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RADIO-SENSITIVITY OF SOYBEAN GENOTYPES TO LOW GAMMA IRRADIATION DOSES

RADIOSENSIBILITÉ DES GÉNOTYPES DE SOJA AUX FAIBLES DOSES D'IRRADIATION GAMMA

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ABSTRACT

To combat the erosion of biodiversity in cultivated plants, gamma-induced mutagenesis is emerging as a technique capable of generating unprecedented genomic diversity. The pending challenge with this technique is in determining the specific radiosensitivity appropriate for each genotype. Soybean (*Glycine max* (L.) Merrill) is one of the crops that can benefit from this innovation. The objective of this study was to establish the optimum radiosensitivity of soybean varieties that are recently developed at Mvuazi research centre. Four gamma irradiation doses (0, 10, 20 and 30 Gy) were applied on three soybean genotypes, Diamuini 2, Lutaladio and Luyindula, sourced from Mvuazi Resaerch centre. Results showed a significant ($P<0.05$) difference for variety x radiation dose interaction; with a slight decline in germination rate, indicating potential for physiological disturbances. The variety Diamuini 2 was the most responsive to gamma irradiation; while variety Lutaladio was the least responsive. Hypocotyl and epicotyl parameters revealed differential sensitivity among plant organs. Hypocotyl length exhibited a highly significant interaction for variety x irradiation dose, highlighting a strong genotype-dependent response. The highest hypocotyl elongation was observed in the Lutaladio variety at 10 Gy, suggesting an optimal stimulation effect at this dose. These findings confirm that low-dose gamma irradiation can induce useful variability in soybean and may serve as an effective tool for

mutation breeding. However, the identification of optimal irradiation doses and the consideration of genotype-specific responses remain critical for maximising beneficial effects; while minimising detrimental impacts.

Key Words: Epicotyl length, *Glycine max*, hypocotyl length, radio-sensitivity, soybean

RÉSUMÉ

Quatre doses d'irradiation gamma (0, 10, 20 et 30 Gy) étaient appliquées afin d'évaluer la radiosensibilité de trois génotypes de soja (*Glycine max* L.). Les résultats ont montré une diminution générale du taux de germination aux doses élevées d'irradiation, bien que certaines combinaisons variétales aient maintenu des taux de germination relativement élevés, suggérant un possible effet d'hormèse aux faibles doses. Le temps de germination n'a pas été significativement affecté par l'irradiation, bien qu'un léger retard ait été observé aux doses élevées, indiquant des potentielles perturbations physiologiques. Les paramètres de croissance ont révélé une sensibilité différentielle selon les organes. La longueur de l'épicotyle n'a pas été significativement influencée par les traitements, alors que la longueur de l'hypocotyle a présenté une interaction variété x dose d'irradiation hautement significative, soulignant une forte dépendance au génotype. L'élongation maximale de l'hypocotyle était observée chez la variété Lutaladio à 10 Gy, suggérant un effet de stimulation optimal à cette dose. La variété Luyindula a démontré une tolérance aux doses élevées d'irradiation, tandis que Lutaladio a montré des performances accrues aux faibles doses. Ces résultats confirment que l'irradiation gamma à faible dose peut induire une variabilité utile chez le soja et constituer un outil efficace pour la sélection par mutation. L'identification des doses d'irradiation optimales et les réponses spécifiques à chaque génotype demeurent essentielles pour maximiser les effets bénéfiques tout en minimisant les effets néfastes. Des études intégrant des évaluations moléculaires et au champ sont recommandées pour mieux exploiter la variabilité induite en amélioration du soja.

Mots Clés: longueur épicotyle, *Glycine max*, longueur hypocotyl, radiosensibilité, soja

INTRODUCTION

Soybeans (*Glycine max* L.), is a growing food security and commercial crop wildwide; particularly in sub-Saharan Africa (SSA), where it provides the richest low cost protein dietary ingredient (Kasongo and Lupungu, 2023). Unfortunately, the sustainability of soybean productivity is threatened by various environmental pressures, exacerbated by climate change (Mazinga *et al*., 2024; Martinez *et al.*, 2026).

To address these challenges, Martinez *et al.* (2021) and, Sierra and Tournebize (2022) advocate for the genetic improvement of legumes as a strategic lever. To combat the erosion of biodiversity in cultivated plants, gamma-induced mutagenesis is emerging as a technique capable of generating unprecedented genomic diversity (IAEA and FAO, 2021; Muduli *et al*., 2024). The problem with this technique lies in determining the specific radiosensitivity of each genotype. Research on the application of low doses of irradiation (generally less than 50 Gy) to legumes has overly focused on the phenomenon of hormesis, where low doses of irradiation act as biological catalysts (Fernani-Meliani *et al*., 2021). Unlike high doses, which induce lethal chromosomal breaks or growth inhibition, low doses stimulate physiological parameters, enhance enzymatic activity, and stimulate initial germination vigour (Fernani-Meliani *et al*., 2021; Zhang *et al*., 2026).

Application of low doses makes it possible to induce useful phenotypic variations; while preserving the overall integrity of the genome (Bakary and Diop, 2024; Rodriguez *et al.*,

2025). Thus, controlling these radiosensitivity thresholds is fundamental for optimising selection protocols and identifying mutants exhibiting traits of precocity or increased stress tolerance. The objective of this study was to evaluate the radiosensitivity and agronomic response of soybean genotypes in order to establish radioactive thresholds for inducing safe mutagenosis necessary for crop improvement.

MATERIALS AND METHODS

Study site. The study was conducted at the Crop Protection Laboratory of the Faculty of Agronomic and Environmental Sciences of the University of Kinshasa in DRC, during March 5 to 15, 2026. The average temperature and humidity prevailing in the Laboratory during the experimental period were 27±0.5°C and 65±2%, respectively.

Biological material and gamma irradiation. The plant material consisted of three soybean genotypes; namely Diamuini 2, Lutaladio and Luyindula in DRC. These genotypes, which resulted from mutations induced at the Kinshasa Regional Center for Nuclear Studies (CREN-K) from the parent TGX1830-7F, were selected for their adaptability to local eco-climatic conditions and their distinct agronomic characteristics (Table 1). Furthermore, these varieties are currently being evaluated for inclusion in the national catalogue of cultivated plants in the DRC. The seeds of these genotypes, certified free of pathogens, came from the INERA/Mvuazi Research Center in DRC.

Seed irradiation was carried out in a Cobalt-60 (Co_{60}) irradiator at CREN-K, calibrated according to the radiological safety standards recommended by the International Atomic Energy Agency (IAEA). The seeds were exposed to four dose levels; namely 0 Gy (control), 10, 20 and 30 Gy. This gradient of moderate doses was chosen in accordance with recent work by Fernani-Meliani *et al.* (2021) and Bakary and Diop (2024) on hormesis and growth stimulation in legume crops.

Experimental procedure. The trial was conducted under laboratory conditions, using a completely randomised design, with twelve treatments, repeated three times. The substrate used consisted of river sand, previously sterilised by autoclaving at 180 °C for 2 hr with dry heat to eliminate biotic pathogens. The seeds were sown in perforated germination trays (ensuring optimal drainage), arranged in four rows per experimental unit. For each variety x dose combination, a batch of 24 seeds was tested, bringing the total number of seeds to 288 for observation.

The evaluation of the radiobiological response of the seeds was based on standard radiosensitivity descriptors. The variables monitored included germination kinetics, expressed by germination time (in days) and germination rate (%); and seedling morphometry (hypocotyl and epicotyl length, measured using a 20 cm graduated ruler). Germination time was assessed by the time

TABLE 1. Phenotypic and agronomic characteristics of the soybean varieties studied

Variety	Cycle (days)	Seed colour	Resistance to dehiscence	Weight of 1000 seeds (g)
Diamuini 2	90-95	Yellow	Average	171
Lutaladio	90-110	Yellow	Forte	157
Luyindula	100-115	Yellow	Forte	157

elapsed between sowing and the appearance of cotyledonary leaves. For each variety x dose combination, the germination rate was calculated 7 days after sowing.

Lengths of epicotyl and hypocotyl were measured 12 days after planting. These variables are regarded as suitable indicators in estimating the damage caused by mutagenic treatments (Horn and Shimelis, 2013). The epicotyl length was measured above the soil surface up to the tip of the primary leaves, while the hypocotyl length was measured from the collar plant to the cotyledonary node of seedlings.

Data recorded were previously subjected to the normality (Shapiro-Wilk) and homogeneity of variance tests, then subjected to the standard analysis of variance procedure using Statistics software (version 8.0) to compare soybean genotypes responses. In cases of significant difference, means comparison was performed using the TukeyHSD test at 5% of probability threshold.

RESULTS

Germination data

Germination period. Germination time was not significantly influenced (P=0.44) by the interaction between variety and irradiation dose (Table 2). Germination times generally ranged from 3.7 to 5 days. However, a slight trend toward longer germination times was observed at higher doses, particularly in the Lutaladio variety at 30 Gy.

Germination time. Although germination time was marginally not significantly responsive to variety x dose interaction, a trend toward a differentiated response of varieties according to irradiation levels existed (Table

TABLE 2. Germination time and rate of soybean variety seeds irradiated at low doses under laboratory conditions

Variety	Radiation dose (Gy)	Settings	
		Germination time (days)	Germination rate (%)
Diamuini 2	0	4.33 ± 0.58	87.5 ± 12.5
	10	4.00 ± 1.00	83.3 ± 14.4
	20	5.00 ± 1.00	75.0 ± 12.5
	30	4.00 ± 1.00	83.3 ± 14.4
Lutaladio	0	4.67 ± 1.15	83.3 ± 14.4
	10	4.33 ± 1.53	87.5 ± 0.0
	20	4.33 ± 0.58	87.5 ± 21.7
	30	5.00 ± 0.00	66.7 ± 7.22
Luyindula	0	4.00 ± 0.00	75.0 ± 12.5
	10	4.33 ± 0.58	87.5 ± 12.5
	20	4.00 ± 1.00	75.0 ± 0.0
	30	3.67 ± 0.58	91.7 ± 7.22
Grand mean		4.31	81.9
P-value (variety x dose)		0.44	0.08

2). In general, a decrease in germination rate was observed with increasing irradiation dose. However, some varietal combinations, notably the Luyindula variety irradiated at 30 Gy, maintain relatively high rates. This reflects the existence of a specific tolerance to certain variety-dose combinations, although the overall effect of the dose remains predominant in reducing germination potential.

Epicotyl and hypocotyl length. Figures 1a and 1b illustrate the effects of low doses of gamma irradiation on epicotyl and hypocotyl length, respectively. There was no significant interaction between the variety and radiation dose ($F = 0.57$; $P = 0.75$) for epicotyl length; implying that the of the main factor effects (variety and dose) acted independently. In general, doses of 20 and 30 Gy tended to result in low epicotyl development values (Fig. 1a). Analysis of the hypocotyl results clearly shows that this morphological parameter response varied considerably depending on the variety-dose combination (Fig. 1b). The Lutaladio variety exposed to 10 Gy, exhibited the best performance; while the other varieties, notably Diamuini 2, showed significantly reduced hypocotyl lengths under this irradiation.

Unlike epicotyl length, the variety x dose interaction for hypocotyl was highly significant ($F = 17.9$; $P < 0.0001$), indicating a strong mutual dependence between these two factors. The TukeyHSD multiple comparison test revealed three distinct homogeneous groups. The first group was characterised by the highest performance, represented exclusively by the Lutaladio variety irradiated at 10 Gy (16.0 cm). The second group corresponded to high intermediate performance (with a hypocotyl of 15.0 cm); this included the Luyindula variety irradiated at 20 Gy; and the unirradiated Lutaladio variety (0 Gy). The third group consisted of combinations with low to moderate performance, with hypocotyl lengths

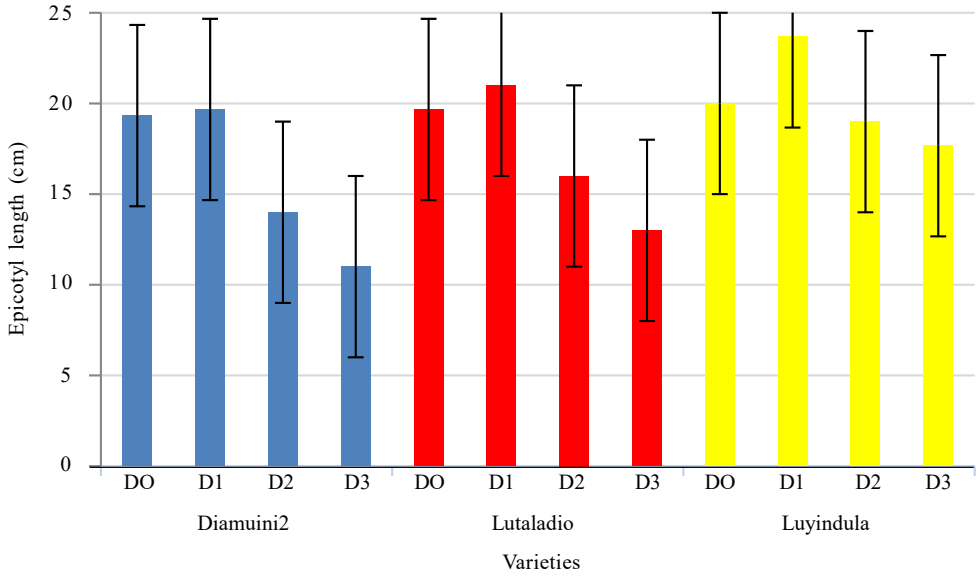

Figure 1a. Soybean varietal x low doses of gamma irradiation doses on epicotyl length under laboratory conditions. D0, D1, D2 and D3 = 0, 10, 20 and 30 Gy irradiation doses, respectively.

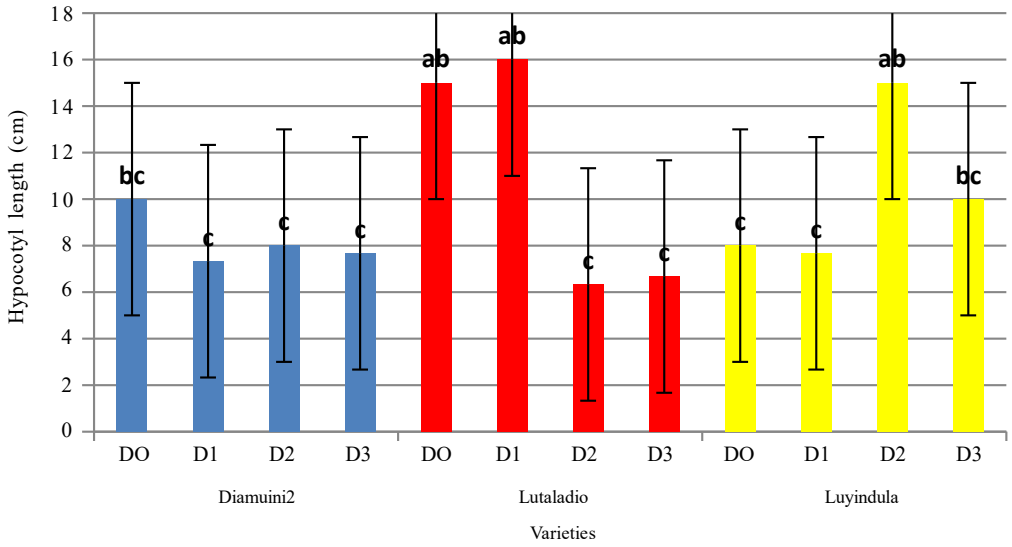

Figure 1b. Soybean varietal x low doses of gamma irradiation doses on hypocotyl length under laboratory conditions. D0, D1, D2 and D3 = 0, 10, 20 and 30 Gy irradiation doses, respectively.

between 6 and 10 cm, including several modalities of the Diamuini 2, and Lutaladio varieties at high doses.

DISCUSSION

Germination rate. The overall decrease in germination rate with increasing irradiation dose, irrespective of soybean variety, is consistent with results of previous studies on legume crops, showing a negative dose-response relationship beyond a certain dose threshold (Oladosu *et al.*, 2021; Wani and Anis, 2022). However, the maintenance of high levels in some varietal combinations (e.g. Luyindula at 30 Gy), suggests a hormesis effect, a phenomenon by which low doses of stress stimulate biological functions (Calabrese, 2021). This implies that low doses of gamma radiation stimulate germination and the development of the first plant roots.

The high germination rate of the Luyindula variety irradiated at 30 Gy (Table 2), suggests increased efficiency of cellular defense systems or ability for soybean to adapt to oxidative stress, possibly linked to efficient regulation of cellular repair mechanisms (Mudibu *et al.*, 2021). In legumes, several studies have demonstrated that doses between 10 and 50 Gy can improve seedling germination rates and vigour (Mudibu *et al.*, 2021; Horn *et al.*, 2022). This stimulatory effect vould be linked to the activation of antioxidant defense systems and to optimisation of metabolic processes (Wi *et al.*, 2021).

The absence of a significant variety x dose interaction for germination time, coupled with a slight increase in this time at high doses (Table 2), suggests a slowing of physiological processes such as imbibition, enzymatic reaction, respiration and cell division. Indeed, Gupta and Datta (2023) argued that ionising radiation induces accumulation of reactive oxygen species, responsible for oxidative damage affecting cell membranes and enzymatic activity; which delays the mobilisation of reserves necessary for germination (Zaka *et al.*, 2022). Furthermore, germination response is highly dependent on the initial quality of the seeds and their physiological state, which may explain the

absence of a statistically significant effect under certain experimental conditions (Al-Salhi *et al.*, 2021).

Epicotyl and hypocotyl length. Epicotyl length was not significantly responsive to the effect of variety x irradiation dose. This contrasted with the significant sensitivity marked by the hypocotyl length. The divergent responses reflects a differential sensitivity of organs to ionising radiation. According to Kovacs and Keresztes (2021), shoot growth is particularly affected by ionising radiation due to its impact on cell division and elongation. At high irradiation doses, significant inhibition in seed germination and seedling growth tends to occur (Beyaz and Yildiz, 2022). The performance observed in Lutaladio irradiated at 10 Gy could correspond to a zone of optimal physiological activation, where repair mechanisms and hormonal regulation (particularly auxins and gibberellins) are stimulated (Singh *et al.*, 2023).

Although the survival rate was not significantly influenced by variety x irradiation dose, the observed differences between varieties confirm the existence of specific genetic tolerance. Literature indicates that survival rate generally decreases with increasing irradiation dose, but that the tolerance threshold depends on genotypes (Mba *et al.*, 2021). Some varieties possess an increased capacity to activate antioxidant mechanisms, allowing them to better resist radiation-induced stress (Wi *et al.*, 2021; Arena *et al.*, 2022).

CONCLUSION

Exposure of three soybean varieties (Diamuini 2, Lutaladio, and Luyindula) to low doses of gamma irradiation revealed a differentiated response among the varieties depending on the applied dose, thus reflecting significant genetic variability in radiosensitivity. In general, a tendency toward decreased germination rates was observed, with increasing irradiation dose, although some variety-dose combinations showed relative tolerance, suggesting the existence of a hormetic effect at low doses. Germination time, on the other hand, was not significantly affected, although a slight slowing of physiological processes was noted at high doses.

Analysis of growth parameters revealed differential organ sensitivity, with the epicotyl being minimally affected by irradiation, unlike the hypocotyl, for which a highly significant interaction between variety and dose was observed. These results highlight the importance of genotype × environment interactions in the response to ionising radiation-induced stress. Notable differences between varieties at high doses were observed, indicating specific varietal tolerance. The Luyindula variety stood out with superior resistance to high doses, while Lutaladio showed an optimal response at low doses (10 Gy).

Overall, these results confirm that low doses of gamma irradiation are a relevant tool for inducing exploitable variability in soybean breeding programs. However, the precise identification of optimal doses and consideration of varietal specificities remain essential to maximise beneficial effects; while limiting negative impacts. Further studies, incorporating long-term molecular and agronomic analyses, would be necessary to better understand the underlying mechanisms and effectively utilise the mutants obtained under field conditions.

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