

## Effects of Gamma Radiation on Germination, Plant Height and Seed Viability of Okra (*Abelmoschus esculentus*)

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### Abstract

This experiment was conducted to evaluate the effects of different doses of gamma radiation on germination at 15 days after sowing, plant height at 15 and 30 days after sowing, and seed viability in okra. Seeds were exposed to 0 (control), 200, 300, 400, and 500 Gy of gamma rays. Significant differences ( $P \leq 0.01$ ) were observed in plant height at 15 days after sowing, and highly significant differences ( $P \leq 0.001$ ) in germination and plant height at 30 days after sowing. However, high gamma doses of 400 and 500Gy markedly decreased the number of viable seeds while increasing unviable seeds. Low doses (200 and 300Gy) had no significant effects on seed viability compared to the control. A strong linear relationship was found between radiation dose and seed viability ( $R^2 = 0.94$ ) and between dose and seed unviability ( $R^2 = 0.95$ ). Increasing radiation doses significantly ( $P \leq 0.05$ ) reduced and delayed seed viability, resulting in a 27% decline (from 100% in the control to 73% at 500 Gy). The results indicate that low doses of gamma radiation are non-detrimental to seed viability, while higher doses substantially impair germination and early growth in okra. Overall, these findings suggest that high gamma doses ( $\geq 500$ Gy) are detrimental to germination and growth, while moderate doses (200-400Gy) maintain viability and can even enhance growth through hormetic effects. Such information is valuable for optimizing gamma irradiation in mutation breeding programs aimed at generating variability without severely compromising seedling performance.

**Keywords:** Gamma radiation, hormetic effects, linear relationship, mutation breeding, okra, seed viability

## تأثير أشعة غاما على الانبات، طول النبات وحيوية البذور في نبات البامية (*Abelmoschus esculentus*)

### المستخلص

أجريت هذه التجربة لتقييم أثر جرعات مختلفة من أشعة غاما على الإنبات بعد 15 يوما من الزراعة، إرتفاع النبات بعد 15 و30 يوما من الزراعة، وحيوية بذور البامية. تعرضت البذور لجرعات صفرية (المجموعة الضابطة) و 200 و300 و400 و500 غراي من أشعة غاما. لوحظت فروقاً معنوية ( $P \leq 0.01$ ) في إرتفاع النبات بعد 15 يوماً من الزراعة، وفروقاً معنوية عالية ( $P \leq 0.001$ ) في الإنبات وارتفاع النبات بعد 30 يوماً من الزراعة. مع ذلك، أدت جرعات غاما العالية (400 و500 غراي) إلى انخفاض ملحوظ في عدد البذور القابلة للحياة، مع زيادة في عدد البذور غير القابلة للحياة. لم يكن للجرعات المنخفضة (200 و300 غراي) أي آثار معنوية على حيوية البذور مقارنة بالمجموعة الضابطة. وجدت علاقة خطية قوية بين جرعة الإشعاع وحيوية البذور ( $R^2 = 0.94$ ) وبين الجرعة وعدم الحيوية ( $R^2 = 0.95$ ). أدت زيادة جرعات الإشعاع بشكل ملحوظ ( $P \leq 0.05$ ) إلى إنخفاض قابلية البذور للنمو وتأخيرها، مما أدى إلى انخفاض بنسبة 27٪ (من 100٪ في المجموعة الضابطة إلى 73٪ عند 500 غراي). تشير النتائج إلى أن الجرعات المنخفضة من أشعة غاما لا تؤثر سلباً على قابلية البذور للنمو، بينما تُضعف الجرعات العالية بشكل كبير الإنبات والنمو المبكر في البامية. بشكل عام، تشير هذه النتائج إلى أن جرعات غاما العالية ( $\leq 500$  غراي) تضر بالإنبات والنمو، بينما تحافظ الجرعات المعتدلة (200 و400 غراي) على قابلية النمو، بل ويمكنها تعزيز النمو من خلال التأثيرات الهرمونية. تعد هذه المعلومات قيمة لتحسين إشعاع غاما في برامج تربية الطفرات الهادفة إلى توليد تباين دون المساس الشديد بأداء الشتلات.

**الكلمات المفتاحية:** إشعاع جاما، التأثيرات الهرمونية، العلاقة الخطية، تربية الطفرات، البامية، حيوية البذور

## Introduction

Okra [*Abelmoschus esculentus* (L.) Moench] belongs to the genus *Abelmoschus*, which belongs to the family Malvaceae that consists of twelve species (Kisher *et al.*, 2016). *A. esculentus*, commonly known as okra or lady's finger, is a warm-season crop cultivated throughout the tropical and warm temperate regions of the world (Surendran *et al.*, 2017). It has the highest chromosome number among vegetables ( $2n=130$ ). It is a self-pollinated vegetable crop that is mainly propagated by seeds (Osawaru *et al.*, 2014).

Okra originated in tropical Africa and is native to northeastern Africa, mainly Ethiopia and Sudan (Oyelade *et al.*, 2003; Santos *et al.*, 2012). The optimum growth temperature range is between 24°-27°C, and it is highly tolerant to high temperatures and drought conditions (Surendran *et al.*, 2017). Okra fruit is primarily eaten raw or cooked, and it is a significant source of vitamins A, B, and C, as well as minerals like phosphorus, potassium, sulfur, calcium, iron, and iodine. However, it is also said to be low in salt, saturated fat, and cholesterol (Kendall and Jenkins, 2004).

Global okra production rose from 1,107,430 tonnes in 1961 to 11,523,290 tons in 2023, with an average annual growth rate of 4.01%. India leads as the world's top okra producer, producing 7.16 million tons annually with an average growth rate of 3.70%, accounting for 62.1% of global production. Nigeria ranks second with an annual production of 1.87 million tons, growing at 5.53% per year and contributing 16.3% to global production. Sudan ranks fourth in okra production, with output having increased from 263,000 tons in 2012 to 303,150 tons in 2023, reflecting an average annual growth rate of 2.31% (FAO, 2024).

In Sudan, okra is the most traditionally popular vegetable; it is grown in all areas of the country throughout the year, where both cultivated and wild types are known (Mohamed, 2023; Schippers, 2002). In addition to the introduced varieties such as Clemson Spineless and Pusa Swani, there are a number of local varieties, e.g., spiny types called Khartoumia, Karrari, Kassala, Medani, Sinnar, and others.

In certain cases, the desired trait does not exist in germplasm collections of a crop, and hence mutation breeding can be efficiently employed as an alternative valuable method to generate and develop new varieties with such desired characteristics (Reddy and Dhaduk, 2014). A mutation is a sudden, heritable change in the DNA of a living cell that is not caused by genetic segregation or recombination. (Van Harten, 1998). Mutation induction has been proven to be an effective method to increase genetic variability in crops (Surendran *et al.*, 2017). Inducing variation through mutation is common and has been found to be successful in okra (Ashadevi *et al.*, 2017).

A primary application of induced mutations is the improvement of polygenic traits in crop plants by introducing desirable mutants directly into commercial cultivars or using them indirectly through crossbreeding (Jadhav *et al.*, 2012). According to the International Atomic Energy Agency (IAEA) database (<http://mvgs.iaea.org>), there are more than 3,300 officially released mutant varieties of 170 different species in more than 60 countries around the world that not only increase biodiversity but also provide material for plant breeding (Jankowicz-Cieslak *et al.*, 2017). Mutation induction can be carried out using chemical or physical mutagens (Shahab *et al.*,

2018). Some of the agronomic traits generated as a result of mutation induction included increasing 3Deoxyanthocyanidin accumulation in leaves (Petti *et al.*, 2014), dwarfism, early flowering, high protein digestibility, and high lysine content, which have been widely used in sorghum breeding (Shu *et al.*, 2011).

Therefore, this study aimed to study the effects of gamma radiation on germination, plant height and seed viability of okra.

### **Study Site**

Experiment was conducted during the winter season of 2017-2018 at Shambat Research Station Farm (latitude 15°36'N and longitude 32°32'E and elevation 380m). Gamma rays radiation was carried out in the International Atomic Energy Agency (IAEA), Austria.

### **Materials and Methods**

#### **Experimental Design**

Experiment was conducted using a randomized complete block design (RCBD) with three replications. Local cultivar of okra seeds was used in this study. Land was prepared by deep ploughing and harrowing twice in opposite directions and then leveled; thereafter, 70 cm ridges were prepared. Seeds were sown at 30 cm intra-row spacing and 2 to 3 seeds per hole, then thinned to one plant per hole. The experiments were irrigated weekly and hand weeded whenever necessary. All the recommended cultural practices were followed to maintain good crop stand.

#### **Genetic Material and Irradiation of Seeds**

A widely cultivated local okra landrace from Kasala was used in this study. Seeds were exposed to gamma radiation doses of 0, 200, 300, 400, and 500 Gy, using a radiation source with a capacity of 3000 Ci and a delivery rate of 7200 r/min.

#### **Data Collection**

Data on morphological traits included germination at 15 days after sowing and plant height at 15 and 30 days after sowing. Twenty-five plants were randomly selected from each plot for assessment of plant height at 15 and 30 days after sowing.

For the assessment of seed viability, fruits from the M<sub>1</sub> generation (plants grown from irradiated seeds) were harvested, and seeds of the M<sub>2</sub> generation (progeny of M<sub>1</sub> plants) were extracted. A total of ninety fruits were then randomly selected for viability testing. The extracted seeds were cleaned, air-dried, and stored under ambient laboratory conditions until analysis. Percentage of the number of seeds was calculated as follow;

Number of viable seeds (%) = (Number of viable seeds/Total number of seeds) × 100

Number of unviable seeds (%) = (Number of unviable seeds/Total number of seeds) × 100

For determining seed germinability, the seeds of the M<sub>2</sub> generation (progeny of M<sub>1</sub> plants) were sown in moist soil under controlled conditions. The number of germinated seeds was recorded daily for 15 days, and the percentage of germination for each treatment was calculated using the formula:

Germination (%) = (Number of germinated seeds/Total number of seeds sown) × 100

Seedling survival was monitored and recorded from the 10th day onward after sowing.

### Data Analysis

All data were subjected to an analysis of variance, with mean comparisons performed using Fisher's protected least significant difference (LSD) test at  $P \leq 0.05$  (Steel and Torrie, 1980). Least square means for all genotypes were generated using analysis of variance (ANOVA) option of GenStat 18th Edition (VSN International Ltd., UK)

### Results

Analysis of variance (Table 1) showed significant differences ( $P \leq 0.01$ ) for plant height at 15 days and highly significant differences ( $P \leq 0.001$ ) for germination and plant height at 30 days.

Table 1: Analysis of variance (ANOVA) of germination and plant height of okra (*Abelmoschus esculentus*) genotypes exposed to five gamma radiation doses in winter season (2017-2018)

Source of variation	Df	Germination	Plant height at 15 DAS (cm)	Plant height at 30 DAS (cm)
Rep	2	1620.6	8.132	710.38
Dose (Gy)	4	9603.2***	8.274**	84.082***
Residual	8	188	1.249	2.56
Total	14			

\*\*= significant difference at  $P \leq 0.01$ , \*\*\*= highly significant difference at  $P \leq 0.001$ .

DAS= days after sowing.

Mean performance of germination at 15 days after sowing and plant height at 15 and 30 days after sowing showed differences among irradiation doses. The 500Gy treatment showed the lowest mean number of germination seeds at 15 days after sowing (95.70) while the control had the highest (237.5), followed by 200Gy (197.5) and 300Gy (152.5), respectively. Regarding plant height at 15 and 30 days after sowing, the control had the highest plant height at 15 and 30 days after sowing (19.8 cm & 57.4 cm, respectively), followed by irradiation dose 400Gy (18.9 & 51.3, respectively), while irradiation dose 300Gy showed the lowest plant height at 15 and 30 days after sowing with 15.7cm and 43.2cm, respectively (Table 2).

Table 2: Mean performance of germination at 15 days after sowing and plant height at 15 and 30 days after sowing of okra seeds planted in winter (2017-2018)

Dose (Gy)	Germination at 15 DAS	Plant height at 15 DAS (cm)	Plant height at 30 DAS (cm)
Control	237.5±7.9	19.8±0.7	57.4±0.9
200	197.5±7.9	17.6±0.7	48.2±0.9
300	152.0±7.9	15.7±0.7	43.2±0.9
400	125.3±7.9	18.9±0.7	51.3±0.9
500	95.70±7.9	16.7±0.7	47.3±0.9
LSD ( $p \leq 0.05$ )	25.8	2.10	3.01
SED ( $p \leq 0.05$ )	11.2	0.91	1.31
CV%	8.50	6.30	3.20
Grand mean	161.6	17.7	49.5
Minimum	91.0	15.1	36.8
Maximum	272	22.8	72.2

DAS= days after sowing.

Differences between viable and unviable seeds are presented in Plate 1. In this study, most seeds were viable, although a few were unviable, especially at the higher irradiation doses (300, 400 and 500Gy). The viable seeds were round and brown to green color, whereas the unviable seeds were small, wrinkled, and dark green to black in color.

To determine seed germinability, seeds were sown in moist soil, and the number of germinated seeds was counted daily for 15 days. The germination percentage for each treatment was then calculated, and seedlings survival was recorded from the 10th day onward after sowing. Results showed that the percentage of viable seed germination was 100%, while unviable seeds did not germinate (0%).

Plate1. Differences between viable and unviable seeds resulting from 400Gy dose



Analysis of variance (Table 3) showed significant differences ( $P \leq 0.001$ ) among viable and unviable seeds. No significant differences in total seed number.

Table 3: Analysis of variance (ANOVA) of seed viability of okra (*Abelmoschus esculentus*) genotypes exposed to five gamma radiation doses in winter season (2017-2018)

Source of variation	Df	Number of viable seeds	Number of unviable seeds	Total number of seeds
Rep	2	810.2	48.13	470.2
Dose (Gy)	4	4457.2***	7272.11***	522.2ns
Residual	443	145.8	12.07	152.9
Total	449			
LSD ( $P \leq 0.05$ )		3.5	1.0	3.60

\*\*\*= highly significant difference at  $P \leq 0.001$ , ns=non-significant difference.

The percentage of viable and unviable seeds is presented in Table 4. The number of viable seeds was highest in the control (62.0) and decreased progressively with increasing radiation doses. Treatments at 200Gy (59.9) and 300Gy (57.9) showed a moderate reduction, while 400Gy (50.0) and 500Gy (45.3) resulted in a pronounced decrease. The 500Gy treatment produced a significantly lower number of viable seeds than all other treatments, as indicated by the mean separation letters (LSD,  $P \leq 0.05$ ). Conversely, the number of unviable seeds increased with increasing radiation dose, ranging from 0.0 in the control to 2.4, 11.6, 21.3, and 35.5 at 200Gy, 300Gy, 400Gy, and 500Gy, respectively. The total number of seeds per treatment varied slightly, ranging from 62.4 at 200Gy to 67.3 at 500Gy.

Table 4: Percentage of seed viability from okra exposed to various doses of gamma radiation

Dose	Viable seed number	Viable percentage	Unviable seed number	Unviable percentage	Total seed number
0	62.0 d	100	0.00 a	0.00	62.0
200	59.9 cd	96.6	1.50 b	2.40	61.4
300	57.9 c	93.3	7.20 c	11.6	65.1
400	50.0 b	80.6	13.2 d	21.3	63.2
500	45.3 a	73.0	22.0 e	35.5	67.3
LSD ( $P \leq 0.05$ )	3.50		1.00		3.60
SE $\pm$	1.30		0.40		1.30
SED ( $P \leq 0.05$ )	1.80		0.50		1.80
CV%	22.0		39.4		19.4
Grand mean	55.0		8.80		63.8
Minimum	15.0		0.00		24.0
Maximum	95.0		36.0		105.0

The percentage of viable and unviable seeds response to gamma radiation doses is presented in Figure 1. Linear regression analysis revealed a strong negative relationship between radiation dose and seed viability ( $R^2=0.94$ ) and a strong positive relationship between radiation dose and seed unviability ( $R^2=0.95$ ), indicating that 94% and 95% of the variation in these parameters, respectively, was explained by the applied radiation doses.

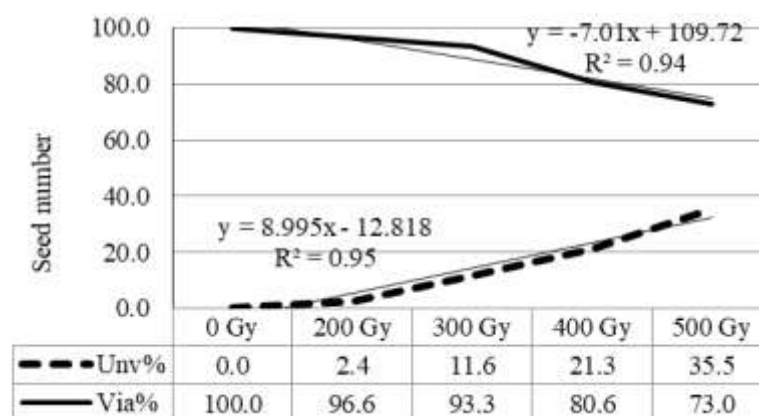


Figure 1: Percentage of viable and unviable seeds of okra in response to gamma radiation doses.



## Discussion

Gamma irradiation significantly affected germination, plant height, and seed viability in okra (*Abelmoschus esculentus* L.) in a dose-dependent manner.

Germinated seeds decreased progressively with increasing irradiation, from the highest in the control (237.5) to the lowest at 500Gy (95.7). This reduction is likely due to radiation-induced damage to embryonic cells or inhibition of key germination enzymes, as reported in okra (Dhankhar and Dhankhar, 2004) and other crops including chickpea (Toker *et al.*, 2005; Umavathi and Mullainathan, 2016), lima bean (Kumar *et al.*, 2003), sweet potato (Tabares and Talavera, 2003), and cowpea (Seema *et al.*, 2003). Likewise, Asare *et al.*, (2017) observed that gamma irradiation above 400 Gy delayed and reduced germination and seedling growth compared with controls.

Reduced growth has been attributed to auxin destruction, changes in ascorbic acid content, and physiological and biochemical disturbances (Gunckel and Sparrow, 1954). However, the stimulating effect of lower doses of gamma irradiation on the growth of okra plants might be due to the stimulation of cell division and processes that affect the synthesis of nucleic acids (Pitirmovae, 1979).

Plant height at 15 and 30 days after sowing showed a non-linear trend. , A decline occurred when the irradiation dose was increased to 200Gy (17.6cm & 48.2cm) and 300Gy (15.7cm & 43.2cm) compared to the control (19.8cm & 57.4cm), while plant height at 15 and 30 days after sowing increased when the irradiation dose was increased to 400Gy (18.9cm & 51.3cm), and then decreased when the irradiation dose was increased to 500Gy (16.7cm & 47.3cm). The highest plant height at 15 and 30 days after sowing was observed for the control which was closely followed by 400Gy, while 300Gy recorded the lowest plant height at 15 and 30 days after sowing.

This non-linear pattern suggests a possible hormetic response, where moderate radiation doses stimulate growth while higher doses are inhibitory, a phenomenon widely reported in plants exposed to abiotic stressors including gamma radiation (Calabrese and Baldwin, 2003; Vaiserman *et al.*, 2021). Similar findings were reported by Hegazi and Hamideldin (2010), who observed maximum plant height at 400Gy compared with 300Gy and 500Gy gamma-ray treatments.

Likewise, high gamma irradiation doses have deleterious effects on plant height (Loch, 1977; IBPGR, 1991; Singh *et al.*, 2000 and Ochatt *et al.*, 2001). The reduction in plant height observed in the current study may be attributed to a reduction in mitotic activity of meristematic tissues (Khalil *et al.*, 1986). Iqbal, 1969 and Walther, 1969 reported that the reduction in plant height may be attributed to damage to the processes of cell division and cell elongation as a result of mutagenic treatment. This is because irradiation causes DNA breakage in plant cells, further leading to various types of damage to plant cell division and development processes, and plant growth (Amirikhah *et al.*, 2019; Li *et al.*, 2021).



Seed viability was significantly reduced at higher doses (400-500Gy), while unviable seeds increased. This decline can be attributed to several biological mechanisms. Ionizing radiation induces lethal mutations in the embryo's DNA, such as chromosomal breaks, deletions, and rearrangements that compromise embryo development (Baek *et al.*, 2005; Kovacs and Keresztes, 2002). It may also damage the endosperm-the primary nutrient source for germination-or disrupt cellular integrity, including membranes and organelles (Melki and Dahmani, 2009; Sangwan and Mehta, 2010). Moreover, gamma radiation triggers oxidative stress through the generation of reactive oxygen species (ROS), which further damage nucleic acids, proteins, and lipids (Kim *et al.*, 2004; Wi *et al.*, 2007; Zaka *et al.*, 2002). For practical purposes, viable okra seeds should be harvested once they reach the proper maturation stage, typically when seeds transition from green to brown, as seeds continue to enlarge on the plant until fully developed. High radiation doses accelerate loss of viability, emphasizing the importance of dose optimization for mutation breeding and seed preservation.

Linear regression analysis revealed a strong negative relationship between radiation dose and seed viability ( $R^2 = 0.94$ ) and a strong positive relationship between dose and seed unviability ( $R^2 = 0.95$ ). This indicates that 94 % and 95 % of the observed variation in these parameters, respectively, were explained by radiation dose. For instance, actual viability declined from 62.0 % to 45.3 %, representing a reduction of ~ 26.9 %.

These results are consistent with earlier studies showing that germination and viability decline proportionally with increasing gamma dose (Asare *et al.*, 2017; Melki and Dahmani, 2009; Toker *et al.*, 2005). The strong linear relationships observed confirm that radiation dose is a key determinant of seed viability, though the magnitude of reduction must be interpreted based on actual percentage changes rather than  $R^2$  values.

Overall, these findings suggest that high gamma doses ( $\geq 500\text{Gy}$ ) are detrimental to germination and growth, while moderate doses (200–400Gy) maintain viability and can even enhance growth through hormetic effects. Such information is valuable for optimizing gamma irradiation in mutation breeding programs aimed at generating variability without severely compromising seedling performance.

### **Conclusion**

Low doses of gamma radiation showed no adverse effects on seed viability in okra, suggesting potential for safe use in mutation breeding. However, higher doses significantly reduced germination and plant growth. Further research is needed to clarify the physiological and molecular mechanisms behind radiation-induced changes and seed aging in okra.

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