

The Effect of Gamma Radiation on Plant Morphological Characteristics of *Zingiber officinale Roscoe*

Shamsiah Abdullah[#], Nor Yusliza Kamaruddin^{*}, Abdul Rahim Harun¹

[#]Agricultural Biotechnology Research Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia

E-mail: shamsiah3938@salam.uitm.edu.my (corresponding author)

^{*}Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia

¹Agrotechnology and Biosciences Division, Malaysian Nuclear Agency, 43000 Kajang, Selangor, Malaysia.

Abstract— Induced mutation through gamma irradiation can cause changes in chromosome and genome, which bring to successful variation in the morphology of plant. This study investigates the effect of gamma radiation on mutation frequency and plant morphology of *Zingiber officinale Roscoe*. The ginger rhizomes of Bentong and Tanjung Sepat cultivars were treated with gamma rays at six different dosages (5, 7, 9, 11, 13, and 15 Gray). The gamma ray was emitted from the Caesium-137 source at the rate of 4.31 Gy per minute at Malaysian Nuclear Agency irradiator facility. Different mutation characteristics and wide of mutation spectrum was observed in all treatments. The spectrums of mutant characters observed were stunted growth, plant stature, leaf deformation, and chlorophyll mutation. The results show that mutation effects on morphological characteristics were varied in respective doses and varieties. Therefore, the results proved that different doses of mutagen caused different effects on plant and it was a variety-dependent.

Keywords—mutation; gamma radiation; mutants; *Zingiber officinale Roscoe*.

I. INTRODUCTION

Zingiber officinale Roscoe or ginger is a monocotyledon in the family Zingiberaceae that consists of a structure of leaves, stems, and rhizomes. In Malaysia, ginger cultivars include Bentong, Tanjung Sepat and China. These local ginger cultivars are named based on the places or localities they are grown. They exhibit different characteristics, for example, the rhizomes grown at Bentong, Pahang is more abundant with a dull, whitish color and it is less fibrous as compared to Tanjung Sepat cultivar which has a yellowish, fibrous and slender rhizome, whereas China cultivar is full of water and slimy rhizomes. There are also other morphological characteristics of ginger that can be used to distinguish between Malaysian gingers; the characteristics of rhizomes such as rhizome size, pungency, fibrous content, and moisture content are commonly used to differentiate the ginger cultivars [1]. However, the metabolic fingerprinting is the most accurate and precise method to differentiate ginger cultivars/varieties in which variations of chemical components are recognized [2].

Ginger is valued all over the world especially in culinary preparation and for its medicinal properties. It has been used as a traditional treatment for curing human ailments such as stomach pain, diarrhea, nausea, and aids the digestive system. The rhizome also has the potential as an edible film used for food packaging [3]. Therefore, there is a need to improve ginger production such as through improved variety. However, the breeding output of ginger is low because of reproductive sterility of the plant with no viable seed. Ginger variability is severely handicapped by poor flowering, low set of seeds, and propagated vegetative through underground rhizome. Furthermore, the genetic complexity becomes an obstacle for conventional breeding practice in ginger.

The mutation breeding is an alternative tool in creating genetic variation in ginger. Induced mutation using gamma-ray radiation is a method that has been applied in plant breeding to increase genetic variations. Mutation through gamma irradiation has caused changes in chromosome and genome which introduces variation in morphology of ginger plant [1]. The central matter of induced mutation is concerned with the viable mutation whether they are

morphological or physiological, and have potential value in plant breeding. Induced mutation either by physical or chemical mutagen is a practical approach in the varietal development of the vegetative propagated plant. It can change one or more characters without changing the entire characters in the genotype [4]. Mutations are artificially induced by exposing plant material to mutagenic treatments in order to increase the genetic base of germplasm for plant breeding [5]. Once the mutagen treatment is applied to plant materials, it breaks the nuclear DNA, and during the process of DNA repair, mutations occur randomly and are heritable [6]. The three main effects of mutagenesis are point mutations, physiological damage, and chromosomal aberrations. Induced mutation generates allelic variants of genes that modulate the expression of traits [7] and [8]. Previous studies showed that induced mutations have successfully created genetic variability in other crops such as in turmeric [9], sesame [10], soybean [11], stevia species [12] and rice [13]. In ornamental horticulture, radiation has been used to alter flower color, flower form, type of inflorescence, fertility or leaf variegation which depends on the objectives of the breeder. Many instances have been reported on the use of radiation-induced mutations to produce novel morphology difference on plant [5], [8], [11].

Several induced morphological mutations have been reported in literature showing alterations in the morphology of various plant parts, and the mutant characters were grouped as plant height, leaf, and stem. For example, the study reported several viable mutants induced by gamma rays in turmeric (*Curcuma longa* L.) which included plant height, number of tillers, early plant maturity, and high yielding mutants [14]. Another study that subjected ginger to gamma radiation, the chlorophyll mutations (albino, xantha, and chlorine) were observed and grouped. The overall mutation spectrum for ginger displayed that xantha occurred with the highest frequency, followed by chlorine and albino [15]. Gamma ray induced morphological mutations have also been seen in turmeric [16]. The types of mutant characters noticed were such as plant height, leaves length and width, and yield of rhizomes. It was found that gamma radiation treatment is very effective and efficient in inducing various types of morphological mutants. Therefore, an induced mutation in ginger using gamma-ray irradiation will proffer solution to creating or inducing desired attributes useful in ginger. The objective of this study was to investigate the effects of gamma rays on plant morphological characteristics and to determine their frequency in both ginger varieties.

II. MATERIALS AND METHODS

Rhizome samples of two ginger cultivars; *Z. officinale* cv Bentong and *Z. officinale* cv Tanjung Sepat were obtained from the Department of Agriculture, Putrajaya, Malaysia. Uniform sized rhizomes of 4-5 cm long from each cultivar were used for treatment, and their moisture content was equilibrated before irradiation. The rhizomes were exposed to gamma radiation doses of 5, 7, 9, 11, 13 and 15 Gy with a dose rate of 4.31 Gy/min using Biobeam GM 8000 Germany irradiator machine at Malaysian Nuclear Agency, Bangi, Selangor, Malaysia. The untreated rhizome of the respective

cultivar was used as the control. All treatments including control were replicated eight times. Treated rhizomes were sown in polyethylene bags containing a mixture of garden loam soil, sand and cocoa peat sowing media at a ratio of 3:2:1 under a rain shelter. The plot size was 3 m x 5 m (15 m²) with inter-row and intra-row spacing of 20.0 cm respectively. The rhizome setts were planted in 2.0 cm depth in the soil, planting the buds upward. The sprinkler irrigations in the rain shelter provide humidity to the plants.

The rhizomes were arranged in Randomized Complete Block Design (RCBD) with eight replications. Mutation effects on the morphology of plant were visually observed over the respective controls. Chlorophyll mutation was recorded when the seedlings were 50 days old. The chlorophyll mutation was classified according to [19]; **Xantha** where mutants were distinguished by their uniform yellowish color with a total absence of chlorophyll and **Chlorine** where mutants had uniform yellow-green color.

The plant growth rate was calculated by taking the change in plant height and dividing it by the amount of time it has been growing. The equation for the growth rate formula was calculated as follows:

$$\text{Plant Growth Rate} = \frac{(S1-S2)}{T} \quad (1)$$

Where, S1=First plant height measurement, S2=Second plant height measurement, and T= The number of weeks between the measurement

The data of all characters recorded were analyzed with SPSS statistical package version 22.0

III. RESULTS AND DISCUSSION

A. Somatic mutation

Almost all mutagenic treatments showed a different degree of mutations with respective dose (5, 7, 9, 11, 13, and 15 Gy). Morphological characters and types of mutation were recorded and explained in Table 1. The example of gamma radiation effects on the plant was dwarfism or stunted growth, leaf deformation and leaf chlorophyll mutation such as variegated leaf with the yellowish line. In both cultivars, irradiated plant at 9, 11, and 13 Gy doses observed high frequencies of mutation such as stunted growth with unique leaf shapes like narrow leaves, uneven lamina, and changes in texture like rough, curly, wrinkled, and twisting midrib leaves.

Interestingly, high frequencies of chlorophyll mutation were detected in almost all irradiated plants, which indicated that the plant was highly mutable. The reduction in chlorophyll mutation frequency at a higher dose might be due to the activation of a suppressor which usually keeps the rate of chlorophyll synthesis under control [17]. However, there is no evident dose-dependent relationship for the occurrence of chlorophyll mutation.

Phenotypic variations were recorded on irradiated ginger plants (Fig. 1). Leaf characters such as incomplete leaf formation and variegated leaves, midrib in twisting form and detachment of leaf from midrib were observed. Some leaflet was rudimentary, rough surface with uneven lamina and leaf margin. At high dose, some irradiated ginger plants showed

yellowish green and imperfect leaflet forms. Phenotypic mutations consist of two types, macro and micro mutation.

The leaf characters were macro mutations which were easily detectable in the individual plant, phenotypically visible and morphologically distinct. It qualitatively inherited genetic changes and occurred in major genes or oligo genes [18]. The leaf characters may occur due to actual mutation like chromosomal alterations or the number of genes with pleiotropic effect, which means that one gene, influences two or more unrelated phenotypic traits [5].

TABLE I
TYPE OF MUTATIONS OBSERVED ON OF BENTONG AND TANJUNGSEPAT
GINGER CULTIVARS AT DIFFERENT GAMMA-RAY DOSES

Cultivar	Dosage (Gy)	Type of mutation
Bentong	0	No mutation
	5	Leaf deformation & variegated leaf, variegated leaf with a yellowish line
	7	Dwarfism with white strip and curly leaves forming
	9	Dwarfism, and small narrow leaves
	11	Dwarfism with uneven lamina
	13	Dwarfism variant with uneven lamina with twisting midrib whole part of leaves
15	Did not survive	
Tanjung Sepat	0	No mutation
	5	Two-midrib leaves, white/yellow strip leaf
	7	Detached leaf from midrib, twisting lamina, Scatter/random variegated
	9	Dwarfism with stunted growth, small narrow leaves, and yellow and white strip Uneven lamina, margin & rough surface
	11	Mosaic variant with bright spot (mottling)
	13	Twisting of midrib, rough surface & yellow coloration
	15	Did not survive

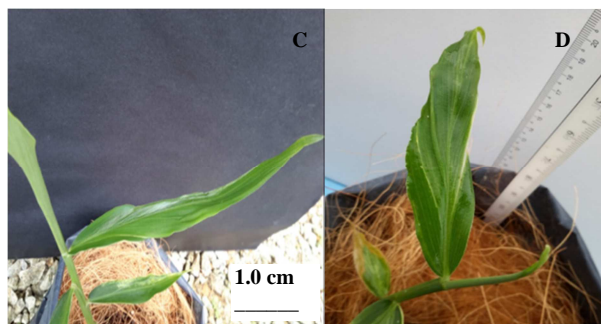
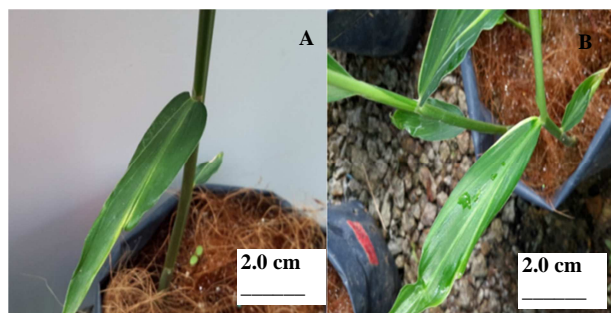


Fig. 1: The effects of gamma rays on leaf shapes: (A) Leaf deformation and variegated type of chlorophyll mutation in Bentong cultivar at 5 Gy irradiation dose, (B) Twisting lamina in Tanjung Sepat cultivar at 5 Gy irradiation dose, (C) Uneven leaf formation in Bentong variety at 5 Gy irradiation dose, (D) Uneven lamina, margin and rough leaf surface in Tanjung Sepat cultivar at 9 Gy irradiation dose.

B. Leaf chlorophyll mutation

The leaf chlorophyll mutant spectrum was found in different doses of gamma rays such as xantha, chlorine and variegated type. Based on the previous study by [7], xantha mutant appeared as yellow to yellowish white and has retarded growth as shown in Fig. 2 (A). For chlorine mutant, the seedling showed light green color leaves, and most of the seedlings died prematurely (Fig. 2). A similar observation was also reported in other work as in [19]. Meanwhile, the previous study in other species also observed variegations in the leaves produced by nuclear or plastid mutations [20].



Fig. 2 The effects of gamma rays on leaf chlorophylls; (A) Twisting of midrib, rough surface and xantha chlorophyll mutation in Tanjung Sepat cultivar at 13 Gy irradiation dose, (B) Dwarf plant, abnormal curly leaves form and chlorine chlorophyll mutation in Tanjung Sepat cultivar at 5 Gy irradiation dose, (C) Detached leaves from midrib with chlorine chlorophyll in Tanjung Sepat cultivar at 5 Gy irradiation dose, (D) Variegated chlorophyll mutation with whitish line in Bentong cultivar at 5 Gy irradiation dose

Based on the observations, the chlorophyll mutation decreased with an increase in the gamma-ray dose which means ginger plant highly mutable. This is because a high

dosage of gamma rays trigger the activation of a suppressor which normally controls the rate of chlorophyll synthesis [17]. Chlorophyll variegated leaves provide additional beauty to plants, and it is one of the remarkable dependable indices for the measure of genetic effects of mutagenic treatments that have been reported in various pulse crops [21]-[23]. However, these chlorophyll mutations do not have any economic importance.

C. Mutants for plant height

Tanjung Sepat cultivar showed better growth performance than Bentong cultivar regarding plant height. Irradiated plants showed the average plant height was in the range of 87.0cm to 89.0 cm while the tall mutants were in the range of 90.0 cm to 94.0 cm (Table 2). Tall mutants were conspicuous by having long internodes and long leaf length (Fig. 3). On the other hands, the short stature, small leaves, short internodes, and slow growth development physically characterized the dwarf mutants. There was a reduction in the number of internodes on the stem axis in the dwarf phenotypes, which means that the intermodal length was reduced in size. Previous research by [10], [24], [25] and [26] also found that the effect of gamma ray on other morphological characteristics were also varied in respective doses. Plant height in the mutants varied due to the increase or decrease of their intermodal length. The frequency of tall mutants was highly observed in Tanjung Sepat cultivar at 5 Gy doses as compared to Bentong cultivar. This was due to the genotype cultivars, which may be varied in the effect of gamma rays on their plant height. Plant height in ginger is a quantitative trait which is predominantly controlled by polygene, and each gene is responsible for contributing small effects, namely genetic additive effect [27]. Furthermore, reduction in growth parameters and dwarfism are also due to disturbance of normal mitosis and frequent occurrence of mitotic aberrations and resulting changes in nutrient levels in plants [26].

TABLE II
THE AVERAGE PLANT HEIGHT OF IRRADIATED AND NON-IRRADIATED GINGER PLANT OF TANJUNG SEPAT AND BENTONG GINGER CULTIVARS

Cultivar	Dosage (Gray)	Plant height (cm)
Bentong	0	55.40±0.43 ^a
	5	51.20±0.49 ^b
	7	37.26±0.68 ^c
	9	21.34±0.30 ^d
	11	10.80±0.66 ^e
	13	5.00±0.14 ^f
	15	NA
Tanjung Sepat	0	87.00.±3.46 ^a
	5	90.20±3.02 ^a
	7	57.00±0.55 ^b
	9	44.60±0.24 ^c
	11	19.20±0.20 ^d
	13	11.00±0.27 ^e
	15	NA

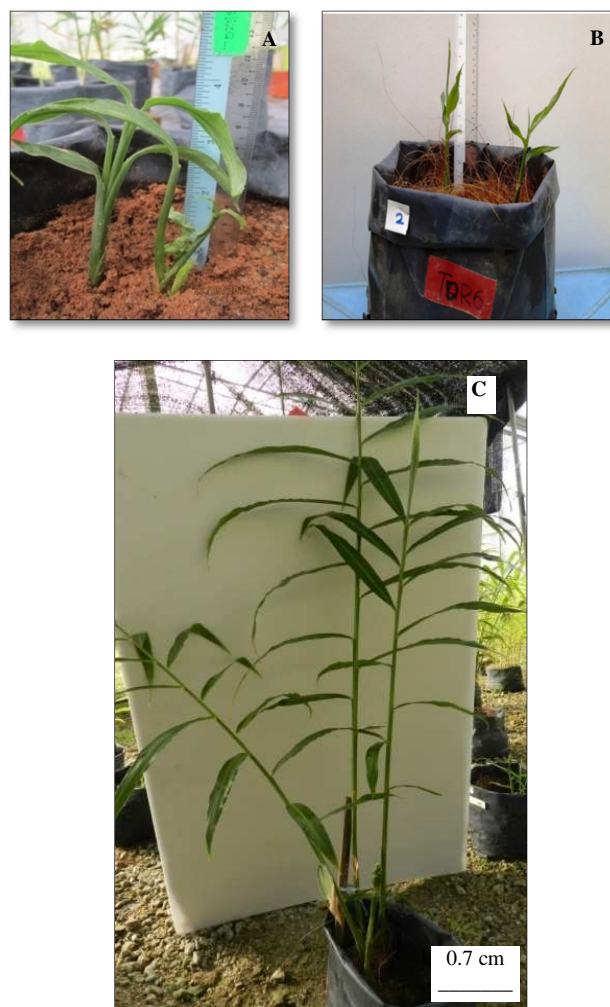


Fig. 3 Showing the plant stature of the irradiated ginger plant. (A): Dwarfism character of irradiated Bentong cultivar at 13 Gy irradiation dose (B): Dwarf variant with a stunted growth of Tanjung Sepat cultivar at 9 Gy irradiation dose (C): Tall mutant plants of irradiated Tanjung Sepat cultivar at 5 Gy irradiation dose

D. Early flowering mutants

Early flowering has been one of the important criteria of breeding, especially for potential ornamental plants. In this study, it was noted that after six months of cultivation, Tanjung Sepat cultivar flowers in about two to three weeks earlier than control plants (Fig. 4 and 5). The inflorescence is a compact spike of 6 to 7 inches long with a cluster of bracts overlapping to form an ovule shape. Most gamma-ray effects on senescence are considered a result of free radicals generated from water and oxygen by the ionizing energy on the cellular components. Membrane deterioration is a general feature of natural senescence and stress-induced aging [28].



Fig. 4 The flower produced in Tanjung Sepat cultivar at 5 Gy irradiation dose, (A) The red color flower, (B) The yellowish-green color flower

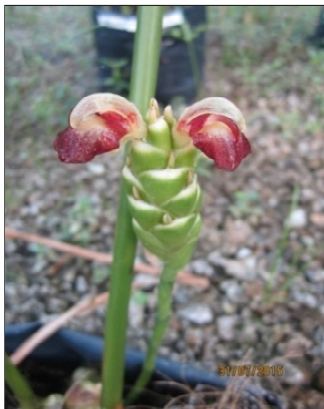


Fig. 5 True flower produced in Tanjung Sepat cultivar at 5 Gy irradiation dose

E. The Yield of Rhizome

In this study, the rhizomes were harvested after nine (9) months of cultivation, and fresh weight of the rhizomes was recorded. From the observation, the interior flesh and epidermis were lighter than the mother rhizomes piece. As shown in Table 3, the present study observed negative effects on the average weight of rhizomes in nearly all gamma-ray doses except for 5 Gy irradiation doses in Tanjung Sepat cultivar where the rhizome weight showed some increment over the control (Fig. 6). Simultaneous decreases in the mean values for the cultivars were observed due to the increase in radiation intensity (Fig. 7). The progressive decrease of a trend for both Bentong and Tanjung Sepat cultivars, in the average value of rhizome weight, was observed at 9 Gy of radiation dose 20.23g and 31.50 g, respectively (Table 3).

Generally, almost all irradiation doses showed adverse effects on the rhizomes' weight. The present results are in agreement with the findings by Giridharan and Balakrishnan [29] and [30] who observed an inhibitory effect on irradiated plants as compared to a control plant group. In addition, the lower dosage could become a stimulatory effect to some agronomical plant traits [27]. However, the increase in the average of rhizomes weight at 5 Gy radiation dose in the present study could happen due to either difference in the genetic material under study or due to agro-climatic variations under which the experiment was carried out [31].

TABLE III
THE AVERAGE RHIZOME WEIGHT OF BENTONG AND TANJUNG SEPAT CULTIVARS

Cultivar	Dosage (Gray)	Rhizome weight
Bentong	0	135.85±2.65 ^a
	5	99.55±1.15 ^a
	7	52.70±2.30 ^b
	9	20.35±1.15 ^c
	11	14.80±0.30 ^d
	13	14.25±0.95 ^d
Tanjung Sepat	0	157.65±2.55 ^a
	5	187.0±2.50 ^a
	7	58.15±2.35 ^b
	9	31.50±1.00 ^c
	11	10.80±0.40 ^d
	13	11.50±1.50 ^d
	15	-

*Data are expressed as mean ± standard error



Fig. 6 Ginger rhizomes of Tanjung Sepat cultivar, (A): Control ginger, (B): Irradiated ginger rhizomes at 5 Gy irradiation dose



Fig. 7 Slender ginger on the left side was irradiated with 5 Gy while ginger on the right side was one of dwarf mutant ginger irradiated with 7 Gy irradiation dose.

F. The Growth Rate of Irradiated Ginger

As gamma irradiation doses increases, the plant growth rate of irradiated ginger decreases (Fig. 8 and 9). All irradiated ginger plant of both varieties exhibited lower growth rate as compared with the control plant. In Bentong cultivar, irradiated ginger at 5 Gy recorded the highest growth rate (6.06 cm/week) and lowest growth rate (0.01 cm/week) was obtained at 15 Gy as against the control, (6.42 cm/week) (Figure 4.5). Surprisingly, for Tanjung Sepat cultivar at 5 Gy exhibited growth rate (10.71 cm/week) which was higher than that of the control plant, (10.48 cm/week) (Fig. 9).

Irradiated ginger plant at high dose (11 to 15 Gy) revealed a gradual decrease in growth rate of both cultivars which may happen due to the effect of high doses of radiation

which disturbed the synthesis of protein, hormone balance, leaf gas exchange, water exchange and enzymatic activity that eventually caused adverse effects on plant growth and development [31]. Similar studies on the effect of gamma radiation on ginger were also observed negative effects on ginger irradiated with a high dose of gamma radiation [1], [30].

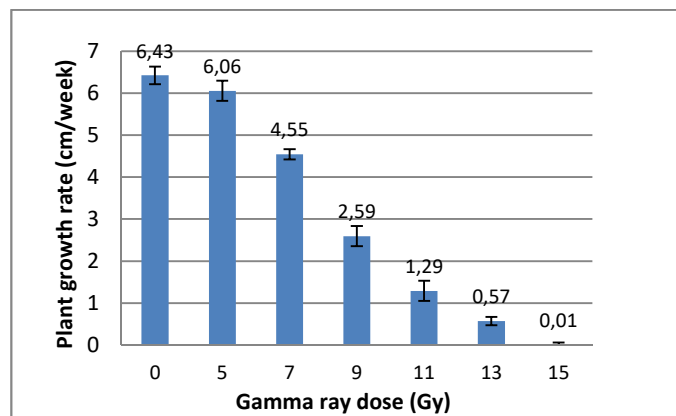


Fig. 8 The average plant growth rate of Bentong cultivar

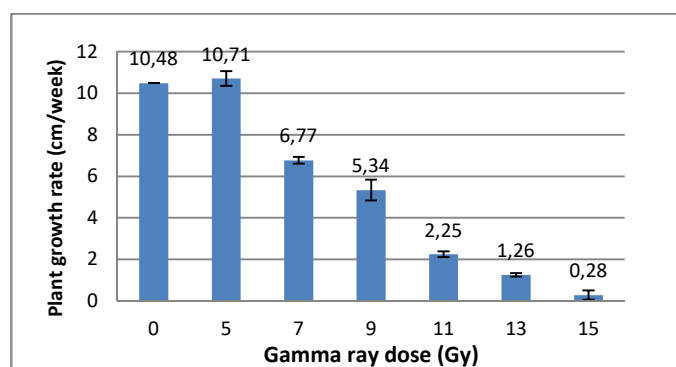


Fig. 9 The average plant growth rate of Tanjung Sepat cultivar

G. Comparison of the effect of gamma rays on different ginger cultivars

Based on the overall observations, Bentong cultivar showed higher sensitivity to gamma-ray doses as compared to Tanjung Sepat. The difference in the sensitivity depended on the different levels of moisture content. From the chemical analysis results, Bentong cultivar recorded higher moisture content (91.47%) as compared to Tanjung Sepat (88.7%). This was because the biological effect of gamma rays was mediated through interaction between water molecules to produce free radicals that damaged or modified the component in plant cells [32]. The hydration has caused an increase in the sensitivity of plant materials as in [12] and [21] thus influencing the ionizing radiation effectiveness through moisture content.

Besides that, the stimulatory effects were obtained at low irradiation dose for both varieties, which produced a distinct character such as tall mutants. In another research, a mutation at low dose occurred in the minor genes that could be observed in the next generation without harmful mutation [33]. Low gamma rays dosage can cause growth stimulation

either by changing the hormonal signaling network in plant cells or increasing the anti-oxidative capacity of the cells to easily overcome daily stress factors. Nonetheless, the present study showed that there was no apparent stimulatory effect on the parameters such as the length and width of leaves, number of shoot and leaves per plants. The overall results also showed that the effects of radiation on rhizomes were proportional to the energy absorbed by the exposed tissue. Consequently, the high dose caused considerably more measurable biological injuries.

The effect of radiation on rhizome yield is proportional to the energy absorbed in the exposed tissue and applied dose. Both cultivars showed the reduction in yield of rhizome produced with increase gamma-ray doses. Consequently, high dose brought more biological injuries and caused a decrease in weight of rhizome. Gamma radiation is high-frequency rays consisting of high-energy protons that penetrated the cell and cause ionization. Hydroxyl radicals are radical products that are generated by a reaction between water and radiation, which caused damage in the DNA chromosome. This toxic substance could react rapidly with macromolecules such as proteins, lipids, and DNA, which destroys cells. Ionization of plant cells disrupts the normal processes of the cell and ultimately affects the ginger rhizome yields.

IV. CONCLUSIONS

The study revealed how plant response to gamma radiation. Results suggested that the mutation effects were cultivar-dependent. The different dose of exposures varies in affecting abnormalities of the ginger plant. Gamma rays induced mutation that could affect growth, leaves, and chlorophyll formation and produce mutant plants that exhibit abnormalities such as a dwarf plant, wrinkled and crumpled leaves, dwarf rhizome, uneven leaf margin, and flower initiation. However, radiation treatment which exceeded 9 Gy lead to slow growth, stunting and finally the plants were dead.

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