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Radio-Sensitivity of Some Groundnut (*Arachis hypogaea* L.) Genotypes to Gamma Irradiation: Indices for Use as Improvement

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Authors' contributions

This work was carried out in collaboration between all authors. Author LTL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors CFB, JM and MNL irradiated seeds used in this experiment. Author AKM managed the analyses of the study. Authors ATM and RMT managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Mutation breeding in crop plants is an effective approach in improvement of crop having narrow genetic base such as groundnuts (*Arachis hypogaea* L.). Determination of effective irradiation dose is prerequisite for mutation breeding and development of genetic variability by induced mutation. Three groundnuts (*Arachis hypogaea* L.) genotypes (JL12, JL24 and Kimpese) were irradiated to the absorbed doses of 100, 200, 400 and 600 Gy for effective dose determination and to compare their sensitivity to different doses of gamma irradiation in a completely random design. It was found that, irradiation reduced significantly germination and survival percentages of seedlings in higher doses and this reduction was more pronounced in JL 24 cultivar. This sensitivity expresses himself distinctly to the rank of stem lengths and roots. Results show a

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negative interrelationship indeed between doses of irradiation applied and lengths of stems and roots. With attention to LD_{50} data, our results indicated that optimum doses were 200 Gy for JL12 and Kimpese, and 100 Gy for JL24. We concluded that JL24 are more sensitive to gamma irradiation than JL12 and Kimpese.

Keywords: Radiation induced mutation; Arachis hypogaea L.; gamma radiation; ^{137}Cesium; primary damage.

1. INTRODUCTION

Groundnut is a predominant oilseed crop in Democratic Republic of Congo (DRC) ranking first among the edible oilseed groups (Kilumba and Lutaladio, 1989; Tshilenge, 2010; Tshilenge et al., 2011). Groundnut kernels are the rich source of edible oil (40-55%) and proteins (22-28%) (Chiou et al., 1995; Dwivedi et al., 1998). The breeding objective in groundnut is to develop varieties with high yield, early maturity, high protein and oil content, resistant to diseases and insect pests (Isleib et al., 2001; Holbrook and Stalker, 2003). To achieve these objectives and bring about desired improvement in crop, the most sophisticated technique of mutation breeding can be exploited by the plant breeders. Mutation breeding is an important method for inducing new variability, which is an essential requirement of any plant breeding programme in the changing agricultural pattern of the day (Bhagwat et al., 1997; Chopra, 2005; Varsha Kumari et al., 2009; Ahmed and Mohamed, 2009).

Before the start of any sound breeding program, knowledge of the relative biological effectiveness and efficiency of various mutagens is useful in mutation breeding (Smith, 1972). Various attempts in this direction have been made by different scientists to determine the most effective mutagenic treatment for the induction of desirable traits in groundnut (Reddy et al., 1977; Patil and Mouli, 1978; Mondal et al., 2006; Mensah and Obadoni, 2007; Benslimani et Khelifi, 2009; Satpute and Suradkar, 2011). Nadaf et al. (2009) developed a high yield groundnut mutant variety G2-58 from a variety GPBD-4, through gamma irradiation (200 Gy) and found that mutant variety G2-58 significantly better than its mother variety GPBD-4 in respect of yield and yield contributing traits. Benslimani and Khelifi (2009) in Algeria used from four local non-dormant groundnut seeds (Berrihane, Boumalek, Tonga and El Frine) to examine varietal differences in radio sensitivity to gamma radiation. They exposed to 50, 100, 150, 200, 300 and 450 Gy doses of gamma irradiation and observed with the increase in radiation dose decrease in germination, seedling height, root length and emergence under field conditions in M1 generation. Plant height and seed fertility decreased with increase in gamma radiation dose in an approximately linear model. They found that LD_{50} values for seed fertility were 450, 300 and 250 Gy for Berrihane, Tonga and Boumalek, respectively. Gamma irradiation absorbed dose of 100 and 200 Gy produced the highest mutation frequency in Berrihane followed by Tonga, Boumalek and El Frine.

Radiation induced mutation have played a significant role for the improvement of groundnut by developing a large number of pod and high yielding varieties in many countries (Ramani and Jadon, 1991; Micke, 1997). As per the International Atomic Energy Agency and Food and Agriculture Organisation (IAEA/FAO) database 72 groundnut varieties have been developed through induced mutations (http://mvgs.iaea.org/Search.aspx) during the period of 1959 to 2010. In DRC, genotype Kimpese is native and qualitative variety of groundnut cultivation and any mutation breeding work for breeding and enrichment of this genotype is accounted to be very important. Also, cultivar JL12 and JL24 are the commercial and high yield groundnut cultivars that are under cultivation in DRC and any favorable change in its germplasm will be so beneficial to increase genetic variation and generate mutants that will be selected in the breeding program to diversify the gene pool. Therefore, the present study has been conducted for evaluation of mutagen (gamma ray) effects and to find out optimum dose of gamma ray for the induce favourable genetic changes in groundnut genotypes Kimpese, JL12 and JL24.

2. MATERIALS AND METHODS

The genetic materials are composed of three groundnut genotypes. They included JL12, Kimpese and JL24. The seeds were obtained from the Research Centre of the National Institute for Agronomic studies and research or 'Institut National pour l'Etude et la Recherche Agronomique (INERA) M'vuazi in the province of Bas-Congo in the DRC. Seeds from selected varieties were irradiated with different doses of gamma radiations with a cesium-137 source using "Lisa 1 conservatome" equipment at the Regional Nuclear Energy Center of Kinshasa (CRENK) in the DRC. The treatments include 0 Gy, 100 Gy, 200 Gy, 400 Gy, and 600 Gy of gamma-rays.

For the sake of studying radiosensitivity, a number of 20 seeds per dose and per genotype including the control are germinated in Petri dishes containing filter paper. They were afterwards soaked in water then placed in a controlled atmosphere room (temp: 25ºC, humidity: 51 %, photoperiod: 16 h/day). The biological effects of the mutagen treatments were studied by surveying the percentage of seed germination, stem and root's length (7 days after germination) (Benslimani and Khelifi, 2009).

The same genotypes and gamma ray doses were retained for the fieldwork. 100 seeds per dose and genotype were irradiated and sown at the Experimental garden of Regional Nuclear Energy Center, Kinshasa (CREN-K) (15º30'E, 04º41'S and 330 m altitude). The region falls within the Aw4 climate type according to Köppen classification characterized with 4 months of dry season (from mid-May to August) coupled with 8 months of rainy season, sometimes interrupted by a short dry season in January/February. Daily temperature averages 25ºC and annual rainfall is close to 1500 mm. Each piece of land corresponded to a treatment (irradiation dose) per genotype. The elementary pieces were laid out in randomized complete block design (RCBD) with two replications by genotype; a total of 6 blocks. Each block comprised the different doses.

Data on the percentage germination, stem length, root length, seedling emergence were subject to two-way analysis of variance (ANOVA). Significant means were separated with the Least Significance Difference (LSD) (Obi, 2002). Linear regression analysis from Statistix Ver. 8 software package was used to estimate the optimum dose causing 50 percent lethality (LD_{50}) for the different groundnut varieties using percentage survival in the field as a standard measure of physical effect. The determination of the optimum doses (Figs. 4, 5 and 6) was based on Gaul, Osborne and Lunden (Mohamed et al., 2005).

3. RESULTS AND DISCUSSION

3.1 Seed Radiosensitivity

<u>3.1.1 Germination rate</u>

Analysis of variance (Table 1) revealed no effect of irradiation dose or genotype effect on percentage of germination. Thus the percentage of germination alone is not a sufficient criterion to select the doses of radiation to which the relevant interest to study other parameters.

Table 1. Germination rate (%) in groundnut varieties upon gamma irradiation

Dose (Gy)	Genotype		
	JL12	Kimpese	JL24
0 Gy	100	90	100
100 Gy	100	100	100
200 Gy	100	100	100
400 Gy	75	100	100
600 Gy	70	100	100
Significance	-	-	-
LSD (0,05)	NS	NS	NS

3.1.2 Average stem length

Analysis of variance indicates a significant genotype effect ($LSD_{0,01}$ = 2.1) for this parameter. In considering the control (0 Gy), comparison reveals two homogeneous groups: group A with genotype JL12 making the average length of the stem highest (6.75 cm) and group B with genotypes that are Kimpese and JL24 mean lengths of stems 4.37 cm and 3.25 cm respectively.

However, in all genotypes, the increase in dose causes a significant decrease in stem length, with the exception of the Dose 2 (200Gy) in the genotype JL 24 (Fig. 1). Comparison of the means in pairs ($LSD_{0,01}$ = 1.6), gives three homogeneous groups. The first, with the control (0 Gy), has lengths ranging from 3.25 to 6.75 cm, followed by a dose (100 Gy) and dose 2 (200Gy) with values ranging from 2.6 cm to 3.5 cm. The third group, with low values (1.5 to 2.1 cm), contains the D3 dose (400 Gy) and D4 (600 Gy).

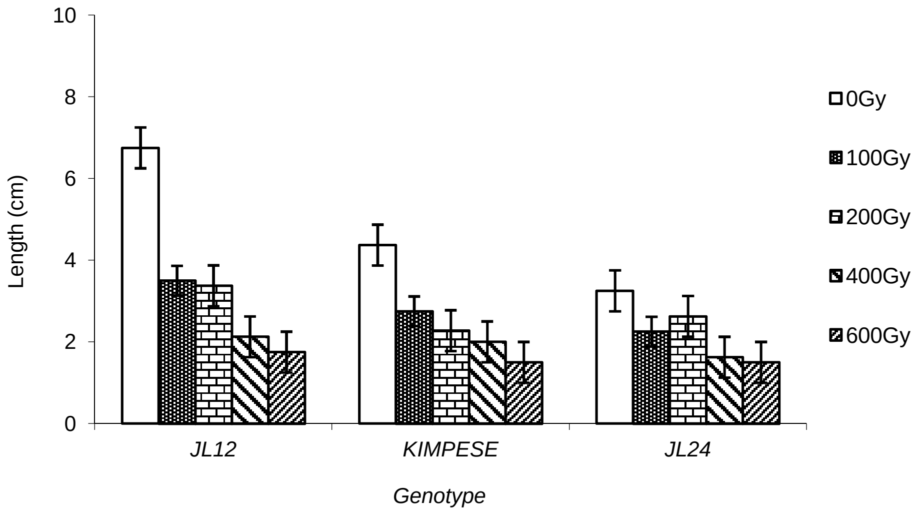

Fig.1. Irradiation doses effect on stem means length

3.1.3 Average root length

Analysis of the average length of roots showed significant differences between genotypes ($LSD_{0,01}$ = 1.7) and between the doses used. In terms of variety, it appears two distinct groups when considering the witness, with JL12 on one side and Kimpese and JL24 the other (Fig. 2). In terms of irradiation doses, as stem length, root length decreased with increasing doses with the exception of dose 2 (200Gy). The dose is highly significant ($LSD_{0,01}$ = 1.2). Comparing two to two doses can prioritize as follows: in the variety JL12, extreme values were recorded in the control (8.1 cm), followed by seeds treated with 200 Gy (3.8 cm) and 100 Gy (3.5 cm), and finally the seeds irradiated at 400 Gy (2.3 cm) and 600Gy (1.7 cm). Among the variety Kimpese, only two groups have been established, it is the group, with values ranging from 4.5 to 5.3 cm, containing the control and the doses of 100 Gy and 200 Gy. The second group contains the seeds irradiated at 400Gy and 600Gy with values ranging from 2.9 to 3.1 cm. Among the variety JL 24, the observed difference is due to the seeds irradiated 600Gy who presented very low values (1.5 cm on average) compared to other treatments studied.

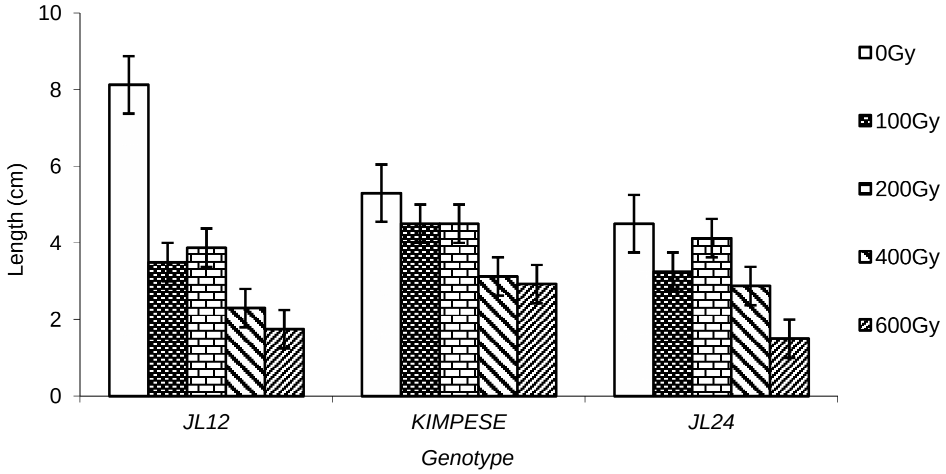

Fig. 2. Irradiation doses effect on root mean length

3.1.4 Irradiation dose effect on stem and root growth

An examination of Fig. 3 shows a reduction of growth of stems and roots of all seedlings from irradiated seeds, regardless of the dose. We also noted a net decrease of the lengths of stems and roots that reach respectively 38.7% and 33.2% at 100 Gy and 38.7% and 25.3% at 200 Gy by against, for doses of 400 Gy and 600Gy, it was noted a decrease in length of stems and roots reaching respectively 57.6% and 49.6% on the one hand and 64.5% and 63.3% of on the other. The slowing of stem and root growth due to increased irradiation doses was observed in different species. The criterion used by Cheah (1988) to determine useful doses is the one inducing approximately a 30% reduction in the stems' length as compared to the control. Konzak and Mikaelsen (1977) recommend a growth reduction ranging from 30% to 50% and advised the use of three irradiation doses (100, 200 and 300Gy). For the continuation field experiment, we selected the same doses as the study of radiosensitivity: 100 Gy, 200 Gy, 400 Gy and 600 Gy.

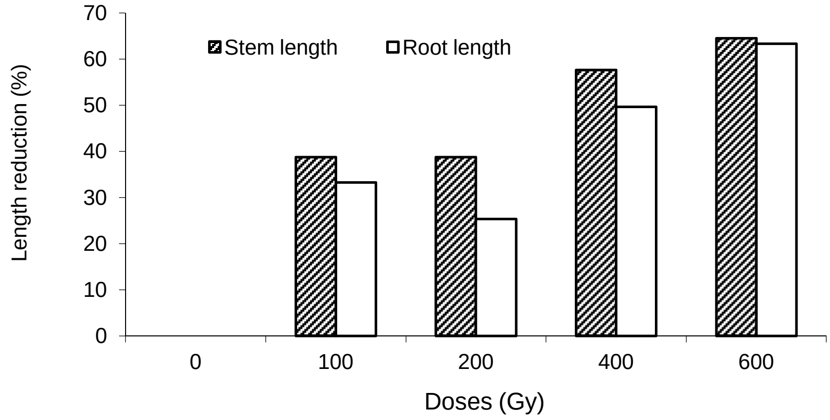

Fig. 3. Effect of radiation on the roots and stem lengths reductions (mean of three genotypes)

3.2 M1 Generation Field Survey

3.2.1. Emergence rate (%)

The emergence decreased after gamma irradiation, but the decrease was related to the increase of dosage and definite pattern was not determined in three groundnut genotypes. In term of resulted information from dose determination, data were classified in four independent groups in any genotypes (Table 2). For the variety JL12, the highest rate is 96% for seeds treated with 100 Gy, followed by seeds irradiated at 200 Gy (90%) and control (88%). It drops to 48% at 600Gy. Among the variety Kimpese, the same observations are made but with germination rate of 68.3% at 100 Gy the percentage of germination recorded for control seeds was 76%. When considering the variety JL24 with a germination percentage significantly lower than other genotypes, it appears a very significant difference between the doses of irradiation. This difference is due to the irradiated plants 100Gy who presented the germination percentage highest (52%).

Table 2. Emergence rate (%) in groundnut varieties upon gamma irradiation

Dose (Gy)	**Genotype**		
	JL12	***Kimpese***	***JL24***
0 Gy	88^{bc}	76^{a}	36^{b}
100 Gy	96^{a}	68.3^{b}	52^{a}
200 Gy	90^{b}	66^{b}	40^{b}
400 Gy	84^{c}	50^{c}	38^{b}
600 Gy	48^{d}	30^{d}	28^{c}
Significance	***	***	***
LSD (0.05)	5.08	5.82	7.2

****, **,*, Significance at P ≤ 0.001, 0.01, 0.05, respectively.*

3.2.2. Survival rate

Optimum dose is dose that cause maximum of mutation with minimum of damage to the plant. LD_{50} for survival of seedlings in three months after sowing of seeds was different and for JL12 and Kimpese varieties were 400 and 600Gy. By cons LD_{50} for JL24 variety was determined about 200 to 600 Gy (Figs. 4, 5 and 6).

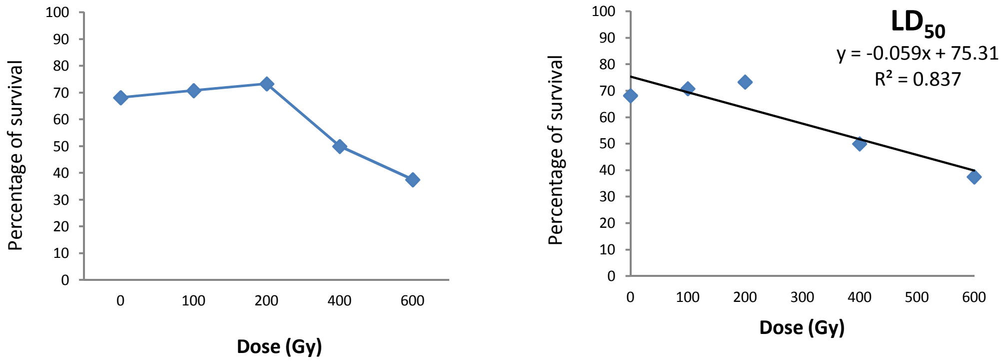

Fig. 4. Survival LD_{50} of seedlings in genotype JL12

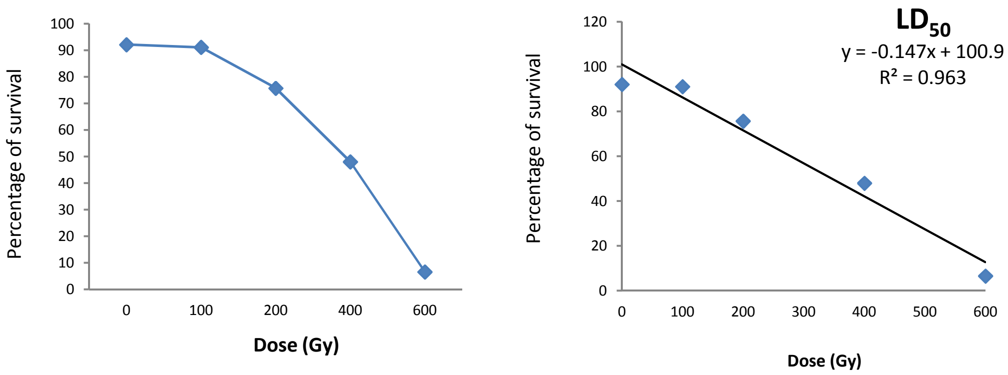

Fig. 5. Survival LD_{50} of seedlings in genotype Kimpese

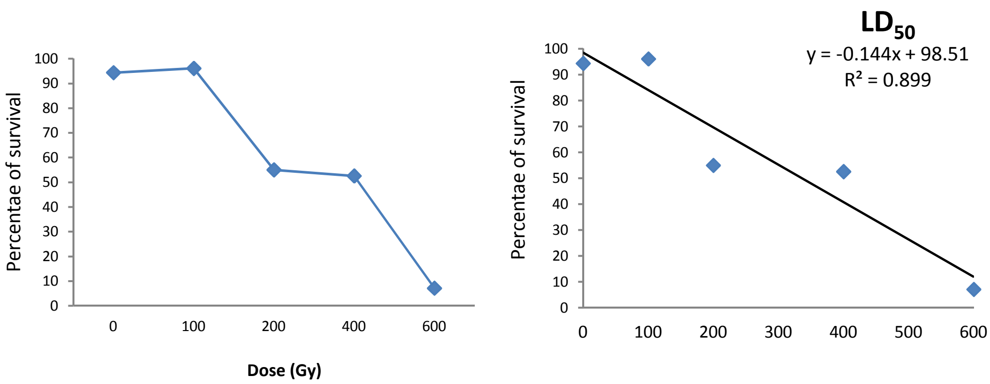

Fig. 6. Survival LD_{50} of seedlings in genotype JL24

There were significant differences in level 5% in dose treatments (between groups). In relation with adventure and survival of seedlings, gamma irradiation in lower doses (100 and 200 Gy) had less minus effects than higher doses specially, 400 and 600 Gy, as survival of seedlings had been reduced significantly in dose 600 Gy in three genotypes. But in this treatment, sensitivity of JL24 and Kimpese cultivars was so higher than JL12, because survival percentage of JL24 and Kimpese seedlings survival in higher doses (400 and 600 Gy) was so less than JL12.

4. DISCUSSION

Benslimani and Khelifi (2009) in their studies on non-dormant groundnut genotypes indicated that LD_{50} values for seed fertility was 450, 300 and 250 Gy for Berrihane, Tonga and Boumalek, respectively, and Gamma irradiation doses 100 and 200 Gy produced the highest mutation frequency for Berrihane followed by Tonga, Boumalek and El Frine. Above issues confirm results of this research. Although LD_{50} in three genotypes was almost the same (between 400 and 600 Gy) but the sensitivity to gamma irradiation in cultivars JL24 and Kimpese was much more severe than JL12.

Adventure and survival of seedlings in cultivar Kimpese in lower gamma doses (100 and 200 Gy) was more than cultivar JL12 and JL24. Specially, in dose 200 Gy; there were considerable differences in survival percentage of seedlings between three genotypes. This subject showed that optimum dose of JL24 (100Gy) was lower than Kimpese and JL12 (200 Gy). By this reason, dose 100 Gy was used for development of M1 generation population of cultivar JL24 whereas optimum dose and LD_{50} in JL12 and Kimpese was 200 Gy. With attention to above results and data from other similar studies that have been done on sensitivity of African groundnut genotypes, it can be found that totally, the sensitivity to gamma irradiation depends on a variety to another.

5. CONCLUSION

This work allowed us to study the radiosensitivity of seeds of three groundnut genotypes. In general, the study shows that groundnut seeds of these three genotypes are sensitive to gamma rays, but this sensitivity varies with the genotype. It was observed that the optimum dose of 200 Gy for Kimpese and JL12, and 100 Gy for JL24.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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