle, epidermis, palisade, and leaves in some individuals which are the result of gamma irradiation, although the range of increase varied and showed no pattern of increasing doses of irradiation. According to Qosim (2006), the plants that have thick cuticle is more likely to have properties more tolerant to drought because a thicker cuticle can reduce the rate of transpiration of water and can reflect sunlight. Cuticle also serves to protect plants from pests and diseases. Stomatal index observations on paradermal slices show that in the gamma-ray irradiation treatment, the stomatal index has a smaller number than the plants without irradiation (control), the smallest density of stomata is also obtained in the plants irradiated with the level of 25 Gy, whose seed is cut into two equal sizes. Mangosteen which has stomata with a high density allows a high gas exchange or absorption of CO<sub>2</sub> so that the photosynthetic rate becomes higher. With a higher rate, photosynthate, which

is the result of photosynthesis process, the plant's growth is more supported. Qosim (2006), states the regeneration mangosteen which has a high density of stomata, palisade parenchyma and a high number of file vessels can be used as indirect selection criteria for efficiency. Harahap (2005) states that the study of anatomical structure of the mutant is very useful to explain the changes in the genetic control of certain processes. Fahn (1991) found a recessive mutant of maize which has survived is found to have changed its anatomy in the form of the obstruction towards the differentiation process on the stem's vessels. Cutter (1969) states that the cells that can grow after irradiation are expected to experience physiological or genetic changes. Irradiated plants that will survive are expected to add diversity to increase the effectiveness of selection.

**Table 5.** The average number of stomata, number of epidermis, stomatal index, and stomatal density of mangosteen leaf.

Individual plants	Average			
	Number of stomata	Number of epidermis	Stomatal index	Stomatal density b/(mm <sup>2</sup> )
<b>Seeds irradiated before cut</b>				
BOI01a	13.00 ± 1.22	205.00 ± 27.07	6.06 ± 1.19	184.03 ± 17.33
BOI03b	14.2 0 ± 1.48	176.20 ± 17.16	7.49 ± 0.93	200.99 ± 20.99
BOI02	13.20 ± 4.38	205.80 ± 16.02	6.09 ± 2.23	186.84 ± 62.02
BOI2	10.00 ± 2.01	182.41 ± 15.46	5.22 ± 1.16	141.54 ± 28.30
BII01b	14.02 ± 0.71	207.02 ± 26.90	6.38 ± 0.61	198.04 ± 10.01
<b>Seeds irradiated after cut</b>				
IOB01b	11.61 ± 1.14	194.81 ± 12.29	5.62 ± 0.38	164.18 ± 16.14
IOB02b	14.22 ± 1.64	204.42 ± 22.81	6.56 ± 1.09	200.99 ± 23.25
IOB03	18.81 ± 2.59	174.60 ± 14.18	9.78 ± 1.62	266.10 ± 36.63
I1B1	13.82 ± 2.38	195.00 ± 28.46	6.67 ± 1.25	195.33 ± 33.79
I2B2	18.63 ± 3.13	213.20 ± 39.51	8.23 ± 2.46	263.27 ± 44.30

Note: IO = 0 Gy irradiation, II = 20 Gy irradiation, I2 = 25 Gy irradiation, I3 = 30 Gy irradiation, BO = seeds intact, B1 = cut two seeds, B2 = cut three seeds.

### Correlation between morphological and anatomical characters

Plants growth and development is influenced by factors that are interrelated, both internal and external. The correlation test between the morphological characters namely the height of the plants, the length, and width of the leaves, with the stomatal index character and the density of stomata, indicates a positive correlation between the height of the plants with the density of stomata, and the length of the leaves with the their width. The existence of a correlation between the height of the plants and the density of stomata indicates that characteristic is influenced by the density of stomata. The level of correlation between the height of the plants and the density of stomata was 90% (Table 6) which means that the density of stomata has 90% role in determining the height of the plants, while the correlation between width and the length of leaves was 74% (Table 6). High density of stomata allows easier process of photosynthesis so that the photosynthate that can be generated will be greater in results, and then the growth and development of the plant are more supported. Positive correlation between the height of the plant and the density

of stomata enable the stomata density parameter to become a criterion to measure the growth of mangosteen.

**Table 6.** The value of correlation between stomatal index, stomatal density, plant height, leaf length, and width.

Anatomical characteristics	Stomatal index	Stomatal density	Plant height	Leaf length	Leaf width
Stomatal index	1	0.02	0.01	-0.16	0.13
Stomatal density		1	0.90*	0.02	0.17
Plant height			1	-0.07	0.07
Leaf length				1	0.74*
Leaf width					1

Results of correlation analysis between variables of the cuticle's thickness, upper epidermis, lower epidermis, palisade and spongy to the morphologic variables of the mangosteen plants showed that plant's height, leaf's width and length are not significantly affected by the thick of the cuticle, upper and lower epidermis, the thickness of palisade parenchyma and the sponges of mangosteen's leaf (Table 7). The height of the was significantly affected by the width of the leaf with a correlation value of 76%.

**Table 7.** The value of correlation between the thickness of cuticle, upper epidermis, lower epidermis, palisade, and sponges and the length, the width of the leaves and the height of the plant.

Karakter	Cuticle	Upper epidermis	Under epidermis	Palisade	Sponges	Leaf length	Leaf width	Plant height
Cuticle	1	-0.17	0.04	0.01	0.08	0.02	-0.1	-0.16
Upper epidermis		1	0.08	-0.04	0.17	0.12	0.12	0.35
Under epidermis			1	0.17	0.1	0.18	0.12	0.29
Palisade				1	0.15	0.25	0.01	0.06
Sponges					1	-0.12	-0.08	-0.07
Leaf length						1	0.24	0.3
Leaf width							1	0.76*
Plant height								1

### CONCLUSIONS AND RECOMMENDATIONS

Gamma-ray irradiations with the doses of 0, 20 Gy, 25 Gy, 30 Gy, 35 Gy, and 40 Gy increase the diversity of morphology of mangosteen by 30%. The highest increase of diversity in the mangosteen obtained in the plants with: (i) the dose of 25 Gy irradiation, (ii) with the seed cut into two equal sizes, and (iii) the cutting of the seeds done after gamma-ray irradiation. The biggest increase of the diversity of the mangosteen is obtained by the method of irradiation on the seeds with the dose of 25 Gy and then are cut across seed into two equal sizes. The density of stomata has a positive correlation with the height of the plants by 90%. The density of stomata can be used as a criterion to estimate the growth of mangosteen. To get mangosteen with greater diversity, it is advisable to perform irradiation

on the mangosteen with the dose of 25 Gy with a more number of the research materials.

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