

## GAMMA IRRADIATION INDUCED CLASTOGENIC ABNORMALITIES IN *Vigna radiata*

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### ABSTRACT

Gamma irradiation is an effective and widely used method in the agricultural sector to alter the traits of plants especially for commercialization purposes and as a mitigation measure to ensure food security in the future. *Vigna radiata* (mung bean) is one of the most important legume crops in Asian countries that is not fully exploited. Therefore, this research aimed to determine the effects of gamma irradiation on the cytology and growth of mung beans. The mung bean seeds were exposed to different doses of gamma radiation; 0, 200, 400, 600, 800, and 1000 Gy. The increasing dose of gamma irradiation caused an insignificant decrease ( $p>0.05$ ) in the mitotic index of *V. radiata* meristematic cells. However, a significant increase at  $p<0.05$  in the percentage of clastogenic chromosomal aberration was observed in the meristematic cells of plants irradiated at 800 and 1000 Gy. The survival percentage, plant height, and root length were inversely proportional to the percentage of chromosomal aberration and clastogenic abnormalities when the irradiation dose exceeded the LD<sub>50</sub> (752.50 Gy). In conclusion, gamma rays greatly induced clastogenic abnormalities which have varying impacts on the cytology and growth of *V. radiata* plants.

**Key words:** Clastogenic, gamma-ray, mutagenesis, *Vigna radiata*

### INTRODUCTION

*Vigna radiata* L. Wilczek or mung bean is a warm-season legume crop from the Fabaceae family with a diploid chromosome number of 22 (Kang *et al.*, 2014). Originated from India, this species was assumed to be derived from *Vigna sublobata* which is a wild variation that exists throughout India and Myanmar before it was introduced to Asia, Africa, Austronesia, the West Indies, and the United States (Akpapunam, 1996; Heuzé *et al.*, 2015). Heuzé *et al.* (2015) stated that this plant grows well in the optimal temperature of 28 to 30°C, especially in tropical and subtropical areas. This drought-tolerant species which is vulnerable to waterlogging and soil salinity grows well on loam or sandy loam soil with a pH ranging from 5 to 8. This minor crop has a high nutritional value for human consumption as it is rich in protein, minerals, and vitamins. According to Pataczek *et al.* (2018), mung beans could provide 24% to 28% of dietary protein and 59% to 65% of carbohydrates based on a dry weight basis besides supplying about 3400 kJ of energy per kilogram of grain. Other than its nutritional properties, mung bean is also utilized as fodder, rotation crop, green manure, and intercropping (Mogotsi, 2006; Kang *et al.*, 2014; Pataczek *et al.*,

2018; Rozaliana *et al.*, 2019). According to Nair and Schreinemachers (2020), the worldwide annual production of mung beans exceeded seven million hectares, which is considered a significant level of production. Despite the introduction of mung beans to a vast area, the major production of this crop is centered in Asia whereby 90% of the production is from India, China, Pakistan, and Thailand (Pataczek *et al.*, 2018). Norkaspi *et al.* (2013) reported that Malaysia spent RM42.09 million on 11 782 tons of imported mung beans in 2011. This demand is expected to get bigger as the population increases. Mung bean is one of the underutilized legume crops that have the potential for commercial exploitation (Ebert, 2014). To maximize the production of this legume crop for commercialization purposes, the traits of the plants could be altered via induced mutagenesis.

Induced mutagenesis using gamma irradiation has been an important tool for plant mutation breeding programs. It is a common and widely used physical mutagen to induce mutagenesis in crops. To date, currently, there are 39 registered mutants of *V. radiata* in the Mutant Variety Database of the International Atomic Energy Agency (IAEA, 2022) with 28 of them being gamma irradiated varieties. Gamma rays are clastogen, meaning it is effective in producing DNA double-strand breaks, which are the precursor lesions for chromosomal aberration

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which will result in morphological, physiological, and biochemical changes in crops (Piri *et al.*, 2011; Cornforth *et al.*, 2021). The breakage of nuclear DNA in the plant after receiving mutagenic treatments will give rise to heritable mutations that occur randomly (Shamsiah *et al.*, 2018). Many studies have been done to determine the morphological and physiological effects of gamma radiation on *V. radiata* (Tah, 2006; Sengupta & Raychaudhuri, 2017; Dhole and Reddy, 2018). However, the impacts of mutagen on cytological aspects of plants are necessary to evaluate to elucidate the response of certain plant genotypes to a particular mutagen. According to Lagoda (2012), tissues or cells with high mitotic rates are more radiosensitive, thus they are more susceptible to the damaging effects of ionizing radiation. However, the study on the clastogenic effects of gamma irradiation on *V. radiata* mitotic cells is inadequate unlike another genus member, *Vigna unguiculata* or cowpea (Badr *et al.* (2014). Badr *et al.* (2014) reported the increase in mitotic activity and enhanced frequency of abnormal chromosomes in meristematic cells of cowpea as the gamma radiation dose increased. The improvement in growth and yield in cowpea plants grown from seeds exposed to 50 Gy and 100 Gy was also found to be associated with the increased mitotic activity. Therefore, the objective of this investigation is to study the effects of different doses of gamma radiation on *V. radiata* cell division, mitotic chromosome, and growth parameters which would be beneficial as a future reference for *V. radiata* crop development.

## MATERIALS AND METHODS

### Sample preparation

About 100 g of mung bean seeds were subjected to gamma radiation of different doses with Caesium-137 (<sup>137</sup>Cs) as the radiation source using the BioBeam GM 8000 facility at the Malaysian Nuclear Agency. The seeds were irradiated at doses of 0, 200, 400, 600, 800, and 1000 Gy. The seeds were placed in the BB13-5 sample beaker (height 29.2 cm × diameter 10.0 cm) and irradiated at a dose rate of 15.7 Gy/min. However, due to the movement restriction in response to the Covid-19 pandemic during the Malaysian Movement Control Order, the macroscopic and microscopic observations were done using aged seeds about 14 months after the seed irradiation.

### Macroscopic observation

The mung bean seeds from each dose of the group were allowed to germinate in plastic basins using germination cubes under a shaded area. Data on germination (%), survival (%), seedling height, and root length (cm) were collected. The number of germinated seeds was recorded daily. After seven days, the measurement of plant height and root

length for each plant was taken. According to Prasath *et al.* (2019), the survival of a plant depends on the emergence of true leaves above the embryonic leaves, therefore the observation of plant survival was done in the second week after planting.

### Microscopic observation

About 2 cm long of root tips from each group were collected and immersed in freshly prepared Carnoy's solution (3:1 v/v, ethanol: glacial acetic acid) for 24 hr. Then, the root tips were hydrolyzed in preheated 1N HCl (60°C) for 10 min. Next, about 2 mm of the root tips were macerated with a dissecting needle on a glass slide and allowed to stain in 2% aceto-orcein for 5 min at room temperature. The microscopic preparations were performed by squash technique. For this purpose, the root tips were squashed down with strong vertical pressure, using the thumb after covering it with a coverslip and paper towel. The pressure was applied to squash the root tip into a single cell layer. Finally, the prepared slide was observed under the light microscope to determine the mitotic index (Equation 1) and percentage of chromosomal aberration (Equation 2).

Equation 1:

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Number of total cells}} \times 100$$

Equation 2:

$$\% \text{ Chromosomal aberration} = \frac{\text{Number of aberrant cells}}{\text{Number of total cells}} \times 100$$

### Statistical analysis

Polynomial regression was used to calculate the median lethal dose (LD<sub>50</sub>) of the gamma irradiation based on the *V. radiata* plant height. The one-way analysis of variance (ANOVA) with the Tukey posthoc test was used for the determination of significant differences at  $p < 0.05$  using SPSS version 25.

## RESULTS

### Germination, survival, plant height, and root length

Table 1 shows that the germination percentage was not dependent on the dose of gamma radiation with germination percentages ranging from 90 to 100%. Irradiated seeds had a lower germination percentage compared to non-irradiated seeds except for those irradiated at 200 Gy and 800 Gy. Plants irradiated at these two doses recorded 100% germination, higher than the germination percentage of non-irradiated seeds at 96.67%.

Table 1 shows the declining survival percentage of *V. radiata* plants as the dose of gamma irradiation increased, except for plants irradiated

at 400 Gy. The non-irradiated plants recorded 95% survival, while the lowest survival percentage at 35% was observed in plants irradiated at 1000 Gy. The plants irradiated at a dose of 400 Gy had a maximum survival percentage of 100%.

The plant height of *V. radiata* seedlings after seven days decreased as the dose of gamma radiation increased, as shown in Table 1. Non-irradiated seedlings recorded the significantly highest average plant height at 17.13 cm compared to the shortest average plant height in plants irradiated at 1000 Gy (2.92 cm). The gamma dose at 600 Gy and higher were significantly able to inhibit the growth of *V. radiata* plants.

The root length of *V. radiata* seedlings after seven days also displayed a similar trend as the plant height. According to Table 1, the root length decreased as the dose of gamma radiation increased, except for the plants irradiated at 200 Gy. The average root length of plants irradiated at 200 Gy was 6.67 cm, which was slightly longer than the average root length of control plants at 5.78 cm. However, the values were not significantly different ( $p>0.05$ ). Significant reduction ( $p<0.05$ ) in root length as compared to control was observed in plants irradiated at 800 Gy and 1000 Gy.

#### Mitotic index and chromosomal aberration

According to Table 2, there were no significant differences between the mitotic index (MI) of meristematic cells of control plants and irradiated plants. A mitodepressive effect was noted with the increment of the dose of gamma irradiation except plants irradiated at 400 Gy. The plants irradiated at this dose had a greater MI than plants irradiated at 200 Gy although the difference was not significant ( $p>0.05$ ). The non-irradiated plants recorded 100% MI while the MI of other groups of irradiated plants ranged from 78.46% (1000 Gy) to 92.63% (400 Gy). There was also no significant difference ( $p>0.05$ ) in the frequency of the mitotic phases in each group. Among the four mitotic phases, most cells observed were undergoing prophase (55.89% to 83.77%) while

the least observed mitotic phase was the telophase (0% to 2.32%).

Based on Table 3, the chromosomal aberration percentage was not dose-dependent. The average percentage of chromosomal abnormalities in the meristematic cells of *V. radiata* ranged from 41.48% (0 Gy) to 94.02% (800 Gy). The non-irradiated plants recorded 41.48% of chromosomal abnormalities while the plants irradiated at dose 200, 400, 600, 800 and 1000 Gy recorded 68.85%, 75.25%, 64.40%, 94.02%, and 85.71% of chromosomal abnormalities respectively. There was a significant increase at  $p<0.05$  in the abnormality percentage observed in the plants irradiated at 400, 800, and 1000 Gy as compared to the control plants. Although the plants irradiated at 400 Gy had the highest MI at 92.63%, this significant increase in the percentage of chromosomal abnormalities in the meristematic cells might have affected the ability of the plants to survive even after the emergence of true leaves. The result showed 41.48% of chromosomal aberrations for non-irradiated seeds, whereas the percentage of chromosomal aberrations for irradiated seeds varied and was not dose-dependent.

The chromosomal aberrations in the meristematic cells of *V. radiata* were further classified into clastogenic and non-clastogenic aberrations (Table 3) based on Renjana, Anjana, and Thoppil (2013) and, Neelamkavil and Thoppil (2018). There were six types of clastogenic chromosomal aberrations observed (Table 4), which were the chromosome bridge (B), chromosome fragmentation (F), sticky chromosome (S), ghost cells (GC), nuclear lesion (NL), and pulverized scattered metaphase (PSM). More types of non-clastogenic chromosomal aberrations were observed in the meristematic cells of *V. radiata* (Table 5); namely C-mitosis (CM), lagging chromosome (L), multipolarity (MP), disturbed prophase (DP), disturbed metaphase (DM), disturbed anaphase (DA), cytoplasmic shrinkage (CS), chromosome loss (CL), and binucleated cells (BC).

Generally, the most frequently observed type of

**Table 1.** The Macroscopic Observations of Gamma Irradiated *Vigna radiata*

Dose (Gy)	Macroscopic Parameters			
	Total Germination (%)	Total Survival (%)	Plant Height (cm)	Root Length (cm)
0	96.67	95.0	17.13±1.48 <sup>a</sup>	5.77±0.53 <sup>a</sup>
200	100.0	85.0	17.10±1.27 <sup>a</sup>	6.67±0.49 <sup>ab</sup>
400	91.67	100.0	15.35±1.77 <sup>a</sup>	5.44±0.55 <sup>ab</sup>
600	90.0	70.0	9.95±1.31 <sup>b</sup>	5.23±0.54 <sup>abc</sup>
800	100.0	65.0	6.68±0.92 <sup>bc</sup>	3.98±0.39 <sup>bc</sup>
1000	95.0	35.0	2.92±0.41 <sup>c</sup>	3.51±0.39 <sup>c</sup>

Note: Means±SE followed by the same letter are not significantly different at  $p<0.05$ .

chromosomal aberrations was the clastogenic type. The frequency of clastogenic aberrations in plants irradiated at 800 and 1000 Gy was significantly different ( $p < 0.05$ ) from the control plants. While less than 50% of clastogenic aberrations were noted in control plants, 91.32% and 76.33% of clastogenic aberrations were noted in 800 Gy and 1000 Gy respectively. Nevertheless, no significant differences ( $p > 0.05$ ) in the frequency of non-clastogenic chromosomal aberrations were observed in all groups of plants.

According to Table 4, the nuclear lesion was the most frequently observed for all groups of plants while pulverized scattered metaphase was the least frequently observed. All six types of clastogenic chromosomal aberrations showed no significant differences in the frequency of aberrations across all groups. Similarly, the difference in the frequency of non-clastogenic aberrations across all groups also was not significant ( $p > 0.05$ ), as seen in Table 5.

#### Median lethal dose ( $LD_{50}$ )

The median lethal dose ( $LD_{50}$ ) of gamma irradiation was determined based on the height of *V. radiata* seedlings. Based on the polynomial regression

( $y = 3E-06x^2 - 0.0151x + 18.232$ ;  $R^2 = 0.9259$ ), the lethal dose that resulted in a 50% reduction in plant height is at 752.50 Gy.

#### DISCUSSION

Despite other previous studies using germination percentage as the indicator of the effectiveness of gamma rays, the values of germination percentage obtained in this study were not statistically significant as compared to the control. A similar result was reported by Borzouei *et al.* (2010) whereby gamma radiation did not cause any statistically significant effect on the final germination percentage of wheat (*Triticum aestivum* L.). Even though the effect of the gamma irradiation on the germination percentage in the current study was not dose-dependent, 800 Gy gamma dose significantly reduced the survival percentage, plant height, and root length of *V. radiata*. These results are inversely proportional to the percentage of chromosomal abnormalities, where at 800 Gy the percentage of chromosomal abnormalities is significantly increased. The irradiation at 800 Gy also significantly increased the clastogenic abnormalities in *V. radiata* with 91.32% as compared

**Table 2.** The Mitotic Index (MI) and Frequency of Mitotic Phases of Gamma Irradiated *Vigna radiata*

Dose (Gy)	Mitotic phase				Mitotic Index (%)
	Prophase	Metaphase	Anaphase	Telophase	Mean $\pm$ SE
0	83.77 $\pm$ 6.34 <sup>a</sup>	5.61 $\pm$ 3.12 <sup>a</sup>	10.61 $\pm$ 6.93 <sup>a</sup>	0.0 <sup>a</sup>	100.00 $\pm$ 0.0 <sup>a</sup>
200	55.89 $\pm$ 8.70 <sup>a</sup>	19.37 $\pm$ 6.45 <sup>a</sup>	22.42 $\pm$ 5.88 <sup>a</sup>	2.32 $\pm$ 1.43 <sup>a</sup>	88.70 $\pm$ 4.78 <sup>a</sup>
400	62.54 $\pm$ 8.62 <sup>a</sup>	7.66 $\pm$ 2.93 <sup>a</sup>	28.42 $\pm$ 9.06 <sup>a</sup>	1.39 $\pm$ 1.02 <sup>a</sup>	92.63 $\pm$ 5.02 <sup>a</sup>
600	81.09 $\pm$ 2.98 <sup>a</sup>	5.05 $\pm$ 1.92 <sup>a</sup>	12.81 $\pm$ 3.14 <sup>a</sup>	1.05 $\pm$ 0.71 <sup>a</sup>	80.54 $\pm$ 7.01 <sup>a</sup>
800	68.35 $\pm$ 9.18 <sup>a</sup>	14.26 $\pm$ 5.88 <sup>a</sup>	16.86 $\pm$ 6.92 <sup>a</sup>	0.53 $\pm$ 0.53 <sup>a</sup>	79.03 $\pm$ 6.65 <sup>a</sup>
1000	63.59 $\pm$ 9.60 <sup>a</sup>	23.22 $\pm$ 6.16 <sup>a</sup>	13.19 $\pm$ 8.42 <sup>a</sup>	0.0 <sup>a</sup>	78.46 $\pm$ 9.52 <sup>a</sup>

Note: Means $\pm$ SE followed by the same letter are not significantly different at  $p < 0.05$ .

**Table 3.** The Frequency of Clastogenic and Non-Clastogenic Chromosomal Aberrations Observed in Gamma Irradiated *Vigna radiata*

Dose (Gy)	Chromosomal aberrations (%)		Abnormality Percentage (%)
	Clastogenic	Non-clastogenic	Mean $\pm$ SE
0	41.48 $\pm$ 13.54 <sup>a</sup>	0.0 <sup>a</sup>	41.48 $\pm$ 13.54 <sup>a</sup>
200	59.02 $\pm$ 6.91 <sup>ab</sup>	9.83 $\pm$ 5.20 <sup>a</sup>	68.85 $\pm$ 6.60 <sup>ab</sup>
400	65.12 $\pm$ 6.54 <sup>abc</sup>	10.14 $\pm$ 3.70 <sup>a</sup>	75.25 $\pm$ 7.35 <sup>b</sup>
600	61.86 $\pm$ 8.07 <sup>abc</sup>	2.54 $\pm$ 1.52 <sup>a</sup>	64.40 $\pm$ 8.73 <sup>ab</sup>
800	91.32 $\pm$ 1.89 <sup>c</sup>	2.70 $\pm$ 1.39 <sup>a</sup>	94.02 $\pm$ 1.77 <sup>b</sup>
1000	76.33 $\pm$ 7.64 <sup>bc</sup>	9.38 $\pm$ 4.59 <sup>a</sup>	85.71 $\pm$ 4.18 <sup>b</sup>

Note: Means $\pm$ SE followed by the same letter are not significantly different at  $p < 0.05$ .

**Table 4.** The Frequency of Different Types of Clastogenic Chromosomal Aberrations

Dose (Gy)	Clastogenic abnormalities					
	B	F	S	GC	NL	PSM
0	0.0 <sup>a</sup>	0.0 <sup>a</sup>	7.14±7.14 <sup>a</sup>	0.0 <sup>a</sup>	34.34±14.63 <sup>a</sup>	0.0 <sup>a</sup>
200	2.12±1.41 <sup>a</sup>	5.00±5.00 <sup>a</sup>	18.66±5.78 <sup>a</sup>	11.30±4.78 <sup>a</sup>	22.81±6.86 <sup>a</sup>	0.0 <sup>a</sup>
400	13.85±7.54 <sup>a</sup>	10.33±7.69 <sup>a</sup>	16.31±8.36 <sup>a</sup>	7.37±5.02 <sup>a</sup>	31.99±8.83 <sup>a</sup>	0.0 <sup>a</sup>
600	2.58±1.34 <sup>a</sup>	1.73±1.33 <sup>a</sup>	6.01±1.53 <sup>a</sup>	19.46±7.01 <sup>a</sup>	33.43±6.03 <sup>a</sup>	0.0 <sup>a</sup>
800	2.02±1.50 <sup>a</sup>	0.0 <sup>a</sup>	17.40±6.11 <sup>a</sup>	19.32±6.86 <sup>a</sup>	53.41±8.26 <sup>a</sup>	0.0 <sup>a</sup>
1000	1.19±1.19 <sup>a</sup>	0.0 <sup>a</sup>	14.67±5.76 <sup>a</sup>	21.54±9.52 <sup>a</sup>	36.98±9.18 <sup>a</sup>	2.78±2.78 <sup>a</sup>

Note: Means±SE followed by the same letter are not significantly different at  $p<0.05$ .

**Table 5.** The Frequency of Different Types of Non-Clastogenic Chromosomal Aberrations

Dose (Gy)	Non-clastogenic abnormalities								
	CM	L	MP	DP	DM	DA	CS	CL	BC
0	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>
200	5.83±5.03 <sup>a</sup>	1.67±1.67 <sup>a</sup>	0.0 <sup>a</sup>	1.54±1.27 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.78±0.59 <sup>a</sup>
400	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.45±0.45 <sup>a</sup>	1.54±1.54 <sup>a</sup>	4.84±2.68 <sup>a</sup>	3.85±3.85 <sup>a</sup>	0.0 <sup>a</sup>	2.33±1.39 <sup>a</sup>	0.0 <sup>a</sup>
600	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	1.87±1.52 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.91±0.91 <sup>a</sup>	0.22±0.22 <sup>a</sup>
800	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.28±0.28 <sup>a</sup>	0.0 <sup>a</sup>	0.65±0.65 <sup>a</sup>	1.10±0.75 <sup>a</sup>	0.0 <sup>a</sup>	0.81±0.59 <sup>a</sup>
1000	0.64±0.64 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	5.83±4.34 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	3.74±2.15 <sup>a</sup>

Note: Means±SE followed by the same letter are not significantly different at  $p<0.05$ .

to the control with 41.48%. Therefore, the clastogenic effects of gamma irradiation affect the survival, height, and root length of the plants. According to Gowthami *et al.* (2017), the chromosomal aberrations, disturbances at the physiological or cellular level, or the combined effect of both damages could be the reasons for the reduced survival percentage caused by mutagens at higher doses. Meanwhile, low doses of gamma irradiation stimulate the germination process and vegetative growth of plants by activating protein synthesis and enhancing enzymatic activation which then enhances the rate of cell division (Jaipo *et al.*, 2019).

The effects of gamma irradiation on plants could also be influenced by hormesis. Hormesis is also known as the stimulatory effect of low doses of radiation on different biological processes such as cell division, growth, and development (Jan *et al.*, 2011; Nielen *et al.*, 2018). Gamma irradiation at low doses alters the hormonal signaling network in plant cells or improves the cells' antioxidative capacity to stimulate the plant's growth and overcome the daily stress factors (Minisi *et al.*, 2013). According to Nielen *et al.* (2018), the hormetic effects usually occur for a short moment, are insignificant, and do not significantly increase the plant yield. In the current study, the hormetic effects of the lower dose gamma irradiation could be observed in the root length and

survival percentage of *V. radiata* plants. The findings on these parameters showed a nonsignificant increase in the groups of plants irradiated at 200 Gy or 400 Gy as compared to the control plants. A similar finding was reported by Asare *et al.* (2017), whereby a low gamma dose showed a stimulatory effect on several growth parameters and plant yield.

The radiosensitivity of plants also played a huge role in determining the effects of gamma irradiation. The sensitivity of plants to mutagens differs depending on their species and genotypes (Quintana *et al.*, 2019). Based on the radiosensitivity test in this study, the LD<sub>50</sub> was determined at 752.50 Gy, in the sensitivity effect can be seen at 800 Gy with a significant reduction of survival percentage, plant height, and root length, and increment in chromosomal abnormalities and clastogenic percentages. Thus, these results are in line with a statement by Kozgar *et al.* (2014), who stated that the cytological analysis can be considered one of the most reliable indices to estimate the sensitivity of plants for different mutagens. Observation of mitotic cells is much easier compared to meiotic as it requires sampling from roots which are available all year round, unlike flowers. Mitotic cells can easily be obtained from the roots of germinating seed which provide an early indicator of plant sensitivity towards the mutagen. The presence of chromosomal aberration in mitotic cells can be used to relate it to

the possibility of a similar occurrence in meiotic cells as proved by Siahpoosh *et al.* (2020), where similar types of chromosomal aberrations can be observed in mitotic and meiotic cells of gamma irradiated *Vicia faba*.

According to Gowthami *et al.* (2017), growth reduction in plants may be attributed to the damage that occurred during cell division. Furthermore, reduced growth caused by gamma radiation also resulted in auxin reduction, disturbed ascorbic acid content, as well as disturbances in the plant's physiology and biochemical contents. In a previous study on gamma irradiated *Vicia faba* by Siahpoosh *et al.* (2020), there was a negative correlation between the number of pods per plant and mitosis anomalies, as a higher percentage of mitosis anomalies greatly reduces the number of pods which will affect the seed yield per plant.

Clastogenic activities such as sticky chromosomes are caused by either increased contraction or condensation of chromosomes or DNA depolymerization and partial dissolution of nucleoproteins which is irreversible and might lead to apoptosis (Sabeen *et al.*, 2020). Chromosome fragmentation reflects probable activation of the endogenous nuclease generating small inter-nucleosomal fragments. Extensive genome rearrangements can be caused by cycles of breakage-fusion-bridging of plant chromosomes. Chromosome bridging that can persist beyond the completion of mitosis into early G1 may cause the formation of micronuclei or abnormally shaped nuclei in daughter cells, or the formation of binucleated cells or polyploid cells (Pampalona *et al.*, 2016). Meanwhile, the failure of the spindle apparatus organization and its normal function is the cause of c-mitosis and vagrant chromosomes. The presence of laggards can be regarded as a contributing factor in promoting pollen sterility in plants (Patil, 1992). The laggard chromosomes and their presence as univalent and unequal separation may result in the production of aneuploid gametes which may be utilized in breeding programs.

The possible presence of reactive oxygen species (ROSs) might cause the higher occurrence of nuclear lesion compared to other types of clastogenic abnormalities. Active oxygen (O) forms emerging under oxidative stress are known to affect the cytoskeleton structure (Egorova *et al.*, 2001). ROSs have toxic properties and cause oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation) or oxidative deactivation of specific enzymes and may also cause damage to DNA or RNA (Sharma *et al.*, 2012). In addition, ROSs may trigger the transcription of specific genes as a response to the stress caused by the treatment. NADPH oxidase has been proposed to play a key role in the production and accumulation of

ROS in plants under stress conditions (Sharma *et al.*, 2012).

The long post-irradiation storage time may deteriorate the quality of *V. radiata* seeds which are associated with reduced food reserve, enhanced enzyme activity, increased fat acidity, and membrane permeability, which then reduced seed germination (Gebeyehu, 2020). Post-irradiation storage of onion (*Allium cepa*) seeds for 24 hr significantly increased the percentage of abnormal seedlings and reduced root and seedling lengths but not the germination activity (Amjad & Anjum, 2003). This was due to the prolonged indirect activity of the radiation, whereby there was a continuous production of free radicals during the storage time. According to Waterworth *et al.* (2019), during the storage period, there was a progressive accumulation of DNA damages that occurs in the embryo, for instance, breakage of DNA single and double strands, base modification, increased base loss, and generation of abasic sites. These prolonged seed damages may influence and increase the occurrence of chromosomal aberration in mitotic cells.

## CONCLUSION

Gamma irradiation induced an inverse relationship between cytological changes and certain growth parameters of *Vigna radiata* as shown in the survival percentage, plant height, and root length. Gamma irradiation was proved to induce more clastogenic abnormalities in *V. radiata* meristem cells as noted in treated groups. Clastogenic abnormalities may lead to stable structural modifications that are transmissible, due to the interaction of reactive free radicals with DNA, which consequently change the structure and function of proteins, and amend the plant's phenotypes.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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