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Effects of gamma irradiation on the growth and yield of *Vigna mungo* (L.)

Rubasinghe RHN, Sutharsan S*, Manokaran T
Department of Crop Science, Faculty of Agriculture, Eastern University, Sri Lanka

Abstract
Agriculture plays an important role in poverty eradication and food security throughout the world. In this regard, this study investigated the effects of gamma irradiation on growth and yield of black gram (*Vigna mungo).* This experiment was conducted at the University farm, Eastern University of Sri Lanka as a field experiment during August 2023 to November 2023. *Vigna mungo* seeds were exposed to Gamma irradiation by using "Gamma chamber 1200 Cobalt- 60" research irradiator. The seeds were subjected to gamma irradiation doses of 20Gy, 40Gy, 60Gy, 80Gy, 100Gy with a control (0Gy). Treated seeds were planted in poly bags with rooting media, thereafter seedlings were transplanted in open field. The experiment was laid out in Randomized Complete Block Design with four replications.

The study revealed that the treatments showed significant difference in treatment T3 (40Gy) in comparison to treatment-control (0Gy) with increased plant height (53.76%), number of leaves (65.44%), Leaflet width (1.96%), leaf area (56.22%), chlorophyl content (11.3%), shoot fresh weight (71.36%), root fresh weight (49.46%), total root length (15.71%), root volume (35.41%), nodulation (112.57%), and yield characteristics as pod diameter (5.09%), total pod fresh weight (149.13%), number of pods per plant (81.55%), 100 seed weight (2.65%), and total yield (166.51%). Further, all mutational characteristics (hairless pods bearing plants, trailer type plant habit and 5-6 pods per cluster) were observed in T3 (40Gy) and it showed highest values in mutation frequency by increased percentages compared to the T6 (100Gy). Therefore, according to this present study, there were significant ($P<0.05$) differences among the treatments on the above-mentioned characteristics and the treatment T3 (40Gy) is more suitable to create desirable characteristic in *Vigna mungo* especially in MI-01 variety.

Keywords: Agriculture, Black gram (*Vigna mungo),* gamma irradiation, gamma rays, mutation breeding, yield

Introduction

Climate change has long-term effects on agriculture and food security, according to the FAO (2016). Population growth and dietary changes will result in a 60% rise in global food demand by 2050 compared to 2006 levels, while climate change will continue to have an impact on food systems globally (Gunaratne *et al.*, 2021) [11]. Induced mutations have played a key role in the development of desirable mutants that have been released for cultivation as new crop varieties in several countries across the world (Penna *et al*., 2023) [20].

One of the best methods for improving traits without changing the crops' well-optimized gene pool is induced mutagenesis (Chaudhary *et al*., 2019) [6]. Maximum variations have developed as a result of radiation. The Mutant Variety Database encounters 3222 entries, 2456 seed propagated plants, and 367 vegetatively propagated plants. The top six crops are rice, barley, chrysanthemum, wheat, soybeans, and maize (Yali & Mitiku, 2022) [31].

Gamma rays which are physical mutagens, are high-penetration, short-wavelength electromagnetic irradiations that are produced when specific elements undergo radioactive disintegration. Depending on the irradiance, it has significant influence on plant growth and development by altering morphological, physiological, metabolic, genetic, and cytological alterations in cells and tissues (Kiani *et al*., 2022) [14].

Grain legumes are a precious gift from nature to humans and are frequently referred to as "poor man's meat" due to their high protein content (16–50%), vital components, dietary fiber (10– 23%), and vitamin content. In addition to protein, grain legumes are a rich source of other nutrients, including mono- and poly-unsaturated fatty acids, sugars, carbs, vitamins, and more than 15 vital minerals (Dutta *et al.*, 2022) [7]. Black gram (*Vigna mungo* L.) is Asia's most traditional and well-known primary pulse crop. The majority of it came from India, with central Asia serving as its secondary origin and extending all the way to Myanmar.Mostly it is cultivated across Asia and Africa. India, Bangladesh, Pakistan, Burma, and Ceylon are among the countries that grow it (Ravi *et al*., 2022) [22].

According to the data of Department of Census and Statistics Sri Lanka (2023), the black gram production is drastically reduced in 2018 than the other years. Therefore, induced mutagenesis can play a major role in black gram to gain desirable mutants for important morphological and quantitative traits and to increase the yield (Tamilzharasi *et al*., 2022) [26]. There is many evidence that there is a wide gap in alteration of bio- chemical, free radicle formation and anti-oxidant activity of black gram, and the gap is very high in Black gram in case of gamma irradiation (Yasmin & Arulbalachandran, 2022) [32, 33]. Even though, there are many research were conducted to evaluate the effect of gamma irradiation on different crops, there are very lack of studies on the effect of gamma irradiation on Black gram especially for MI 01 variety. High yielding and drought tolerant black gram variety with the use of gamma irradiation will be use full to ensure the food security.

Therefore, this study was undertaken with the following objective: To evaluate the effect of gamma irradiation on the growth and yield of *Vigna mungo*, Variety MI 01.

Methodology

The field experiment was carried out in the Crop farm, Faculty of Agriculture, Eastern University, Sri Lanka, Palachcholai during the period of August to November

2023. The study location receives the annual mean rainfall varies from 1400mm to 1680mm and the annual mean temperature ranges from 27.5°C − 31.7°C. 76% is the annual humidity in this area. The major soil type in the area is Sandy Regosol (Department of Meteorology, 2023).

Black gram seeds of MI-01 variety was selected for this experiment and selected seeds were irradiated with different doses of gamma radiation ranging from 0Gy to 100Gy according to the different treatments such as T1 (0Gy), T2 (20Gy), T3 (40Gy), T4 (60Gy), T5 (80Gy) and T6 (100Gy). Gamma irradiation was done by using the "Gamma chamber 1200 Cobalt 60" research irradiator at Horticultural Crops Research and Development Institute, Gannoruva, Sri Lanka. Irradiated seeds were sown in labeled poly bags filled with rooting media (top soil: cow dung in the ratio of 1:1) after soaking 8 hours. Treated seeds were sown in the depth of 2-3cm and poly bags were kept under the shade house (40%) to reduce the irradiation treatment shock. $0.75m^2$ size of 24 beds were prepared in the field by using mammoty and meter scale according to the different treatments and replications. 16 planting holes were made in each bed with the spacing of 30cm×10cm. Thereafter, seedlings were transplanted to main field from the shade house 2 weeks after sowing. Those were transplanted to field without disturbing the root system. This field experiment was laid out in Randomized Complete Block Design with 6 treatments and 4 replications. Management practices such as irrigation, fertilizer application, weed management and pest and disease management were done according to the recommendation of Department of Agriculture. Growth characteristics such as plant height (cm), leaf area (cm^2), fresh weight of shoots (g) and yield characteristic as number of pods per plant, 100 seed weight (g) and total yield (kg/ha) were collected. Furthermore, mutational characteristics such as hairless pods bearing plants, trailer type plant habit, 5-6 pods per cluster bearing plants were observed. Collected data were statistically analyzed by using Minitab (version 17) software and mean comparison within treatments was performed by Tukey's Test at 5% significant level.

Results and discussion

Plant height

Figure 1 Shows effects of Gamma irradiation on plant height of *Vigna mungo* at 4WAP,6WAP, 8WAP and 10WAP. It was found that there were significant differences between the applied gamma radiation doses on plant height during 4WAP to 10WAP at 2 weeks' interval. The highest plant height value was observed in the treatment T3 (40Gy) which were recorded as 14.12%, 47.18%, 53.76% and 66.36% during 4WAP to 10WAP compared to control T1 (0Gy). It is obvious that with the increasing doses of gamma irradiation, the plant height values were getting reduced.

Besides genomic modifications, radiation exposure can also affect to the changes in cell cycle patterns, hormonal balance, metabolic pathways and enzymatic alterations (Majeed, 2018) [16]. According to the study on Arabidopsis by Piri *et al*., (2011) [21], gamma radiation can impede plant growth because plants have a signal transduction mechanism that tracks cellular damage. This chanism prevents cell division whenever there is harm to the cell's structure.hese obtained results in the experiment, are in agreement with Goyal & Khan, (2020) [10] who reported that increased doses of gamma radiation deteriorate the plant height significantly and reduced mitotic activities in meristematic tissues and the minimum moisture content are obviously responsible for the reduction in plant height in higher doses of gamma radiation (Goyal & Khan, 2020) [10]. Furthermore, the results are proved with the findings of Abd El-Rahman *et al*., (2016) [1].

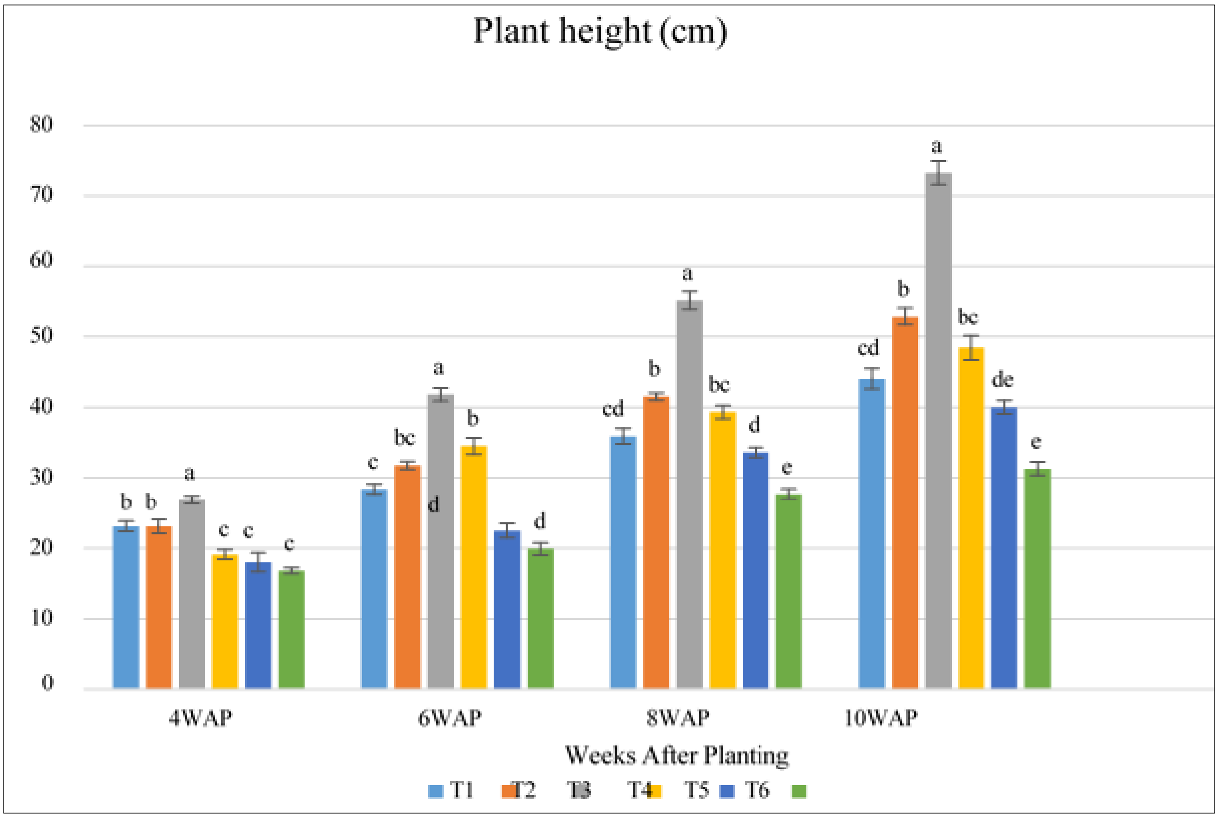

Fig 1: Effects of Gamma irradiation on plant height of *Vigna mungo* at 4WAP,6WAP, 8WAP and 10WAP.

Leaf area

Table 1 shows effects of different doses of gamma radiation on leaf area of *Vigna mungo*.

It was revealed that, 56.22% of increased leaf area was recorded in the treatment T3 (40Gy) compared with the control (T1). Furthermore, it is obvious that the leaf area was reduced by 38.58% in the treatment T6 (100Gy). This might be caused due to the reduction of values in the leaflet width, leaflet length and number of leaves with the increasing of gamma radiation doses.

Alterations in these plant growth parameters, which were raised from seeds exposed to different low dosages of gamma radiation prior to sowing, might be due to the concomitant induced changes in the endogenous phytohormones in plants, such as IAA, GA3, and BA, which could be stimulated by the low dosages of gamma radiation (Mohammad, 2011) [17].

The results obtained from this experiment, are in the same direction with the results obtained with green bean by Soliman, (2003) [25] and with the study by Yadav, (1997) [30], who was stated that lower doses of gamma radiation are favorable for leaf area and higher doses are reduced.

Table 1: Effects of different doses of gamma radiation on leaf area of *Vigna mungo*

Treatments	Leaf area (cm2)
T1 (0Gy)	2257.1 ± 92.1^{b}
T2 (20Gy)	2489.9 ± 68.5^{b}
T3 (40Gy)	3526.1± 86.7^{a}
T4 (60Gy)	2298.7 ± 68.7^{b}
T5 (80Gy)	1803.8± 85.0^{c}
T6 (100Gy)	1386.3 ± 54.8^{d}
F- Test	*

Shoot fresh weight

Effects of different doses of gamma radiation on shoot fresh weight of *Vigna mungo* is shown in table 2. The results showed that different doses of gamma radiation significantly (P<0.05) affected the shoot fresh weight of *Vigna mungo*.

The highest value for shoot fresh weight was observed in the treatment T3 (40Gy) which was 76.55% of increased value compared to control (T1). Significantly, T6 (100Gy) resulted the lowest value in shoot fresh weight and it was 22.73% in reduced shoot fresh weight compared to control.

Higher doses of gamma radiation might reduce the shoot fresh weight since they produced minimum number of leaves, less leaf size and minimum plant height. These results could have been caused by the biological activities of plants being negatively impacted by increasing the mutagen dose. Low doses of gamma-radiation may increase the enzymatic activation and awakening of the young embryo, which results in stimulating the rate of cell division and affects for the vegetative growth. (Chandrakumar & Ragini, 2008) [5]. Majeed, (2018) [16], reported that reduction in fresh weights of shoot might be due to reduced plant stature or reduced moisture content due to radiation stress at higher doses of gamma radiation. These results are in harmony by the study with the results obtained from Faba bean by EI-Gazzar *et al*., (2016) [8] who stated that the maximum shoot fresh weight was recorded in 40Gy in Faba bean and by Yasmin & Arulbalachandran, (2022) [32, 33] on *Lepidium sativum*.

Table 2: Effects of different doses of gamma radiation on shoot fresh weight of *Vigna mungo*

Treatments	Shoot fresh weight (g)
T1 (0Gy)	53.36 ± 1.94^{cd}
T2 (20Gy)	76.02 ± 0.99^{b}
T3 (40Gy)	94.21± 1.93^{a}
T4 (60Gy)	61.30± 1.02^{c}
T5 (80Gy)	51.06± 1.92^{d}
T6 (100Gy)	41.23± 1.84^{e}
F- test	*

Number of pods per plant

Effects of different doses of gamma radiation on number of pods per plant of *Vigna mungo* was shown in Table 3. It was shown that highest value for number of pods per plant was recorded in the treatment T3 (40Gy) which was 81.55% compared to control. The gamma irradiation induced changes in the source–sink relationship, which ultimately determines the yield. Low dose gamma radiation in inducing favorable morphological and physiological changes with an intent of improving yield and quality of grains, (Singh & Datta, 2009) [24]. And it was revealed that, the lowest value for the number of pods per plant was observed in the treatment T6 (100Gy) and it was 54.61% compared to control. This result is in agreement with the finding that, the radiation can affect the reproductive organs of the plant, hindering their ability to produce seeds, flowers, or fruits such as pods. Reduction in the number of pods in black gram could be attributed to the direct damage caused by the higher doses of gamma radiation, affecting the plant's ability to develop and sustain healthy pods for seed production (Hegazi & Hamideldin, 2009) [12].

And, the findings of Kafi & Borzouei, (2010) [12] which was that increasing doses of gamma radiation reduces the number of pods per plant in cereals and legumes are broadly supported to the current results.

Table 3: Effects of different doses of gamma radiation on Number of pods per plant of *Vigna mungo*

Treatments	Number of pods per plant
T1 (0Gy)	68.3 ± 2.32^{c}
T2 (20Gy)	101.0 ±4.02^{b}
T3 (40Gy)	124.0 ± 5.60^{a}
T4 (60Gy)	53.5 ± 1.55^{cd}
T5 (80Gy)	48.0 ± 1.08^{d}
T6 (100Gy)	31.0 ± 1.96^{e}
F- Test	*

100 seed weight

The table 4 shows the Effects of different doses of gamma radiation on 100 seed weight of *Vigna mungo*. According to the results, there is a statistically significant difference (P<0.05) among the treatments. Treatment T3 (40Gy) was shown the highest value for 100 seed weight compared to the other treatments and it was 2.65% compared to control.

Gamma radiation generate free radicals in cells by interacting with atoms and molecules. Almost all structural and functional organic compounds, including proteins, lipids, and nucleic acids, can react with these free radicals. They result in metabolic abnormalities by causing unsaturated fatty acids to peroxidize, which produces alkoxyl and peroxyl radicals (Roy, 2016) [23]. Mundeep, (2018) [18] has reported that lower doses of gamma irradiation significantly enhanced the crude protein content,

potassium, phosphorus, magnesium, manganese and vitamins (Mundeep, 2018) [18]. Therefore, it can be concluded that higher radiation affects to the decremental to the weight of seeds.
These results are supported by Irfaq & Nawab, (2002) who stated that increase in dose of radiation might decrease the grain weight of green bean (Irfaq & Nawab, 2001) [13].

Table 4: Effects of different doses of gamma radiation on 100 seed weight of *Vigna mungo*

Treatments	100 seed weight (g)
T1 (0Gy)	6.02 ± 0.09^b
T2 (20Gy)	6.29 ± 0.07^b
T3 (40Gy)	6.70 ± 0.16^a
T4 (60Gy)	6.18 ± 0.10^b
T5 (80Gy)	5.30 ± 0.08^c
T6 (100Gy)	4.56 ± 0.10^d
F- Test	*

Mutation frequency

Effects of different doses of gamma radiation on mutation frequency of *Vigna mungo* is shown in Table 5. According to the results increased percentages for those three mutants were observed in the treatment T3 (40Gy) compared to the T6 (100Gy). It was noted that, there was no any mutational effects in the treatment T1 (0Gy) and it supports for the conclusion which gamma radiation has high effectiveness and efficiency to make desirable mutants in plant.

Table 5: Effects of different doses of gamma radiation on mutation frequency (%) of *Vigna mungo*

Treatments	Plants having Trailer plant habit	Plants bearing hairless pods	Plants having 5-6 pods per cluster
T1 (0Gy)	0	0	0
T2 (20Gy)	40.66	6.25	0
T3 (40Gy)	32.11	25.32	37.56
T4 (60Gy)	0	0	10
T5 (80Gy)	0	0	32.69
T6 (100Gy)	25	12.5	32.5

Plate 1 Shows the Hairless pods bearing plants were observed in the treatments T2 (20Gy), T3 (40Gy) and T6 (100Gy). This characteristic might be useful for the plant breeding programs in order to generate new varieties and for the easiness of harvesting (Norihiko *et al*., (2010) [19]. Further, the ability of hairless pods to retain water might vary, which could affect the plant's capacity to withstand water stress. Further, Kumar *et al*., (2006) [15] also noted that in farmers and consumers' perspective, the hairless pods are more preferred and easier for them. Therefore, by these studies it is concluded that, hairless pods due to gamma radiation is a new pathway for the advanced black gram breeding programs.

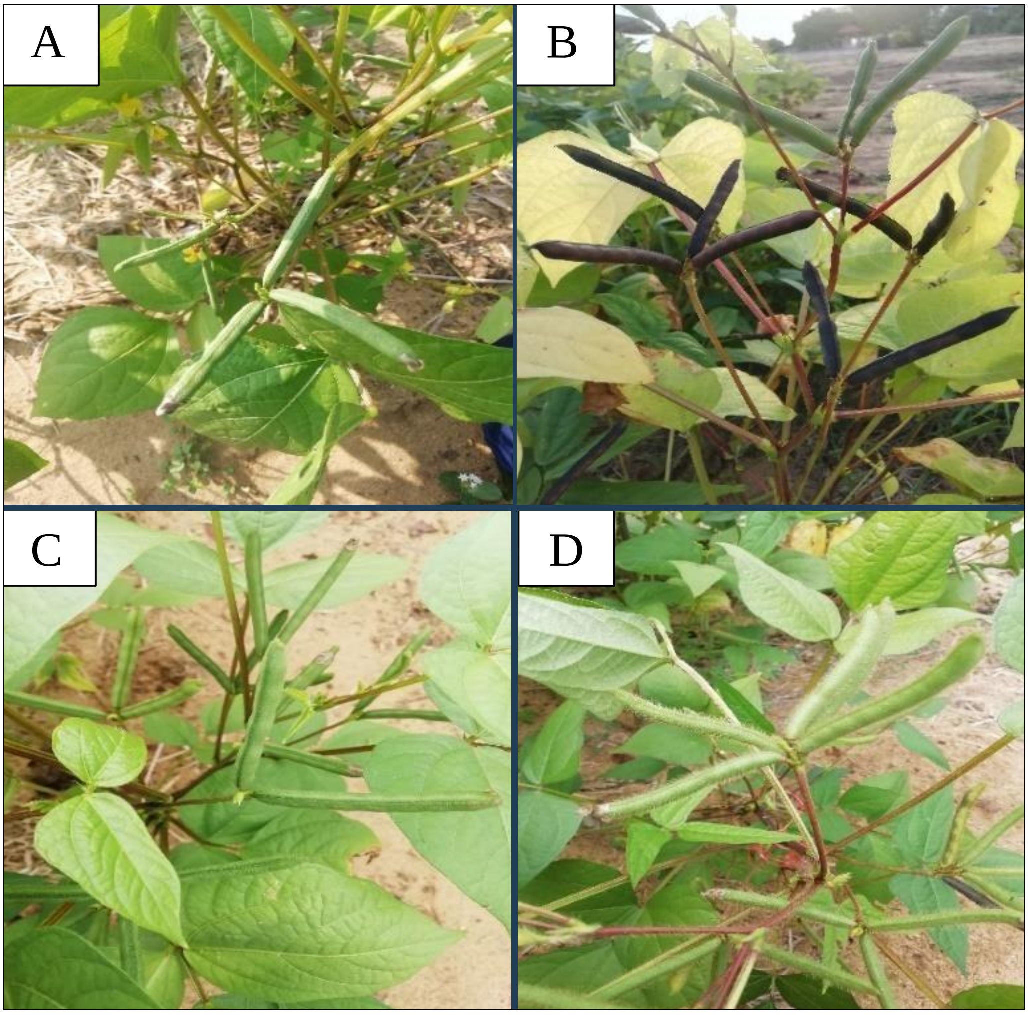

Plate 1: (A). hairless pods in T2 (20Gy), (B). hairless pods in T3 (40Gy), (C). Hairlesspods in T6 (100Gy), (D). Pods with hairs (T1-Control)

Plate 2 shows the trailer type plant habit and the normal plant habit in black gram. Trailer type plant habit was observed in the treatments T2 (20Gy), T3 (40Gy) and T6 (100Gy). Radiation- induced mutations can alter growth patterns and branching architecture, potentially affecting the distribution of flowers, pods along the plant by contributing more sites for pod formation. This alteration might impact the overall pod production and ultimately for the increasing of total yield (Wi & Chung, 2007) [29]. These results are in agreement with the findings by Vanniyarajan *et al*., (2016) who stated that among different gamma radiation has been commonly used, and numerous mutants have been produced in black gram.

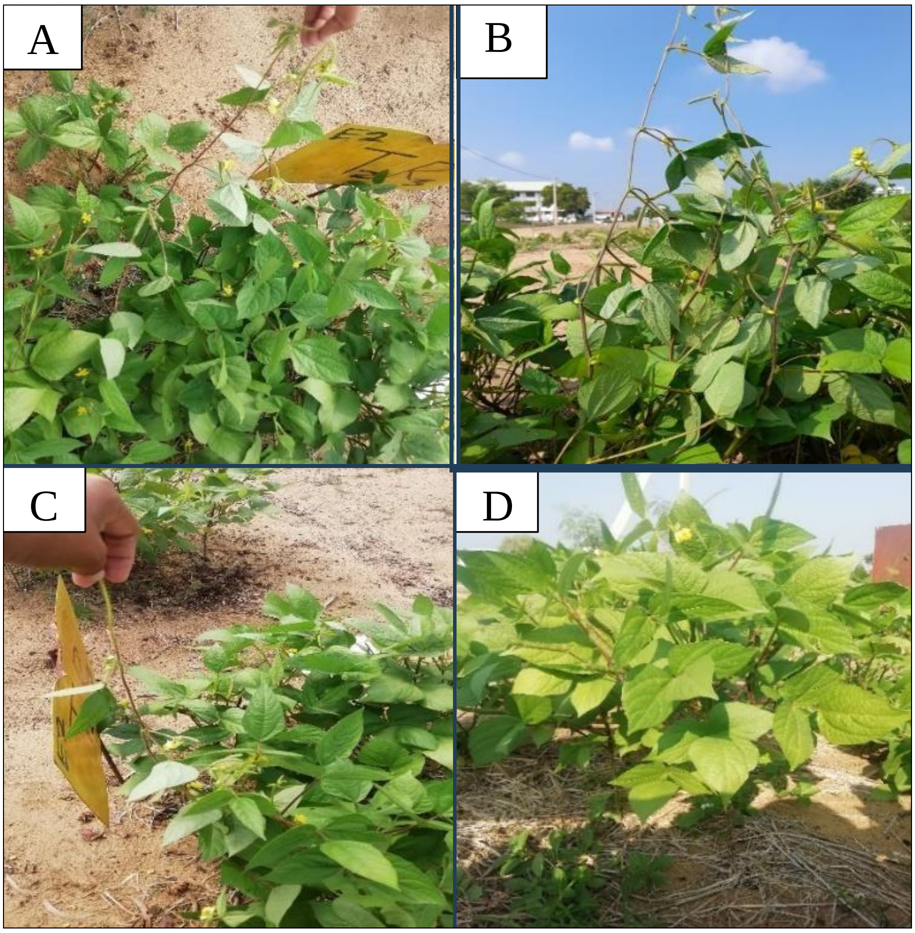

Plate 2: (A). Trailer type plant habit in T2 (20Gy), (B). Trailer type plant habit in T3 (40Gy), (C). Trailer type plant habit in T6 (100Gy), (D). Normal plant type (T1- control)

Plate 3 showed the 5-6 pods per cluster in Black gram. This habitat was observed in the treatments T3 (40Gy), T4 (60Gy), T5 (80Gy) and T6 (100Gy). However, in T1 (0Gy) bears only 2- 3 pods (Maximum). Induced mutation due to gamma radiation might cause for the alteration of the number of pods per cluster. These results might be due to mutations of polygenes governing the quantitative traits in legumes (Vanniarajan, & Chandirakala, (2022) [27]. Furthermore, the results in this study are in agreement with the study on Cluster bean by Babariya *et al*., (2008) [3].

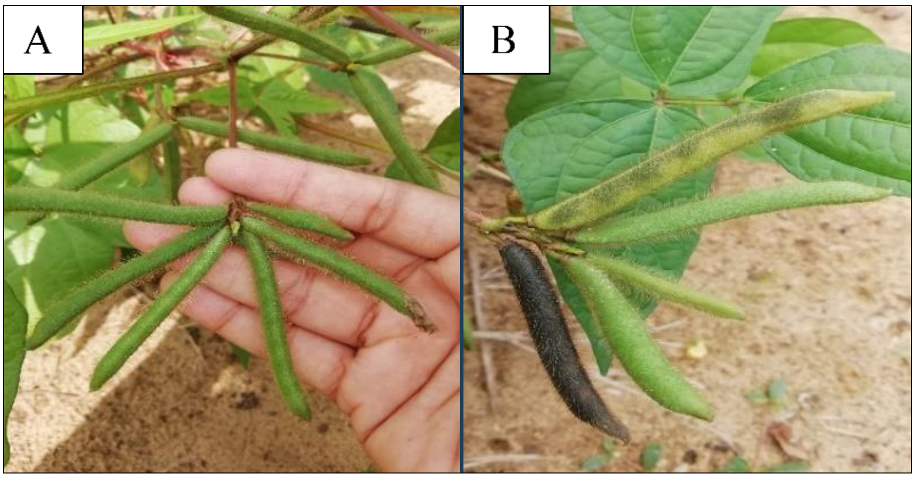

Plate 3: (A) & (B). 5-6 Pods per cluster

Total yield

Effects of different doses of gamma radiation on total yield of *Vigna mungo* was shown in figure 2. It was revealed that, the highest value for total yield was observed in the treatment T3 (40Gy) which was 166.51% of increased value, compared to control. According to the results, when increasing the dose of gamma radiation, the values for total yield were decreased gradually. The increasing value in the treatment T3 (40Gy) might be caused the highest value in 100 seed weight, maximum value in number of pods per plant and enhanced values in Number of seeds per pod.

Yasmin & Arulbalachandran, (2022) [32, 33] have reported that compared to control, the photosynthetic pigments such as chlorophyll a, b contents were decreased with the increasing dose of gamma rays. Roy, (2016) [23] were observed that yield biomass was increased in lower doses of gamma radiation in desi cotton. And also, some plant characteristics including number of pods per plant were increased than control of black gram with effect of gamma rays (Arulbalachandran *et al.*, 2009) [2].

Further, it is obvious that lowest value for total yield was recorded in the treatment T6 (100Gy) and it was 49.13% compared to control. These results are in supported with the findings by Badr, (2014) [4] who noted that effective plant physiological functions, adequate mineral and water uptake, and photosynthetic rate have been correlated with total yield. A disturbance in a plant's physiological activity can result in a reduction in photosynthesis, which in turn can lead to a reduction in total yield. At the highest dose of gamma radiation causes to physiological disturbance to decrease these traits. And the study by Abd El-Rahman *et al.*, (2016) [1] also supported with these results.

The results of this experiment are supported with the research study of Mohammad, (2011) [17] that higher total yield for green bean observed in the dose of 40Gy. Furthermore, highest values in the total yield in the treatment T3 (40Gy) might cause due to the greater number of pods per cluster and alteration of branching architecture due to trailer type planting habit as an effect of mutational frequency.

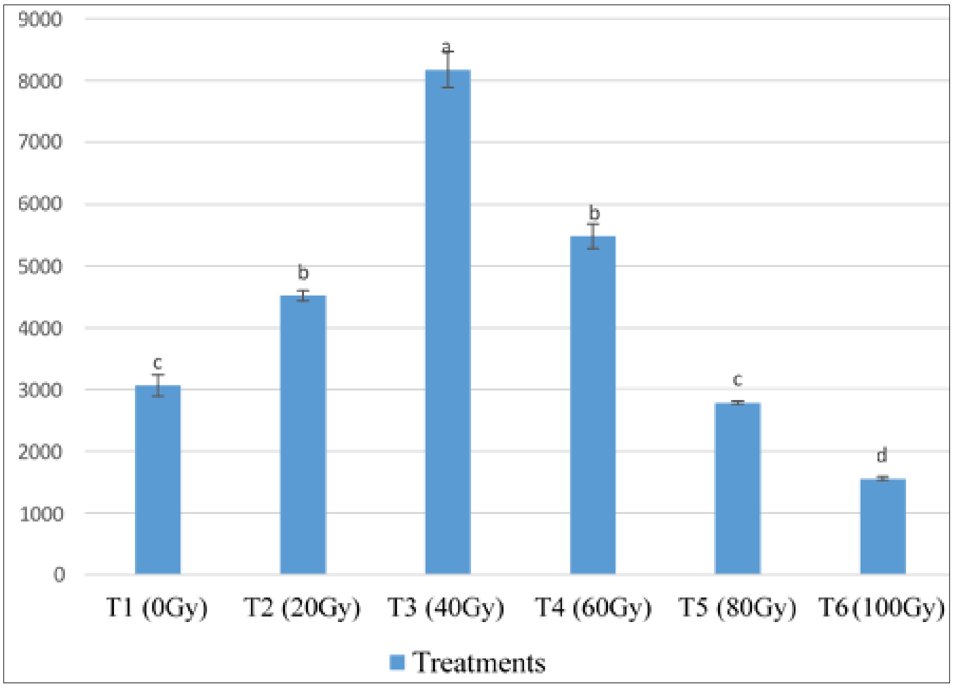

Fig 2: Effects of Gamma irradiation on Total yield (kg/ha) of *Vigna mungo*.

Conclusion

Various doses of gamma radiation were significantly influenced the most of the growth and yield characteristics of *Vigna mungo* in MI- 01 variety. In this investigation, the statistical analysis of data proved that, T3 (40Gy) proved significantly increased values in growth characteristics as plant height (53.76%), number of leaves (65.44%), leaflet width (1.96%), leaf area (56.22%), chlorophyl content (11.3%), shoot fresh weight (76.55%), root fresh weight (49.46%), total root length (9.33%), root volume (37.5%), nodulation (112.57%), and yield characteristics as pod diameter (5.09%), total pod fresh weight (149.13%) number of pods per plant (81.55%), 100 seed weight (2.65%), and total yield (166.51%) compared to control (T1- 0Gy). Furthermore, all mutational characteristics (hairless pods bearing plants, trailer type plant habit and 5-6 pods per cluster) were observed in T3 (40Gy) and it showed highest values in mutation frequency by increased percentages compared to the T6 (100Gy).

In this regard, treatment T3 (40Gy) showed more superior performances of growth and yield characteristics compared to the other treatments. Furthermore, incremental of the doses of gamma radiation showed the reduction in growth and yield characteristics of Black gram (*Vigna mungo).* Therefore, according to present study, treatment T3 (40Gy) is more suitable than the other treatments to create desirable characteristics in *Vigna mungo* especially in MI- 01 variety to improve the yield.

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