



Radiosensitivity and Biological Effects of Gamma and X-Rays on Germination and Seedling Vigour of Three *Coffea arabica* Varieties

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Received: 11 January 2021 / Accepted: 16 March 2022 / Published online: 31 March 2022
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Abstract

Effects of gamma and X-ray treatments were studied on three varieties of *Coffea arabica* (Kent, Mundo Novo and Geisha) to determine their radiosensitivity and relative biological effects. The coffee varieties seeds were subjected to 0, 50, 100, 150, 200 and 400 Gy of gamma and X-rays from Cobalt 60 (⁶⁰Co) source irradiation. The irradiated seeds were pre-germinated in Petri dishes placed in a germination chamber, whilst some were sown in the greenhouse for germination studies. Data were collected on germination date and rate, root and hypocotyl length to determine the relative biological effectiveness of treatments and the optimum dose. The results showed varieties responding differently to the irradiations and doses. There was a decrease in germination with increasing doses of the irradiation. The X-ray-treated seeds had less germination percentage and seedling vigour measured at 28 days after treatment compared to the gamma-irradiated seeds. The irradiation effects on germination suggest that lower doses of X-rays give the same Relative Biological Effects as higher gamma doses for both growth chamber and greenhouse germination for Geisha at LD₅₀, where the effects were similar for the two irradiations. Whereas 50–100 Gy stimulated germination and seedling vigour, 150 Gy adversely affected germination and no germination occurred at 200–400 Gy. The study concluded that all the coffee varieties evaluated are sensitive to gamma and X-ray irradiation in terms of germination, seedling vigour and biological effects with an optimum dose of 50–100 Gy. Therefore, both gamma and X-rays could be utilized in a future mutational breeding programme for coffee seedlings.

Keywords *Coffea arabica* · Crop improvement · Lethal dosage · Mutagenesis · Physical mutagens

Introduction

Coffee is a perennial tropical crop that belongs to the genus *Coffea*, in the family Rubiaceae. The family comprises about 650 genera and 13,000 species (Rova et al. 2002). *Coffea* species are native to Africa; Madagascar and the Mascarenes

and there are published taxonomies for the genus (Coste 1992; Fazuoli et al. 2000; Maurin et al. 2007; Davis et al. 2011). Coffee is the fourth most valuable traded agricultural commodity, one of the most consumed commodities in the world and the second most valuable commodity exported by developing countries (FAO 2015) with over 75 million people depending on it for all or most of their livelihood (Pendergrast 2009; FAO 2021). Thus, as an important source of income and employment, coffee production contributes significantly to the economies of producing countries (Orozco-Castillo et al. 1996; Anthony et al. 2001; Déchamp et al. 2015).

Coffea arabica and *Coffea canephora* are the most widely used commercial varieties of coffee (Berthouly and Ettiene 1999). *Coffea Arabica* indigenous to Ethiopia is an autogamous tetraploid ($2n = 4x = 44$) with a remarkably narrow genetic base (Wrigley 1995; Anthony et al. 2001). Because of its superior quality and comparatively low caffeine content, *C. arabica* accounts for about 75% of world coffee production (Coste 1992; Orozco-Castillo

Handling Editor: Ranjit Riar.

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et al. 1996). It is very popular with different folks who used it for the stimulatory effect. The economic potential of *C. arabica* besides its autogamous and perennial character has led to the establishment of its homogeneous plantation in many countries of the world (Carneiro 1999; Perfecto and Armbrecht 2003), with sales values of several US billion dollars (Viniestra-Gonzalez 2000). The soils, nutrient and environmental conditions required for *C. arabica* cultivation have been reported (Wellman 1961; CRIN 1989; www.ico.org). In 2007, amongst west and central African producing countries, Nigeria ranked second after Sierra Leone with an estimated yield of 1440 kg/Ha and 1636 kg/Ha, respectively (FAO 2015).

Over the years, coffee improvement is slow and little attention is given to the development of new cultivars despite its global economic status. The narrow gene pool of *C. arabica*, lack of funding and the longer time required for conventional breeding and progeny evaluation, which could last decades are the major constraints for improvement (Mishra and Slater 2012). Besides, its self-fertilization habit promotes low genetic diversity. Therefore, there is a need to explore artificial sources of inducing variability to harness alternative and verifiable methods of tree crop breeding which could reduce the breeding cycle in the crop. To this effect, mutagenesis could play a role.

Physical mutagens such as gamma rays and X-rays influence plant growth and development by inducing cytological, genetical, biochemical and physiological changes in cells and tissues (Islam et al. 2014; Bado et al. 2017; Quintana et al. 2019; Wanga et al. 2020). Mutation breeding through irradiation has been applied on some crop species ranging from cereals, vegetables, root and tree crops etc. (Brunner and Keppel 1991; Noor, 2009; Mba et al. 2010; Animasaun et al. 2014; Ge et al. 2015; Bado et al. 2017; Lamo et al. 2017; Wanga et al. 2020; Andrew-Peter-Leon et al. 2021). Also, studies have shown the effect of irradiations on seed germination (Mensah et al. 2005; Warghat et al. 2011; Animasaun et al. 2014; Deshmukh et al. 2018). Kumar et al. (2007) reported the germination inhibitory effects of seeds exposed to higher doses of irradiations. However, lower exposures may sometimes be stimulatory (Thapa 2004).

Tree crops improvement by conventional breeding is less desirable because of the breeding cycle. Notwithstanding, mutation breeding has been employed in the production of improved and useful mutants of some tree crops (IAEA 2015) and about 3200 mutant varieties of crops are available on the database (www.mvgs.iaea.org). But *Coffea* spp. with its industrial and economic importance has not been included in any mutagenesis for its improvement. Moreover, coffee seeds germinate slowly in the field, taking an average of 50–60 days or more during the low-temperature season. This also poses a challenge for conventional crop improvement. Hence, mutation breeding

may be inevitable to enhance the variability and genetic resources of coffee. This, of course, would be significant in developing improved cultivars.

Plants respond to irradiation with various biological changes (Forster and Shu 2012), which may involve biological injuries, inhibition or disruption of the enzymatic pathway, reduction in growth and yield, alteration of the life cycle sequence, photochemical damage and induction of mutations in the DNA sequence (Spinoso-Castillo et al. 2021). Thus, the determination of an optimal dosage or time of exposure for mutagenic improvement in crop plants is important (Animasaun et al. 2014). Therefore, the present study tests the biological effects (sensitivity) of *Coffea arabica* to X-ray and Gamma irradiations with the view to determine the optimum dosage and relative biological effect of the irradiation sources on the seedling survival and vigour.

Materials and Methods

Sample Collection and Preparation

Three varieties of *Coffea arabica*, Kent, Mundo Novo and Geisha, were used for this study. The varieties were amongst 112 accessions of *C. arabica* introduced to Nigeria from Kenya in 1964, maintained on the conservation filed of Cocoa Research Institute of Nigeria (CRIN), Mambilla sub-station, Taraba State of Nigeria. The field is 1800 m above sea level, Latitude 6.71 °N and Longitude 11.25 °E. The annual rainfall is about 1905 mm and the average annual temperature is 16 °C (CRIN 1989). Fresh seeds samples were harvested from the Mambilla sub-station plots and wet processed at Plant Breeding Section in CRIN, Ibadan, Nigeria. Good beans (seeds) with no infection or injury were sorted out and stored in a seed room at 4 °C in vacuum desiccators over glycerol (60% v/v) or room temperature for 4–7 days to reduce the moisture content to 12–14% to prepare for irradiation.

Seeds Irradiation

Irradiations of the seeds were carried out at Plant Breeding and Genetics Laboratory (PBGL), Seibersdorf, Austria from Gamma and X-ray sources. The gamma rays were from a Gamma chamber GC4000 (10 kCi) having Cobalt 60 (⁶⁰Co) source operated at 8 Gy/s, whilst 250 kVp X-ray was generated from a 15-mA Cobalt 60 source for 5 min. The seeds were treated with irradiation doses of 0, 50, 100, 150, 200 and 400 Gy.

Germination Studies

100 seeds without parchment from each variety were soaked in water for 72 h, shortly after the irradiation. The seeds were surface sterilized in 70% alcohol (20 s) and 20% of clorox solution for 8 min with occasional vigorous shaking as described by Wang et al. (2009). The seeds were rinsed in demineralized water and placed on soaked filter paper (no.860, Schleicher & Schuell, Dassel, Germany) in 88-mm Petri dishes in four replicates. The plated Petri dishes were kept in the growth chamber at 27 °C for 12-h period. The filter papers were moistened every three days. Germination and development were monitored in the Petri dishes until the emergence of the radicle.

Another 100 irradiated seeds with parchment were soaked for five days in warm tap water for imbibition. The seeds are sown into a growing plastic tray (600 × 400 × 80 mm) filled with a mixture of peat moss and garden soil (1:4) for each variety in three replicates laid out in the greenhouse at Seibersdorf. Seeds were planted in 6 rows representing the treatments in terms of dosage of radiation (0, 50, 100, 150, 200 and 400 Gy), a distance of 10 mm and a depth of 60 mm. The temperature of the greenhouse was kept at 27 °C for this work, the soils were watered every two days to avoid over damping of the germinating seeds. Germination and development were monitored until the emergence of the seedling.

Data were collected on germination and the seedling vigour on 14, 21 and 28 days after treatment (DAT) from the Growth Chamber experiment as described by Brazil (2002). Also, germination studies were carried every week till seven weeks after sowing (WAS) on the greenhouse seedlings. Data were taken on the survival percentage and hypocotyl length. Hypocotyl and root length were measured from the seedling following the methods of Tshilenge-Lukanda et al. (2012). The germinations data were obtained in three replicates.

Data Analysis

The collected data were analysed. The LD₅₀ and LD₃₀ (dosage required to reduce the population by 50% and 30%, respectively); GRD₅₀ and GRD₃₀ (dosage required to reduce the root and hypocotyl length by 50% and 30%, respectively) were determined. Also, the relative biological effect (RBE) of the dosage on the varieties and seedling morphological data were analysed using SPSS 17 and GenStat 1998 version. Results are presented in tables and figures for discussion.

Results

Growth Chamber and the Greenhouse Studies

Germination of the *Coffea arabica* seeds treated with gamma and X-rays started 7 days after treatment (DAT) in the growth chamber with a visible radicle protruding out of the stony endocarp (Fig. 1). The irradiated seeds sprouted at a different time. The radicle emergence was observed at 7 DAT in the control (0 Gy) set-up, whilst it took 7, 9, 11, 14 and 15 DAT in 50, 100, 150, 200, and 400 Gy-treated seeds, respectively. The number of germinated seeds and the speed of germination varied significantly for the two treatments and the applied doses (Table 1). At 14 DAT, germination only occurred in seeds treated with 0 and 50 Gy of gamma rays in Kent and Mundo Novo varieties, whilst 0, 50 and 100 Gy-treated seeds of Geisha variety germinated within the same period. The significant highest number of germinations occurred in Geisha seeds treated with 0 and 50 Gy gamma irradiation. Meanwhile, no germination was observed in 150–400 Gy-treated seeds for the three varieties. At 21 DAT, germination was recorded for 0–150 Gy-treated seeds of Kent and Mundo Novo. Whereas germination occurred in 0–100 Gy gamma-irradiated seeds of Geisha, 200 and 400 Gy seeds recorded no germination amongst all the varieties. This trend was similar at 28 DAT, whilst 50 Gy enhanced optimum germination, doses above 100 adversely affected seed sprouting. At 150 Gy, germination was less than 50% in Kent and Mundo Novo varieties, but none in Geisha. No germination was recorded for 200–400 Gy. The trend of germination for the X-ray-treated seeds were like that of the gamma-irradiated seeds as shown in Table 1. Although, 50 Gy X-ray produced a similar germination effect as the 0 Gy in Kent and Mundo Novo, high and similar germination effects were found in Geisha at 0, 50 and 100 Gy. For the three varieties, 200–400 Gy X-ray had no germination.

Germination of the seeds (with parchment) in the greenhouse showed varying effects of the irradiation sources and doses on the coffee varieties as shown in Fig. 2. Germination occurred for both irradiation sources and treatments except in higher dosages of 200 and 400 Gy. However, germination commenced at 4 WAS, although few, for Mundo Novo and Geisha varieties irradiated at 200 Gy of X-ray. Germination equilibrium was attained at 5 weeks after sowing (WAS). Although germinations were delayed at treatments higher than 100 Gy, the peak of germination was at different weeks for the treatments and the doses. The 0 Gy-irradiated Kent seeds attained germination peak at 5 WAS, whilst it was 4 WAS for Mundo Novo and Geisha varieties. The three varieties had the highest seed germination (above 90%) at 50 Gy dose, both

Fig. 1 Germination of coffee treated with different doses of Gama and X-ray irradiation. *R* sprouting radicle; *C* cotyledon (The radicle emergence was observed at 7 DAT in the control (0 Gy) set-up, whilst it took 7, 9, 11, 14 and 15 DAT in 50, 100, 150 200 and 400 Gy-treated seeds, respectively)

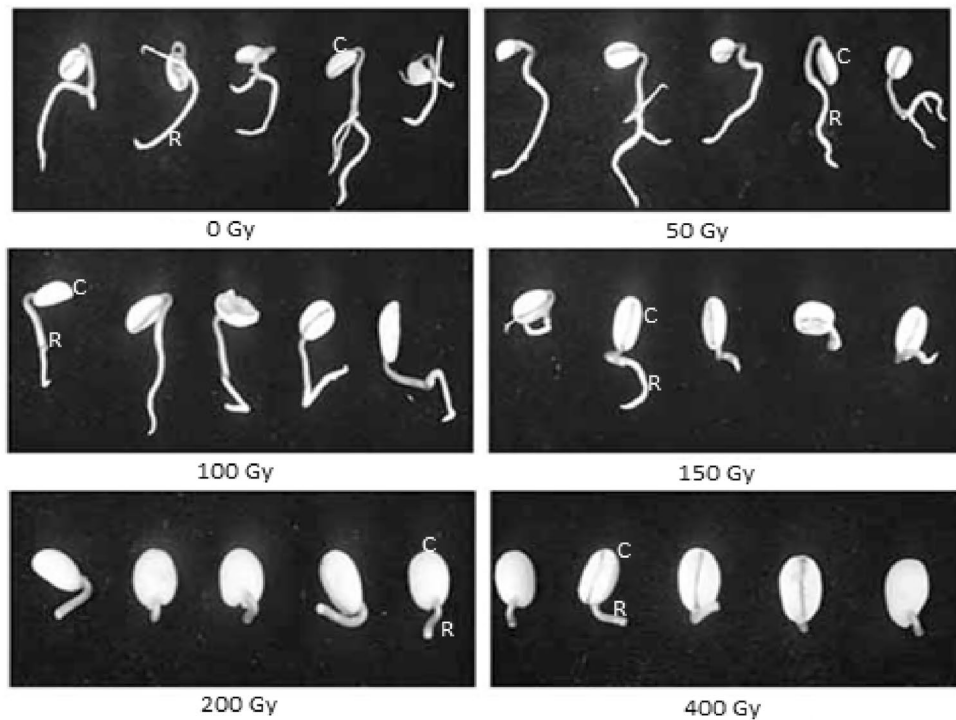


Table 1 Effect of different doses of Gamma and X-ray irradiation on germination of three varieties of *Coffea arabica* in the Growth Chamber

Variety	Treatment	Gamma ray			X-ray		
		14 DAT	21 DAT	28 DAT	14D AT	21 DAT	28 DAT
Kent							
	0 Gy	30.0 ^b	85.3 ^a	90.1 ^b	30.3 ^b	90.5 ^b	90.1 ^{ab}
	50 Gy	18.4 ^{ce}	77.6 ^{cd}	95.4 ^{ab}	19.8 ^{cd}	75.2 ^{cd}	80.7 ^c
	100 Gy	0.0 ^e	70.1 ^d	80.0 ^c	0.0 ^e	70.7 ^d	75.2 ^{cd}
	150 Gy	0.0 ^e	35.3 ^{gh}	50.3 ^f	0.0 ^e	10.4 ^j	25.6 ^{hi}
	200 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	0.0 ^k	0.0 ^h
	400 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	0.0 ^k	0.0 ^h
Mundo Novo							
	0 Gy	10.3 ^d	100.8 ^a	100.4 ^a	15.4 ^{cd}	100.7 ^a	100.4 ^a
	50 Gy	15.7 ^{cd}	100.5 ^a	100.1 ^a	10.2 ^d	80.3 ^c	100.8 ^a
	100 Gy	0.0 ^e	77.4 ^{cd}	80.5 ^c	0.0 ^e	75.4 ^{cd}	90.6 ^b
	150 Gy	0.0 ^e	25.9 ^{hi}	45.4 ^{fg}	0.0 ^e	15.3 ^{ij}	30.3 ^h
	200 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	0.0 ^k	0.0 ^h
	400 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	0.0 ^k	0.0 ^h
Geisha							
	0 Gy	40.4 ^a	100.4 ^a	100.6 ^a	35.2 ^{ab}	100.4 ^a	100.4 ^a
	50 Gy	36.6 ^{ab}	100.1 ^a	100.7 ^a	42.6 ^a	95.7 ^{ab}	100.7 ^a
	100 Gy	15.4 ^{cd}	100.7 ^a	100.3 ^a	10.5 ^d	100.9 ^a	100.1 ^a
	150 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	15.3 ^{ij}	60.4 ^c
	200 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	0.0 ^k	0.0 ^h
	400 Gy	0.0 ^e	0.0 ^k	0.0 ^h	0.0 ^e	0.0 ^k	0.0 ^h

Values with the same letters within the same column under the same treatment are not statistically significant at 5% LSD

DAT Day after treatment of the seeds with different doses of gamma or X-ray irradiations

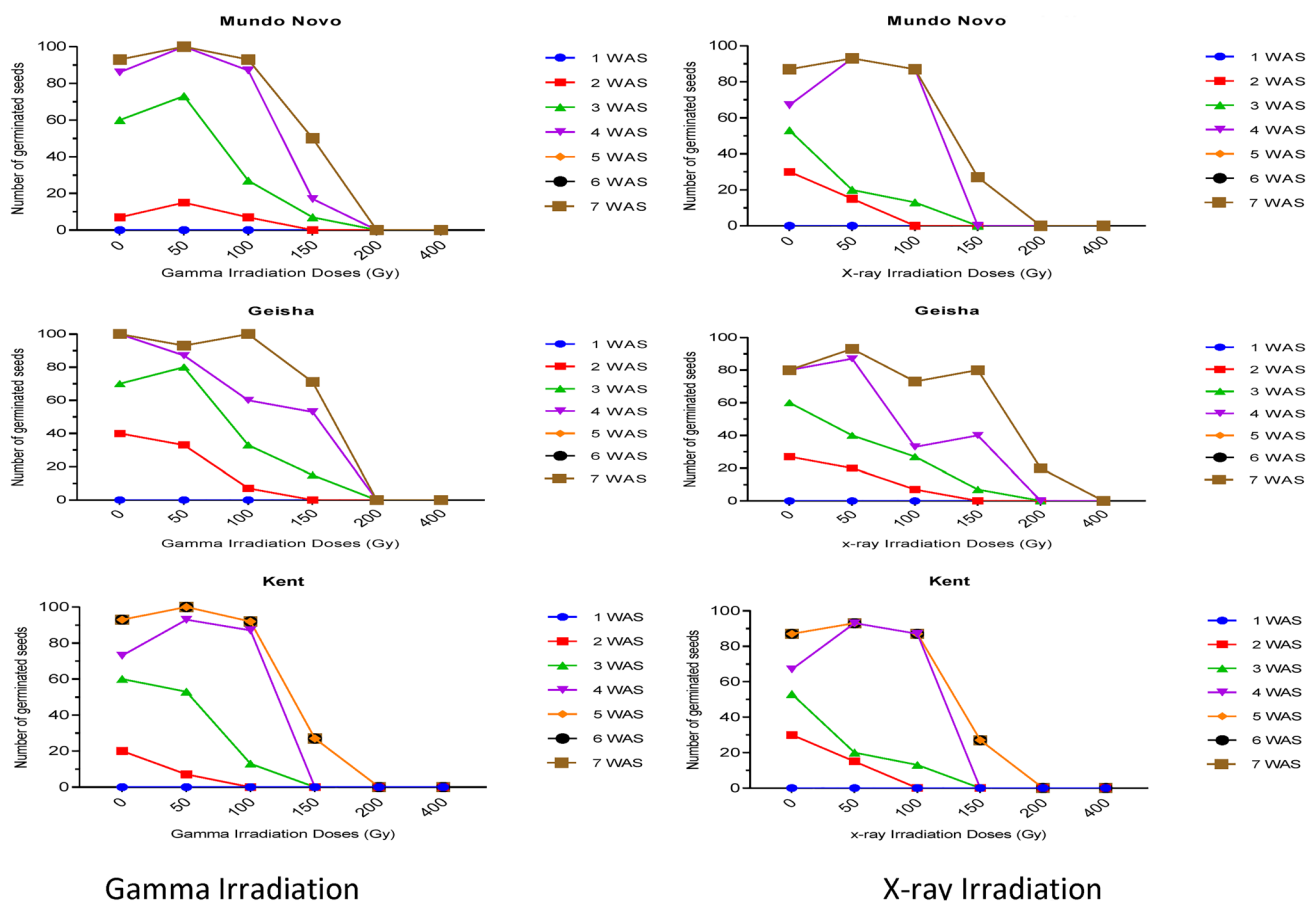


Fig. 2 Germination of three *Coffea arabica* varieties treated with different doses of gamma and X-ray irradiations evaluated under greenhouse conditions. WAS: Weeks after sowing

for gamma and X-ray irradiations, 100 Gy also stimulate germination in the coffee varieties. Meanwhile, 150 Gy of gamma irradiation had 50, 70 and 27% germination at 7 Was for Mundo Novo, Geisha and Kent, respectively, 200 and 400 Gy gamma-irradiated seeds did not germinate. At 7 WAS 100% germination was achieved in Mundo Novo and Kent treated with 50 Gy gamma irradiation, but in Geisha, it was obtained with 0 and 100 Gy. In contrast, none of the doses had 100% germination in any of the varieties amongst the X-ray-irradiated seeds. Notwithstanding, 50 Gy X-ray stimulated the highest germination (93%) in the varieties (Fig. 2). The ability of 200 Gy X-ray to induce germination in coffee varieties except Kent is in contrast to no germination observed in the Growth Chamber in 200–400 Gy of both irradiations. However, there was no germination at 400 Gy in all the varieties.

Seedling Vigour

The seedling vigour (Hypocotyl and Root length) as shown in Table 2 varied significantly with the treatments, doses

and varieties. The roots did not develop at 200 and 400 Gy gamma irradiation despite hypocotyl emergence. Some roots developed from the 150 and 200 Gy-treated seeds are friable. The longest root length (3.08 cm) was recorded in 0 Gy Mundo Novo, whilst the shortest (0.43 cm) was obtained from 150 Gy gamma-treated Mundo Novo, which is not different (at LSD of 0.05) from the values obtained in Kent and Geisha varieties for 150 Gy. For the X-ray-irradiated seedlings, the highest root length (3.03 cm) was recorded at 50 Gy on the Geisha variety and the shortest (0.42 cm) occurred at 150 Gy in the Kent variety. The values are not different from the performing of Mundo Novo and Geisha variety irradiated at 150 Gy (Table 2). The longest hypocotyl length (2.60 cm) was recorded at 50 Gy on Geisha variety and the shortest (0.70 cm) was observed at 400 Gy in Kent, Mundo Novo and Geisha (Table 2).

The Biological Effect of the Treatment on Seeds

Table 3 shows the sensitivity effect of irradiations on germination of irradiated seeds of three *Coffea arabica* varieties

Table 2 Effect of gamma and X-ray irradiation doses on seedling vigour of three varieties of *Coffea arabica*

Variety	Treatments	Gamma ray		X-ray	
		RL (cm)	HL (cm)	RL (cm)	HL (cm)
Kent	0 Gy	2.42 ^d	2.28 ^c	2.38 ^d	2.17 ^c
	50 Gy	2.53 ^d	2.28 ^c	2.29 ^{de}	2.15 ^c
	100 Gy	2.08 ^e	1.35 ^e	1.86 ^e	1.01 ^{ab}
	150 Gy	0.45 ^h	1.13 ^g	0.42 ^h	0.92 ^b
	200 Gy	0.00 ⁱ	0.95 ⁱ	0.00 ⁱ	0.45 ^c
Mundo novo	0 Gy	3.08 ^a	2.45 ^b	2.99 ^b	2.80 ^a
	50 Gy	2.90 ^b	2.60 ^a	2.89 ^b	2.65 ^b
	100 Gy	1.45 ^f	1.25 ^f	1.44 ^f	1.30 ^e
	150 Gy	0.43 ^h	0.98 ^{hi}	0.44 ^h	1.10 ^f
	200 Gy	0.00 ⁱ	0.83 ^j	0.00 ⁱ	0.77 ^{gh}
Geisha	0 Gy	2.70 ^c	2.58 ^a	2.80 ^c	2.90 ^a
	50 Gy	2.80 ^{bc}	2.45 ^b	3.03 ^a	2.80 ^a
	100 Gy	1.20 ^g	1.48 ^d	1.50 ^{fg}	1.50 ^d
	150 Gy	0.50 ^h	1.05 ^{gh}	0.50 ^h	1.45 ^{de}
	200 Gy	0.00 ⁱ	0.90 ^{ij}	0.00 ⁱ	1.29 ^e
	400 Gy	0.00 ⁱ	0.70 ^k	0.00 ⁱ	1.15 ^{ef}

Values with the same letters within the same column under the same treatment are not statistically significant at 5% LSD

RL Root length, HL Hypocotyl length measured in centimetre on the germinated seeds

from which lethal dose for 50% (LD_{50}), lethal dose for 30% (LD_{30}), the growth reduction dose for 50% (GRD_{50}) and the growth reduction dose for 30% (GRD_{30}) were determined. The comparison of relative biological effects (RBE) on the root and hypocotyl of the coffee seedlings revealed that the required dosage to reduce the population by 30% (LD_{30}) was lower than the required dosage to reduce the population by 50% in all the varieties. LD_{30} was highest in the Geisha variety and lowest in the Kent variety under gamma and X-ray irradiation. For the relative biological effect was 1 ($RBE = 1$) at LD_{50} for Geisha variety. The biological effect of radiation on the varieties' root as computed in the table was more or less than 1. The growth reduction dosage GRD_{30} ranged between 57.6 and 88.5 Gy, whilst GRD_{50} ranged 121.9–155.6 Gy in treatment with gamma irradiation. For the X-ray-treated seeds, GRD_{30} was between 74.3 and 109.2 Gy and GRD_{50} fell between 170.7 and 241.6 Gy. Geisha variety was found to be the highest in both treatments. The survival effect and relative biological effect in terms of root and hypocotyl development of the varieties was highest in Kent. Meanwhile, the biological effect of radiation on the variety root was more or less than 1. The higher the biological effect, the higher the fitness of the variety to tolerate the irradiations.

The optimum dosage for germination and seedling (Fig. 3) describes the irradiation effect on the germination, the root and hypocotyl length. The optimum irradiation dose for germination lies between 50 and 100 Gy as the peak of germination was obtained within the dosage range. Doses above 100 Gy are counterproductive and

Table 3 Comparison of the biological effect of irradiation source on germination, root length and hypocotyl length of three varieties of *Coffea arabica*

Variety	Gamma ray (Gy)		X-Ray (Gy)		RBE	
	LD_{30}	LD_{50}	LD_{30}	LD_{50}	LD_{30}	LD_{50}
Germination						
Kent	21.00	107.80	15.20	100.00	1.38	1.08
Mundo Novo	80.40	158.30	65.90	143.50	1.22	1.10
Geisha	91.70	150.00	83.30	150.00	1.10	1.00
Root length (cm)						
Kent	8.85	15.56	7.51	14.53	0.12	1.07
Mundo Novo	4.72	12.19	5.16	12.54	0.91	0.97
Geisha	5.76	13.01	6.81	13.83	0.84	0.94
Variety	Gamma ray (Gy)		X-Ray (Gy)		RBE	
	GRD_{30}	GRD_{50}	GRD_{30}	GRD_{50}	GRD_{30}	GRD_{50}
Hypocotyl length (cm)						
Kent	11.54	22.74	7.86	17.07	1.47	1.33
Mundo Novo	9.63	20.02	7.43	17.87	1.30	1.12
Geisha	9.14	19.47	10.92	24.16	0.84	0.81

LD_{50} Lethal dose to reduce the population by 50%, LD_{30} Lethal dose to reduce the population by 30%, RBE Relative biological effect, GRD_{50} Growth reduction dose for 50%, GRD_{30} Growth reduction dose for 30%

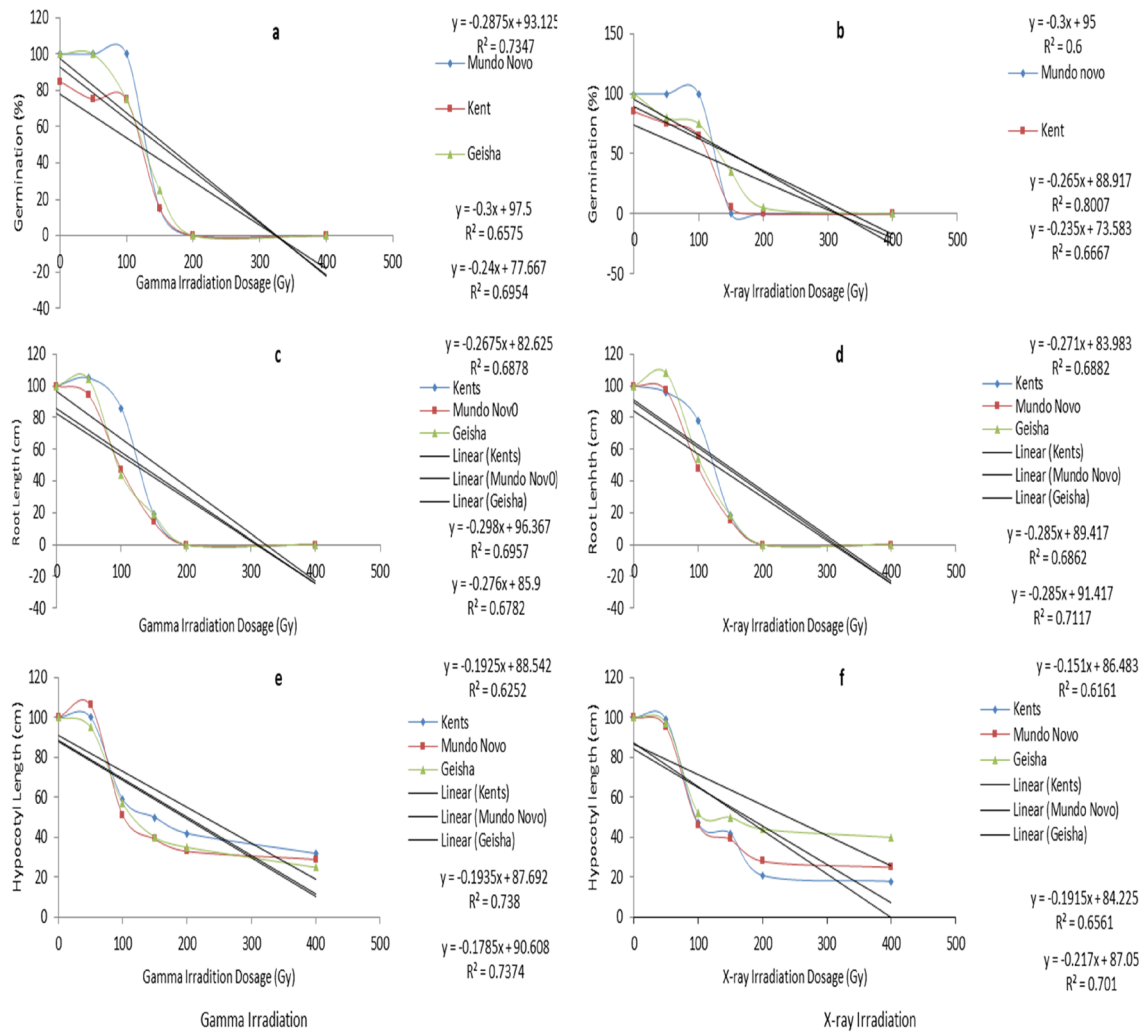


Fig. 3 Determination of optimum dose and relative biological effect of Gamma and X-ray irradiations on germination, root and hypocotyl length of three varieties of *C. arabica*

negatively affect germination. The equation got for the seedling vigour (roots) for gamma irradiation were Geisha ($y = -0.276x + 85.9$), Kent ($y = -0.298x + 96.367$) and Mundo Novo ($y = -0.2675x + 82.625$). The GRD_{30} and the GRD_{50} ranged from 57.6–88.5 Gy and 121.9–155.6 Gy, respectively, in gamma irradiation treatment. For both irradiation types, 50–100 Gy was most effective, with fewer negative effects on root development. For the X-ray-treated seeds, the equation obtained were Geisha ($y = -0.285x + 89.417$), Kent ($y = -0.285x + 91.417$) and Mundo Novo ($y = -0.271x + 83.983$). GRD_{30} and GRD_{50} ranges were 51.6–75.1 Gy and 125.4–145.3 Gy, respectively. In terms of hypocotyl development which is an important component of seedling vigour, 0–50 Gy of both gamma and X-ray treatments had an optimum effect on the three varieties of *C. arabica*.

Discussion

The germination study of the three varieties of *Coffea arabica* seeds subjected to different doses of gamma or X-ray irradiation showed a slow and non-uniform germination pattern for the treatments and the varieties. The germination results got from the present study were congruent to earlier reports of da Silva (2002; Spinoso-Castillo et al. 2021). Usually, seed germination begins with water uptake (imbibition) by the seed and ends with the elongation of the embryonic axis. However proper germination takes place when hypocotyl and roots developed from the elongated embryo (Brasil 2002). All the varieties under investigation had high in vitro germination at 28 days after treatments for irradiation doses between 0 and 100 Gy.

Meanwhile, in the greenhouse experiment, the germination peak was between 5 and 7 weeks after sowing (WAS). Coffee seeds germinate slowly (Boliva-Gonzalez et al. 2018); however, in the current study, many of the treated seeds germinated between 4 and 6 WAS depending on the varieties. For instance, the Kent variety was less tolerant of irradiation in terms of germination. This suggests that the varietal factor is important in seed propagation. Besides, seed treatments could either enhance or delay germination. It has been demonstrated that seed germination is adversely affected by high doses of irradiation (Mensah et al. 2005; Warghat et al. 2011; Animasaun et al. 2014; Deshmukh et al. 2018).

The positive effects of low irradiation doses of 50–100 Gy on coffee seed germination showed the dose can activate enzymatic or physiological changes that favour the germination process (Islam et al. 2014; Spinoso-Castillo et al. 2021). The 50 Gy of gamma and X-ray irradiations stimulated 100% germination in Geisha and Mundo Novo and above 80% was achieved in the Kent variety. This is likely because of stimulation of the embryo radicle, which enhanced their germination even better than the control at 95% and 90, respectively. Although some argued that exposure of plant materials to low doses of irradiation may sometimes be stimulatory and promote growth (Thapa 2004; Oladosu et al. 2016), higher doses or longer time of exposure may be injurious, negatively affecting germination, genetic composition, or biochemical/physiological activities of the cells or tissue. None or poor germination recorded at higher doses (200–400 Gy) of both gamma and X-ray in this study was due to inhibitory effects on the seeds. It is known that both gamma and X-ray irradiations can penetrate the seeds, and they could therefore cause impairment of the metabolic process of *de novo* synthesis regulating and controlling germination and growth; hence, the low and delayed germination and poor seedling vigour associated with high doses of gamma and X-ray exposures (Forster and Shu 2012).

Coffee seed germination requires optimum conditions, even at that, germination is usually slow and may take between 50 and 60 days to attain a germination peak. However, the percentage of germinated seeds at 7 WAS recorded in the present study may be ascribed to varietal factor, more so, *C. arabica* accounts for the highest percentage of the world coffee production (Berthouly and Ettiene 1999; Orozco-Castillo et al. 1996; Perfecto and Armbrecht 2003). The ability of the varieties to germinate in less time could also be an added advantage to its choice in plantation establishment. The optimum condition created by the growth chamber resulted in radicle sprouting within 14 days after plating in tandem with the earlier work of Brasil (2002). The results for gamma irradiation showed that germination was not different at 100% ($p < 0.05$) for Geisha and Mundo Novo varieties. In terms of sensitivity to irradiation, Geisha

is more sensitive than Mundo Novo and Kent. The latter two varieties are much more tolerant to irradiation, as they recorded better germination and seedling vigour. The varieties are more sensitive to gamma irradiation than the X-ray. This may likely be due to the penetrating power and ionization capacities of the rays. Whereas 150–400 Gy of gamma irradiation was lethal for Geisha germination, radicle sprouting occurred in Kent and Mundo Novo at the dosage. However, 150 Gy X-ray treatment recorded germination in all the varieties, although delayed. This further gave credence to the opinion that there is a differential sensitivity of species to irradiation (Pathirana et al. 2002; Khan and Goyal 2009; Animasaun et al. 2014; Quintana et al. 2019).

The relative biological effectiveness of the two irradiations on coffee varieties revealed that low doses (50 Gy) may stimulate root and hypocotyl growth, but the effect is higher in Mundo Novo and Geisha. Doses above 100 Gy are detrimental to seedling vigour. This finding agreed with the report of Quintana et al. (2019) that coffee varieties are sensitive to irradiation doses. Similar observation has been reported early by a number of workers (Mensah et al. 2005; Chowdhury and Tah 2011; Shagufta et al. 2013). The authors alluded that higher irradiation doses or a long time of exposure could cause a significant decrease in root development and seedling vigour. Since roots are primarily concerned with material assimilation and translocation from the soil or growth media, friable and fragile roots could not perform the function well and the stunted growth of the plant. The relative biological effect greater than 1.0 shows similar activities of the irradiation type, whereas a lower value than 1.0 indicates that the X-ray biological effect on the varieties is more than the gamma ray. The interaction of the variety with atoms or molecules of mutagens releases free radicals, which are responsible for the differences in the germination and the seedling vigour of the varieties. The relative biological effect (> 1.0) of the varieties in response to the irradiation doses showed that the varieties are sensitive to the irradiations, although Mundo Novo and Geisha are more sensitive than Geisha.

Despite the potential of physical mutagenesis in crop improvement (Shamsi and Sofjay 1980; Pathirana et al. 2002; Shamusuzzaman et al. 2005; Amanda et al. 2008; Warghat et al. 2011; Forster and Shu 2012), higher irradiation doses are detrimental (Mensah et al. 2005; Khan and Goyal 2009; Chowdhury and Tah 2011; Shagufta et al. 2013; Andrew-Peter-Leon et al. 2021). There is a need therefore for the knowledge of varietal sensitivity and optimal dose to achieve desirable effects in crop mutagenesis as the success of mutagenesis or otherwise to a greater extent depends on the utilization of the optimal dose. This will be the basis of a rationale for its application in crop improvement. From the current study, a low dose of both irradiations stimulated germination in the three varieties of *C. arabica*. Also,

100 Gy performed well compared to 0 Gy as the control. A high dose of mutagenic treatment likely destructed growth promoters, increase growth inhibitors and metabolic status of the seedling (Mba et al. 2012; Gudkov et al. 2019). Therefore, the present results suggest that doses between 50 and 100 Gy of gamma and X-ray could achieve optimum germination and seedling vigour in the coffee varieties as it stimulates early emergence, increased percentage germination and vigorous seedlings. Animasaun et al. (2014) noted that gamma irradiation within the range of 20–40 Gy is optimum for germination, vigour and yield in *Digitaria* spp. The reduced percentage of germination and seedling survival at a high dosage of irradiation possibly indicates the greater sensitivity of the crop due to the occurrence of more genetic chromosomal and physiological disturbances at these concentrations. Thus in the current study, 50–100 Gy possibly enhanced germination and vigour because of activation of RNA or protein synthesis at the early stage of germination.

Conclusion

The sensitivity of the three varieties used in this study shows the variation in response to the different dosages of gamma and X-ray irradiations. Geisha varieties had the lowest sensitivity to the irradiations compared to the other two varieties. 50–100 Gy of both irradiations stimulates radicle sprouting and seedling vigour, whilst doses above 100 Gy are detrimental to the germination and seedling fitness of the varieties. The germination pattern and seedling development of the coffee irradiated with gamma rays were like that of the X-ray. The study concludes that varieties response to irradiation varied and that low doses of 50–100 Gy could be employed for germination inducement in coffee arabica varieties.

Acknowledgements The authors are thankful to the staff and members of the Plant Breeding and Genetics Laboratory, International Atomic Energy Agency, Vienna, Austria for their technical support.

Author Contribution KED conceptualized the work and conducted the irradiation and manuscript development; DAA analysed the data, interpreted the data and developed the final manuscript; OTM alongside KED conceptualized the work and the execution and manuscript proof; SB and BPF designed the experiment, alongside KED conducted the irradiation and analysed the data. All the authors are involved in manuscript development.

Funding No funding was received for this work.

Declarations

Conflict of interest The authors have no conflict of interest to declare whatsoever.

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