

The Effect of Using Gamma Rays on Morphological Characteristics of Ginger (*Zingiber officinale*) Plants

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Abstract: Ginger (*Zingiber Officinale*) was used to determine the effect of different exposure durations of Gamma ray on its growth. The exposure durations used were 30, 60, 90, 120 and 150 seconds. The research was carried out in the Greenhouse of Institute of Biological Sciences at the University of Malaya. The parameters used were germination rate, survival rate, and morphological studies (Seedlings height, the number of leaves, leaves length, leaves width and the number of roots). From the results, the average germination rate showed decrement with increment of Gamma ray exposure duration in which the average germination rate for the Control group was 43.68%, while for the Treatment group, the average germination rate was 40.35% for the Ginger with 30 seconds exposure duration, 32.75% for the Ginger with 60 seconds exposure duration, 25.70% for the Ginger with 90 seconds exposure duration, 25.92 % for the Ginger with 120 seconds exposure duration, and finally 24.48% for the Ginger with 150 seconds exposure duration. The survival rate also showed decrement with increment of exposure duration in which the survival rate for the Control plants was 95.24 %, but for Treatment ones, survival rate was 94.74% for the Ginger with 30 seconds exposure duration, 92.30% for the Ginger with 60 seconds exposure duration, 84.62% for the Ginger with 90 seconds exposure duration, 76.92% for the Ginger with 120 seconds exposure duration, and 75% for the Ginger with 150 seconds exposure duration. The growth of Ginger also showed decrement with increment of exposure duration. Gamma ray also caused some abnormalities in the Ginger. Some of the abnormalities were the formation of dwarf Ginger plants, Ginger plant with crooked stems and corrugated leaves. Generally, all the parameters showed decrement with increment of exposure duration of Gamma ray.

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1. Introduction

Zingiber officinale or Ginger are among the important herbs in the world. It is a vegetative plant that comes from the family of *Zingiberaceae*. This family is the largest family in the order of *Zingiberales* that include approximately 50 genera and more than 1000 species. Of the more than 50 genera, 47 of which are perennial plants and have the structure of rhizomes. Ginger is usually distinguished based on the presence of the labellum, which is formed from the merger of two sterile stamens and also by the presence of essential oils in the tissues. Plants in this family are commonly used as ornamental, medicinal purposes and it has also been used as a spice for over 2000 years (Bartley and Jacobs, 2000). This crop has been used in Ayurvedic and Chinese medicine for the treatment of asthma, diabetes, nausea and pain (Ali et al., 2008). In the United States, 38% of adults use complementary and alternative medical (CAM) treatments to improve or treat musculoskeletal and muscle pain conditions in which Ginger is included as a remedy (Barnes et al., 2008; Chrubasik et al., 2007). Ginger is among the 10 most common natural products used as a CAM treatment (Barnes et al.,

2004). It is a herbaceous plant which can reach a height of up to 90cm when it is fully grown and has a number of lateral shoots formed in the cluster. It also holds 15-30 cm long and 2-3cm wide. Rizomnya is an aromatic fragrance which produces smell and is yellowish brown in color. It has leaves measuring 1.9cm wide and 17.8cm long. The flowers are yellowish green and rarely visible.

Ginger is a creeping plant, has long life, and is perennial. It has a thick rhizome which produces thick ubertuber between 30 to 100cm in height. The leaves are elongated or "lance-shape" and light Green in color. Long leaves are 15-20cm in size with oblong, protruding ribs that cover the flower cluster of yellowish green and purple patch. Ginger is planted in the fertile and well drained soil (Spice encyclopedia, 2003). Ginger requires tropical climate with two rainy seasons and dry summer. Old enough rhizomes which are about 9 months can be used for cultivation. Before planting, they are kept under shade and wind for 2 to 3 weeks for bud growth. Rhizomes are cut into 5cm along with at least two buds. Ginger is the best to be planted in February and March, while the next drought can reduce wilt disease. The recommended planting

distance is between 60cm and 30cm in the row line. Shading is given to the new shoots that appear for a period of 2 to 4 weeks after planting. The shoot can come out after ten days from planting and harvested after 9 or 10 months of cultivation. Ginger plants require rainfall of 200 to 260cm a year and a little shade. The best type of soil for planting is peat soil that is well-drained, loose, friable, and does not restrict the growth of rhizomes. PH of the soil for planting Ginger is 5.5 to 6.5 while the most suitable pH for peat soil is 3.8. In addition, Ginger is a plant that has quite different characteristics according to the region. For example, Ginger is grown in Bentong, Pahang, Malaysia location has a large and fleshy rhizomes, while in Taiwan, it has rhizomes with full of water, slimy, and moderately spicy.

Ginger root is commonly used throughout the world as a flavor in foods. Its root extracts contain polyphenol compounds (6-gingerol and its derivatives) which have a high antioxidant activity (Chen et al., 1986; Herrmann, 1994). Zingiberol, zingiberene, bisabolene, α -curcumene, linalool, cineole, gingerol, and gingerone are organic compounds present in ginger (Xu, 1990). In India, Ginger is usually fried and eaten as a spice in curries. In Indonesia, it is roasted to be added to the fish and meats as a flavor. In Chinese society, it is widely used and usually boiled or fried for consumption. Similarly, in Japan, it is used for dishes such as sushi. In Europe, it is used as an ingredient to fight dental plaque and in England, most pubs provide Ginger in a container so that customers can mix it into the ginger beer to form a "ginger ale", a type of alcoholic beverage containing ginger as a flavor ingredient.

Apart from being a cooking and food, Ginger also has a lot of nutrients for good health. This crop can reduce dandruff problems. Rhizomes parts can be dried and used as drugs. Ginger is also used to warm up, freshen up, add appetite, and increase the production of saliva which is an important ingredient in the process of food digestion. Furthermore, it has also been used as a remedy for congestion in the sinuses or shortness of breath in the chest. In addition, it is often used as an additive in various types of tonic or stimulant or as antidote to overcome abdominal pain. In fact, in the Philippines, ginger is used to chew to avoid impeded by demons or evil spirits (Spices encyclopedia, 2003).

2. Materials and Methods

2.1. Plant Materials

Ginger rhizomes were collected from the Institute of Biological Sciences, Faculty of Science, University of Malaya and gamma ray radiation were prepared from the Department of Physics, University of Malaya. Ginger rhizomes used were old in which they looked like yellowish brown and quite dark. All

plants were grown in the Greenhouse of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur.

2.2. Gamma Radiation Method

Old ginger rhizomes were treated with radiation in different exposure periods including 30, 60, 90, 120, and 150 seconds while the dose rate used was fixed at the rate of 0.1613 Gy per second. Gamma radiation was derived from Gammacell 220 (Figure 1).

2.3. Cultivation Method

Plants were imposed by radiation and grown in a greenhouse using polybags with the size of 15 x 30 cm. Ginger was irradiated and fermented in black plastic bags for 2 days to ensure that all rays and gamma particles were absorbed by the cells of the ginger. Each polybag included ginger rhizomes measuring approximately 5.5cm to 11.5cm long. Parameters used in this study were the rate of bud growth, the number of days for the first shoots, first leaf, and second leaf to grow, length and width of leaves, the number of leaves per plant, plant height, number of roots, germination and survive rate.

Observations for each parameter were made every week. In addition to the morphological characteristics, the observation of abnormalities (if any) were also investigated.

3. Results

3.1. Germination rates

In ginger control group, 4 days after cultivation, all the buds in rhizome produced the first shoots while the first root was produced after 6 days of cultivation. On the day of 9, it was found that 3 buds shoots produced the first leaf, followed by the second leaf on day 12. On the day of 15, it was found that all the buds shoot produce the forth leaves. For the ginger treated with exposure periods of 30 seconds, all rhizomes produced shoot buds on the 6th day after planting. On the 8th day, the rhizomes that were treated by exposure duration of 60 seconds and 90 seconds had issued the first buds of shoots. After 10 days of planting, all the gingers treated with gamma rays had issued the first buds of shoots. After 2 weeks of planting, ginger rhizomes treated with 60 seconds exposure period produced roots. But for other ginger rhizomes (exposure duration of 30, 60, 90, 120 and 150 seconds), root production occurred after 3 weeks of planting.

On the 20th day of cultivation, two shoots from among the ginger treated with 30 seconds exposure period had issued the first leaf, while a ginger shoots from among those treated with 60 seconds exposure period had issued the first leaf. After 4 weeks cultivation, all the shoots bud of ginger (the duration of exposure to gamma rays 30, 60, 90, 120 and 150 seconds) had put the forth leaves.

From the data taken, the average germination rate was decreased with increasing time of exposure to gamma. In Control group, the average germination rate was 43.68% (Table 1), for a period of 30 seconds of exposure, the average germination rate was 40.35%, 32.75% for 60 seconds, and 25.70% for 90 seconds (Table 2). But, for a period of 120 seconds exposure, the average germination rate was increased a little and reached to 25.92%, and in 150 seconds exposure, the average germination rate was 24.48% (Table 2).

Table 1. Germination rates for Ginger (Control)

Rhizome length (cm)	Expected buds	Grown sprouts	Germination rate (%)
11.5	8	4	50.0
12.7	9	4	44.4
10.3	7	3	42.9
9.8	7	3	42.9
11.2	9	4	44.4
10.4	8	3	37.5

The average germination rate = 43.68 %

Table 2. Average rate of germination in different gamma ray exposure periods

Duration of exposure (seconds)	The average germination rate (%)
30	40.35
60	32.75
90	25.70
120	25.92
150	24.48

3.2. Ginger plant survive rate

After 3 weeks of planting (Table 3), the survive rate of ginger in Control was similar to that of

Table 3. The survive rate of Ginger plants after 3 weeks

Gamma ray exposure period (seconds)	The number of shoot buds	The number of alive seedlings	Plants survive rate (%)
0	21	21	100.0
30	19	19	100.0
60	13	13	100.0
90	13	12	92.3
120	13	12	92.3
150	12	11	91.7

Table 4. The survive rate of Ginger plants after 4 weeks

Gamma ray exposure period (seconds)	The number of shoot buds	The number of alive seedlings	Plants survive rate (%)
0	21	20	95.24
30	19	18	94.74
60	13	12	92.30
90	13	11	84.62
120	13	11	84.62
150	12	10	83.33

Table 5. The survive rate of Ginger plants after 5 weeks

Gamma ray exposure period (seconds)	The number of shoot buds	The number of alive seedlings	Plants survive rate (%)
0	21	20	95.24
30	19	18	94.74
60	13	12	92.30
90	13	11	84.62
120	13	10	76.92
150	12	9	75.00

treated in the 30 and 60 seconds exposure (100%). While in the ginger treated with gamma for 90 and 120 seconds, survive rate was 92.3% and finally for Ginger treated with 150 seconds, the lowest survive rate (91.7%) occurred.

After 4 weeks of planting, plants' survive rate for each exposure period decreased (Table 4). Ginger survive rate in Control group was 95.24% while in the 30 and 60 seconds exposure periods, the rates were 94.74% and 92.30% respectively. For 90 seconds and 120 seconds, survive rates were the same (84.62%). Finally, ginger plants treated with gamma rays at 150 seconds exposure period showed the lowest survive rate of 83.33%. The number of dead plants in 30 seconds and 60 seconds was one piece. While for plants treated with 90, 120 and 150 seconds exposure, the number of dead plants for each dose were 2 pieces.

After 5 weeks of planting, survive rate for each exposure period continued to decline (Table 5). Ginger plants in Control group showed the survive rate of 95.24%. For 30, 60, 90, and 120 seconds exposure, survive rates were 94.74%, 92.30%, 84.62%, and 76.92%, but this value was enhanced in 150 seconds exposure (83.33%). The number of dead plants in 30 and 60 seconds was one piece. While for plants treated with 90 seconds exposure period, the number of dead plants was 2 pieces. Plants treated with 120 and 150 seconds exposure showed the highest number of death (3 pieces).

3.3. Morphological studies of Ginger

After 2 weeks of cultivation, the average height was reduced with increment time of exposure to gamma ray. In Ginger control group, the highest average height (2.10cm) was detected. In 30 seconds exposure to gamma ray, the average height was 1.04cm. In 60 seconds exposure, the height was decreased (0.82cm) and in 90 seconds exposure, the average height reached to 0.27cm. For the ginger treated with exposure duration of 120 and 150 seconds, the same average height was obtained (0.16cm) (Table 6).

After 3 weeks of planting, it was found that the average height of plants fell down in each additional period of exposure to gamma rays. In other words, plants growth was decreased with increasing duration of exposure while the ginger control group showed the highest average height (11.20 cm). In addition, after 3 weeks of cultivation, there were still seedlings which had to grow roots and leaves. For a period of 150 seconds exposure, all the shoots still had not issued any roots or leaves, but the growth rate was very low with an average height of 0.27cm (Table 7).

When 4 weeks of planting was completed, all plants had leaves and roots, but 2 plants that were treated with 150 seconds exposure, had produced leaves. In terms of growth, plants treated with the 120 and 150 seconds exposure showed very slow growth compared with the control plants and those treated with the duration of exposure 30, 60 and 90 seconds. The average height of the ginger was decreased by the time of exposure to gamma rays (Table 8), but the control group showed the highest average height (24.10 cm), followed by 21.21cm in 30 seconds

exposure. Ginger plants treated with the duration of exposure of 60, 90 and 120 seconds showed the average height of 13.62cm, 11cm and 7.65cm respectively. Ginger plants treated with 150 seconds exposure period showed the lowest average height (5.25cm). After 4 weeks grown, all plants had abnormalities in each exposure period (Table 8).

After 5 weeks of planting, all plants produced leaves and roots with at least three veins in a leaf and root. In terms of growth, it was found that plants treated with the 120 and 150 seconds exposure showed very slow growth compared with control plants and those treated with the duration of exposure 30, 60 and 90 seconds. The average plant height was decreased with increasing exposure period (Table 9), while ginger control group showed the highest average height (45.13cm). In 30 seconds exposure period the average height was 42.34cm. Ginger plants treated with the duration of exposure 60, 90 and 120 seconds showed the average height of 22.65cm, 13.75cm and 11.14cm respectively. Ginger plants treated with 150 seconds exposure period showed the lowest average height of 10.08cm (Table 9). At this stage, it appeared that some ginger plants already showed abnormalities in morphology. For the ginger treated with exposure periods of 30 and 60 seconds, abnormalities occurred such as rhizomes had peeled skin. Plants treated with 90 seconds exposure, rhizomes skin was flaky. While ginger plants treated with the 120 and 150 seconds exposure, the rhizomes skin looked peeled, leaves were wrinkled and crinkly and stems were bent. In addition, plants looked like dwarf (Table 10).

Table 6. Morphological studies of Ginger after 2 weeks cultivation

Gamma ray exposure period (seconds)	Plant height (cm)	Number of leaves per plant	Leaf length (cm)	Leaves width (cm)	Number of roots per plant
Control	2.10	-	-	-	-
30	1.04	-	-	-	-
60	0.82	-	-	-	-
90	0.27	-	-	-	-
120	0.16	-	-	-	-
150	0.16	-	-	-	-

Table 7. Morphological studies of Ginger after 3 weeks cultivation

Gamma ray exposure period (seconds)	Plant height (cm)	Number of leaves per plant	Leaf length (cm)	Leaves width (cm)	Number of roots per plant
Control	11.2	3	4.5	0.9	3
30	8.31	3	2.8	0.3	3
60	6.87	2	3.4	0.8	3
90	2.55	2	2.5	1.0	3
120	0.37	-	-	-	-
150	0.27	-	-	-	-

Table 8. Morphological studies of Ginger after 4 weeks cultivation

Gamma ray exposure period (seconds)	Plant height (cm)	Number of leaves per plant	Leaf length (cm)	Leaves width (cm)	Number of roots per plant
Control	24.10	3	9.98	1.84	4
30	21.21	3	11.62	1.98	5
60	13.62	2	6.84	0.98	3
90	11.00	2	3.9	0.68	3
120	7.65	2	1.86	0.54	2
150	5.25	1	0.7	0.46	2

Table 9. Morphological studies of Ginger after 5 weeks cultivation

Gamma ray exposure period (seconds)	Plant height (cm)	Number of leaves per plant	Leaf length (cm)	Leaves width (cm)	Number of roots per plant
Control	45.13	5	18.5	2.83	5
30	42.34	5	17.3	2.9	5
60	22.65	4	13.6	2.0	4
90	13.75	3	7.5	1.0	3
120	11.14	3	5.5	0.8	3
150	10.08	2	3.4	0.4	3

Table 10. Abnormalities in Ginger plant

Gamma ray exposure period (seconds)	abnormality
0	-
30	Rhizomes with exfoliate skin
60	Rhizomes with exfoliate skin
90	Rhizomes with exfoliate skin, some plants had twitching leaves
120	Rhizomes with exfoliate skin, leaves were wrinkled and crumpled and plant looked like dwarf
150	Rhizomes with exfoliate skin, leaves were wrinkled and crumpled and plant looked like dwarf



Figure 1. Gammacell 220



Figure 3. Ginger growth at different exposure rates



Figure 2. Ginger plant that grows



Figure 4. Abnormalities in ginger

Discussions

Based on the results obtained, the average germination rate was decreased in ginger for every increase the duration of exposure to gamma rays. However, the decrease in the average germination rate was not as serious as the maximum exposure period of 150 seconds, causing only to a drop in average germination rate (44%) compared with untreated ginger with gamma rays. This might be due to the source of ginger that was derived from cultivated areas and different varieties, although it was purchased from the same place. Therefore, ginger had showed varied germination rates. Germination rate differences might also be caused by age and level of maturity of different ginger plants.

In terms of survive rate; this parameter was decreased by increasing duration of exposure to Gamma rays. Each exposure dose of gamma ray decreased the survive rate each week up to 5 weeks after planting. In other words, the death occurred at each dose of Gamma rays. For example, 120 and 150 seconds exposure showed the highest rate of death while exposure to other doses showed a low mortality rate. The mortality rate was increased with increasing duration of exposure to Gamma rays which was probably due to the damage that occurred in the cells and caused to death.

Theoretically, it is well-known that every organism whether plant, animal, human, and even microorganisms have a DNA repair mechanism called "DNA Repair Mechanism". This mechanism acts repair in damaged DNA when cells are exposed to any source of radiation such as Ultraviolet rays, X-rays, Gamma rays and so on. But, when cells are exposed too long to radiation sources or when intensity is too high, then, the mechanism is no longer able to correct all the severe cell damage caused by the radiation source. As a result, the exposed cells die. Even if they are alive, they form mutated cells that produce a mutant. When too many cells die, the ginger is no longer able to live perfectly and dies in a few days after planting. Most of the dead consisted of ginger treated with exposure duration that exceeds 90 seconds.

According to Konzak et al. (1984), the most useful dose for most of breeding programs is the dose that gives survive rate of 50 to 60% of the seedlings that are planted in the field or crop plot. Therefore, it can be said that the doses used in this study were less useful but this statement cannot not absolute because such studies often influenced by environmental factors, weather, climate, soil, disease and so on. Furthermore, the data in this study was only presented in the last 5 weeks cultivation in the greenhouse. It might give a value that meets the statement issued by Konzak (1984), if the data was taken for longer

periods of time e.g. up to 8 or 10 weeks. But, looking at the limited time factor, the data could only be taken for a period of up to 5 weeks cultivation. This was important because we should have applied the doses which would have provided optimal mutation frequency rather than the maximum mutation frequencies to achieve a high frequency of beneficial mutations and minimize the occurrence of mutation and mutation drastically unwanted (Konzak, 1984).

In terms of morphological traits, based on the data, the average height was declined with increasing duration of exposure to gamma rays. Data on the height of the Ginger plant could also be used as a measure for the growth rate. Growth rates were decreased by each increase in the duration of exposure to Gamma rays which might be because of mutations that had occurred in DNA and chromosomes of cells. Mutations may result in DNA synthesis at the level of interphase cells which disrupt plant buds, lead to the process of cell division through mitosis, and cause cell differentiation process to be interrupted. Thus, the growth rate became less and less with increasing dose of mutagen. We also knew that the doses of the potent mutagen may cause severe damage to the plant cells. It was also probably one of the factors that affected the growth rate of the plants treated with mutagen because severe cell damage had somehow interfered with the growth of the ginger plant. According to Konzak (1984), the most useful dose for most of the breeding objective is the dose that gives 25% reduction in seedlings height planted in the greenhouse. But from the research done, it was quite difficult to get a timely value proposition because it was influenced by a variety of errors and factors like varieties, species, weather; climate and so on. Whatever the case, it should be noted that the selection of appropriate doses is necessary because the doses should provide the optimal mutation frequency rather than the maximum mutation frequencies to achieve high-frequency useful mutations and minimize the occurrence of harmful mutations (Konzak, 1984).

Similarly, the growth of other organs such as roots leaves and so on was affected. High doses caused to a relatively low organ growth. For example, in ginger plants treated with the 120 and 150 seconds exposure, they just showed the growth of roots and leaves after 4 weeks of cultivation. This was much different from ginger control group that they grew forth leaves and roots after 3 weeks of planting. This might also be caused by mutations that occurred in the cells of ginger. Mutations may cause DNA synthesis at the level of interphase cells to be disturbed and also mitotic cell division and cell differentiation process can be interrupted. Therefore, it slowed down the growth and production of organs such as leaves and

roots. Thus, after 5 weeks of planting, there were Ginger plants that were dwarf because of stunted growth resulted from a mutation.

After 5 weeks of cultivation, abnormalities such as skin peeled rhizomes were unfolded when Ginger plants were treated with the duration of exposure of 30 and 60 seconds. When plants were treated with the exposure duration of 90 seconds, rhizome skin looked like flaky and leaves were wrinkled. While for plants treated with the 120 and 150 seconds exposure, the rhizomes skin looked like peeling, leaves were wrinkled and crinkly and stems that were bended. In addition, the plant also looked like a dwarf.

Conclusions

Gamma rays are a type of mutation that can affect growth, germination, survive of ginger plant (*Zingiber officinale*) and cause to produce the mutant plants with specific abnormalities. Different durations of exposure are very in affecting germination and survive rate of Ginger plant. When the exposure is increased, it gives a decline in germination and survive rate.

Duration of exposure affected the growth when the Ginger treatment with gamma ray exceeded 90 seconds, then Ginger growth became stunted and very slow. Exposure to gamma rays could also produce mutants with abnormalities such as a dwarf plant, the wrinkled and crumpled leaves and the bent stems.

For future studies, it is recommended that the duration of exposure used must be between 60 to 150 seconds and doses less than these ranges will not have any significant changes compared to the control and doses above these ranges may cause the plant to have a very slow growth and death. Mutation breeding studies using *in vitro* techniques are also strongly encouraged through *in vitro* methods. The specimen can be increased to enhance the accuracy of data collected.

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