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ESTABLISHING LETHAL AND GROWTH REDUCTION DOSES OF FAST NEUTRON IRRADIATED COWPEA [*VIGNA UNGUICULATA* (L.) WALP.]

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ABSTRACT

Mutation breeding using physical mutagens such as fast neutron irradiation offers a promising approach for creating genetic variability in cowpea (*Vigna unguiculata* L. Walp.), an important staple legume in sub-Saharan Africa. However, determining optimal mutagen doses that balance mutation induction with plant survival is critical for successful breeding programs. This study was conducted to establish the median lethal dose (LD_{50}) and median growth reduction dose (GR_{50}) of fast neutron irradiation in two cowpea varieties, SAMPEA 21 and SAMPEA 20T. Uniform seeds of both varieties were exposed to fast neutron irradiation at the Center for Energy and research Training (CERT) for varying durations corresponding to doses ranging from 0.00 to 16.40 µSv. The M_1 generation was raised using a randomized complete block design with two replications. Data collected on germination percentage, survival percentage, shoot length, stem girth, number of leaves, leaf area, and number of branches were subjected to probit regression analysis and dose-response modeling. Results revealed a progressive, dose-dependent decline in germination, survival, and all growth parameters with increasing irradiation doses. SAMPEA 21 exhibited greater radiosensitivity, with LD_{50} and GR_{50} values consistently lower than those of SAMPEA 20T. The LD_{50} was estimated at 1.70 µSv for SAMPEA 21 and 5.38 µSv for SAMPEA 20T. Growth reduction doses (GR_{50}) for shoot length were 1.65 µSv and 4.85 µSv for SAMPEA 21 and SAMPEA 20T, respectively. Number of branches and leaf area were the most sensitive growth parameters, while shoot length showed moderate tolerance. The regression models exhibited high coefficients of determination (R^2 = 0.5713–0.9753), indicating strong dose-response

relationships. The established LD_{50} and GR_{50} values provide essential benchmarks for optimizing mutation breeding protocols in cowpea. The differential radiosensitivity between varieties underscores the need for genotype-specific dose optimization. These findings contribute to the efficient induction of beneficial mutations while minimizing plant mortality and growth impairment, thereby enhancing the effectiveness of cowpea improvement programs.

Keywords: Cowpea, fast neutron, growth reduction dose, lethal dose, mutation breeding, radiosensitivity

INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] Is a diploid leguminous crop (2n = 2x = 22) belonging to the family Fabaceae, sub-family Faboideae, tribe Phaseoleae, and genus Vigna (Herniter *et al*., 2019; Timko and Singh, 2008). Africa is widely considered the center of origin for cowpea, with wild relatives found predominantly in Madagascar and tropical Africa (Steele, 1976). The crop is cultivated across tropical and subtropical regions, with Nigeria being the world's largest producer, contributing approximately 3.58 million metric tons from 4.3 million hectares (FAOSTAT, 2023). Cowpea plays a vital role in the livelihoods of millions, particularly in marginal environments, due to its high adaptability to heat, drought, and salinity stresses (Davis *et al*., 2007).

The nutritional significance of cowpea cannot be overstated. Its grains are rich in protein (approximately 25%), carbohydrates, dietary fiber, vitamins, antioxidants, polyphenols, and polyunsaturated fatty acids (Ngoma *et al*., 2018; Ketema *et al*., 2020; Nkomo *et al*., 2021). Cowpea complements cereal-based diets by providing essential amino acids such as lysine and tryptophan, which are often deficient in cereals (Okwu and Njoku, 2009). Agronomically, the crop contributes to soil fertility through biological nitrogen fixation, forming symbiotic relationships with Bradyrhizobium spp. and fixing between 140–240 kg of nitrogen per hectare annually (Enyiukwu and Awurum, 2013; Heuza *et al*., 2015).

Despite its importance, cowpea productivity remains constrained by several factors, including narrow genetic variability, low yielding potential of existing genotypes, susceptibility to insect pests such as the legume pod borer (Maruca vitrata) and bruchid beetles (Callosobruchus spp.), and vulnerability to diseases including blight, root rot, and viral infections (Raina *et al*., 2020; Addass *et al*., 2011; Chikwendu *et al*., 2014). The consistent use of conventional breeding approaches has reduced genetic variability, which is the main prerequisite for crop improvement programs (Holme *et al*., 2019). Therefore, new breeding techniques such as induced mutagenesis are required to accomplish the goals of increased genetic variability.

Mutation breeding is a powerful strategy for inducing heritable genetic changes that can be harnessed to improve crop plants (Ali and Suryakant, 2024; Raina *et al*., 2016). Induced mutagenesis offers an opportunity to improve a single character without altering the entire genetic constitution. Up to 2022, 3,402 mutant varieties, including 468 legumes, have been developed worldwide; however, only 16 cowpea mutant varieties with improved agronomic traits have been officially released (IAEA, 2022). This highlights the potential for further exploitation of mutation breeding in cowpea improvement. (Shu *et al*., 2012; Maghuly *et al*., 2018)

Fast neutron irradiation, a form of physical mutagenesis with high linear energy transfer (LET), induces mutations by breaking DNA strands and causing chromosomal aberrations (Zhang *et al*., 2002). Unlike low-LET radiation such as gamma rays, fast neutrons cause more complex DNA damage, including double-strand breaks, deletions, and translocations, which can result in novel genetic variations (Hwang *et al*., 2014; Viana *et al*., 2019). Fast neutron irradiation has been used effectively to generate mutants with desirable traits in various crops, including rice (Li *et al*., 2023), soybean (Wu *et al*., 2022), wheat (Zhang *et al*., 2021), sorghum (Musa *et al*., 2022), and cowpea (Ibrahim *et al*., 2024; Ahmed *et al*., 2023).

Determining appropriate mutagen doses is critical for achieving effective mutagenesis while minimizing adverse effects on plant growth and survival. The median lethal dose (LD_{50}), defined as the radiation dose that results in 50% mortality or reduction in germination, and the median growth reduction dose (GR_{50}), defined as the dose that causes 50% reduction in growth parameters, provide valuable information on the sensitivity of crop varieties to mutagenic treatments (Chavan and Desai, 2018). Such data guide the selection of optimal mutagen levels that maximize mutation induction while maintaining plant viability, thereby enhancing the efficiency of mutation breeding programs. The LD_{50} and GR_{50} are particularly important in mutation breeding because they help researchers select sub-lethal doses that induce maximum genetic variability with minimal biological damage (Oladosu *et al*., 2016). Given the limited research on fast neutron-induced mutagenesis in cowpea, especially involving multiple varieties, this study was designed to establish the LD_{50} and GR_{50} of fast neutron irradiation in two improved cowpea varieties. The specific objectives were: (1) to determine the LD_{50} (median lethal dose) of fast neutron on cowpea, and (2) to determine the GR_{50} (median growth reduction dose) of fast neutron that causes reduction in growth parameters of cowpea. The findings are expected to provide essential baseline information for optimizing mutation breeding protocols in cowpea, ultimately contributing to the development of improved varieties with superior agronomic performance (Oladosu *et al*., 2016).

MATERIALS AND METHODS

Study Area

The research was conducted at the Zoological Garden of the Department of Biological Sciences, Kaduna State University, Kaduna (10°20′N and 7°45′E) in the northern Guinea savanna agro-ecological zone of Nigeria. The area has a mean annual rainfall of approximately 1,173.47 mm for Kaduna town specifically, with a spread from 859 mm to 1,530 mm.

Source of Cowpea Varieties

Uniform and healthy seeds of two cowpea varieties, SAMPEA 20T and SAMPEA 21, were obtained from the Institute for Agricultural Research (IAR), Samaru, Ahmadu Bello University, Zaria, and Kaduna State. SAMPEA 20T is a variety released in 2019 with resistance to the legume pod borer, early maturity (65–75 days), and a potential yield of 2.9 t/ha. SAMPEA 21 is a variety released in 2022 with resistance to Striga and bacterial blight, medium maturity (70–85 days), and a potential yield of 2.0 t/ha.

Treatment of Seeds with Fast Neutron

Healthy, dry, and uniform seeds of the two varieties were grouped into batches of 50 seeds and irradiated using fast neutron for durations of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 seconds. The irradiations were carried out at the Centre for Energy Research and Training (CERT), Ahmadu Bello University, Zaria, in 2025. Fast neutron irradiation was conducted under controlled reactor conditions, and neutron dosimetry was carried out to confirm the delivered fluence per treatment level (Yahaya *et al*., 2023; Ibrahim *et al*., 2024). The corresponding doses in microSieverts (µSv) for SAMPEA 21 were 0.00, 0.85, 0.99, 1.36, 1.50, 1.82, 2.46, 3.08, 7.82, 7.90, and 11.10 µSv. For SAMPEA 20T, the doses were 0.00, 0.70, 1.02, 1.84, 6.73, 10.60, 11.20, 11.87, 12.00, 13.20, and 16.40 µSv. Control seeds remained untreated with fast neutrons to serve as baseline (Raina *et al*., 2022; Yahaya *et al*., 2023).

Experimental Design and Procedures

Raising the First Mutant (M_1) Generation

The treated seeds and the control of each variety were sown on 3 m row plots at a distance of 75 cm inter-row and 30 cm intra-row spacing to raise the M_1 generation. The experiment was set out in a Randomized Complete Block Design (RCBD) with two replications. Each replication consisted of 11 plots per replication and 22 plots per variety. Recommended agricultural practices such as weeding and plant protection methods were carried out during the growth of the crop.

Data Collection

M_1 Evaluation

Data were collected on a plot basis on the M_1 plants for the following traits:

Seed germination

Seed germination of each replication was recorded on the 18th day after sowing, and the average was recorded as expressed below:

Seed germination (%) = (Number of germinated seeds / Total seeds sown) × 100

Seedling survival

This was observed after six weeks of seedling emergence, and percentage survival was calculated as follows:

Seedling survival (%) = (Number of surviving plants / Number of germinated seeds) × 100

The following data were also collected on the M_1 plants to estimate growth reduction:

Shoot length (cm)

This was the height measured from the base up to the apex, and the mean of five randomly selected plants was recorded.

Stem girth (cm)

This was the circumference measured using a measuring tape, and the mean of five randomly selected plants was recorded.

Number of leaves

This was the total number of leaves per plant counted, and the mean of five randomly selected plants was recorded.

Leaf area (cm^2)

Leaf length and leaf breadth per plant were measured, and the mean of five randomly selected plants was recorded. Leaf area was obtained by multiplying leaf length and leaf breadth.

Branches per plant (number)

This was the total number of branches per plant counted, and the mean of five randomly selected plants was recorded.

2.6 Data Analysis

2.6.1 Analysis of Variance

Data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 9.13 (SAS Institute, 2011). Mean separation was done using Duncan Multiple Range Test (DMRT) at a 5% level of probability.

Determination of Lethal Doses (LD_{50})

The lethal dose (LD_{50}) was determined as the radiation dose that resulted in a 50% reduction in seed germination. A dose-response curve was plotted with radiation dose on the x-axis and survival percentage on the y-axis. Probit regression analysis was used to determine the LD_{50} dose from the germination data. The LD_{25} (dose causing 25% mortality) and LD_{75} (dose causing 75% mortality) were also estimated using the same regression equation (Raina *et al*., 2022).

Determination of Growth Reduction Doses (GR_{50})

Dose-response curves were plotted to illustrate the relationship between radiation dose and growth reduction, and the GR_{50} value was estimated using appropriate statistical and regression analysis methods. Linear regression equations were generated for each growth parameter, and the GR_{25}, GR_{50}, and GR_{75} values were estimated from the regression equations (Chavan and Desai, 2018). The coefficient of determination (R^2) was computed to assess the goodness of fit of the regression models.

RESULTS AND DISCUSSION

Effect of Fast Neutron Irradiation on Germination and Survival

The germination and survival percentages of SAMPEA 21 and SAMPEA 20T exposed to different fast neutron irradiation doses are presented in Tables 1 and 2, respectively. For SAMPEA 21, germination percentage values ranged from 12.50% to 93.75%. The highest germination percentage was observed in the control (0.00 µSv) at 93.75%, followed by 0.85 µSv (81.25%), while the lowest germination

percentage was observed at 11.10 µSv (12.50%). Survival percentage values ranged from 0.00% to 98.57%. The highest survival percentage was observed in the control (98.57%), while no survival was recorded at doses of 7.82 µSv, 7.90 µSv, and 11.10 µSv. Survival over control ranged from 0% to 100%, with survival reduction over control increasing progressively from 40.58% at 0.85 µSv to 100% at doses ≥ 7.82 µSv.

SAMPEA 20T, germination percentage values ranged from 0.00% to 100.00%. The highest germination percentage was observed in the control (100.00%), followed by 0.70 µSv (81.25%), while no germination was observed at 16.40 µSv. Survival percentage values ranged from 0.00% to 95.00%. The highest survival was observed in the control (95.00%), followed by 1.02 µSv (64.00%), while no survival was recorded at doses of 12.00 µSv, 13.20 µSv, and 16.40 µSv. Survival over control ranged from 0.00% to 100.00%, with survival reduction over control ranging from 35.07% at 1.02 µSv to 100% at doses ≥ 12.00 µSv.

Lethal Dose of Fast Neutron Irradiation of Cowpea

The mean lethal dose (LD_{50}) of SAMPEA 21 irradiated with fast neutron from this study was estimated from the resulting regression equation of the percentage survival rate (Figure 4.1), the same regression equation was used to determine a step below (LD_{25}), a step above (LD_{75}) and the mean lethal dose (LD_{50}). For the SAMPEA 21 variety, the established lethal dose values were -1.67 µSV for LD_{25}, 1.70 µSV for LD_{50}, and 4.91 µSV for LD_{75}, respectively.

The mean lethal dose (LD_{50}) of SAMPEA 20T irradiated with fast neutron from this study was estimated from the resulting regression equation of the percentage survival rate (Figure 4.2), the same regression equation was used to determine a step below (LD_{25}), a step above (LD_{75}) and the mean lethal dose (LD_{50}). For the SAMPEA 20T variety, the established lethal dose values were 0.63 µSV for LD_{25} 5.38 µSV for LD_{50}, and 10.13 µSV for LD_{75}, respectively.

Effects of Fast Neutron Radiation on Growth Parameters of Cowpea

Effect of fast neutron irradiation on various quantitative traits of SAMPEA 21 variety of cowpea are presented in Table 4.3. Shoot length showed significant differences among the doses and ranged from 10.12–24.80 cm. The highest shoot length was recorded at 0.00 µSV (24.80 cm), followed by 0.85 µSV (20.05 cm), while the lowest shoot length was recorded at 1.82 µSV (10.12 cm). Stem girth showed significant differences among the doses and ranged from 1.27–3.68 cm. The highest stem girth was observed at 0.00 µSV (3.68 cm), followed by 0.85 µSV (2.85 cm), while the lowest stem girth was

recorded at 1.82 µSV (1.27 cm). The number of leaves showed significant differences among the doses and ranged from 12.00–26.50. The highest number of leaves was observed at 0.00 µSV (26.50), followed by 0.85 µSV (18.50), while the lowest number of leaves was recorded at 1.82 µSV (12.00). Leaf area showed significant differences among the doses and ranged from 12.37–27.05 cm². The highest leaf area was recorded at 0.00 µSV (27.05 cm²), followed by 0.85 µSV (20.60 cm²), while the lowest leaf area was observed at 1.82 µSV (12.37 cm²). The number of branches showed significant differences among the doses and ranged from 3.00–12.75. The highest number of branches was observed at 0.00 µSV (12.75), followed by 0.85 µSV (7.75), while the lowest number of branches was recorded at 1.82 µSV (3.00).

Effect of fast neutron irradiation on various quantitative traits of SAMPEA 20T variety of cowpea are presented in Table 4.4. Shoot length showed significant differences among the doses and ranged from 6.75–26.00 cm. The highest shoot length was observed at 0.00 µSV (26.00 cm), followed by 0.70 µSV (18.00 cm), while the lowest shoot length was observed at 10.60 µSV (6.75 cm). Stem girth showed significant differences among the doses and ranged from 1.20–2.50 cm.

The highest stem girth was recorded at 0.00 µSV (2.50 cm), followed by 0.70 µSV (1.85 cm), while the lowest stem girth was observed at 10.60 µSV (1.20 cm). Number of leaves showed significant differences among the doses and ranged from 12.08–25.00. The highest number of leaves was observed at 0.00 µSV (25.00), followed by 0.70 µSV (18.50), while the lowest number of leaves was observed at 10.60 µSV (12.08). Leaf area showed significant differences among the doses and ranged from 7.90–26.00 cm². The highest leaf area was observed at 0.00 µSV (26.00 cm²), followed by 0.70 µSV (18.90 cm²), while the lowest leaf area was observed at 10.60 µSV (7.90 cm²). Number of branches showed significant differences among the doses and ranged from 5.00–11.00. The highest number of branches was observed at 0.00 µSV (11.00), followed by 0.70 µSV (10.00), while the lowest number of branches was observed at 10.60 µSV (5.00).

Determination of Growth Reduction Doses (GR_{50})

The mean growth reduction dose (GR_{50}) of SAMPEA 21 was estimated from the resulting regression equations of the growth parameters measured (Table 5). The regression equations and coefficients of determination (R^2) for each parameter showed strong dose-response relationships. For shoot length, the regression equation was $y = -8.1134x + 25.82$, with $R^2 = 0.9478$. The GR_{25}, GR_{50}, and GR_{75} values for shoot length were 0.89 µSv, 1.65 µSv, and 2.42 µSv, respectively. For stem girth, the regression equation was $y = -1.2292x + 3.8141$, with $R^2 = 0.9477$, and the GR values were 0.86 µSv (GR_{25}), 1.61

µSv (GR_{50}), and 2.35 µSv (GR_{75}). For number of leaves, the regression equation was y = -8.2879x + 25.761, with R^2 = 0.9720, and the GR values were 0.86 µSv (GR_{25}), 1.61 µSv (GR_{50}), and 2.35 µSv (GR_{75}). For leaf area, the regression equation was y = -7.5872x + 27.365, with R^2 = 0.9342, and the GR values were 0.93 µSv (GR_{25}), 1.82 µSv (GR_{50}), and 2.72 µSv (GR_{75}). For number of branches, the regression equation was y = -5.0603x + 12.449, with R^2 = 0.9753, and the GR values were 1.20 µSv (GR_{25}), 1.20 µSv (GR_{50}), and 1.83 µSv (GR_{75}).

For SAMPEA 20T (Table 6), the regression equations showed moderate to strong dose-response relationships. For shoot length, the regression equation was y = -1.4035x + 19.803, with R^2 = 0.7448, and the GR values were 0.22 µSv (GR_{25}), 4.85 µSv (GR_{50}), and 9.48 µSv (GR_{75}). For stem girth, the regression equation was y = -0.0661x + 1.9747, with R^2 = 0.6003, and the GR values were 1.16 µSv (GR_{25}), 8.42 µSv (GR_{50}), and 15.68 µSv (GR_{75}). For number of leaves, the regression equation was y = -0.8248x + 19.626, with R^2 = 0.5713, and the GR values were 1.06 µSv (GR_{25}), 8.64 µSv (GR_{50}), and 16.22 µSv (GR_{75}). For leaf area, the regression equation was y = -1.272x + 20.065, with R^2 = 0.7119, and the GR values were 0.44 µSv (GR_{25}), 5.55 µSv (GR_{50}), and 10.66 µSv (GR_{75}). For number of branches, the regression equation was y = -0.4836x + 9.6006, with R^2 = 0.7723, and the GR values were 2.79 µSv (GR_{25}), 8.48 µSv (GR_{50}), and 14.17 µSv (GR_{75}).

The findings of this study clearly demonstrated that fast neutron irradiation exerted a pronounced, dose-dependent effect on germination, plant survival, and early vegetative growth of both cowpea varieties. As irradiation dose increased, there was a consistent decline in germination percentage, survival rate, and all measured quantitative growth traits. This trend aligns strongly with established principles of radiation biology, which explain that high-linear energy transfer (LET) radiation such as fast neutrons causes complex and often irreparable DNA damage, including chromosomal breaks, deletions, and translocations, leading to impaired mitosis, metabolic dysfunction, and reduced plant viability (Ahloowalia *et al*., 2022; Kharkwal and Shu, 2023). The high germination and survival percentages observed in the control treatments of both varieties confirm the inherent genetic viability of the seeds and validate the experimental conditions. Therefore, the observed reductions in irradiated treatments can be attributed primarily to the biological effects of fast neutron exposure rather than environmental or handling factors.

Similar dose-dependent reductions in germination and survival following neutron or gamma irradiation have been widely reported in cowpea, soybean, rice, and other legumes, reinforcing the reliability of the present findings (Bado *et al*., 2022; Raina *et al*., 2024). For instance, Oladosu *et al*. (2016) reported comparable reductions in germination and seedling survival in gamma-irradiated rice, while Singh *et al*. (2021) observed dose-dependent decreases in germination and early growth in neutron-irradiated chickpea. These consistent patterns across different crops and radiation types underscore the universal biological effects of ionizing radiation on seed viability and early plant development.

A comparative assessment of the two varieties revealed that SAMPEA 21 was more sensitive to fast neutron irradiation than SAMPEA 20T. This was clearly reflected in the lower LD_{50} range recorded for SAMPEA 21 (1.70 µSv) compared with the higher LD_{50} range observed for SAMPEA 20T (5.38 µSv). Such varietal differences in radiation sensitivity are well documented in mutation breeding studies and are often attributed to genotypic variation in DNA repair efficiency, antioxidant enzyme activity, cell cycle regulation, and overall physiological resilience (Horn *et al*., 2023; Oladosu *et al*., 2022). The relatively higher tolerance of SAMPEA 20T suggests that this variety possesses more effective mechanisms for mitigating radiation-induced oxidative stress and repairing DNA lesions. Ionizing radiation is known to generate reactive oxygen species (ROS) that damage cellular membranes, proteins, and nucleic acids. Varieties with stronger antioxidant defense systems, including higher activities of superoxide dismutase, catalase, and peroxidase, are therefore better equipped to survive and recover from radiation stress (Kumari *et al*., 2023). Similar genotypic differences in tolerance have been reported in cowpea and other legumes exposed to physical mutagens, where tolerant genotypes maintained higher survival and growth rates at equivalent doses (Raina *et al*., 2024).

The LD_{50} values established in this study provide essential benchmarks for mutation breeding in cowpea. The LD_{50} is particularly important because it helps researchers' select sub-lethal doses that maximize mutation induction while maintaining sufficient plant survival for the recovery of useful mutants (Oladosu *et al*., 2016). The LD_{50} of 1.70 µSv for SAMPEA 21 and 5.38 µSv for SAMPEA 20T fall within the range of values reported for other legume species. For instance, Bado *et al*. (2022) reported LD_{50} values ranging from 150–300 Gy for gamma irradiation in various cowpea varieties, while Raina *et al*. (2024) reported LD_{50} values between 200–400 Gy for gamma rays in chickpea. The relatively lower doses required for fast neutron irradiation compared to gamma rays reflect the higher biological effectiveness of fast neutrons due to their higher LET (Viana *et al*., 2019).

The significant reductions observed in shoot length, stem girth, number of leaves, leaf area, and number of branches with increasing fast neutron doses indicate that irradiation adversely affected overall plant vigor and architectural development. These growth parameters are critical indicators of vegetative performance and are closely linked to photosynthetic efficiency, assimilate production, and eventual yield potential. The progressive decline in shoot length and stem girth suggests that fast neutron irradiation interfered with cell division and elongation in meristematic tissues. Radiation-induced chromosomal aberrations often disrupt normal mitotic processes, resulting in reduced cell proliferation and stunted growth (Kharkwal and Shu, 2023). Reduced stem girth further implies impaired vascular tissue development, which can limit the translocation of water, nutrients, and photosynthates throughout the plant.

Similarly, the reduction in leaf number and leaf area observed at higher irradiation doses indicates compromised photosynthetic capacity. Leaves are the primary sites of photosynthesis, and any reduction in their number or surface area directly affects carbohydrate production and biomass accumulation (Taiz *et al*., 2015). Several studies have reported comparable reductions in leaf traits following neutron or gamma irradiation in cowpea, rice, and wheat, attributing these effects to damage to leaf primordia and disruption of hormonal balance, particularly auxin and gibberellin biosynthesis (Bado *et al*., 2022; Shu *et al*., 2022). The decline in the number of branches with increasing irradiation dose further highlights the sensitivity of axillary bud development to radiation stress. Branching is an important agronomic trait in cowpea, as it influences canopy structure, pod bearing capacity, and yield. Excessive reduction in branching at higher doses therefore suggests that such irradiation levels may not be desirable for mutation breeding aimed at yield improvement.

The linear regression analysis and growth reduction doses (GR_{25}, GR_{50}, and GR_{75}) provided quantitative estimates of mutagenic sensitivity in the two cowpea varieties. The wide range of GR values recorded across growth parameters indicates differential sensitivity among traits. Shoot length consistently recorded lower GR_{25} and GR_{50} values, suggesting that it is one of the most sensitive indicators of radiation-induced damage. Similar observations have been reported in cowpea and other legumes subjected to physical mutagens (Dhanavel *et al*., 2008; Roychowdhury *et al*., 2004). The high coefficients of determination ($R^2 = 0.9342–0.9753$) for SAMPEA 21 indicate that the regression models explained over 93% of the variation in growth parameters, confirming strong dose-response relationships. This high predictive power makes these models useful for estimating growth reduction at intermediate doses not directly tested in the experiment.

In SAMPEA 20T, the higher GR_{50} and GR_{75} values compared to SAMPEA 21 suggest a relatively higher tolerance to fast neutron irradiation. This difference may be attributed to inherent genetic buffering capacity, chromosomal stability, or differences in DNA repair mechanisms between the two varieties. Lancu and Soare (2012) reported that varietal differences in mutagen sensitivity are common and must be considered when designing mutation breeding programs. The moderate R^2 values observed in SAMPEA 20T (R^2 = 0.5713–0.7723) further indicate greater variability in response to irradiation, suggesting that growth reduction was not uniform across all traits. This variability is desirable in mutation breeding, as it increases the likelihood of recovering beneficial mutations in later generations.

The GR_{50} values for different growth parameters provide insights into the relative sensitivity of specific traits. In SAMPEA 21, the GR_{50} values ranged from 1.20 µSv (number of branches) to 1.82 µSv (leaf area), with shoot length (1.65 µSv), stem girth (1.61 µSv), and number of leaves (1.61 µSv) showing intermediate sensitivity. This ranking indicates that branching was the most sensitive trait, followed by stem girth and leaf number, while shoot length and leaf area showed relatively higher tolerance. In SAMPEA 20T, the GR_{50} values ranged from 4.85 µSv (shoot length) to 8.64 µSv (number of leaves), with leaf area (5.55 µSv), number of branches (8.48 µSv), and stem girth (8.42 µSv) showing intermediate values. The different rankings of trait sensitivity between the two varieties suggest that the genetic background influences the response of specific traits to radiation stress.

The differential sensitivity of growth parameters has practical implications for mutation breeding. Traits that are highly sensitive to irradiation (e.g., number of branches in SAMPEA 21) may require lower doses to avoid excessive growth reduction, while more tolerant traits (shoot length in SAMPEA 20T) may tolerate higher doses, allowing for greater mutation induction. Breeders should consider the specific traits they wish to improve when selecting irradiation doses. For example, if the goal is to improve branching and yield potential, lower doses closer to the GR_{25} for branching may be appropriate. Conversely, if the goal is to generate a wide range of mutations without severe growth reduction, doses around the GR_{50} for shoot length may be more suitable.

The comparison of LD_{50} and GR_{50} values reveals interesting relationships between lethality and growth reduction. In SAMPEA 21, the LD_{50} (1.70 µSv) was very close to the GR_{50} for most growth parameters (1.61–1.82 µSv), indicating that growth reduction and lethality occurred at similar dose levels. This suggests that in this variety, the doses that cause significant mortality also cause substantial growth reduction, leaving a narrow window for mutation induction. In contrast, in SAMPEA 20T, the LD_{50}

(5.38 µSv) was closer to the GR_{50} for shoot length (4.85 µSv) and leaf area (5.55 µSv) but much lower than the GR_{50} for stem girth (8.42 µSv), number of leaves (8.64 µSv), and number of branches (8.48 µSv). This indicates that in SAMPEA 20T, doses that cause 50% mortality may still allow 50% or more of the growth in certain traits, providing a wider window for mutation induction. The broader tolerance range in SAMPEA 20T makes this variety more suitable for mutation breeding using fast neutron irradiation, as higher doses can be used to induce more mutations while maintaining acceptable levels of growth and survival.

The established LD_{50} and GR_{50} values in this study provide essential benchmarks for optimizing mutation breeding protocols in cowpea. These values can guide breeders in selecting irradiation doses that balance mutation induction with plant survival and growth. The findings confirm that moderate doses of fast neutron irradiation are more suitable for mutation breeding aimed at cowpea improvement. The differential radiosensitivity between varieties underscores the need for genotype-specific dose optimization, rather than a one-size-fits-all approach. This is particularly important for breeding programs working with diverse germplasm, as different genotypes may respond differently to the same irradiation dose.

The results of this study contribute to the growing body of knowledge on radiation biology in crop plants and provide practical guidelines for mutation breeding in cowpea. The LD_{50} and GR_{50} values established here can be used to design efficient mutation breeding programs, reducing the time and resources spent on trial-and-error dose optimization. Furthermore, the high coefficients of determination for the regression models indicate that these equations can be used to predict growth reduction at doses not directly tested, enabling breeders to make informed decisions about dose selection for their specific breeding objectives.

Table 1. Germination and survival percentages of SAMPEA 21 exposed to different fast neutron irradiation doses

Dose (µSV)	Percentage germination (%)	Percentage survival (%)	Survival over control (%)	Survival reduction over control (%)
0.00	93.75[a]	98.57[a]	100.00	-
0.85	81.25[b]	58.57[b]	59.42	40.58
0.99	68.75[c]	58.00[b]	58.84	41.16
1.36	62.50[c]	56.00[bc]	56.81	43.19
1.50	62.50[c]	52.00[c]	52.75	47.25
1.82	50.00[d]	25.00[d]	25.36	74.64
2.46	50.00[d]	20.00[e]	20.29	79.71
3.08	50.00[d]	10.00[f]	10.15	89.85
7.82	31.25[e]	0.00[g]	0.00	100.00
7.90	25.00[e]	0.00[g]	0.00	100.00
11.1	12.50[f]	0.00[g]	0.00	100.00
Mean	53.41	34.38		
CV (%)	8.35	6.34		

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 2. Germination and plant survival percentages of SAMPEA 20T variety of cowpea exposed to different fast neutron irradiation doses

Dose (µSV)	Percentage germination (%)	Percentage survival (%)	Survival over control (%)	Survival reduction over control (%)
0.00	100.00[a]	95.00[a]	100.00	-
0.70	81.25[b]	58.57[b]	59.42	40.58
1.02	62.50[c]	64.00[bc]	64.93	35.07
1.84	62.50[c]	56.00[bc]	56.81	43.19
6.73	62.50[c]	52.00[c]	52.75	47.25
10.60	50.00[c]	25.00[d]	25.36	74.64
11.20	50.00[c]	20.00[d]	20.29	79.71
11.87	25.00[d]	20.00[d]	20.29	79.71
12.00	25.00[d]	0.00[e]	0.00	100.00
13.20	12.50[de]	0.00[e]	0.00	100.00
16.40	0.00[e]	0.00[e]	0.00	100.00
Mean	48.30	35.51		
CV (%)	11.84	11.61		

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

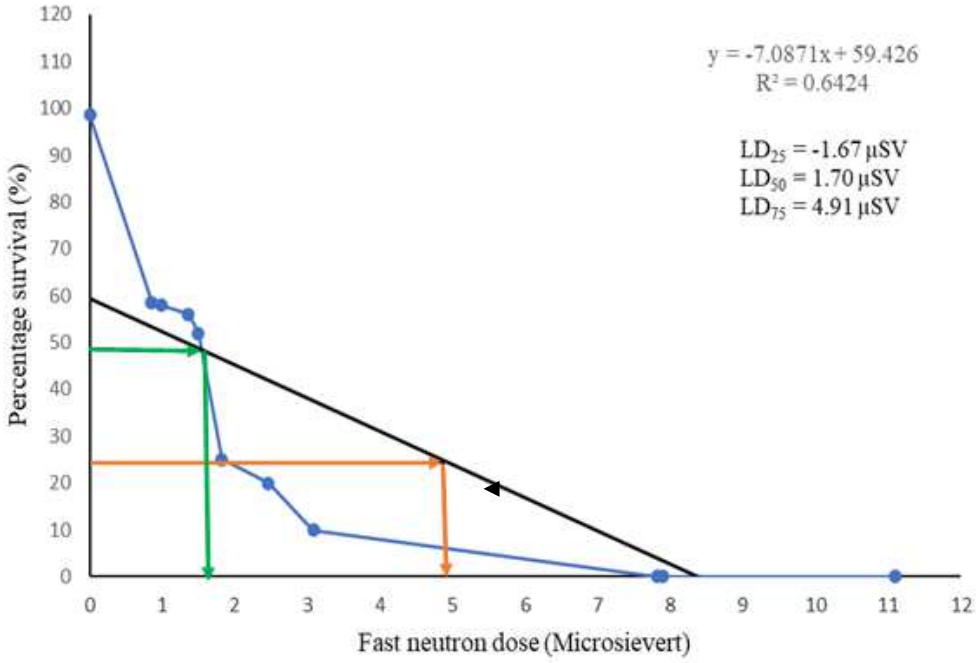


Figure 1. Lethal dose determination of SAMPEA 21 variety of cowpea irradiated with different doses of fast neutron irradiation

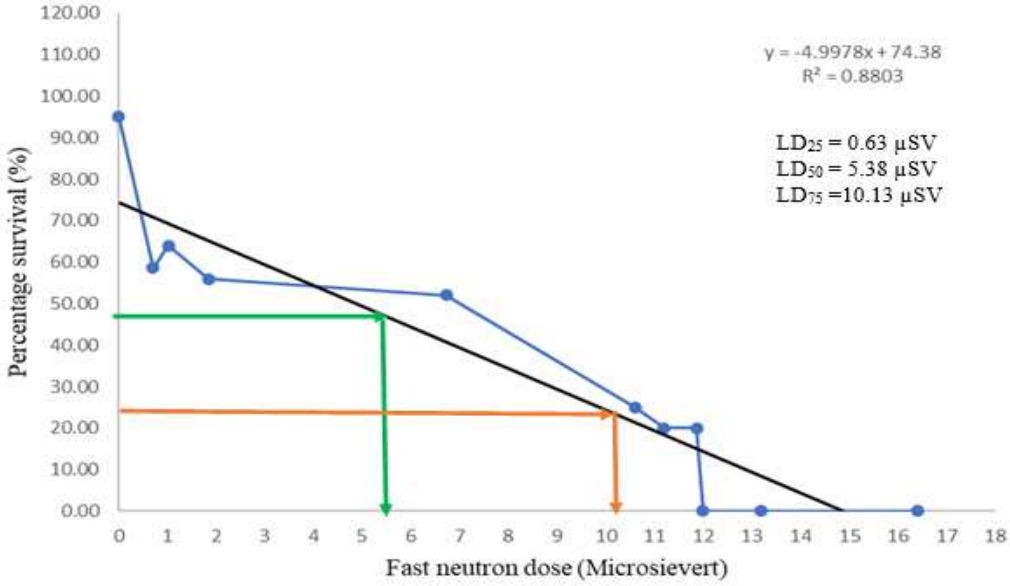


Figure 2. Lethal dose determination of SAMPEA 20T variety of cowpea irradiated with different doses of fast neutron irradiation

Table 3. Effect of fast neutron irradiation on various quantitative growth traits of SAMPEA 21 variety of cowpea

Dose (µSV)	Shoot length (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Number of branches
0.00	24.8[a]	3.68[a]	26.50[a]	27.05[a]	12.75[a]
0.85	20.05[ab]	2.85[b]	18.50[ab]	20.6[ab]	7.75[b]
0.99	18.35[b]	2.65[bc]	16.50[bc]	19.75[b]	6.75[bc]
1.36	16.3b[c]	2.31[cd]	14.45[cd]	19.55[b]	6.15[bc]
1.50	12.4[c]	2.11[d]	12.58[d]	15.4[c]	5.30[cd]
1.82	10.12[d]	1.27[e]	12.00[d]	12.37[d]	3.00[d]
Mean	17.00	2.48	16.75	19.12	6.95
CV	11.11	7.39	8.32	12.94	12.99

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 4 Effect of fast neutron irradiation on various quantitative traits of SAMPEA 20T variety of cowpea

Dose (µSV)	Shoot length (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Number of branches
0.00	26.00[a]	2.50[a]	25.00[a]	26.00[a]	11.00[a]
0.70	18.00[b]	1.85[ab]	18.50[b]	18.90[b]	10.00[ab]
1.02	15.50[c]	1.70[b]	16.50[bc]	16.32[bc]	8.50[bc]
1.84	14.50$_c$	1.50[b]	15.00[cd]	13.80[cd]	7.00[cd]
6.73	8.75[d]	1.30[b]	13.45[d]	10.90[de]	6.00[de]
10.60	6.75$_d$	1.20[b]	12.08[d]	7.90[e]	5.00[e]
Mean	14.92	1.68	16.75	15.64	7.92
CV	6.36	15.66	6.84	7.67	8.78

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 5 Linear regression equations, coefficients of determination (R^2) and growth reduction dose at 50% (GR_{50}) of SAMPEA 21

Parameter	Equation	R^2	LD_{25} (µSV)	LD_{50} (µSV)	LD_{75} (µSV)
Shoot length (cm)	y = -8.1134x + 25.82	0.9478	0.89	1.65	2.42
Stem girth (cm)	y = -1.2292x + 3.8141	0.9477	0.86	1.61	2.35
Number of leaves	y = -8.2879x + 25.761	0.972	0.86	1.61	2.35
Leaf area (cm^2)	y = -7.5872x + 27.365	0.9342	0.93	1.82	2.72
Number of branches	y = -5.0603x + 12.449	0.9753	1.20	1.20	1.83

KEYS: µSV: MicroSievert, LD_{50}: Lethal Dose 50%, R^2: Coefficient of Determination, cm: centimeters, GR_{50}: Growth Reduction 50

Table 6 Linear regression equations, coefficients of determination (R^2) and growth reduction dose at 50% (GR_{50}) of SAMPEA 20T

Parameter	Equation	R^2	LD_{25} (µSV)	LD_{50} (µSV)	LD_{75} (µSV)
Shoot length (cm)	y = -1.4035x + 19.803	0.7448	0.22	4.85	9.48
Stem girth (cm)	y = -0.0661x + 1.9747	0.6003	1.16	8.42	15.68
Number of leaves	y = -0.8248x + 19.626	0.5713	1.06	8.64	16.22
Leaf area (cm^2)	y = -1.272x + 20.065	0.7119	0.44	5.55	10.66
Number of branches	y = -0.4836x + 9.6006	0.7723	2.79	8.48	14.17

KEYS: µSV: MicroSievert**,** LD_{50}: (Lethal Dose 50%), R^2: Coefficient of Determination**,** CV (%): Coefficient of Variation**,** GCV (%): Genotypic Coefficient of Variation**,** (g): grams, (cm): centimeters, GR_{50}: Growth Reduction 50%

CONCLUSION

The results of this study clearly demonstrated that fast neutron irradiation caused significant dose-dependent reductions in germination, survival, and growth parameters of two cowpea varieties. It was established that the lethal dose (LD_{50}) values for the two cowpea varieties are 1.70 µSv for SAMPEA 21 and 5.38 µSv for SAMPEA 20T, indicating that SAMPEA 21 is more radiosensitive than SAMPEA 20T. The growth reduction dose (GR_{50}) analyses revealed differential sensitivity among the traits, with branching and leaf production being the most affected parameters, while shoot length showed relatively moderate tolerance. The GR_{50} values ranged from 1.20 to 1.82 µSv for SAMPEA 21 and from 4.85 to 8.64 µSv for SAMPEA 20T across different growth parameters. The regression models exhibited high coefficients of determination, indicating strong dose-response relationships. These findings confirm that moderate doses of fast neutron irradiation, particularly doses around the LD_{50} and GR_{50} values established in this study, are more suitable for mutation breeding aimed at cowpea improvement. The differential radiosensitivity between varieties underscores the need for genotype-specific dose optimization in mutation breeding programs.

RECOMMENDATIONS

1. Irradiation doses close to the LD_{50} and GR_{50} values established in this study should be prioritized in future mutation breeding programs, balancing mutation induction with plant survival and growth. For SAMPEA 21, doses between 1.50–1.82 µSv would be appropriate, while for SAMPEA 20T, doses between 5.00–8.50 µSv would be more suitable.
2. Subsequent generations (M_3 and beyond) should be evaluated to identify stable and heritable mutations that may contribute to improved agronomic traits such as yield, stress tolerance, and disease resistance.
3. Molecular and cytogenetic analyses should be incorporated in future studies to quantify mutation frequency and characterize induced genetic variations, providing deeper insights into the genetic basis of the observed phenotypic changes.
4. Additional cowpea genotypes should be included in future studies to broaden understanding of genotypic variation in mutagen sensitivity and support national cowpea breeding programs.
5. The regression equations generated in this study should be used to estimate growth reduction and optimize dose selection for cowpea mutation breeding, reducing the time and resources required for empirical dose optimization.

Declarations

Funding

Not applicable

Conflicts of interest/Competing interests

The authors declare that they have no competing interests. No financial or non-financial conflicts of interest exist that could influence the interpretation or presentation of the research findings reported in this manuscript.

Ethics approval

Not applicable. This study did not involve human participants, vertebrate animals, or any biological materials requiring ethical approval. The research was conducted on cowpea seeds (*Vigna unguiculata* L. Walp.), which are plant materials not subject to ethical review requirements.

Consent to participate

Not applicable. This study did not involve human participants; therefore, consent to participate is not required.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data, images, or identifiable information requiring consent for publication.

Availability of data and materials

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request. All data pertaining to germination percentages, survival rates, growth parameters (shoot length, stem girth, number of leaves, leaf area, and number of branches), and dose-response analyses for both cowpea varieties (SAMPEA 21 and SAMPEA 20T) are presented within the manuscript in tabular and graphical formats.

Code availability

Not applicable. No custom software applications or computational codes were developed or utilized for the analysis in this study. Statistical analyses were performed using standard statistical software (SAS version 9.13), and regression models were generated using conventional statistical procedures.

Authors' contributions

- **Ibrahim Ashiru**: Conceptualization, methodology, investigation, data curation, writing original draft preparation, project administration
- **Umar U. Uwais**: Methodology, formal analysis, investigation, writing review and editing

- **H. Ibrahim**: Investigation, data collection, validation, writing review and editing
- **Almustapha Zakari**: Investigation, data curation, validation, writing—review and editing
- **Abdullahi A. Dabo**: Investigation, resources, writing review and editing
- **Aminu Isyaku**: Investigation, methodology, validation, writing review and editing

All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Figure

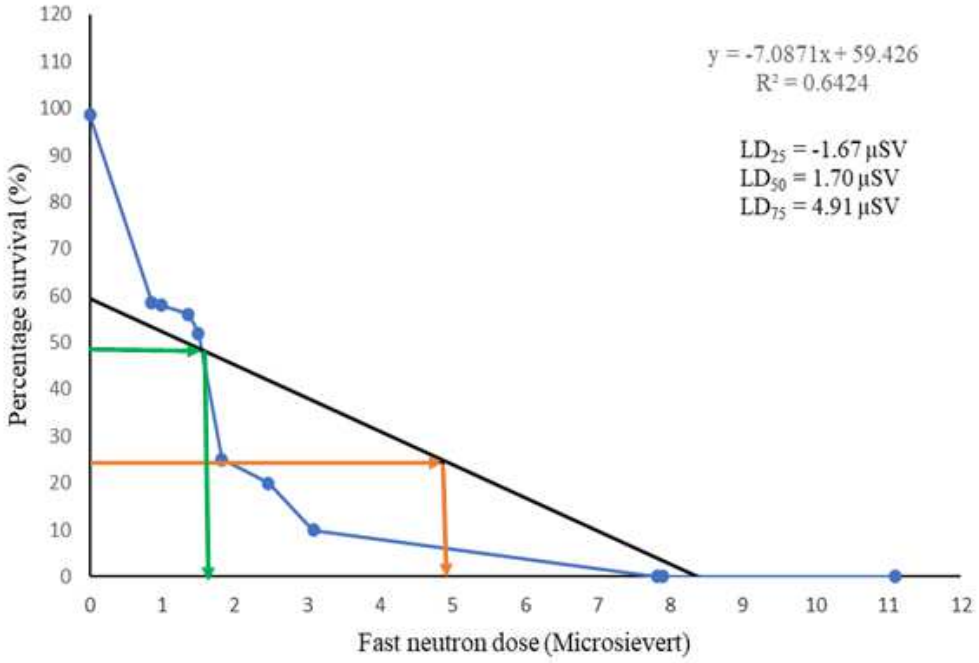


Figure 1. Lethal dose determination of SAMPEA 21 variety of cowpea irradiated with different doses of fast neutron irradiation

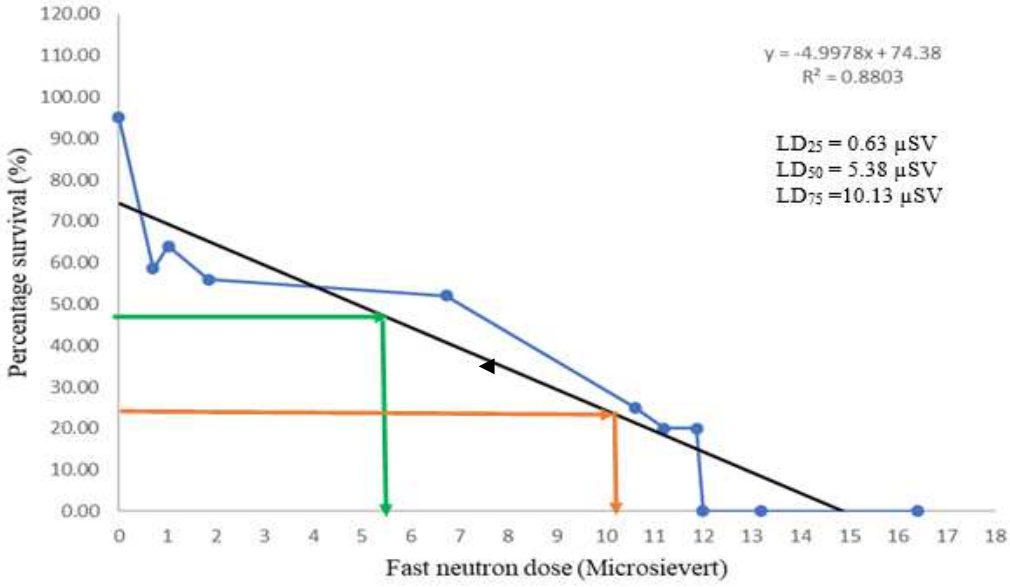


Figure 2. Lethal dose determination of SAMPEA 20T variety of cowpea irradiated with different doses of fast neutron irradiation

Table

Table 1. Germination and survival percentages of SAMPEA 21 exposed to different fast neutron irradiation doses

Dose (µSV)	Percentage germination (%)	Percentage survival (%)	Survival over control (%)	Survival reduction over control (%)
0.00	93.75[a]	98.57[a]	100.00	-
0.85	81.25[b]	58.57[b]	59.42	40.58
0.99	68.75[c]	58.00[b]	58.84	41.16
1.36	62.50[c]	56.00[bc]	56.81	43.19
1.50	62.50[c]	52.00[c]	52.75	47.25
1.82	50.00[d]	25.00[d]	25.36	74.64
2.46	50.00[d]	20.00[e]	20.29	79.71
3.08	50.00[d]	10.00[f]	10.15	89.85
7.82	31.25[e]	0.00[g]	0.00	100.00
7.90	25.00[e]	0.00[g]	0.00	100.00
11.1	12.50[f]	0.00[g]	0.00	100.00
Mean	53.41	34.38		
CV (%)	8.35	6.34		

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 2. Germination and plant survival percentages of SAMPEA 20T variety of cowpea exposed to different fast neutron irradiation doses

Dose (µSV)	Percentage germination (%)	Percentage survival (%)	Survival over control (%)	Survival reduction over control (%)
0.00	100.00[a]	95.00[a]	100.00	-
0.70	81.25[b]	58.57[b]	59.42	40.58
1.02	62.50[c]	64.00[bc]	64.93	35.07
1.84	62.50[c]	56.00[bc]	56.81	43.19
6.73	62.50[c]	52.00[c]	52.75	47.25
10.60	50.00[c]	25.00[d]	25.36	74.64
11.20	50.00[c]	20.00[d]	20.29	79.71
11.87	25.00[d]	20.00[d]	20.29	79.71
12.00	25.00[d]	0.00[e]	0.00	100.00
13.20	12.50[de]	0.00[e]	0.00	100.00
16.40	0.00[e]	0.00[e]	0.00	100.00
Mean	48.30	35.51		
CV (%)	11.84	11.61		

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 3. Effect of fast neutron irradiation on various quantitative growth traits of SAMPEA 21 variety of cowpea

Dose (µSV)	Shoot length (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Number of branches
0.00	24.8[a]	3.68[a]	26.50[a]	27.05[a]	12.75[a]
0.85	20.05[ab]	2.85[b]	18.50[ab]	20.6[ab]	7.75[b]
0.99	18.35[b]	2.65[bc]	16.50[bc]	19.75[b]	6.75[bc]
1.36	16.3b[c]	2.31[cd]	14.45[cd]	19.55[b]	6.15[bc]
1.50	12.4[c]	2.11[d]	12.58[d]	15.4[c]	5.30[cd]
1.82	10.12[d]	1.27[e]	12.00[d]	12.37[d]	3.00[d]
Mean	17.00	2.48	16.75	19.12	6.95
CV	11.11	7.39	8.32	12.94	12.99

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 4 Effect of fast neutron irradiation on various quantitative traits of SAMPEA 20T variety of cowpea

Dose (µSV)	Shoot length (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Number of branches
0.00	26.00^{a}	2.50^{a}	25.00^{a}	26.00^{a}	11.00^{a}
0.70	18.00^{b}	1.85^{ab}	18.50^{b}	18.90^{b}	10.00^{ab}
1.02	15.50^{c}	1.70^{b}	16.50^{bc}	16.32^{bc}	8.50^{bc}
1.84	14.50_{c}	1.50^{b}	15.00^{cd}	13.80^{cd}	7.00^{cd}
6.73	8.75^{d}	1.30^{b}	13.45^{d}	10.90^{de}	6.00^{de}
10.60	6.75_{d}	1.20^{b}	12.08^{d}	7.90^{e}	5.00^{e}
Mean	14.92	1.68	16.75	15.64	7.92
CV	6.36	15.66	6.84	7.67	8.78

Means with same letter(s) in a column are not significantly different according to Duncan Multiple Range Test (DMRT) at 5% level of probability

Table 5 Linear regression equations, coefficients of determination (R^2) and growth reduction dose at 50% (GR_{50}) of SAMPEA 21

Parameter	Equation	R^2	LD_{25} (µSV)	LD_{50} (µSV)	LD_{75} (µSV)
Shoot length (cm)	y = -8.1134x + 25.82	0.9478	0.89	1.65	2.42
Stem girth (cm)	y = -1.2292x + 3.8141	0.9477	0.86	1.61	2.35
Number of leaves	y = -8.2879x + 25.761	0.972	0.86	1.61	2.35
Leaf area (cm^2)	y = -7.5872x + 27.365	0.9342	0.93	1.82	2.72
Number of branches	y = -5.0603x + 12.449	0.9753	1.20	1.20	1.83

KEYS: µSV: MicroSievert, LD_{50}: Lethal Dose 50%, R^2: Coefficient of Determination, cm: centimeters, GR_{50}: Growth Reduction 50

Table 6 Linear regression equations, coefficients of determination (R^2) and growth reduction dose at 50% (GR_{50}) of SAMPEA 20T

Parameter	Equation	R^2	LD_{25} (µSV)	LD_{50} (µSV)	LD_{75} (µSV)
Shoot length (cm)	y = -1.4035x + 19.803	0.7448	0.22	4.85	9.48
Stem girth (cm)	y = -0.0661x + 1.9747	0.6003	1.16	8.42	15.68
Number of leaves	y = -0.8248x + 19.626	0.5713	1.06	8.64	16.22
Leaf area (cm^2)	y = -1.272x + 20.065	0.7119	0.44	5.55	10.66
Number of branches	y = -0.4836x + 9.6006	0.7723	2.79	8.48	14.17

KEYS: µSV: MicroSievert**,** LD_{50}: (Lethal Dose 50%), R^2: Coefficient of Determination**,** CV (%): Coefficient of Variation**,** GCV (%): Genotypic Coefficient of Variation**,** (g): grams, (cm): centimeters, GR_{50}: Growth Reduction 50%