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Assessment of radio-sensitivity for three cowpea genotypes to gamma irradiation

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Cowpea (*Vigna unguiculata* [L.] Walp) is an important food legume. However, production is limited by various biotic and abiotic constraints in sub-Saharan Africa and reduced genetic variability hinders crop improvement efforts. Gamma ray irradiation has been used as a mutagen to create variability but optimal dose determination is a prerequisite to maximize the recovery of mutant plants. In this study, three genotypes (KVx396-4-5-2D, Tiligré and Moussa local) from Burkina Faso were treated with four gamma irradiation doses (150, 200, 250 and 300 Gy) using a labelled cobalt source (60CO) and evaluated for seed germination and seedling survival. Results showed that germination and survival rates were severely decreased at treatments greater than 150 Gy and no germination was observed at the 300 Gy dose. The optimum dose (LD 50%) varied for the genotypes and ranged from 129 to 163 GY. Seed characteristic and quality were factors contributing to this variation.

Key words: *Vigna unguiculata*, γ-rays, mutagenesis, variability, radiosensibility.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is an important source of dietary protein that is rich in leucine, isoleucine, lysine, tyrosine and phenylalanine (Elhardallou et al., 2015). Extracts of cowpea have been shown to possess antioxidant and anti-free radical properties (Zia-Ul-Haq et al., 2013) and oil from seeds was shown to regulate blood glucose and lipid levels in rats (Ashraduzzaman et al., 2011). Cowpea also plays an agronomic role in fixing 52 to 56% atmospheric nitrogen, thus increasing soil mineral nitrogen from 13 to 40% (Bado, 2002). However, cowpea production is hindered by biotic and abiotic stresses in sub-Saharan Africa including Burkina Faso. Gains in productivity have not been sufficient due to low genetic variability. Mutagenesis is one method to introduce genetic variability. Gamma ray irradiation has been used to broaden genetic variability (Kumar and Shunmugavalli, 2018; Olasupo et al., 2018). The successful use of γ-rays to generate genetic variability in groundnut has been reported by Gunasekaran and Paravadai (2015) and in cowpea by Micke (1993). The use of gamma radiation requires knowledge of appropriate dosage for variability induction (Horn and Shimelis, 2013). The

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Table 1. Characteristics of cowpea genotypes.

Characteristics	Genotype		
	KVx396-4-5-2D	Tiligré	Moussa local
Pedigree	(IAR1696 x KN1) x Gorom local	KVx640 x (B301x 994) x KVx640	Landrace
Water content at 30°C (%)	8.3	7.9	8
Seeds color	White	White	White
Texture	Rough	Rough	Rough
WHS (g)	13.3	18.0	16.5
Port	Creeping	Semi-erect	Creeping

WHS (g): Weight of one hundred seeds; IAR1696: Cowpea line from IITA (International Institute of Tropical Agriculture, Mali); KN1 and KVx640: Cowpea line from Kamboinsé (Burkina Faso), Gorom local: Landrace from Burkina Faso.

objective of this study was therefore to evaluate the gamma irradiation sensitivity levels for three cowpea varieties to (i) determine the effect on seed germination and seedling survival and (ii) identify the optimum dose plant survival.

MATERIAL AND METHODS

Plant material and seeds treatment

The characteristics of the three cowpea genotypes, Tiligré, Moussa local and KVx-396-4-5-2D, used in the study are presented in Table 1. Seeds were packaged in four batches of 500 g which were treated with four doses of gamma irradiation (150, 200, 250 and 300 Gy) supported by the laboratory of the International Atomic Energy Agency (IAEA) of Seibersdorf, Austria. The source of gamma radiation was marked cobalt (60CO).

Radiated seeds were kept in a cold room at a temperature of 20°C for 3 weeks with 160 seeds of each treatment and variety evaluated. The same number of non-irradiated seeds was as a control.

Experimental site and M1 management

The experiment was conducted at the INERA research station at Kamboinsé, in Burkina Faso (12°26'59.4``N, 1°33' 08.0``W; 296 m above sea level). The 160 seeds of each treatment were sown in two 24 m rows of in a 24×25 m^2 plot. Planting was on July 12, 2016 using 0.30×0.80 m intra and inter row spacing at the rate of one seed per hole.

Data collection

Seed germination was recorded 7 days after sowing. Plant survival was recorded at 50% flowering. Germination and survival rate were calculated using following formula:

Germination rate (%) = $\frac{number\ of\ germinated\ seeds}{number\ of\ irradiated\ seeds\ planted} x100$ (Olasupo et al., 2016).

Survival rate (%) = $\frac{number\ of\ survived\ seedling}{number\ of\ germinated\ seeds} x100$ (Olasupo et al., 2016).

The lethality rate was calculated by the following formula:

Lethality rate (%) = $\frac{\text{control} - \text{treated}}{control} x100$ (Bashir, 2012).

The (LD50%) for each genotype was estimated through the simple linear regression model considering 100% of seed germination and survival rate of the control.

Data analysis

The SAS version 8 software and Excel software were used to perform a linear regression analysis subjecting data to a variance analysis, where the influence factor was the radiation dose with a significance level of 0.05. The lethal dose LD50% was estimated using the resulting regression equation from germination and survival percentage.

RESULTS AND DISCUSSION

Effect of γ-radiation on cowpea germination and seedling survival in M1 generation

Seed germination and plant survival data are presented in Table 2. Results reveal a positive significant effect of gamma rays (P<0.05). Doses ranging from 150 to 300 Gy resulted in increased lethality from 22 to 100% of germination and survival percent (Figure 1). The maximum seed germination and survival rates were greater at the 150 Gy dose. At this dose, Tiligré showed the highest germination rate; whereas, Moussa local showed the highest survival rate The Tiligré plants were attacked by wet rot, resulting in lower survival rates. Doses greater than 200 Gy resulted in no survival except in Moussa local. Indeed, it inhibited seed germination. If the seed germinated, it showed a high risk of mortality. The effect of gamma radiation was manifested in the hypocotyl and in the cotyledon presenting necrotic damage, and in leaf by the deficient of chlorophyll (Figure 2). So, seedlings only survived for a maximum for two weeks. However, Badr et al. (2014) found that 300 Gy was the toxic dose in cowpea while Horn and Shimelis (2013) showed toxic doses greater than 400 or 600 Gy depending on cowpea genotypes. These results differ

Table 2. Effect of gamma mutagen on seed germination and seedling survival of cowpea varieties

Variety	Doses	Lethal seed (%)	Germination (%)	Lethality survival (%)	Survival (%)
KVx396-4-5-2	0	0	100	0	100
	150	43.74	56.26	39.68	60.68
	200	66.07	33.93	100	0
	250	95.53	4.47	100	0
	300	100	0	100	0
P value			0.0016		0.0207
Moussa local	0	0	100	0	100
	150	40.82	59.18	29.31	70.69
	200	70.4	29.6	75.86	75.86
	250	90.81	9.19	100	0
	300	100	0	100	0
P value			0.0013		0.0116
Tiligré	0	0	100	0	100
	150	22.11	77.89	43.21	56.21
	200	67.31	32.69	100	0
	250	91.34	8.66	100	0
	300	100	0	100	0
P value			0.013		0.0176

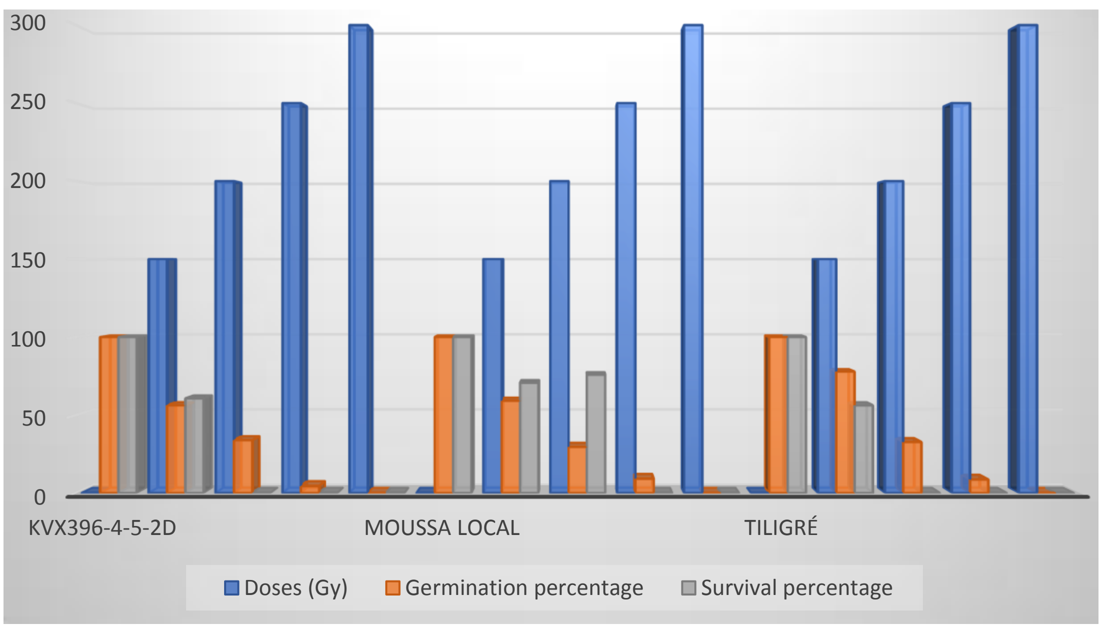
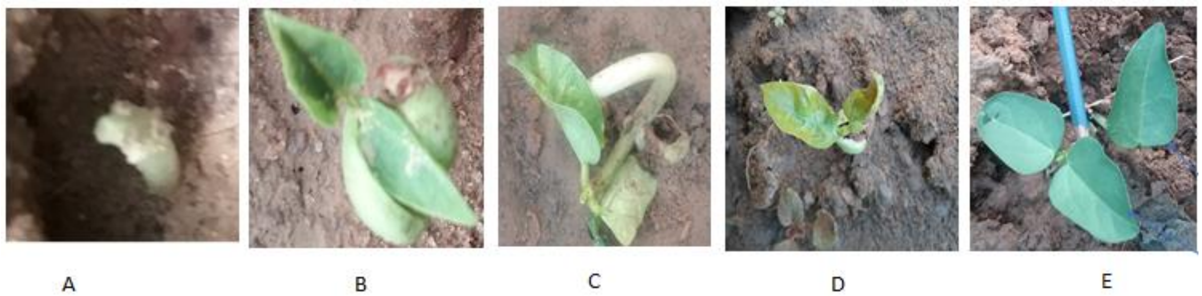


Figure 1. Percentage germination and survival of irradiated seed.

from those of Essel et al. (2016) which showed no significant effect of increasing doses of gamma irradiation on cowpea germination. The radio-sensitivity difference observed in cowpea genotypes could be related to the water content of the seeds (Mba et al., 2010) or the nature of their seed coat (Olasupo et al., 2016). According to Mba et al. (2010), the water content below 12 to 14% threshold promotes the accumulation of free radicals that are toxic to cells (Mahama, 1980). According to Olasupo et al. (2016), rough texture seeds are more



A: leafless stem; B: rotten cotyledon; C: foliage leaves showing early dommages; D:curly leaves and chlorophyll deficient; E: control (1) and plant with one leaf (2)

Figure 2. Irradiation damage.

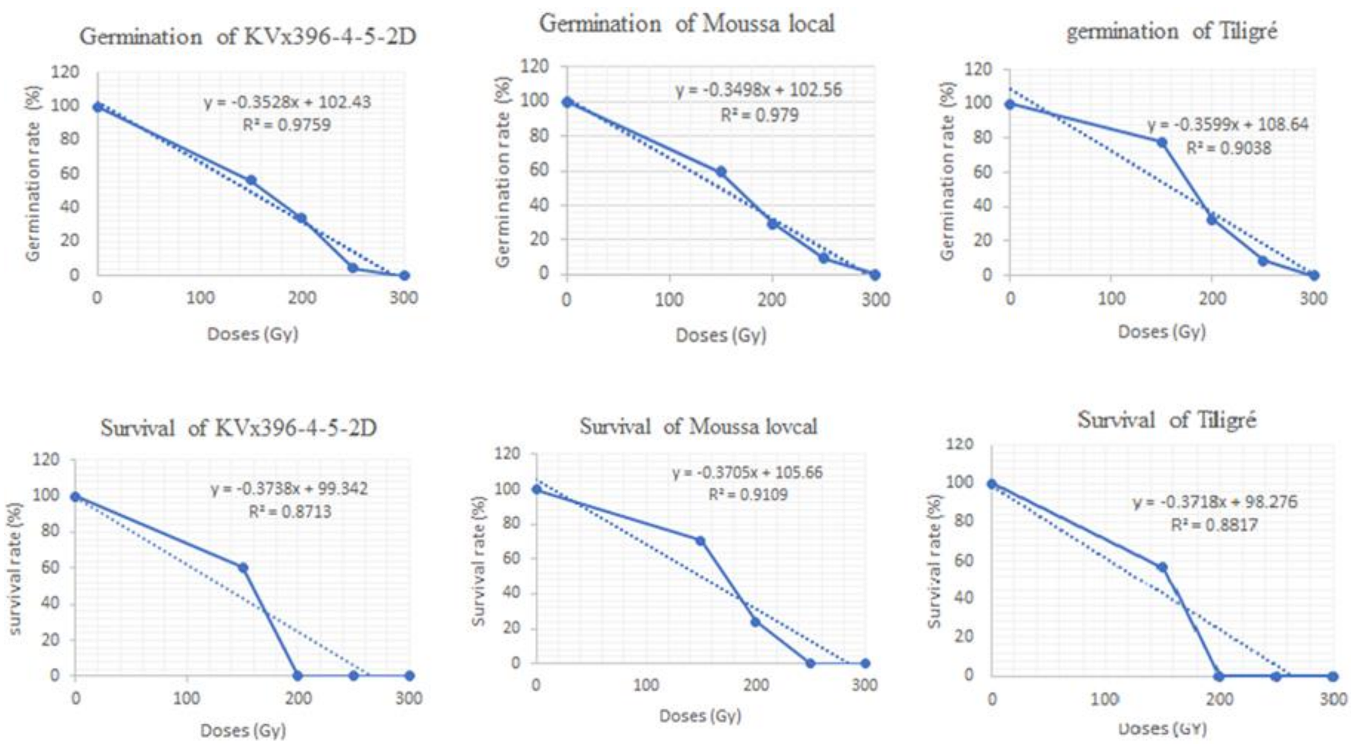


Figure 3. The regression of germination and survival rates for gamma irradiation.

radio-susceptible than smooth-texture seeds. As a result, the water content and the rough texture of seeds could partially justify the radio-sensitivity in this study. Gamma radiation impacts the germination potential and also the quality of germinated seedling. These results were similar with the others researches who reported that increasing dose irradiation decreased the seed germination and seedling growth (Verma et al., 2017; Tshilenge-Lukanda et al., 2013). The reduction of seed germination and plants survival had been explained by the inhibition of physiological and biological process for seed germination and plant survival.

Optimal dose determination

Figure 3 shows linear regression of seed germination rate and seedling survival to irradiation doses. A variation in the estimated LD50% was observed among the three cowpea varieties. The lowest LD50% values for seed germination and seedling survival were recorded for KVx396-4-5-2D, while Moussa local recorded the highest LD50% values (Tables 3 and 4). Olasupo et al. (2016) presented a wide variation of LD 50% ranged from 326 to 1053 Gy for seed germination and from 148.8 to 620.2 Gy for seedling survival in cowpea. The variation in

Table 3. The optimal dose (LD50%) of seeds germination of cowpea varieties.

Variety	Linear equation	LD50% (Gy)	R^2 (%)
KVx396-4-5-2D	Y=-0.35X+102.43	148.6	97.6
Moussa local	Y=-0.35X+102.56	150.3	97.9
Tiligré	Y=-0.36X+108.64	162.9	90.4

LD50%: Optimal dose 50%, R^2: coefficient of determination.

Table 4. The optimal dose (LD50%) for plants survival of cowpea varieties.

Variety	Linear equation	LD50% (Gy)	R^2 (%)
KVx396-4-5-2D	Y=-0.37X+99.34	132.0	87.1
Moussa local	Y=-0.37X+105.66	150.2	91.1
Tiligré	Y=-0.37X+98.28	129.8	88.2

LD50%: Optimale dose 50%, R^2: Coefficient of détermination.

LD50% was a result of difference in cowpea seeds characteristics (Olasupo et al., 2016).

Conclusion

Moussa local and Tiligré genotypes were less radiosensitive compared to KVx396-4-5-2D. The harmful effect of gamma radiation manifested in cowpea seedling was the necrotic damage and the chlorophyll deficient. Plant radiosensibility dependent upon several factors, some related to seeds characteristics. Doses less than 160 Gy would be better for inducing variability.

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ETHICS

The authors have not declared any conflict of interests.

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