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RESEARCH PAPER

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Effects of ^{60}Co gamma radiation doses on seed germination of *Jatropha curcas* L.

Baudouin K. Nyembo*[1], Alexandre N. Mbaya[1], Calvin C. Ilunga[2], Jean-Louis N. Muambi[1], Luc L. Tshilenge[3]

[1]*Department of Phytobiology, General Atomic Energy Commission/Regional Center of Nuclear Studies, Kinshasa, DR-Congo*

[2]*Department of Biotechnology, General Atomic Energy Commission/Regional Center of Nuclear Studies, Kinshasa, DR-Congo*

[3]*Department of Genetics and Plant Breeding, General Atomic Energy Commission/Regional Center of Nuclear Studies, Kinshasa, DR-Congo*

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Abstract

This study aimed to assess the effects of different gamma radiation doses from Cobalt -60 isotopic source on seed germination and early growth parameters of *Jatropha curcas* L. Healthy and dry seeds were subjected to three doses of gamma rays (100, 200 and 300 Gy). The experiment was conducted using randomized complete block design, with three replicates. The significantly maximum germination percentage (89.85 %), seedling survival (92.3 %), seedling collar diameter (0.892 cm), plant height (17.30 cm), number of leaves (7) were observed at 30 days after germination. The results revealed that seed germination percentages and seedling shoot length decreased with increasing dose of gamma-rays. Higher gamma-ray dose (300 Gy) in particular had a pronounced effect on these germination parameters than others, probably because high-dose inhibited cell division due to free radicals and DNA system damage. The LD_{50} for seeds germination rates was obtained at 254 Gy. These results implied that germination traits of *Jatropha curcas* seeds were sensitive to increase in gamma-ray.

* **Corresponding Author:** Baudouin K. Nyembo ✉ jbaudyne@gmail.com

Introduction

Jatropha curcas L. belongs to the *Euphorbiaceae* family, a multipurpose, perennial, drought resistant shrub or tree which is widely distributed in the wild or semi-cultivated areas in Central and South America, Africa, India and South East Asia (Martinez-Herrera *et al.,* 2006; Tatikonda *et al.*, 2009). That is one of the important oil seed crops and a potential source of vegetable oil as a replacement for petroleum and in particular, the production of biodiesel (King *et al*., 2009). It is gaining a lot of economic importance because of its several potentials in industrial application and medicinal values. It is established different parts of this biodiesel producing plant including leaves, oil, sap, stem; roots and bark have numerous health benefits (Prasad *et al*., 2012; Agbogidi *et al*., 2013).

In Democratic Republic of Congo (RDCongo), the vernacular names of this plant are Nakakula, Mitanda, Ludimba by the Luba; Mupuluka, Dasikamabete, Mpulungu, Mukadipemba, Ngubanguedi, by the Kongo (Kambu, 1990).

Jatropha is a fast growing plant and produce seeds after approximately two years depending on many factors such as rainfall conditions and either the plant is propagated from cuttings or from seeds (Heller, 1996). The first developmental stage of the plant is germination, a critical stage in the life cycle of plants and this growth stage is strongly influenced by environmental factors (De Villiers *et al*., 1994). The studies on seed germination are needed to contribute to the knowledge of *Jatropha curcas* cultivation.

Improved varieties of *J.curcas* with desirable traits for specific growing conditions are not available, which makes growing *Jatropha* a risky business. This plant can be improved through application of mutation breeding to bring the change in the desired traits (Divakara *et al.,* 2010; Surwenshi *et al*.,2011; Nayak *et al.,* 2012). Mutation breeding which is efficient and much cheaper method than others can play an important role in crop improvement either directly or by supplementing the conventional breeding. Mutagenic agents, such as radiation and certain chemicals, can be used to induce mutations and generate genetic variation in seed germination, growth and yield traits of *J.curcas* (Dhakshanamoorthy *et al.*, 2010; Chiangmai *et al.*, 2014). The doses of 10, 15, 20 and 25 Gy of gamma applied to cutting because of its higher water content were able to increase genetic variability in *J. curcas* (Dwimahyani and Ishak, 2004). Sarhan *et al.* (2015) induced mutation in *Jatropha curcas* L. with gamma rays 20 and 25 Kr dose in order to increase the tolerance and resistances salinity of it. Pandey (2016) reported the effect of gamma rays on initial development of *Jatropha curcas* especially on the cotyledonary leaves. Irradiation dose of 225 Gy was good to be given in acute, intermittent, and split-dose methods on the performance of Jatropha plants (Surahman *et al*., 2018).

The main objective of the present investigation was to study the effects of different gamma radiation doses from Cobalt -60 isotopic source on seed germination and early growth of *Jatropha curcas* L.

Materials and methods

Seed source

The seeds of *J.curcas* were collected from the experimental garden of the Regional Center for Nuclear Studies of Kinshasa (CREN-K) and were used as basic material. The agro-climatic conditions of the Kinshasa region where the seeds were collected, are 15°30'E, 04°41'S and 330 m altitude.

The region falls within the Aw4 climate type according to Koppen 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 25°C and the average annual rainfall is approximately 1500 mm.

Irradiation study

In this study, gamma – rays were generated from Cobalt-60 isotopic source installed at FAO/IAEA Plant Breeding and Genetics laboratory, Seibersdorf, Austria (PBGL). Healthy and dry seeds were irradiated with different doses of gamma rays namely 100,200,300 Gy. The untreated seeds were used as control.

Sowing of seeds

The gamma rays treated as well as untreated seeds were sown at 1cm depth in black polyethylene bag containing 3 Kgs of growing medium (CREN-K soil). The emergence of shoot above ground level was considered as germinated and it was converted into percentage. Germination percentage =100 × (Number of seeds germinated / Number of seeds sown). The seedlings are grown in controlled environment (28 °C) and were regularly watered .The experiment was conducted using completely randomized design, with three replicates. Observations on seed germination and survival of germinated seedlings were recorded regularly. The survival percentage at 30 days after germination (DAG) was calculated as follows: Survival (%) = 100× (Number of survival plant at 30 DAG / number of seeds germinated). Medial lethal dose of ionizing radiation required to cause 50 % mortality of the tested seeds (LD_{50}) was standardized.

Based on germination rate 50 %, LD_{50} dose of (60) gamma radiation for the seeds was calculated using linear regression equation. The plants were allowed to grow and data were evaluated for seedling growth parameters such as collar diameter, plant height and leaf number.

Statistical analysis

The analysis of variance (ANOVA) was used to determine the differences in average of all tested parameters between irradiated and non-irradiated seeds. All calculations were done in SPSS version 20 and Microsoft Excel.

Results and discussion

Jatropha curcas sample

The physical properties of *Jatropha curcas* are shown in Table 1 and fig. 1.

Table 1. Physical characteristics of *Jatropha curcas* seed.

Sample	Weight (g)	Length (mm)	Width (mm)
J.curcas	0.879±0.031	19.12±0.76	11.13±0.30

The value of whole seed weight (0.879 g) is higher than that found by Martinez-Herrera *et al.*, (2006)(0.72 g). Dry seed weight is the idiotype commonly used as an indicator of superior variety of Jatropha plant as it is correlated with oil production (Das *et al.*, (2010).

Values are means of 100 separate seeds.

Seed germination percentage

The germination test was conducted at 28 °C. Martins *et al.*, (2008) and Dias *et al.*, (2007), reported that *Jatropha curcas* seeds did not germinate at temperatures below 20 °C or above 45 °C. To date, there is no consensus as to the most suitable temperature for the germination of these seeds.

Table 2. Growth performance of *J.curcas* plants derived from seeds irradiated with different dose of gamma rays (30 DAG).

Dose of gamma rays Gy	Germination %	Survival rate %	Plant height cm	Collar diameter cm	Leaf number
Untreated	89.8	92.3	17.30±1.79	0.892	7
100	78.2	85.4	14.24±2.33	0.818	6
200	50.4	67.2	12.17±2.14	0.652	6
300	39.2	55.9	8.57±2.28	0.527	5

Values are Triplicate

± Standard Deviation.

The evaluations of the test were made at 5 and 12 days after sowing, there was stabilization of scores from the 12th day.

According to Oliveira *et al.* (2014), the first count and the final count of germination in *Jatropha curcas* seeds should be performed at 7 and 12 days after sowing.

Fig. 1. Dry seeds of *Jatropha curcas*.

Germination % of *Jatropha curcas* seeds differed significantly among treatments and is presented in Table 2. The results revealed that the highest seed germination % (89.8 %) was observed in seeds untreated and lowest (39.2 %) in those treated by 300 Gy. After gamma irradiation, some seeds were unable to germinate due to disturbances in the metabolic pathway. Gamma-rays affected the biochemical reactions that regulate the metabolism of the germination process.

The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kova'cs and Keresztes, 2002.). It supports the claim of (Chaudhuri,2002;Zaka *et al.*, 2004;Patade *et al.*, 2008) that low dose of gamma irradiation stimulates cell division and high-dose inhibits cell division due to free radicals and DNA system damage. Noticeable variations were observed in germination percentage after gamma irradiation.

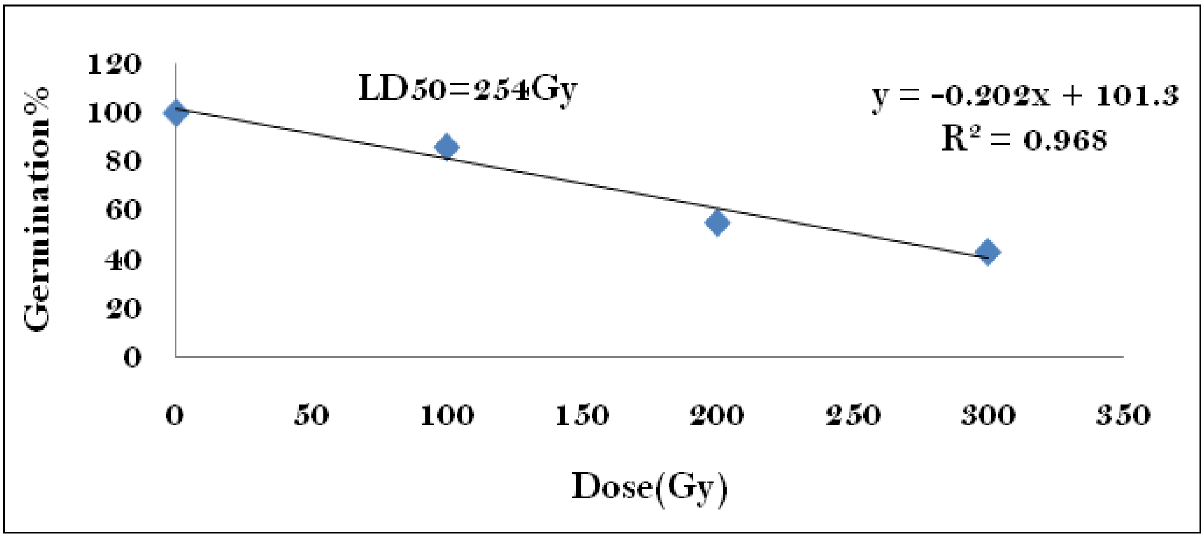

Fig. 2. Radio sensitivity test of *Jatropha curcas* after acute gamma irradiation, seed germination.

Radio-sensitivity test

Gamma rays derived from Cobalt-60 source belong to ionizing radiation and are the most energetic form of such electromagnetic radiation, having the energy level from around 10 kilo electron volts (keV) to several hundred keV. Therefore, they are more penetrating than other types of radiation such as alpha and beta rays. They cause cellular damage, thus induce high variants at phenotypic levels (Kova´cs and Keresztes, 2002). Radio-sensitivity test involves the determination of the dose that causes 50% reduction of vegetative growth of the treated plants material (LD_{50}) when compared to the control treatment (Azhar *et al.*, 2009).

Healthy and dry seeds were used as basic material. Higher dose of gamma rays (100-300 Gy) was applied to the seed, because of its lower water content. Songsri *et al.* (2011) reported that *J.curcas* seeds treated with gamma radiation higher than 600 Gy resulted in complete mortality. Fig. 2 shows the dose response after acute gamma irradiation and 254 Gy was the amount of ionizing radiation that caused 50 % mortality of the tested seeds (LD_{50}). In previous studies, Wang *et al.* (2009) found that LD_{50} doses of gamma radiation were 178 to 198 Gy for seeds of *Jatropha curcas* from different source in China and India. Azhar *et al.* (2009) indicated that LD_{50} value for seeds from Malaysia was obtained at 297 Gy. LD_{50} dose was estimated roughly as 425 Gy for five Jatropha genotypes collected from different part of Thailand by Songsri *et al.* (2011). In this study, the LD_{50} dose 254 Gy was lower than those reported by Azhar *et al.* (2009) and Songsri *et al* (2011) but higher than those found by Wang *et al.*(2009). The variations in LD_{50} doses of different studies might be possibly due to seed moisture rather than seed source. Seeds with low moisture seem to be more tolerant to gamma radiation than the seeds with high moisture (Songsri *et al.* 2011).

Fig. 3. The healthy seedling at 30 DAG of *J.curcas* subjected to ^{60} Co gamma rays.

Growth Performance and Survival percentage

Growth performance of the seedlings was measured 30 days after sowing. The effects of irradiation on the growth are visible and heights were statistically significant among treatments (Fig.3 and Table 2).

The control had the highest measurement (17.30 cm). Shakoor *et al.* (1978) and Khalil *et al.* (1986) attributed decreased shoot and root lengths at higher doses of gamma rays to the alteration of metabolic processes due to nucleic-acid disruption, which in turn disturbed the hormonal action. This reduced the mitotic cell division in the apical meristem. Data showed that irradiation with 100,200,300 Gy resulted in survival % of 85.4, 67.2 and 55.9 % respectively compared to control 92.3 %.

The survival of *J. curcas* seedlings decreased with increasing doses of gamma rays. Experiment results were in harmony with Songsri *et al.* (*2011*), Nayak *et al.* (2015) and Sharkh *et al.* (2016) where they concluded that higher doses of gamma radiation resulted in the significant reductions in germination, survival % and plant height.

Analysis of variance showed significant differences at the 0.05 level among gamma ray treatments for germination, survival % and plant height at 30 days after sowing.

Conclusion

Based on the results obtained from the present investigation it can be concluded that germination traits of *Jatropha curcas* seeds were sensitive to increase in gamma-ray. The high dose treatments (200 and 300 Gy) showed an inhibitory effect on all the parameters studied like germination, survival percentage and plant height as compared to the control. The inhibitory effect at a higher dose may be due to DNA damage such as breakage, inversion deletion of DNA structure.

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