Effects of gamma irradiation on agromorphological characteristics of chili (Capsicum annuum L.) var. Kulai

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Effects of gamma irradiation on agromorphological characteristics of chili (*Capsicum annuum* L.) var. Kulai

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Abstract

The increasing demand for chilli has drawn the interest of chilli breeders to improve the production of this crop. Mutation breeding is applied in many crops improvement programs as it can rapidly create the variability of inherited traits in crops. This study evaluates the effects of irradiation doses of radioactive cobalt (60Co) γ rays on seed performances, morphological characteristics and yield of chilli (*Capsicum annum* L.) var. Kulai grown under laboratory and glasshouse conditions. The chilli seeds were treated with gamma radiation at 0, 20, 40, 60, 80, 100, 200, 300, 400, 500 and 600 Gy. The effect of gamma-rays was assessed towards germination, survival rate and several morphological characteristics. From the observation, seeds from all treatments were germinated (100%) in 10 days. However, the gamma radiation affects the survival rate, fruit length, fruit weight, plant height, and most of the results were lower upon exposure to higher doses particularly 100 Gy and above. Germination rate, plant height, plant survival rate and other morphological characteristics of the irradiated plant were observed to improve at lower doses (40, 60 and 80 Gy). Apparently, lower gamma-ray doses (<100 Gy) were more suitable to study the effect on seed germination as well as other morphological characters of *Capsicum annuum* L. especially Kulai variety. Mutagenic dose of 300 Gy was estimated as the LD50 for Kulai variety. The findings of this study provide useful information for mutation breeding in *Capsicum annuum* L. using gamma radiation for future breeding programmes in chilli.

1. Introduction

Capsicum annuum L. or commonly known as bell pepper, hot pepper or chilli, is one of the important vegetable crops of the world. Like other crops, chilli is also influenced by climate change. Changes in the temperature, precipitation and sunlight affect the agricultural ecosystem, giving rise to pests and diseases and changes in biodiversity have severely affected the productivity of the chilli (Bhutia *et al*., 2018). Pests and diseases are the common and persistent problems that hinder chilli production. The varietal effect also influences chilli productivity. Local varieties such as Kulai are among the popularly grown variety due to their economic value and high germination percentage (Omar *et al.*, 2008). These local varieties are genetically heterogeneous and susceptible to pests (Aisha *et al.* 2018). Lack of suitable variety and available variety of capsicum seems to be susceptible to diseases and other environmental factors thus making the industry turn to the imported varieties of hybrid seeds (Suhana *et al.*, 2016). Therefore, the development of a new chilli variety that is resistant or tolerant to this condition is crucial to coping with new threats.

The natural way of hybridization requires many years of study in order to get the desired characteristic of a plant, but the success rate is considered low. On other hand, genetic engineering, the more targeted crop improvement method, is currently not acceptable in many countries. Mutation breeding in crop plants is a technique that could result in mutant varieties with desirable traits (Jain and Suprasanna, 2011; Kamaruddin and Abdullah, 2017; Tatar *et al.*, 2020). In mutation breeding, the prime strategy that has been done by upgrading the well-adapted plant varieties by altering

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one or two major agronomic metrical traits which enhance their quality or limit their productivity. The success of the mutation breeding technique resulted in positive and negative based on gamma radiation rate, the number of screened plants and the mutation efficiency. It is a tool to create genetic variability by altering one or a few traits of well-adapted cultivars while maintaining their major genetic composition. Gamma radiation exposure to plants is known to produce morphological mutants, physiological mutants and biochemical mutants (Kim *et al.* 2021). In wheat, gamma radiations have improved morphological characteristics of plants such as germination, plant height, number of seeds and seed yield per plant (Jamil and Khan, 2002). Sumira *et al.* (2011) found that irradiation with lower doses of gamma rays has dramatically improved the vegetative traits on pre-flowering, flowering and post-flowering (seed to seed) of *Psoralea corylifolia* L. after irradiation. Kobori *et al.* (2010) reported that the germination percentage of the seeds and growth rate of sprouts of *Cicer arietinum* (kabuli chickpea) were inversely related to the irradiation doses.

Several mutagenesis works on *Capsicum annuum* L. has discussed the effect of gamma irradiation on morphological characteristics of this crop. Jo *et al.* (2016) identified individuals in each population of *Capsicum annuum* with various developmental mutations through phenotypic analysis and categorized the mutations into four groups (mutations in plant architecture and development, leaf, flower, or fruits). Aisha *et al.* (2018) revealed that low doses of gamma radiation stimulated some of the growth parameters, while high doses severely affect the evaluated parameters of Chili Bangi3 and Chili Bangi5 varieties. Recently, Kayode-Samuel *et al.* (2021) reported the morphological changes in three chilli cultivars. They found that doses between 150 to 250 Gy give higher desirable mutations in the varieties. Therefore, mutation induction using gamma-ray could be one of the promising methods to induce mutation that will lead to the development of chilli varieties that are resistant and tolerant to pests, diseases and other environmental factors suitable for local production. From the study, the optimum gamma-ray dose will be determined for Kulai variety. The finding will provide useful information for breeding programs of the variety or other variety in the same family. Therefore, this study focused on the determination of different gamma radiation dose effects on several morphological characteristics of *Capsicum annuum* var Kulai.

2. Materials and methods

2.1 Plant material

This study was conducted using *Capsicum annuum* L. *var*. Kulai seeds. The seeds were obtained from Malaysia Agricultural Research and Development Institute (MARDI).

2.2 Gamma irradiation

Seeds were treated with gamma radiation at 20, 40, 60, 80, 100, 200, 300, 400, 500 and 600 Gy of ^{60}Co γ at 15 Gy/min using Biobeam GM8000 (Germany) with Caesium-137 as a source at the Malaysia Nuclear Agency. The non-treated (0 Gy) seeds of chilli were considered as control treatment.

2.3 Survival rate and radiosensitivity test

After irradiation, twenty-five seeds were sown in three replications per treatment on blotting paper using the sandwich blotter technique as described by Myhill and Konzak (1967). Seeds were placed between two wet blotters, which were supported vertically between slots in Poly Vinyl Chloride (PVC) racks. The racks were placed in plastic trays containing enough water to immerse the lower edge of the filter papers. Germination percentages were recorded 10 days after sowing, meanwhile, shoot length and root length were measured 14 days after sowing. The experiment was laid out in a completely randomized design with three replications The seedling survival rate at the 15 and 30 days after planting (DAP).

2.4 Planting and observation

The one-month-old healthy seedlings with 4 to 5 true leaves were transferred into a polybag (40×40) containing only sand to ensure no other factors influence the plant growth. Water was applied daily to maintain soil moisture. The shoot length, root length, plant height and fruit length were measured using a meter ruler. The number of leaves, the number of branches, the number of seeds per fruit, the number of fruit, length of fruit and days to flowering were determined manually.

2.5 Data analysis

Analysis of variance (ANOVA) was used according to a Completely Randomized Design. Data were analysed by using Computer software Minitab version 16 and Statistical Package for the Social Science (SPSS). Where the main effects were significant, mean separation was carried out using Tukey's Test Fisher's comparison ($P<0.05$). Comparison of means was performed using the least significant difference (LSD). In the table, means followed by the same letter are not significantly different at the 5% level.

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3. Results and discussion

3.1 Survival rate

Seed germination rate and days to germination were observed in all treatments (Table 1). The results showed the number of days to germinate varies between treatments. Plants treated with 20 Gy took less than 6 days (5.1±0.56) to emerge followed by plants treated with 40 Gy and 60 Gy with 5.7±0.69 and 5.6±0.72 respectively. The results showed a significant difference with control. Seeds exposed to higher gamma doses (300 – 600 Gy) took seven days or more to emerge. From the overall observation, the number of days increases as the doses increase.

Table 1. Seed germination rate and number of days to germinate in all treatments

Gamma Dosage (Gy)	Germination rate (%)	Number of days to germinate (day)
0	100^{a}	$5.4±0.72^{de}$
20	100^{a}	$5.1±0.56^{e}$
40	100^{a}	$5.7±0.69^{d}$
60	100^{a}	$5.6±0.72^{d}$
80	100^{a}	$6.4±0.69^{c}$
100	100^{a}	$6.6±0.65^{c}$
200	100^{a}	$6.7±0.72^{c}$
300	100^{a}	$7.8±0.72^{c}$
400	100^{a}	$8.4±0.72^{b}$
500	100^{a}	$9.4±0.70^{a}$
600	100^{a}	$9.7±0.72^{a}$

Values with different superscript within the same column are significantly different (P<0.05)

The germination results obtained are in contrast with findings by Omar *et al.* (2008). They found that chilli seeds radiated with a lower dose of gamma-ray (<300 Gy) did not produce good results. Therefore, they conducted a study using 300 to 800 Gy. In their study, a decrease in germination from 42.22% to 15.56% was observed on 300 Gy to 600 Gy seeds, while 800 Gy seeds failed to germinate 13 days after planting. A similar decreasing trend was reported in other crops such as rice (Harding *et al*., 2012), groundnut (Aparna *et al*., 2012), physic nut (Songsri *et al*., 2011), cucumber and okra (Jaipo *et al.,* 2019). Perhaps, the major source of the declining trend in the germination of plants may have been due to the phenomenon of seeds without properly developed embryos which can be linked to RNA activation of protein synthesis during the preliminary phase of germination (Chopra, 2005; Omar *et al*., 2008). Mokobia and Anomohanram (2005) recorded in *Zea mays*, 90% germination was observed at 240 Gy as well as other crops in Nigeria such as *Abelmoschus esculentus* Moench and *Arachis hypogeal* L. that were exposed to 150, 300, 500, 700, 900, 1000 Gy. In our study, 100% of germination was observed in all treatments.

Observation on seedling survival rate at 15 and 30 days after planting showed a significant difference between treatments (Table 2). Seedlings from control treatment (0 Gy), showed highest survival rate of 80.8% (15DAP) and 77.2% (30DAP) followed by 20 Gy (75.4% and 70.5%) and 40 Gy (71.8% and 68.3%). The rate decreases as the dose increases and the number of seedlings surviving decreases from doses 500 Gy and 600 Gy. Gamma dose higher than 300 Gy had reduced the survival rate to more than 50% while no seedling survived when exposed to 500 Gy or higher. Sood *et al.* (2016) claimed that only at a certain amount of radiation the seedling could thrive at the early stage of development but it may not survive after some period of time, apparently due to breakage of DNA and incapability of reconstructing it. On the other hand, a higher dose of exposure induced the inhibition of growth of the seedlings attributed to the G2/M cell cycle arrested during somatic cell division and/or severe damages throughout the whole genome (Wi *et al.,* 2007). In order to avoid excessive loss of actual experimental materials, LD50 (the safe dose at which half of the planting material survived) were determined. The result obtained was 305 Gy which indicates a safe dose at which half of the irradiated chilli plant survived.

3.2 Shoot and root length

Based on Table 2, there are significant differences in seedlings length (shoot and root) between treatments. The results showed that the performance of seedling growth is gamma dose-dependent. Higher doses (>200 Gy) of gamma have affected the length of seedlings negatively compared to lower doses (20 to 100 Gy). This observation is similar to the study of Mendoza *et al.* (2012) who found that increased gamma doses gradually decrease the seedling height of *Capsicum annumm* L *var.* Chile de Agua grown in the greenhouse. Similar observations were also found in other plants such as cowpea (Gnanamurthy *et al*., 2013). The inhibitory effect of mutagens on seedling length can be seen from the length of root and shoot decreasing when the concentration of gamma-ray radiation increased. An earlier study by Gaul (1977) reported that the reduction in root and shoot length was attributed to the effects of mutagens on the physiological system. Overall reduction of several plant characteristics with a rising trend in the dosage of gamma radiation could be attributable to the loss of water and nutrient absorption due to an extreme impact of irradiation on the root growth and development (Aisha *et al.* 2018). Moreover, a study by Zata *et al.* (2004) mentioned that these outcomes achieved by irradiation treatments were due to in vivo division rate as

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Table 2. Effect of gamma doses on survival rate, shoots and root length of seedling

Gamma Doses (Gy)	Seedling survival rate (%) Day after Planting (DAP)		Shoot length (cm)	Root length (cm)
	15	30		
0	80.8^{a}	77.2^{a}	4.8±3.49^{a}	16.8±5.80^{ab}
20	75.4^{ab}	70.5^{ab}	3.4±0.98^{a}	15.2±1.43^{a}
40	71.8^{ab}	68.3^{ab}	5.1±4.038^{ab}	15.2±1.84a^{b}
60	69.2^{b}	66.8^{b}	4.4±3.29^{b}	13.4±1.39^{b}
80	66.0^{bc}	64.2^{bc}	4.9±0.89^{ab}	14.6±1.38^{ab}
100	58.0^{cd}	56.9^{cd}	4.4±0.90^{b}	13.4±0.88^{b}
200	55.8^{d}	53.3^{de}	2.1±0.78^{c}	12.3±1.65^{c}
300	50.8^{e}	50.4^{e}	2.3±0.80^{c}	12.3±1.72^{c}
400	40.2^{f}	34.8^{f}	2.2±0.60^{c}	12.1±1.54^{c}
500	-	-	-	-
600	-	-	-	-

Values with different superscript within the same column are significantly different ($P<0.05$)

well as activation of auxin.

Next, higher doses of gamma radiation resulted in plant height and root length reduction because of inhibitory effects, especially on physiological and physical traits. Bhosale and More (2014), reported higher doses of gamma rays (40 Kr) has caused fluctuation in the seedling height and seedling injury. Another study also determined that seedling height was dependent on gamma radiation dose. Kostov *et al*. (2007) and Abdullah *et al*. (2018) who tested gamma radiation on tomato and ginger obtained similar findings. Plant height increment was calculated to assess the effect of gamma doses on the overall height of the plant after 70 days of planting (Figure 1). The highest percentage of height increment was obtained from plants exposed with 80 Gy followed by 40 Gy and 60 Gy with percentages of 220 and 207 respectively. Plant exposed with doses 500 Gy and 600 Gy were discarded since no plant survived after 14 days of planting.

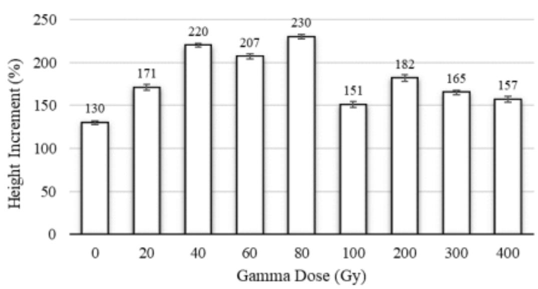

Figure 1. Plant height increment 70 days after planting (70DAP)

The results recorded variations in plant height due to exposure to higher doses of gamma rays. This indicates that increasing the amount of gamma-ray dose may incur damage in seedlings. Poor crop growth performance was denoted as a result of higher dosage of exposure towards the plant as the mutagenic agent can cause a blockage of cellular DNA which leads to the plant growth stopping or slowed down (Roslim *et al*., 2015; Gaswanto *et al*., 2016). A previous study reported by Asmahan and Nada (2006), Fahad (2009) and Hegazi and Hamideldin (2010) found the increase in gamma irradiation doses tends to increase certain morphological traits of plants such as plant height. In contrast, Jamil and Khan (2002) found that 5 and 10 Krad radiation had caused a slight reduction in plant height while other doses seemed to have no significant effect on plant height. Previous studies strengthened the findings of this experiment where mutagens may cause both positive and negative genetic variability in plant height.

3.3 Effects on flowering, branches, leaves and seed

Results on the number of days to flowering, the number of branches, leaves and seed count showed variations between treatments (Table 3). These findings indicate that the plants tested with gamma rays are often in a positive or negative way which leads to significant variance in each treatment. Plant exposed with 20 Gy showed no significant difference with control treatment on days to flower. Surprisingly, early flowering was observed in 40 Gy and 80 Gy as these irradiated plants started to flower in less than 48 days (as in control), which were between 45 - 46 days. Different flowering days may relate to the seed metabolism and initiation of DNA synthesis due to the consequences of gamma rays as stated by Shah *et al*. (2008). In the meantime, the higher amount of gamma irradiation contributed to an average decline in all variables analyzed. This may be partially due to the fact that cells which comparatively more chromosomal damage at extreme exposures of gamma radiation are at huge risk due to the diplontic segment which cannot integrate well with normal cells

Table 3. Effect of gamma doses on number day to flowering, number of branches, leaves and seed count

Dosage (Gy)	Number of days to flowering (day)	Number of branches	Number of leaves	Mean seed count per fruit
0	49.6±1.1[e]	7.4±0.9[b]	57.4±2.7[a]	33.8[de]
20	49.8±1.1[e]	7.4±0.9[b]	56±2.5[a]	34.0[de]
40	46.0±0.7[f]	9.2±1.3[b]	57.2±2.6[a]	35.8[cd]
60	45.6±0.9[f]	9.4±1.5[b]	52.8±2.8[ab]	37.2[bc]
80	45.2±0.9[f]	7.0±1.0[a]	53.6±2.0[ab]	41.2[a]
100	52.4±0.7[d]	6.6±0.7[b]	52.4±2.1[ab]	42.2[a]
200	54.6±0.7[c]	6.4±0.7[b]	49.8±2.9[b]	39.8[ab]
300	55.8±0.7[b]	6.2±0.6[b]	53.2±2.4[ab]	33.8[de]
400	58.0±0.7[a]	6.2±0.6[b]	54.6±2.0[ab]	31.4[e]
500	-	-	-	-
600	-	-	-	-

Values with different superscript within the same column are significantly different ($P<0.05$)

eventually causing a slight delay in flowering. Generally, the determined plant category experienced a flowering rate of 50 per cent faster than in the indeterminate category, which could also result in delays in the ripening process.

Observations on the number of leaves show that the plant irradiated with gamma doses 60 Gy, 800 Gy, 100 Gy, 300 Gy and 400 Gy had no significant difference between these treatments. The highest number of leaves was obtained in the control treatment (57.4±2.7) followed by 40 Gy (57.2±2.6) and 20 Gy (56±2.5). Although the height of the plant had increased, the number of leaves showed no significant difference from the control treatment (0 Gy). Moreover, physiologically impaired seedlings affected by gamma-ray irradiation are generally associated with mutation frequency.

The number of branches was also observed to have no significant influence on the number of leaves. The highest number of branches was observed on the treatment 60 Gy (9.4±1.5) which was higher than treatment 0 Gy and 20 Gy however the number of leaves was lower compared to these treatments. Past research concluded that mutagenesis in crop plants significantly influences the morphological and physiological parameters which in turn enhances yield, days to maturity and resistance to pests and diseases (Gopalakrishnan and Selvanarayanan, 2009; Tomlecova, 2010). In a previous study by Gnanamurthy *et al.* (2013), reduction in leaf numbers per plant was observed in all mutagenic treatments except for the control. Another factor found that would increase the number of leaves was wider row spacing and a higher number of shoots. Increasing the number of shoots will increase the number of leaves on the plants. Similar compensatory growth was recorded in sweet pepper cultivars (Vann *et al.*, 1986; Cebula, 1995).

The seed count of capsicum was different based on their variety and size. Chilli fruits were cut in half to determine the number of seeds in one pod. Based on Table 3, the highest number of seeds obtained was in treatment 100 Gy (42.2) and the lowest number of seeds was in treatment 400 Gy (31.4). According to a previous study by Daniel *et al.* (2014), among 5 varieties of capsicum investigated var annuum showed the lowest number of seeds which was 41.3 per fruit. The number of seeds per fruit may be due to internal structure and the size of chilli fruit. In contradiction with Zaki *et al.* (2013), this study found a huge difference in the number of chilli seeds and this may be due to the thick wall of capsicum and most of the fruit sizes used in this experiment were quite smaller as compared to the previous one.

3.4 Yield performance

In this study, the effects of gamma radiation on the yield performance were measured based on the number of fruits per plant, the weight of fruit and the length of fruit. From the results in Table 4, the highest number of fruits was observed for the treatment 80 Gy and 40 Gy (8/plant) which is higher than the control treatment. For higher gamma-ray doses, the number of fruit decreases. A similar trend was also obtained in the weight of fruit where the highest weight is observed in treatment 80 Gy (9.6 g) and the length of fruit can reach up to 12 cm as in doses of 60 Gy and 80 Gy.

Table 4. Effect of gamma radiation on the number of fruits, weight of fruit and length of fruit

Gamma Dosage (Gy)	Number of fruit	Fresh weight of fruit (g)	Length of fruit (cm)
0	7.2±1.06[ab]	9.6±1.5[a]	7.5±0.9[abc]
20	7.4±1.06[ab]	7.4±1.1[ab]	7.0±0.8[abc]
40	7.6±0.74[a]	8.8±1.8[a]	9.5±1.0[ab]
60	6.2±0.91[cd]	9.4±1.9[a]	11.6±0.9[a]
80	7.8±0.74[ab]	9.6±1.0[a]	11.0±1.1[a]
100	6.6±0.74[bcd]	7.4±1.4[ab]	7.5±1.2[abc]
200	7±0.74[abc]	6.36±1.9[b]	6.5±1.1[ab]
300	6.2±0.74[cd]	4.78±1.4[c]	5.0±1.1[ab]
400	4.6±0.74[e]	5.42±1.5[bc]	4.8±1.0[bc]

Values with different superscript within the same column are significantly different ($P<0.05$)

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FULL PAPER

Each treatment produces a different number of fruits per plant including the weight of fruits. The treatment using 60 Gy produced only six chilli fruits but the fruit weighs (9.4 g) was similar to that of the control which had produced seven fruits. It is shown that each treatment produced different sizes and weights of fruits. Cebula (1995) mentioned that the lesser number of shoots, the heavier the fruits produced. The weights obtained were similar to the fruits produced in the non-irradiated which was 9.6 ±1.51 g per fruit. While in other treatments, the weight obtained was in the range of 4.78 to 8.8 g per fruit. The fresh weight of mature fruit is an important characteristic in this study because heavier fruit correlates to higher yield. The high yield contributed by the weight is basically due to the size of fruits which comprises width, length and number of seeds. Similarly, Taleb (2012) also denoted that the high number of leaves per plant would result in high plant fresh and dry weight even though their treatment were affected by organic matter source treatments.

The results showed that all treatments were significantly different in fruit length. The variations in fruit length might be due to genetic alterations caused by gamma radiation. According to Daniel *et al.* (2014), the changes in fruit length might be a result of gene mutation or due to genetic constitution variation where there are three to ten pairs of genes with a heritability value of 40 to 50% in capsicum. However, agronomic and environmental conditions might also affect the fruit length. Hence, further investigation at the molecular level is crucial to confirm this observation. The effects in a large range of plants that are exposed to gamma rays have been described by many researchers (Kovacs and Keresztes, 2002; Kim *et al*., 2005; Wi *et al*., 2005). The effects that are commonly observed in the low or high doses of irradiated plants are enhancement or will be inhibition of germination (Kim *et al*., 2005; Wi *et al*., 2005).

4. Conclusion

Based on the results, gamma-ray affects the germination rate, days to germination, plant height, plant survival, fruit weight, fruit size, number of leaves and branches. Increasing doses of gamma irradiation above 300 Gy, caused severe damage on the shoot length, root length but no effect on the germination. The optimum dose determined for improving the efficiency of chilli Kulai is 300 Gy. This optimum mutagen dose determined could be useful in chilli varietal improvement programmes. Further analysis on the molecular is useful to confirm the genetic variation on the observed plant.

Conflict of interest

The authors declare no conflict of interest.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8