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Detection of changes in growth, yield and genetic variation using RAPD markers among M_1V_2 and M_1V_3 generations of irradiated ginger (*Zingiber officinale* Roscoe)

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Abstract

The effects of gamma-rays on the growth and yield of twelve mutant lines of *Zingiber officinale* Roscoe from 2 varieties; namely Bentong and Tanjung Sepat were analysed for a second (M_1V_2) and a third generation (M_1V_3). Mutant rhizomes have previously been exposed to different doses (0, 5, 7, 9, 11, and 13 Gy) of gamma-rays that were propagated until the third generation. In the study, the phenotypic characteristic and genetic variation study using Randomly Amplified Polymorphic DNA (RAPD) of ginger mutant lines of M_1V_2 and M_1V_3 were analysed and compared with the first generation (M_1V_1). Parameters such as sprouting rhizome, plant height, the average number of leaves and shoots, average length and width of leaves, and average weights of rhizomes per plant for mutant lines of both varieties were collected. It was observed that the increase in dosage had a negative effect on the growth performance of plants. The number of leaves and shoots, length and width of leaves and average weight of rhizomes for both generations were decreased as the dose increased. The plants from the treatment 5 Gy dose produced the highest ginger rhizome yields of 65.45±1.35 g and 165.0±2.30 g in the Bentong and Tanjung Sepat varieties respectively, after nine months of cultivation in M_1V_2. While in M_1V_3, the rhizome yield at 5 Gy showed that the highest rhizome yields were reduced in both varieties of Bentong (45.45±1.25 g) and Tanjung Sepat (125.0±2.30 g). The PCR-based RAPD analysis showed 98.29% of polymorphism which indicated genetic variations between ginger mutant lines. The information provides an important input in determining resourceful management strategies for genetics improvement of ginger.

1. Introduction

Zingiber officinale Roscoe or ginger belongs to the family Zingiberaceae. It is a monocotyledon perennial herb that consists of a structure of leaves, stems, and rhizomes. The commercialized plant part is the rhizome, which is a relatively short horizontal underground stem. Ginger is one of the important tropical horticultural plants and is widely used worldwide as a spice, in culinary preparations and as traditional medicine. There are four main ginger varieties cultivated in Malaysia which are Bentong, Tanjung Sepat, Bara, and China (Suhaimi *et al.,* 2012).

The production of local ginger in comparison with other export crops is relatively low because of its inherent poor yields which can be attributed to the lack of new varieties (Zakir *et al.* 2018). The ginger plant is usually propagated by vegetative means of underground rhizomes with a slow multiplication rate, resulting in low variability. The breeding of ginger is limited by a sterile plant reproduction system with no viable seed, poor flowering and a low set of fruit and seed. Genetic complications encountered in conventional breeding have led the breeders to apply induced mutation as alternative improvement tools for vegetatively propagated plants. Based on the FAO/IAEA (2016) database, more than 3000 mutant varieties of food crops and over 500 mutant varieties of vegetatively propagated plants have been released, resulting from inducing mutation as a breeding tool. Induced mutation has been identified as the most effective technique to create morphological and genetic variability in plants mainly in those with limited genetic backgrounds. It provides a solution to induce desired attributes that are useful especially in ginger improvement. Gamma-rays which belong to ionising radiations are highly effective in triggering natural genetic resources and creating mutants in plants as they can induce a high number of mutations

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in plants (Ilyas and Naz, 2014). Induced mutation using gamma-ray offers the opportunity to increase the genetic variation of ginger species. Genetic variations produced using mutation can create high potential variations to be commercialised for future use.

This research evaluated the phenotypic changes of the second (M_1V_2) and third (M_1V_3) generations of mutant ginger lines derived from two irradiated ginger varieties, Bentong and Tanjung Sepat. The tendency of a population to maintain its genotypes over a generation was assessed on (M_1V_3) and compared to M_1V_1 by Kamaruddin and Shamsiah (2017) which high polymorphism was detected between the mutant lines. Therefore, this study investigates the phenotypic and genetic variation of mutant lines from two ginger varieties, Bentong and Tanjung Sepat.

2. Materials and methods

The study was conducted at Universiti Teknologi MARA, Kampus Puncak Alam, Malaysia to observe the morphology characters of mutant lines of two ginger varieties in second (M_1V_2) and third (M_1V_3) generations. Analysis on the genetic stability was performed using Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique on third-generation only.

The first generation of the ginger plant was obtained from Kamaruddin *et al.* (2016) and similar planting conditions were followed. Matured rhizome at the age of nine months was used for planting the second (M_1V_2) and the third (M_1V_3) generation of mutant lines. There were 12 mutant lines designated as TS0, TS5, TS7, TS9, TS11, TS13, TS15, BT0, BT5, BT7, BT9, BT11, BT13 and BT15.

These mutant lines were derived from two varieties, which were Bentong (BT) and Tanjung Sepat (TS), previously irradiated with gamma-ray from Caesium-137. Both mutant varieties of *Zingiber officinale* Roscoe had been exposed with different dosages of gamma-ray at 5, 7, 9, 11, 13 and 15 Gy at a dose rate of 4.31 Gy per minute (Kamaruddin *et al.* 2016).

2.1 Preparations of planting media

The planting media used in this research was a mixture of garden loam soil, sand and cocoa peat sowing media at the ratio of 3:2:1 in 12×12 cm of the polybag. The soil preparation was done 3 days before planting. The soil pH was measured. About 10.0 g of soil mixture was diluted in 10.0 mL distilled water and mixed evenly. The pH value was set at 5.8.

2.2 Data collection and analysis

The rhizome sprouting was calculated as the number of rhizomes germinated/number of rhizomes sown) × 100. The plant height was measured from the soil surface to its highest point. The number of fully emerged leaves was counted and expressed as the number of leaves per plant. The number of shoots involved of the total count of the shoot in the whole plant. The size of leaves (length and width) was a measurement from the leaf tip to the leaf base and the width of the leaf blade at the longest axis and widest point of the leaf were measured with a measuring tape. The ginger rhizome yield was calculated as the average weight of harvested ginger rhizomes after nine months of cultivation. The data collected on plant height, leaves length and width, the number of shoots and leaves, ginger rhizomes, yield were analysed using SPSS statistical package version 32.0. The results were expressed as mean ± standard error (SE). Statistical differences between experimental groups were assessed by analysis of variance (ANOVA), using the SPSS software package version 24. The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significant differences among the treatment and the value of $p<0.05$ was considered to be significant.

2.3 Genetic variations analysis

The DNA extraction protocol was carried out based on the handbook Qiagen Plant Mini Kit 250 with a slight modification. Approximately 100 mg of young leaves were ground in a sterile mortar to a fine powder in liquid nitrogen and homogenized. The powder was transferred to a new 2 mL polypropylene tube and 400 µL Buffer AP1 and 4 µL RNase A were added to the sample. Then the mixture was vortexed and incubated for 30 mins at 65°C. The tube was inverted 2–3 times during incubation. After that, 130 µL Buffer AP3 was added. The mixture was mixed and incubated for 5 mins on ice. Then, the lysate was transferred into a QIAshredder spin column placed in a 2 mL collection tube and centrifuged for 2 mins at 14,000 rpm. Approximately, 1.5 volumes of Buffer AP3 was added and mix well. About 650 µL of the solution was transferred into a DNeasy Mini Spin column in a 2.0 mL collection tube and centrifuged for 1 min at 8000 rpm. The flow-through was discarded and 500 µL Buffer Aw has added again and centrifuged again for 2 mins at 14,000 rpm to dry the membrane. At the recovery DNA stages, the spin column was transferred to a new 1.5 mL microcentrifuge tube and 100 µL Buffer AE was added for elution. Then the mixture was incubated for 5 mins at room temperature and centrifuged for 1 min at 8000 rpm. The spin-column was transferred to a new 2 mL microcentrifuge, eluted with 50 µL Buffer AE and incubated for 10 mins at room temperature (15–25°C). Amplified products were scored

using PYELPH (Version 1.4) software which showed sensitivity in detecting the presence (1) or absence (0) of homologous bands separately for each primer. This was used for estimating the polymorphic loci and average linkage or Unweighted Pair Group Mean Averaging (UPGMA) dendrog of the populations based on Nei (1972) genetic distance using the POPGENE (Version 1.32) (Yeh and Boyle, 1996) software package. To determine the pairwise similarity, a similarity index "S" was calculated from band-sharing data of each fingerprint using the formula 2Nxy/(Nx)x(Ny), where, Nx = the total number of fragments detected in individual 'x', Ny = The total number of fragments showed by an individual 'y' and Nxy = The number of fragments shared by individuals 'x' and 'y' by Nei and Li (1979).

3. Results and discussion

3.1 Rhizome sprouting

The sprouting response (day) and the number of sprouting rhizome (%) of mutant lines from both varieties were analysed for the second (M_1V_2) and the third (M_1V_3) generations as presented in Table 1 and Table 2. The mutant lines of the Bentong (BT) variety sprouted between 14 to 52 days while the Tanjung Sepat (TS) variety sprouted as early as 10 days up to 52 days. From the observation, the number of sprouting decreased with the increase in doses of gamma radiation for both generations. M_1V_2 of the BT mutant lines showed that BT05 had the highest number of sprouting (64%) on day 14 and BT15 showed the lowest number of sprouting (15%) on day 43.

In contrast, the M_1V_2 generation of Tanjung Sepat (TS) mutant lines showed that ginger of TS5 resulted in the greatest number of sprouting (90%) at day 12 and the lowest number of sprouting was observed in TS11 (40%) at day 26.

The number of sprouting for both varieties in the third generation (M_1V_3) of ginger mutant lines displayed in Table 2, showed similarity trend with M_1V_2, which means in both varieties, the number of sprouting is lesser in the mutant lines derived from a higher irradiated dosage of gamma-ray. BT mutant ginger progeny in the third generation showed a higher number of sprouting in BT5 (62%) at 12.2±0.50 (day) and a lesser number of sprouting in BT15 (12%) at 39.8±0.97 (day). While in TS mutant lines, the lowest percentage was observed in TS11 (37%) and the highest in TS5 (88%).

Exposure to higher gamma-rays doses may cause inhibitory effects on the plant cell. This could be due to injury in rhizome tissue and the severity of damage depending on the dosage used in gamma radiation treatment. According to Preuss and Britt (2003), high radiation doses trigger a phase in cell cycle arrest during somatic cell division which could bring to inhibition of

Table 1. Effect of Gamma-rays on the Rhizome Sprouting in Second Generation (M_1V_2)

Gamma Dosage (Gy)	Bentong		Tanjung Sepat	
	Sprouting response (day)	Number of sprouting (%)	Sprouting response (day)	Number of spouting (%)
0	11.2±0.29	100	9.2±0.30	100
5	14.2±0.50	64	12.5±0.20	90
7	15.6±0.50	62	16.5±1.05	85
9	22.3±0.52	52	19.0±0.60	73
11	25.6±0.49	57	26.0±0.82	40
13	40.8±0.92	40	36.0±1.02	52
15	43.8±0.97	15	43.0±0.98	52

Values are expressed as mean ± standard error

Table 2. The effect of gamma-rays on rhizome sprouting in Third Generation (M_1V_3)

Gamma Dosage (Gy)	Bentong		Tanjung Sepat	
	Sprouting response (day)	Number of sprouting (%)	Sprouting response (day)	Number of spouting (%)
0	10.2±0.29	100	8.2±0.30	100
5	12.2±0.50	62	11.5±0.20	88
7	13.6±0.50	59	15.5±1.05	80
9	21.3±0.52	50	16.0±0.60	70
11	20.6±0.49	51	24.0±0.82	37
13	33.1±0.92	38	30.0±1.02	50
15	39.8±0.97	12	41.0±0.98	51

Values are expressed as mean ± standard error

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plant sprouting and development process. Additionally, delayed sprouting time and reduction in the number of rhizome sprouting is correspond with the frequency of chromosomal damage as gamma radiation doses increased, thus the survival of plants up to the maturity phase could be influenced by the amount of chromosomal damage (Ling *et al.* 2008). Similarly, the sprouting response of vegetatively propagated crops such as cassava (Ndofunsu *et al.,* 2015), and pigeon pea (Sangle *et al.,* 2011) decreased in high doses.

3.2 Plant Height

The height of the plant was lower in higher doses treated lines. From the observations, the mean value of plant height in mutant lines was lower than control plant in both varieties. Ginger mutant lines in both generations especially from BT11 and BT13 showed a significant reduction in plant height as compared to other doses (Figure 1). While Ginger mutant lines of BT15 showed relatively lower height increment of plant height and the plants were not survived after 14 days of planting.

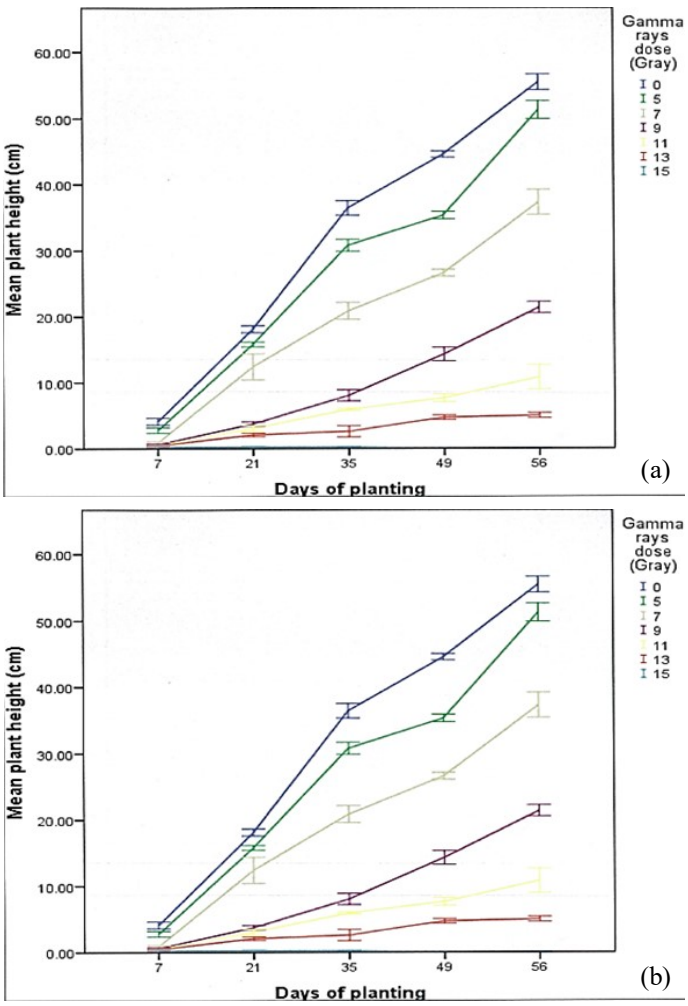

Figure 1. Height increment of ginger mutant lines of bentong variety in 56 days of planting in M_1V_2 (a) and M_1V_3 (b) generations

A similar observation was obtained for ginger mutant lines of Tanjung Sepat variety (TS), the plant height is lower in mutant lines of lower treatments (Figure 2). The maximum plant height was observed at TS5 while the minimum was at TS15. Interestingly, the plant height of mutant lines ginger T5 showed no significant difference with the control plant ($P<0.05$). However, mutant lines TS11, TS13 and TS15 displayed significant differences in plant height.

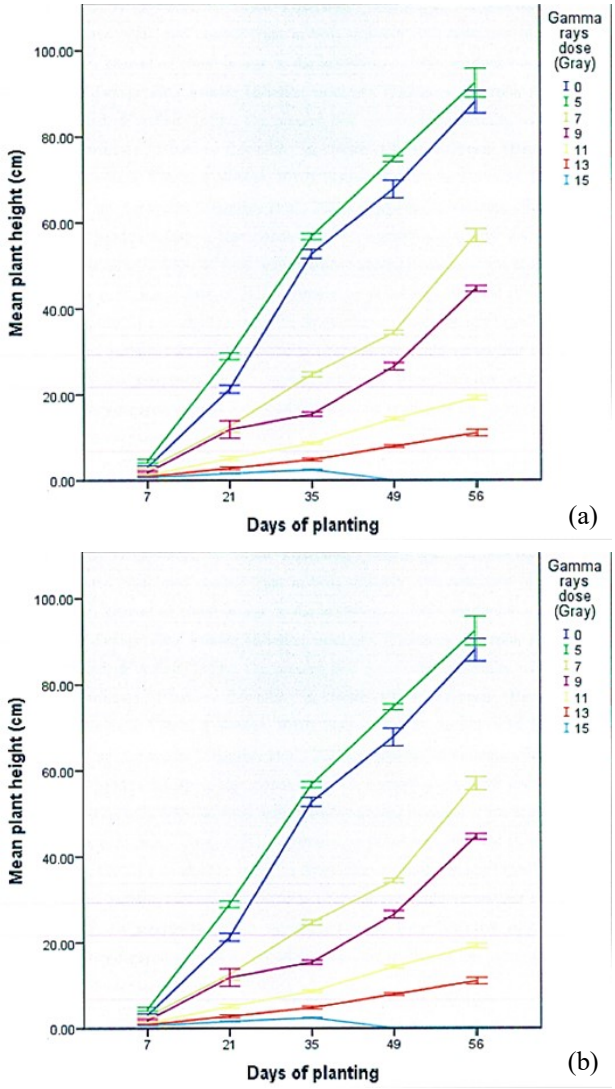

Figure 2. Height increment of ginger mutant lines from tanjung sepat variety during in 56 days of planting in M_1V_2 (a) and M_1V_3 (b) generations

3.3 Leaves length and width

The magnitudes of ranges for leaves length and width of mutant ginger were significantly decreased with the increase of applied treatment (Table 3). Almost all mutant lines plants showed a significant difference in their leaves length and the maximum decrease in leaves length was observed in BT9 and above. It was noticed that mutant lines ginger of BT5 exhibited greater leaves length and width as compared to other doses in both generations. Meanwhile, TS5 recorded no significant difference in leaves length and width with the control plant.

Reports from Gunckel and Sparrow (1961); Ikram *et al.* (2010) and Kamaruddin and Shamsiah (2018) stated

Table 3. The average leaves length and width of mutant

Dosage (Gray)	Mean of Leaves Length (cm)	Mean of Leaves Width (cm)	Mean of Leaves Length (cm)	Mean of Leaves Width (cm)
	M_1V_2		M_1V_3	
B0	3.80±0.37[a]	1.80±0.20[a]	6.2±0.37[a]	2.07±0.24[a]
B5	4.40±0.42[b]	1.90±0.20[a]	5.6±0.24[b]	1.92±0.32[a]
B7	4.01±0.44[c]	1.80±0.10[b]	4.6±0.40[c]	1.78±0.23[b]
B9	3.20±0.73[d]	1.70±0.20[c]	4.5±0.66[d]	1.74±0.22[c]
B11	3.30±0.86[d]	1.62±0.20[c]	3.0±0.70[d]	1.72±0.15[c]
B13	3.10±0.74[d]	1.60±0.20[c]	2.8±0.37[d]	1.71±0.11[c]
TS0	3.80±0.37[a]	1.48±0.22[a]	5.6±0.25[a]	2.4±0.29[a]
TS5	4.00±0.31[a]	1.69±0.38[a]	5.8±0.29[a]	2.9±0.30[a]
TS7	3.80±0.37[b]	1.55±0.25[bc]	5.0±0.20[b]	2.2±0.28[bc]
TS9	3.00±0.23[b]	1.44±0.22[ab]	4.4±0.34[b]	2.4±0.35[ab]
TS11	2.80±0.38[c]	1.41±0.21[bc]	4.2±0.31[c]	2.2±0.31[bc]
TS13	2.00±0.31[bc]	1.34±0.18[c]	3.8±0.11[bc]	2.2±0.30[c]

Values are expressed as mean±standard error. Values with different superscript within the column are significantly different at 0.05 level of significance.

that higher exposures of gamma-rays produce generally negative effects on plant growth and development although the effect of dose rate on mutation frequency might differ among plant species. These effects include cytological, anatomy, genetically, biochemical, physiological and morphogenetic changes in cells and tissues. Generally, the leaf is responsible for helping the plant to produce food by converting the energy from sunlight into chemical energy so that plant can consume by the plant itself.

3.4 Number of leaves and shoots

The number of shoots and leaves are widely used as an index in determining the biological effects of various physical mutagens. Based on the results obtained, there were significant differences in the number of shoots and leaves per plant produced in all treatments (Table 4). All mutant lines displayed a lower number of leaves as compared to the control plant. Both BT5 and TS5 at 5 exhibited a higher number of leaves per plant of 10.20±0.94 (M_1V_2), 10.8±1.96 (M_1V_3) and 11.6±1.21 (M_1V_2), 10.4±1.07 (M_1V_3) and were significantly different ($p<0.05$) among treatments. Meanwhile, the highest number of shoots of BT mutant lines were BT5 (1.80±0.80) and was not significantly different from the control plant. A similar observation in Tanjung Sepat mutant lines, TS5 recorded a higher number of shoots as compared with the control plant with no significant difference ($P<0.05$).

Table 4. Average number of leaves and shoot per plants.

Dosage (Gray)	Mean of Leaves Length (cm)	Mean of Leaves Width (cm)	Mean of Leaves Length (cm)	Mean of Leaves Width (cm)
	M_1V_2		M_1V_3	
B0	08.40±0.82[a]	01.61±0.50[a]	11.6±1.86[a]	01.80±0.37[a]
B5	10.20±0.94[b]	01.80±0.80[a]	10.8±1.96[b]	01.50±0.44[a]
B7	05.60±0.47[bc]	01.50±0.48[a]	08.60±1.07[bc]	01.10±0.37[a]
B9	05.20±0.46[cd]	01.00±0.44[ab]	05.80±0.46[cd]	00.50±0.37[ab]
B11	04.00±0.31[d]	00.40±0.20[ab]	04.60±0.39[d]	00.40±0.24[ab]
B13	03.60±0.21[d]	00.20±0.14[ab]	03.00±0.22[d]	00.20±0.17[ab]
TS0	08.80±0.97[a]	01.72±0.52[a]	11.40±2.14[a]	02.42±0.26[a]
TS5	11.60±1.21[b]	01.89±0.86[bc]	10.40±1.07[b]	02.20±0.24[bc]
TS7	05.40±0.74[b]	01.00±0.51[cd]	09.80±0.91[b]	01.80±0.33[cd]
TS9	04.40±0.50[b]	00.70±0.39[b]	08.60±0.92[b]	01.20±0.28[b]
TS11	03.20±0.45[b]	00.60±0.38[bc]	05.20±0.80[b]	00.50±0.10[bc]
TS13	02.60±0.37[b]	00.40±0.24[bc]	05.20±0.76[b]	00.20±0.05[bc]

Values are expressed as mean±standard error. Values with different superscript within the column are significantly different at 0.05 level of significance.

3.5 Yield of rhizomes

The rhizome was harvested after nine months of cultivation and the weight of the fresh rhizome was measured and observed (Table 5). From the observation in M_1V_2 generation, BT5 produced the highest rhizome yield per plant (65.45±1.35 g) among mutant lines however the weight was 50% of control treatment and the lowest rhizome yield per plant (11.25±0.12 g) was recorded in BT13. Surprisingly, in the Tanjung Sepat variety, treatment at TS5 showed the highest in rhizome yield per plant (165.0±2.30 g) which was higher than the control plant (134.65±2.00 g) and treatment TS11 obtained the lowest in rhizome yield per plant (11.80±0.50 g). A similar pattern was observed in M_1V_3 generation. The higher weight of rhizome was obtained in treatment TS5 (125.45±23 g) and the lowest was treatment TS11 (10.8±0.3 g). For Tanjung Sepat mutant line, the rhizome weight of TS5 was significantly higher than control treatment (114.65±0.20 g).

Table 5. The average weight of rhizomes of mutant lines of Bentong and Tanjung Sepat varieties

Treatment	Mean of rhizome weight per plant (g)	Mean of rhizome weight per plant (g)
	M_1V_2	M_1V_3
BT0	133.00±2.00[a]	111.00±1.02[a]
BT5	65.45±1.35[a]	45.45±1.25[a]
BT7	42.70±1.20[b]	33.70±0.20[b]
BT9	19.22±1.02[c]	16.22±0.02[c]
BT11	13.80±0.65[d]	12.80±0.12[d]
BT13	11.25±0.12[d]	8.25±0.25[d]
TS0	134.65±2.00[a]	114.65±0.20[a]
TS5	165.00±2.30[a]	125.00±2.30[a]
TS7	48.15±2.12[b]	44.15±1.12[b]
TS9	23.50±1.20[c]	13.50±0.20[c]
TS11	11.80±0.50[d]	10.80±0.32[d]
TS13	12.50±0.36[d]	11.50±0.40[d]

Values are expressed as mean±standard error. Values with different superscript within the column are significantly different at 0.05 level of significance.

The gamma radiation also affects the yield as the higher gamma radiation dose, the lower weight of ginger rhizome, which can be attributed to a reduction of plant growth, leaf area and size and growth of rhizome. The reduction in most parameters was in accordance with what has been found by Mehetre *et al*. (1994) who confirmed that yield reduction was resulting from injury effect in seeds and usually showed inhibitory effects on seeds of angiosperms and gymnosperms. These results might be due to the stimulating effects of gamma-radiation on activating RNA synthesis or protein synthesis. Gamma-rays can induce free radicals, and these molecules are strong oxidative stress factors that damage lipids, proteins, and DNA within plant cells (Moghaddam *et al.,* 2011). These observations indicate that treatment with high doses of gamma irradiation has harmful effects on plant growth and development through the increase of free radicals. When plants are exposed to gamma irradiation, free radical concentrations increase with increasing absorbed doses (Marcu *et al.,* 2013).

3.6 DNA band analysis using Randomly Amplified Polymorphic DNA (RAPD) markers

RAPD molecular DNA markers were used to reveal the genetic variation in both varieties. In this study, a total number of 116 reproducible RAPD bands were scored across two varieties of ginger for all primers, the results were used to evaluate genetic diversity relationships among mutant lines. The overall polymorphism percentage was 98.29% and only 2.8% was monomorphic bands (Table 6). The high polymorphic value indicated there were high alterations in the sequence on the primer binding site in mutant lines of gingers. The number of bands (DNA fragments) per primer ranged from six (OPA-12) to 23 (RN-08), the average number being 12.89 per primer and 12.67 polymorphic bands per primer (Table 6). All primers showed the ability to detect distinct, clearly resolved and good polymorphic amplified products within the mutant lines.

Table 6. Total number of amplified fragments and number of polymorphic fragments generated by PCR based RAPD using random decamers in M_1V_3

Primer's Name	No. Amplified Products	No of Polymorphic Products	Polymorphic Loci (%)
OPA 12	5	6	
OPA 27	6	6	
OPA 28	13	14	
OPN 10	20	20	
OPN 15	14	14	
OPP 16	9	8	98.29
OPU 03	10	10	
RN 08	20	18	
S 11	19	18	
Total	116	114	
Average	12.89	12.67	

In this study, differences were observed in RAPD profiles among ginger mutant lines. According to Mullainathan and Saraswathy (2014) polymorphism on RAPD banding pattern can be explained as a result of mutations that bring to structural changes in plant DNA after the impact of gamma radiation. Genetic mutations like point mutations or changes in a DNA base also cause differences in the DNA template, resulting in random PCR product banding patterns. A similar opinion

by Micke and Donini (1993) stated that gamma radiation cause destruction and grouping formation led to chemical changes resulting in the distribution in organic bases pairing of DNA. It caused gene mutations or chromosomes rearrangement to occur in the cell genotype.

Mutation can cause changes in genome sequence which affect primer annealing sites and therefore also change DNA bands in the RAPD profile (Sianipara *et al*., 2015). The effect of radiation towards DNA was analysed by detecting changes of DNA bands profile between parent plant (control ginger) and mutant lines of gingers sample. One base difference in genome sequence may inhibit the annealing of primers.

The genetic distance of different mutant lines of ginger varieties is shown in Figure 3. The maximum genetic distance of Bentong mutant lines variety was found between mutant ginger lines at BT5 with BT11 (0.63) while the minimum genetic distance (0.22) was found between control and mutant line BT7. Two main clusters were delineated from the dendog (Figure 4). However, BT5 was found to be quite divergent and did not fall in any of the major clusters. The first major cluster was divided into two minor clusters. Another minor cluster contained mutant ginger lines at BT13 solely formed sub-minor I while mutant ginger lines BT9 and BT11 formed sub-minor II. One minor cluster holds control of BT0 and BT7.

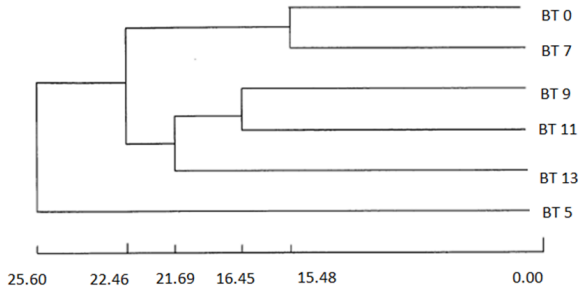

Figure 3. Genetic distance of ginger mutant lines of Bentong variety.

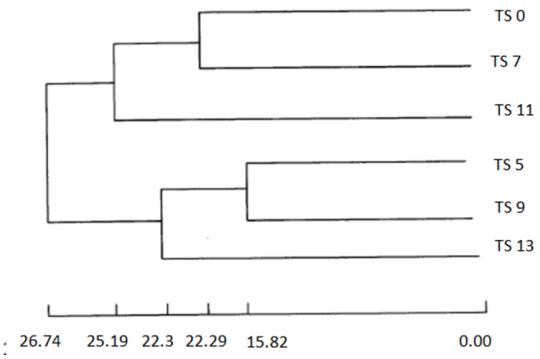

Figure 4. Genetic distance of ginger mutant line of Tanjung Sepat variety.

In the Tanjung Sepat variety, the maximum genetic distance (0.62) was found between control TS0 and TS13 while minimum genetic distance (0.30) was found between ginger mutant lines TS5 and TS9 (Figure 4). The dendrog indicated that six mutant lines of ginger could be grouped into two major clusters (Figure 4). The first major cluster comprised of mutant lines ginger of 11 Gy only and other minor cluster consists of control and mutant lines ginger TS7 Second major cluster comprised of ginger mutant lines TS13 only and other minor cluster were ginger mutant lines ginger TS5 and TS9.

Overall, the proximity matrix based on RAPD analysis of mutant lines from Bentong and Tanjung Sepat varieties showed the control ginger was more closely genetically related to mutant ginger derived from treatment 7 Gy. Almost all mutant lines ginger showed high genetic variation between them which revealed significant differences existed in the genotypic variations. Both varieties showed the trend of genetic variability was not exactly proportional to the doses of gamma-rays when genetic distances were examined. A similar finding was discovered by Teng *et al*. (2008) in chrysanthemum, Dhillon *et al.* (2014) in Jatropha and Tabasum *et al*. (2011) in rice.

The finding of this research provides insight for the RAPD analysis which was found to be useful for detecting the mutation changes in ginger plants DNA induced by gamma-rays. The mutant lines of ginger showed differences in morphological traits as well as genetics as showed by the DNA polymorphism profile amplified by the RAPD marker. Thus, it can be concluded that DNA polymorphism detected by RAPD analysis offers a useful molecular marker for the identification of mutants in gamma-ray plants.

Overall, both varieties showed the trend of genetic variability was not exactly proportional to the doses of gamma-rays when genetic distances were examined. This is because the trend of genetic variability sometimes was proportional to the doses of gamma-rays within a certain range (Teng *et al*., 2008). However, the findings of this research provide insights for the RAPD analysis which had been found to be useful for detecting the mutation changes in plant DNA induced by gamma-rays. The mutant showed the differences in morphological traits from the DNA polymorphism analysis in the PCR profile amplified by RAPD marker.

4. Conclusion

Overall, ginger mutant lines of Bentong variety derived from lower gamma radiation doses (5, 7 and 9 Gy) showed a significant difference in plant morphology and growth performance among ginger mutant lines. In

contrast, Tanjung Sepat variety mutant lines derived from treatment 5 Gy, showed better performance in plant height, leaves length and width, number of leaves and shoots and the highest rhizome yield in both generations, M_1V_2 and M_1V_3. The genetic variation analysis through RAPD-PCR provides strong evidence for the existence of a high level of variability among mutants.

Conflict of interest

The authors declare no conflict of interest.

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