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Response of some cowpea genotypes to radiosensitivity using ^{60}Co gamma radiation

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A B S T R A C T

Mutagenesis is one of the most effective methods for crop improvement as it expands the genetic pool, offering more opportunities for selecting desirable traits, especially in cowpea, which is predominantly self-pollinating. For effective mass irradiation at acute doses, the sensitivity of cowpea genotypes to gamma rays needs to be determined. The objective of this study was to determine the lethal dose (LD_{50}) at 50% germination and reduction in the appearance of cowpea growth (RD_{50}) when exposed to gamma radiation. Five cowpea genotypes, namely, Hansadua, WC-36, ACC122WxWC-10, IT97K-819, and WC-10, were irradiated with gamma radiation from a ^{60}Co radioactive source at 0-1200Gy with an interval of 100 Gy. The results showed significant wide variations in the responses of genotypes. Hansadua, an improved cultivar, had the lowest LD_{50} and RD_{50} values of 531.0 and 452.0 Gy, respectively, indicating its high sensitivity to gamma radiation. Thus, suggesting a relatively lower dose is required to kill half of the population and more so, a tendency to produce more useful mutants at lower doses of radiation from which selection could be made. In addition, the highest values of LD_{50} and RD_{50} were observed for ACC122WxWC-10 at 903.0 and 694.0 Gy, respectively. This implies that the ACC122WxWC-10 genotype was the least sensitive to gamma radiation, as more radiation was required to reduce the growth of the control population to half. In addition, there were progressive reductions in other parameters such as plant height, root length, shoot weight, and whole plant weight in all genotypes as the radiation dose increased.

Keywords: *Vigna unguiculata,* Breeding, Mutagenesis, Seed germination, Agriculture

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Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is a vital grain legume extensively cultivated across diverse agro-ecological zones in Africa and tropical regions worldwide. While it is also grown in temperate climates such as California's Central Valley and the Mediterranean basin (Ba *et al.*, 2004), Africa remains the primary production hub. The crop is essential for supporting subsistence farmers and rural livelihoods, particularly in low-input farming systems. In 2017, Africa contributed a staggering 98% of global cowpea harvest across 12.5 million hectares, yielding nearly 7.1 million of the total 7.4 million tons of dried cowpeas produced worldwide (www.iita.org/cropsnew/cowpea/; accessed 15/07/2020).

Cowpea is a food crop, forage, and vegetable source, especially in tropical regions (Steele, 1972). With protein-rich green leaves, pods, and grains, cowpea offers a dietary protein source. Its grains contain approximately 50-60% carbohydrates, while the leaves hold

27-34% proteins (Sebetha *et al.*, 2015). This nutritional profile is especially valuable for rural populations lacking access to alternative protein sources. Cowpea is cultivated for fodder in Sahelian areas of West Africa and fiber in dry Asian areas (Steele, 1972; Ba *et al.*, 2004).

As a leguminous crop, cowpea has the unique ability to harness atmospheric nitrogen through interaction with rhizobia, soil-borne bacteria. This characteristic is particularly important for small-scale farmers who cannot afford synthetic fertilizers. Despite its resilience to moderate drought levels, cowpea productivity remains constrained by the poor fertility of the soils in many production areas. For instance, the average yield in Ghana hovers around 1.41 MT ha^{-1}, representing 56% of its potential yield (MoFA, 2016; Gyasi, 2016). Factors like persistent drought, use of low-yielding cultivars, and susceptibility to pests and diseases limit its productivity. In the context of climate change, developing cowpea varieties resistant to these stresses emerges as an environmentally sustainable approach to enhancing yields (Kouressy *et al.*, 2008). Therefore, creating cowpea genetic materials that are resistant to stresses and has desired traits becomes pivotal for productivity improvement.

While cowpea's genetic diversity is limited (Sharawy and El-Fiky, 2003; Asare *et al.*, 2010; Wamalwa *et al.*, 2016), variability is essential for successful crop breeding (Cooper *et al.*, 2001; Mudibu *et al.*, 2012; Horn and Shimelis, 2013). Cowpea's predominantly self-pollinating nature and early flower fertilization pose challenges to generating new variations. Meanwhile, plant breeding can only result in significant improvements when the breeder has access to sufficient variation for a given trait (Swarup *et al.*, 2021; Yali and Mitiku, 2022). Mutagenesis, involving mutagens like gamma rays, has been suggested as an efficient and cost-effective means of increasing genetic variability (Yali and Mitiku, 2022). Since its discovery by Müller (1927) and Stadler (1928), mutagenesis has been applied to modify agronomic traits, enhance stress tolerance, and boost yields in existing varieties and landraces. This approach holds promise for crops like cowpea, which inherently exhibit limited genetic variation. Mutations, according to Yali and Mitiku (2022), are heritable alterations in an organism's phenotype. Heritability of different traits after the mutation process has been found to be variable for different traits and at different generations after mutagenesis. For example, the heritability of iron, calcium, and protein content was reported to be high in the M3 generation mutant (Waghmode *et al.*, 2020), while the heritability of M4 generation was reported to be high in yield characteristics (Vasisth *et al.*, 2022; Adhi *et al.*, 2024).

In addition to creating genetic mutations and chromosomal changes, mutagens induce physiological damage to genetic materials, termed mutagenic sensitivity (Bashir *et al.*, 2013). Factors influencing mutagenic sensitivity include mutagen dose (Shah *et al.*, 2008; Laskar *et al.*, 2015). Therefore, determining optimal doses is crucial to induce maximum variability without damaging genetic material. This study aimed to introduce genetic variation in cowpea using gamma rays and identify optimal mutation doses.

Materials and Methods

Genetic materials and irradiation

The research was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI) of the Ghana Atomic Energy Commission. The study involved five distinct cowpea genotypes: two landraces (WC-10 and WC-36) and an inbred line (ACC122WxWC-10) sourced from Uganda; IT97K-819, an inbred line acquired from the International Institute of Tropical Agriculture (IITA); and Hansadua, an improved variety obtained from the Crops Research Institute of the Council for Scientific and Industrial Research (CRI-CSIR).

Seeds of the cowpea genotypes were subjected to gamma irradiation using a range of 13 irradiation doses from 0 Gy to 1200 Gy, spaced at an interval of 100 Gy. Each of the five genotypes had 10 seeds irradiated with 12 radiation doses, ranging from 100 to 1200 Gy. An additional set of 10 seeds for each genotype served as non-irradiated controls. This resulted in three replications per genotype per radiation dose, amounting to 30 seeds for each genotype and radiation dose combination. To achieve the desired moisture content of approximately 8%, irradiated seeds were placed in separate zip-lock bags within a desiccator for three days. The gamma irradiation was carried out using a category IV gamma irradiation facility at the Ghana Atomic Energy Commission, utilizing a ^{60}Co source at a dose rate of 303 Gy/hr.

Sowing, experimental design, data collection, and analysis

Seven days following irradiation, the treated seeds were sown in polythene nursery pots filled with smooth-textured loamy soil, each measuring 13 cm in width and 15.5 cm in depth. The experiment was conducted in a screen house, with the pots arranged in a Completely Randomized Design. Harvesting was done after 21 days of sowing, and the parameters such as germination, seedling height, fresh whole plant weight, fresh root weight, root length, and fresh shoot weight were recorded.

Germination counts were conducted on days 3, 5, 8, 13, and 18 after sowing, while other parameters were measured at 21 days after sowing. Plant height was measured from the soil surface to the tip of the primary leaf, and root length was measured from the taproot tip to the soil level. Germination rates were calculated as percentages. Per cent differences compared to the control were computed for plant height.

Data was subjected to standard analysis of variance procedures using Genstat 12th edition version 12.1 statistical software. This facilitated genotype comparisons and identification of optimal lethal dose aiming at LD_{50} (dose at 50% germination inhibition) and RD_{50} (dose causing 50% reduction in growth). The LD_{50} values were determined based on seed germination percentages, pinpointing the dose at which the 50 percent point intercepts the curve.

Results

The irradiation doses significantly affected the performance of cowpea genotypes. The LD_{50} values varied from 531 Gy in Hansadua to 903 Gy in ACC122WxWC-10 (Figure 1; Table 1). In decreasing order, LD50 values of 903, 858.7, 762, 705, and 531 Gy were achieved for ACC122WxWC-10, WC-36, IT97K-819, WC-10, and Hansadua, respectively. Figure 2a shows the emergence reduction curve (RD_{50}) for the cowpea genotypes. Emergence reduction defines the dose that reduces the growth and seed production of an M_1 population by 50% and varies between the genotypes (Table 1). The RD_{50} values were 694, 662, 591, 590.5 and 452 for ACC122WxWC-10, WC-36, IT97K-819, WC-10 and Hansadua, respectively. Thus, Hansadua had the least LD_{50} and RD_{50} values of 531 and 452 Gy and ACC122WxWC-10 recorded the highest LD_{50} and RD_{50} values of 903 and 694 Gy.

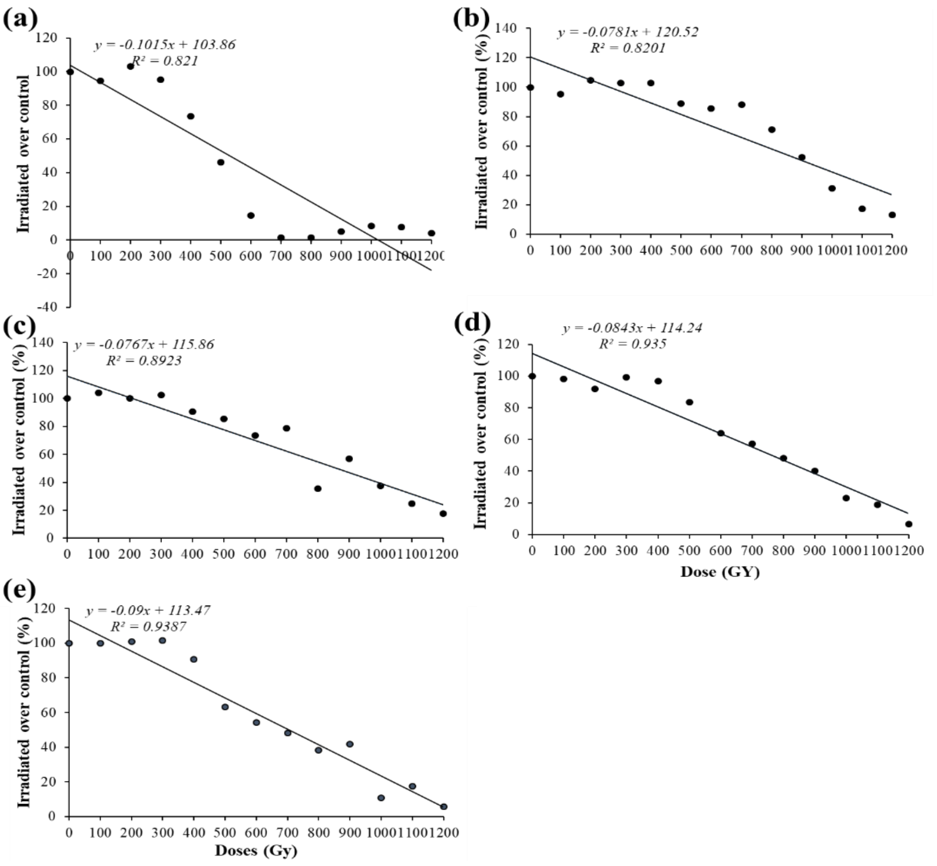

Fig. 1. Radio-sensitivity curves for five cowpea genotypes: (a) Hansadua, (b) ACC122WxWC-10, (c) WC-36, (d) IT97K-819 and (e) WC-10.

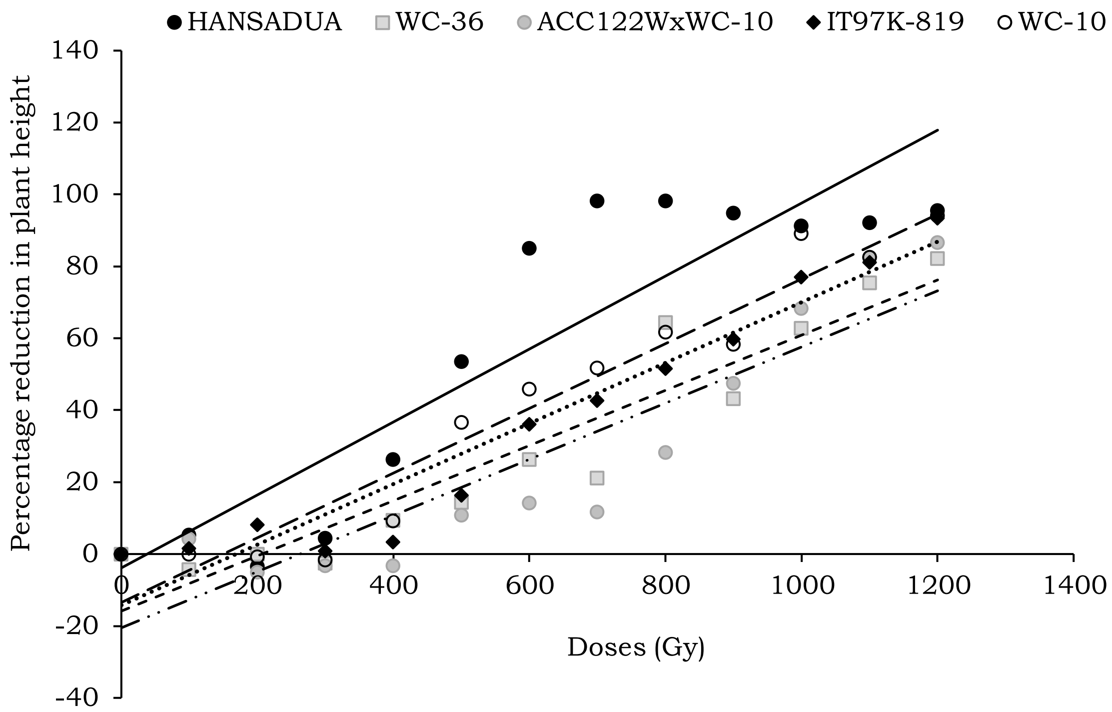

Fig. 2. Emergence reduction curves for five cowpea genotypes.

Table 1. LD_{50} and RD_{50} values of five cowpea genotypes.

Genotype/Optimum doses	LD_{50} (Gy)	RD_{50} (Gy)
Hansadua	531.0	452.0
WC-36	858.7	662.0
ACC122WxWC-10	903.0	694.0
IT97K-819	762.0	590.5
WC-10	705.0	591.0

Significant differences (P< 0.001) were found among radiation doses and their effect on germination. The germination percentage in the radio-sensitivity test is expressed as a percentage of the control or unirradiated. The results indicated a rise in germination percentage from control (0 Gy), which peaked at 300 Gy and gradually declined to 800 Gy. There was a slight rise at 900 Gy, which declined to 1200 Gy (Fig. 3).

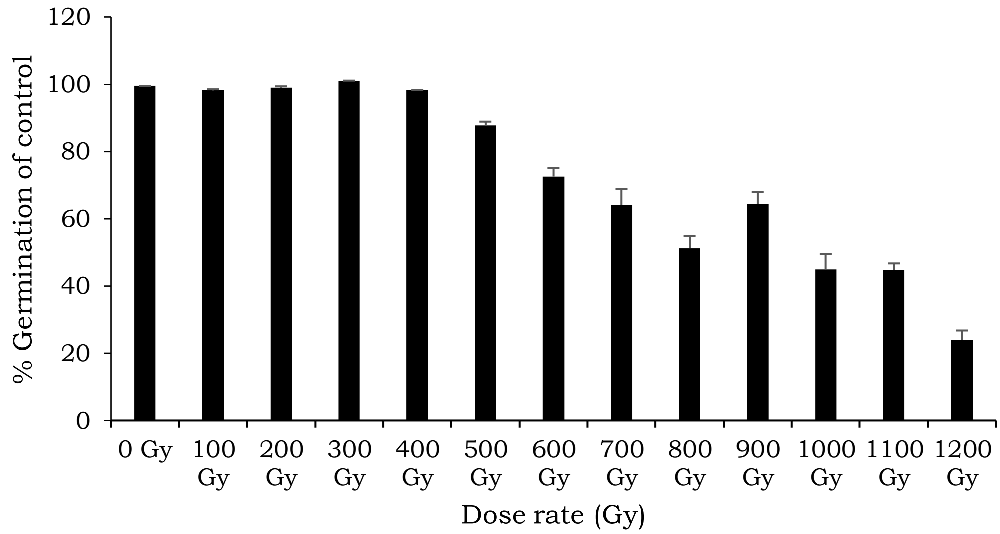

Fig. 3. Mean seed germination (%) of five genotypes at various doses.

There were varied responses of seed and other plant propagules to various gamma-ray doses administered. Gamma-ray doses between 0-300 Gy had no significant effect on germination. There were no significant differences in plant height between irradiation doses 0-200 Gy for plant height and root length (Figure 4), shoot weight and whole plant weight between 0-100 Gy. The differential effect of gamma rays was observed beyond 300 Gy in germination, 200 Gy in plant height, and 100 Gy in root length, shoot weight, and whole plant weight. The results also showed varying effects of different radiation doses on pod architecture, as shown in Figure 5. Variations in pod architecture included reduced pod length, increased pod length, curved pods, and misshapen pods in some treatments.

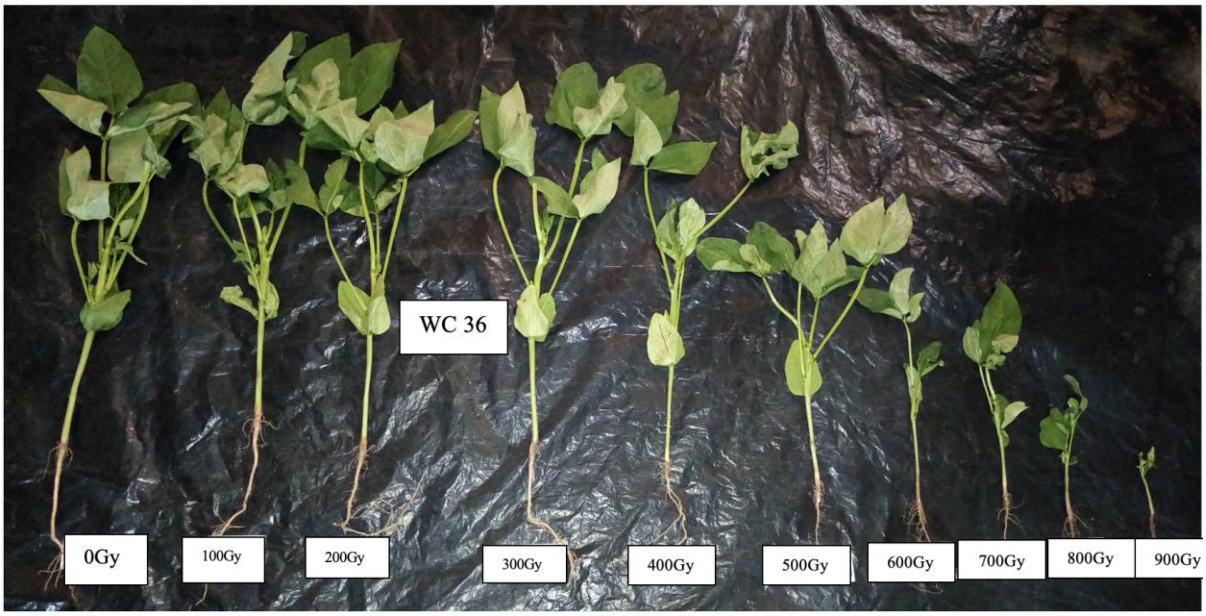

Fig. 4. Effect of different gamma radiation doses on the plant height of WC36 cowpea genotype.

Table 2. Growth traits of cowpea plants exposed to different radiation doses.

Doses	Germination	Plant Height (cm)	Root Length (cm)	Shoot Weight (g)	Whole plant weight (g)
0	8.0 a	20 a	21.5 a	16.5 a	18.0 a
100	7.8 a	19.8 a	20.3 a	16.4 a	15.9 a
200	7.8 a	19.6 a	17.7 b	13.6 b	14.4 b
300	7.8 a	19 b	16.5 b	12.6 b	10.9 b
400	7.2 b	12.4 c	12.0 c	7.6 c	3.2 c
500	6 c	12.2 c	10.9 c	7.4 c	6.9 c
600	4.6 d	11.2 d	9.4 d	4.8 d	7.9 d
700	4.4 d	6.8 e	6.6 e	2.9 d	2.3 d
800	3.2 e	4.6 f	5.0 e	1.7 e	2.1 e
900	2.8 e	2.8 g	1.9 f	0.9 e	1.7 e
1000	2 f	0.8 h	0.4 f	0.3 e	0.0 e
Mean	5.6	11.7	11.1	7.7	7.6
STDEV	2.3	7.2	7.3	6.2	6.4
CV%	40.7	61.6	65.4	79.8	84.0
LSD	0.6	0.7	1.9	2.6	2.7

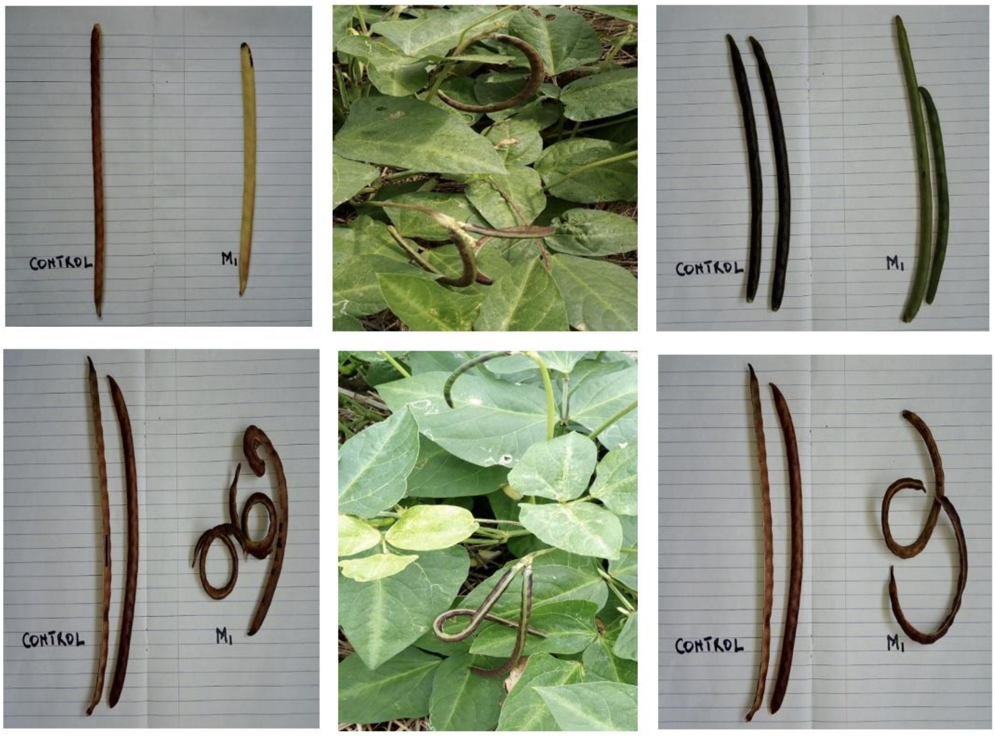

Fig. 5. Effect of gamma rays on pod architecture as shown by M1 mutants.

Discussion

This study investigated the responses of five cowpea genotypes to twelve gamma radiation doses, including a control (non-irradiated), to determine the LD_{50} and RD_{50} values for use in mass irradiation experiments. The observation that seedling survival was minimal at radiation doses of 1000 Gy and higher indicated that high doses of mutagens could inhibit seed germination across genotypes. Increased radiation doses were associated with more pronounced adverse effects on plant height and other traits. The LD_{50} and RD_{50} values increased similarly, though LD_{50} values were generally higher. Hansadua was the most sensitive among the genotypes, while ACC122WxWC-10 demonstrated the highest tolerance (Figures 1 and 2). Hansadua exhibited the most significant reduction in plant height at minimal radiation doses, whereas ACC122WxWC-10 was the least affected. These findings align with those of Bhagwat and Duncan (1998), who explored mutation breeding in bananas (*Musa* spp., AAA Group) using chemical mutagens to induce Fusarium wilt resistance.

Plant species, genera, and, to a lesser extent, genotypes and varieties exhibit varying levels of radiosensitivity due to differences in genetic, physiological, morphological, and other biological factors, such as ontogeny. Environmental conditions like oxygen and water content also play a crucial role in modulating seed and plant responses to ionizing radiation and chemical mutagens. Also, seed texture, coat color, and testa size influence the effects of mutagens on plant propagules. Olasupo *et al.* (2016) observed that cowpea accessions with rough testa surfaces and thin testas were more radiosensitive to gamma irradiation. In contrast, those with smooth testas were more tolerant, showing higher seed germination and survival rates. Seed weight also affects radiosensitivity, with lighter seeds being more radiosensitive than heavier ones.

Germination percentage exhibited an exponential increase, reaching 64.31% at 900 Gy, possibly due to mutagen dose saturation, before declining to 23.94% at 1200 Gy (Figure 3). This suggests that germination is still possible at 1200 Gy in cowpea. The mixed responses of cowpea genotypes to irradiation underscore the need for independent radiosensitivity testing before mass irradiation. Gamma-ray exposure at 300 Gy had the most pronounced effect on mean germination across the five cowpea genotypes, indicating that this dose is sufficient to induce mutations in cowpea. This observation corroborates the findings of Girija and Dhanavel (2009), who reported that 300 Gy effectively produced a high frequency of mutants in cowpeas, including chlorina and xanthan types.

The study demonstrated a consistent decline in seed germination, plant height, root length, shoot weight, and overall plant biomass across all cowpea genotypes as gamma radiation doses increased (Table 2). This pattern aligns with findings by Manju and Gopimony (2009), who proposed that reductions in plant survival reflect post-germination mortality driven by cytological and physiological disturbances caused by ionizing radiation. Similar observations were reported in rice (*Oryza sativa*) varieties, where plant height and growth decreased significantly with exposure to radiation doses up to 600 Gy (Harding *et al.*, 2012). These observations can be attributed to the destruction of the growth hormone auxin, as suggested by Sparrow and Evans (1961), likely resulting from ionizing radiation-induced genetic damage, including chromosomal aberrations (Horn, 2016). This inverse relationship between gamma-ray doses and germination percentage has been corroborated by multiple studies in various species, including fenugreek (*Trigonella foenum-graecum*) (Bashir *et al.*, 2013) and *Moluccella laevis* (Minisi *et al.*, 2013), where higher doses of gamma radiation consistently reduced germination rates and plant survival.

Gamma radiation doses between 0 and 200 Gy produced similar effects on plant height across genotypes, but doses above 200 Gy led to significant reductions in plant height, corroborating findings by Songsri *et al.* (2019) in *Jatropha curcas*. For doses below 100 Gy, there were no significant changes in root length, shoot weight, or whole plant biomass in cowpea. However, high doses of radiation have been associated with toxicity, as described by Mudibu *et al.* (2012), which leads to adverse effects such as chromosomal aberrations, lethality, reduced fertility, and developmental anomalies like chlorophyll-deficient chimeras. These findings align with reports from Verma *et al.* (2017), which further confirmed that higher radiation doses result in substantial reductions in plant growth parameters, survival rates, and reproductive success due to genotoxic effects and cytological disruptions.

For optimal mutant induction in crop improvement programmes, Spencer-Lopes *et al.* (2018) recommended that irradiation levels be maintained within ±20% of the experimentally determined optimal dose. Owoseni *et al.* (2007) and Mba *et al.* (2010) suggested a narrower range of ±5 units for achieving desired mutagenic outcomes. To maximize the effectiveness of radiation-induced mutagenesis, plant breeders must also consider factors beyond radiation doses, such as the survival and reproductive capacity of M_1 plants, to ensure viable seed production at maturity. Determining appropriate dose ranges for inducing beneficial mutations remains crucial for advancing crop improvement initiatives (Ahloowalia *et al.*, 2004; Jain, 2010).

Conclusion

The study confirmed that varying doses of gamma radiation had a considerable impact on different parts of the cowpea plant. Gamma rays differentially affected germination, plant height, root length, shoot weight and whole plant weight. The LD_{50} and RD_{50} of cowpea differ with the genotypes, and Hansadua had 531 Gy followed by WC-10 with 705, IT97K-819 with 762, WC-36 with 858.7 and ACC122WxWC-10 with 903 Gy in increasing order of LD_{50} values. The same order of genotypes had increasing RD_{50} values of 452, 590.5, 591, 662 and 694 Gy, respectively. Hansadua had the least LD_{50} and RD_{50} values of 531.0 and 452.0 Gy, respectively, thus indicating that it was most sensitive to gamma radiation because a minimal dose was required to kill half of the population. Also, the highest values of LD_{50} and RD_{50} were observed for ACC122WxWC-10 at 903.0 and 694.0 Gy, respectively. This implies that the ACC122WxWC-10 genotype was least sensitive to gamma radiation because higher doses of irradiation were needed to reduce the growth of the control population to half. The experimentally selected dose of gamma radiation may help as a dose to induce mass mutagenesis in cowpeas for breeding studies.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of interest

The authors declare no conflict of interest.

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