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EFFECTS OF X AND GAMMA RAYS ON THE RADIOSENSITIVITY TEST OF TWO MALAGASY UPLAND RAIN FED RICE VARIETIES

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ABSTRACT

Finding the appropriate irradiation dose rate is a critical step before initiating a mutation plant breeding program. The objective of this research is to identify the most effective irradiation type for generating genetic diversity in two Malagasy upland rice varieties, namely 3729 and 3737, which are susceptible to the parasitic plant *Striga asiatica*. The seeds of these rice varieties were subjected to different irradiation doses, ranging from 0, 75, 150, 300, 450, and 600 Gy, using Gamma and X-rays. The germination and survival rates of the rice seeds were evaluated based on the LD50 value, while the height and root length were assessed using GR30. Based on the LD50 values for germination and survival rates, as well as the GR30 values for shoot and root length reduction, irradiation doses ranging from 75 to 100 Gy were found to be favorable for inducing mutations in the genomes of both rice varieties using X-rays. For gamma rays, the suitable irradiation doses were determined to be between 75 and 200 Gy. It should be noted that gamma irradiation exhibited greater effectiveness in inducing genetic variation in all tested rice varieties compared to X-rays.

KEYWORDS: induced mutation, gamma-ray, X-ray, *Oryza sativa*, LD50, GR30.

INTRODUCTION

Rice (*Oryza sativa* L.) holds significant importance as one of the world's major food crops. However, its production is hindered by various biotic and abiotic stresses. In Madagascar, rice is the primary staple crop and food source, followed by cassava and maize (Bosser, 1969). The Malagasy population ranks as the second-largest consumer of rice globally, with an average annual consumption of 138 kg per person, which remains high (UPDR/FAO, 2000). Nonetheless, domestic production fails to meet the demands of the growing population (Randrianarisoa, 2005). Traditional rice cultivation methods yield an average of 2.8-3 t/ha through low-yield irrigated rice production alone (Rakotofiringa and Tokarski, 2007). Several factors contribute to insufficient rice production in agricultural areas, including natural disasters, climate change (such as droughts, floods, cyclones, and grasshopper invasions), poverty, rapid population growth, traditional practices, and inappropriate economic policies (Sourisseau et *al.*, 2016). To address the need for increased rice yields, it is crucial to develop rainfed rice cultivation on plateaus named "Tanety"in Malagasy terms, which are typically used for maize crops and pastures (Rakotoarisoa, 2016). However, rainfed rice crops in Madagascar face numerous challenges, including poor soils, water scarcity, viral and insect infestations, diseases, and particularly the presence of the parasitic plant *Striga asiatica* (Kuntze), as well as the lack of improved rice varieties. Enhancing rice varieties is a vital approach to improving food security (Arouna, 2017). Currently, mutagenesis-based crop improvement methods serve as effective tools for rapidly increasing genetic variability within plant genomes and inducing direct genotype changes in cereals (Van Harten, 1998; Chahal and Gosal, 2002). Radiation-induced mutation using gamma and X-rays is a practical method for augmenting genetic variability within the genome and improving agronomic traits, such as yield, disease and pest resistance, in food crops, ornamental plants, and export crop varieties (IAEA, 1977; Van Harten, 1998; Mohamad et *al*., 2005; FAO, 2011), especially in response to climate change (Micke et *al*., 1990; IAEA, 1995). Numerous studies have demonstrated the advantages of using physical mutagens in plant breeding for field crops such as wheat, rice, barley, cotton, and beans (Ahloowalia and Maluszynski, 2001).

Radiosensitivity testing serves as a relative measure to assess the effects of radiation on irradiated objects (Morishita et *al.,* 2003; Albokari et *al.,* 2012), considering parameters such as LD50 and GR30, and aids in determining optimal irradiation doses for inducing genetic variability within the rice genome. With the aim of contributing to rice production improvement and ensuring food security in Madagascar. This study aims to compare the effects of gamma and X-rays on the growth and development of two Malagasy upland rain fed rice varieties.

MATERIALS AND METHODS

Plant material

Two rain fed rice varieties called Marotia or 3729 belonging to *japonica* subspecies and Telorirana or 3737, *indica* subspecies, known as sensitive to the parasitic plant *Striga asiatica*, and among the most appreciated by consumers and most cultivated in the Middle West regions of Madagascar were used in this study.

METHODS

Pre-treatment methods

Before subjecting the upland rain fed rice seeds to irradiation, a pre-treatment process was carried out. The harvested seeds had a moisture content of approximately 70 to 72%. To reduce the moisture content or relative humidity (RH) of the seeds, they were pre-treated by placing them in a desiccator containing 60% glycerol for a period of five days. This pre-treatment process aimed to achieve a moisture content or RH level of 12-14% in the seeds before they were exposed to irradiation.

Irradiation treatment

In this study, a total of seventy-five (75) seeds from each upland rain fed rice variety were selected for each treatment. The selected seeds were then exposed to varying doses of gamma rays and X-rays using a Cobalt-60 gamma irradiator. The doses of irradiation applied were 75, 150, 300, 450, and 600 Gy. The same set of doses was used for both gamma rays and X-rays. For each variety and irradiation dose, a total of four hundred fifty (450) rice seeds were used, with 25 seeds per dose of irradiation per variety. These seeds were divided into three replications, resulting in a total of three sets of experiments for each treatment. The irradiation process was conducted at the FAO/IAEA Plant Breeding Unit located in the Seibersdorf laboratory in Austria.

Germination test and rice culture on a hydroponics system

After irradiation, all the irradiated rice seeds of each variety were planted in Petri dishes containing moistened filter paper soaked with distilled water. The Petri dishes were then placed in an incubator set at a temperature of 25±2°C to facilitate germination of the seeds. For the hydroponic system, young seedlings at the age of seven days were carefully transplanted into trays filled with Yoshida's solution, a liquid nutrient medium commonly used for rice cultivation (Yoshida, 1965). There were six doses of irradiation in each tray, making it one replication. In total, three replications were carried out for each variety and each irradiation dose, resulting in a comprehensive experimental setup to assess the effects of irradiation on rice seedling growth and development.

Parameters considered

To assess the radiosensitivity of the rice plants and determine the optimal irradiation doses for inducing genetic mutations, several parameters were measured. Firstly, the germination rate and survival rate of the irradiated rice seeds were recorded. The germination rate represents the percentage of seeds that successfully sprouted, while the survival rate indicates the percentage of seedlings that survived after irradiation. Additionally, the maximum length of shoots and roots was measured to evaluate the growth and development of the rice plants. These measurements were taken in a hydroponic system, where the seedlings were grown in trays filled with Yoshida's solution. To determine the suitable irradiation doses, two specific values were considered: the lethal dose at 50% (LD50) and the 30% growth reduction (GR30). The LD50 represents the dose at which 50% of the irradiated seeds or seedlings fail to germinate or survive, indicating the lethal effect of radiation. On the other hand, the GR30 indicates the dose at which there is a 30% reduction in the length of shoots and roots compared to the non-irradiated control plants. By analyzing the LD50 and GR30 values, the favorable irradiation doses for inducing genetic mutations within the rice plant genome can be determined. These values are essential for establishing the effective doses of radiation that promote genetic variability and desired traits in the rice varieties (Van Harten, 1998).

Data analysis

The collected data, including seeds germination, shoot length, root length, and survival plant, were analyzed to assess the significance of quantitative changes caused by the irradiation dose and type using statistical methods. Firstly, the normality and homogeneity of variance assumptions were tested using the Shapiro-Wilk test. This test helps determine if the data follow a normal distribution and if the variances are equal across different groups or treatments. Once the assumptions were met, a two-way ANOVA was performed. This analysis allows for assessing the significance of the effects of irradiation dose and irradiation type on the various parameters measured in the rice genotypes. It helps determine if there are significant differences between the treatment groups and if these differences can be attributed to the irradiation factors. To further analyze and compare the treatment means, the Tukey Honesty Significance Difference Test (HSD) was employed. This post-hoc test helps identify specific differences between treatment groups and determine which groups are significantly different from each other. To visualize the relationship between the radiation dose and the variable rates studied, such as germination rate, shoot length, root length, and survival plant, curves were created using simple linear regression. This graphical representation helps understand the trend and pattern of the data in response to the radiation dose. All the statistical analyses were performed using Prism version 9 software, which provides tools for data analysis and visualization.

RESULTS AND DISCUSSION

Before giving the results on each parameter, the following tables resume the means square values and significance tests for all tested parameters.

The analysis of variance revealed that the irradiation type (IT) affects the irradiation dose (IR) on seed germination (Gr), plant survival (Svr), shoot reduction (Shr), root reduction (Rr) rate of the two rice varieties 3729 and 3737 (Table 1 and 2). Significant difference was observed between these parameters studied (Table 1 and 2). The growth reduction (GR) was measured by the shoot reduction (Shr).

Table I: Mean-square values and significance tests for germination, survival, shoot reduction, and root reduction rate of the rice variety 3729.

Source of variation		Mean square (3729)			
	DF	Gr	Svr	Shr	Rr
Irradiation dose (ID)	5	6880***	8122***	5964***	5666***
Irradiation type (IT)	1	1714**	4301***	1212***	1471**
ID x IT	5	85.63***	383.9***	113.6***	69.54**
Residual	10	7.689	4.706	2.720	10.49

*DF=degrees of freedom, *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level*

Table II: Mean-square values and significance tests for germination, survival, shoot reduction, and root reduction rate of the rice variety 3737.

Source of variation		Mean square (3737)			
	DF	Gr	Sr	Shr	Rr
Irradiation dose (ID)	5	5666***	8291***	8722***	10074***
Irradiation type (IT)	1	1471**	1340**	335.1**	251.3*
ID x IT	5	69.54**	84.10***	44.91**	52.44***
Residual	10	10.49	1.935	7.999	3.925

*DF=degrees of freedom, *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level*

1- Effect of X and Gamma rays on seeds germination of 3729 and 3737 varieties

The critical gamma and X-rays irradiation results have shown highly significant differences in germination rate among the 0, 75, 150, 300, 450, and 600 Gy treatments doses. Whatever the rice genotype tested, the result reveals a gradual diminution in the germination rate with increased Gamma-and X-rays irradiation doses (Figure 1(A) and (B)). Highly significant interaction effects ($p < 0.01$) were recorded between irradiation dose and irradiation type on rice seed germination (table 1 and 2). This indicates that the genotype, the irradiation dose, and the irradiation type significantly influence the optimum dose of gamma radiation in the tested rice.

The germination rate varies from 4.8 to 100% at 7 days after sowing. Regardless of the type of irradiation used, the germination rate always reached 100% for all controls (0Gy). At the dose of 75Gy, the germination rate has reached 86.21% using gamma-ray and 72% for X ray for 3729 rice variety. Statistically, there is a difference in these values. This difference is also found in 3737 rice variety for each dose and for each type of irradiation used. From 75 Gy, the curves show both for X and gamma rays and for 3737 and 3729 varieties that the higher the irradiation dose, the lower the germination rate (Fig 1 A and B). At 600 Gy, gamma ray can germinate 20% of seeds instead of 5% for X ray (for 3729 variety) and it is 25% for gamma and 10% for X ray with 3737 variety. The LD50 was recorded at 242 Gy for X ray and 341Gy for gamma ray with 3729 variety. Using 3737 variety, this LD50 was identified at 274 Gy for X ray and 380 Gy with gamma ray. From this study, it was demonstrated that the low doses of 75 to 300 Gy for gamma and 75 to 150 Gy for X rays were found favorable for seed germination for the two rice rain fed varieties respectively 3729 and 3737. The high doses of 600 Gy using gamma or X rays had reduced significantly the germination percentage with the respective values 20% and 5% (Photo 1). The effectiveness of gamma and X irradiation on the percentage of germination after seven days was evident, even reductions are not significant for the two varieties, and this result is similar with that obtained by Harding et *al.*, (2012) who worked on rice varieties cultivated. During the observations, it was remarked that by increasing the irradiation dose, the germination percentage of the tested rice varieties decreases, which indicates that with high doses of irradiation, seeds lose their faculty of germination, because more DNA were damaged that would result in a large number of undesirable mutations (Britt, 1996; Alboukari et *al.*, 2012). The germination time of the control rice seeds is faster than that of the irradiated ones, as mentioned by Van Harten (1998) who demonstrated that the cut at the ATGC base of their DNA can no longer reorganize due to the high irradiation dose. This delay may be due to the mechanism of chromosome rearrangement after breaking the linkages following irradiation. The observation of Seung (2007) and Mba et *al.*, (2012) have shown that among the physical mutagens, gamma ray is the most efficient and energetic form to induce mutation because they are more penetrating and their effect is more beneficial than other radiations such as X ray.

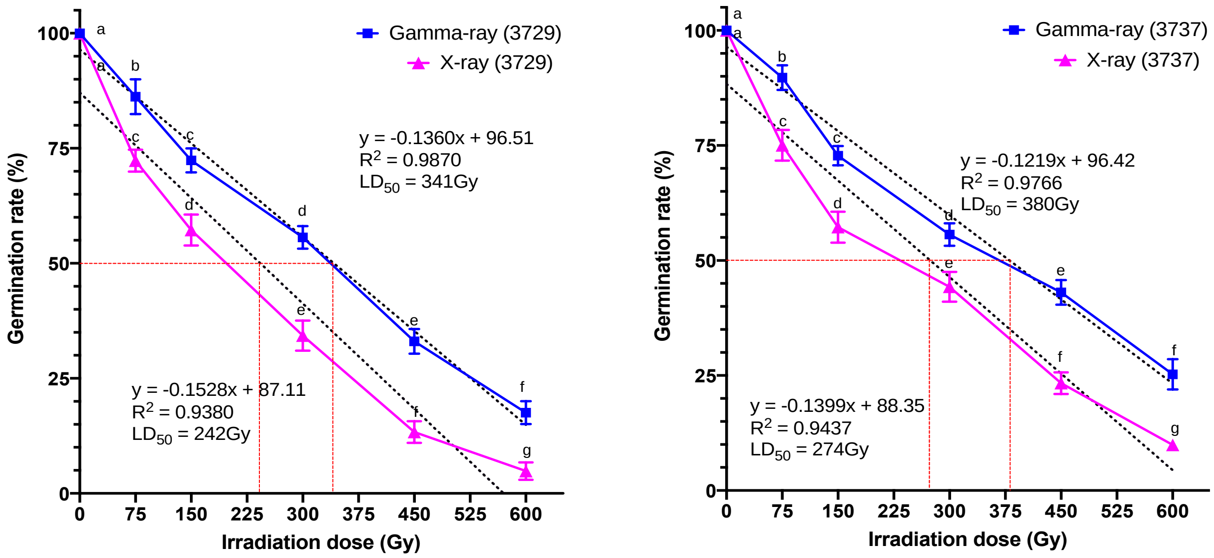

Figure 1: Effects of Gamma and X-rays irradiation on seeds germination of two rain fed rice varieties 3729 and 3737.

2- Effect of X and Gamma rays on survival rate of 3729 and 3737 varieties

After two months of sowing, the gamma and X-rays irradiation results have shown that the survival rate varies from 4,5 to 100%. There are highly significant survival rate differences among the 0, 75, 150, 300, 450, and 600 Gy treatments doses. For the two rice genotypes tested, the result exposes a gradual reduction in survival rate with increased Gamma-and X-rays irradiation doses (Figure 2 (A) and (B)). Highly significant interaction effects ($p < 0.01$) were recorded between irradiation doses and irradiation types on plant survival rate. This suggests that the genotype, th1e irradiation dose, and the irradiation type are significantly different. Regardless the type of irradiation used, the germination rate has always reached 100% for all controls (0Gy). At the dose of 75Gy, the survival rate reaches 82% using gamma ray and 60% for X ray for 3729 rice variety, and for 3737, it was 85% for gamma ray and 68% for X ray then the survival rate decreases up to 0% at 450 Gy for X ray, and it is 10% at 600 Gy using gamma ray. This identical situation is also found in 3737 rice variety at 450 Gy (0%) for X ray and 15% at 600 Gy for gamma ray. The lethal dose at 50% or LD50 is recorded at 169 Gy (for X ray) and at 310 Gy for gamma using 3729 variety. For 3737 the LD 50 was identified at 230 Gy for X ray and 308 Gy for gamma. No survival plant was observed at the 450 Gy for X ray but very low percentage (10 and 15%) at 600Gy using gamma ray both for the two rice varieties (Figure 2 (A) and (B)). A significant difference effect was observed between gamma and X rays on rice survival plant. With low doses of 75 to 150 Gy, the 3729 and 3737 rice seedling varieties irradiated with gamma ray have shown well-developed growth compared to those from X ray (photos 2 to 6). The results of this study have also shown that the rain fed rice variety belonging to *indica* subspecies (3737) has well supported high irradiation dose than the *japonica* subspecies (3729). Several studies confirm these results like that obtained by Rakotoarisoa (2008) by testing the radiosensitivity of five Malagasy rice varieties such as Mailaka, Rojofotsy, Soameva and IR58614 (*indica* ssp), Malady (*japonica* sub species), which mentionned that the rice varieties belonging to *indica* subspecies tolerate high irradiation doses using gamma ray compared to *japonica* subspecies. Similar results have been reported in rice by Raveloarisolo and Andrianaivo (2002); Razafinirina, 2011; Andrianjaka, 2011; Zhang et *al.*, 2019; Abdelnour-Esquivel et *al.*, 2020 and Haque et *al.*, 2021) in their studies. At the M1 generation, the plant survival rate can reach 82.02% and 85% for gamma ray and 60 and 68% for X ray using the low dose of 75 Gy with 3729 and 3737 varieties respectively. This result is similar to that obtained by Razafinirina (2011), who tested gamma ray sensitivity on rice and maize varieties in germination and survival plant rate which were higher for low doses. Parween and Siddiqi (2012), who have revealed that low irradiation doses provide favorable effects on the survival of the *Zingiber officinale*. Several studies have already been carried out and have shown the effectiveness and benefits of using gamma rays because of their beneficial effects on improving characteristics like germination, root and shoot length, and survival (Kon et *al.*, 2007; Harding et *al.*, 2012, FAO/IAEA, 2018). In this study, for the survival of rice plants, the use of low dose of gamma ray 75 to 300Gy is favorable compared to X ray where the favorable doses are from 75 to 150Gy.

Photos comparing the effect of Gamma and X-ray irradiation on rainfed rice plants of variety 3737

Photo 1: Seeds germination of 3737 rice variety.

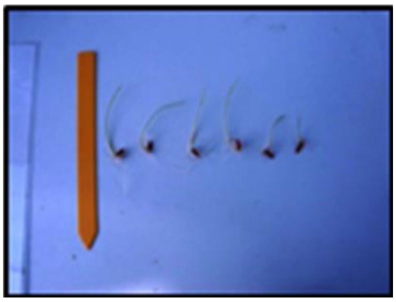

Photo 2: 3737 rice seedling measurement.

Photo 3: Shoot length and survival plants, 3737 rice variety from gamma ray.

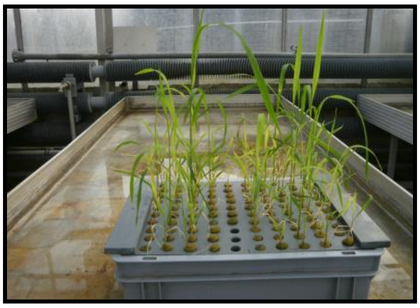

Photo 4: Shoot length and survival plants 3737 rice variety from X ray.

Photo 5: Root length of 3737 rice variety from gamma ray.

Photo 6: Root length of 3737 rice variety from X ray.

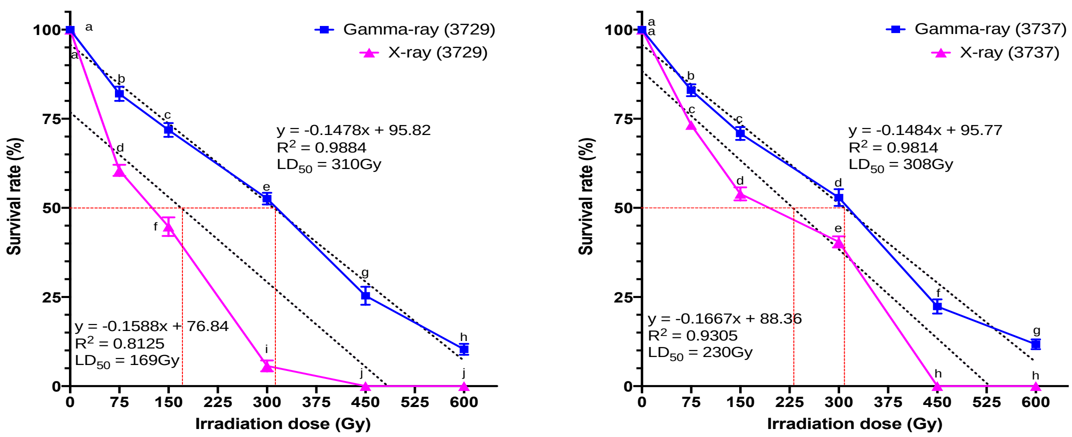

Figure 2: Gamma and X-rays irradiation effects on plant survival rate of two rain fed rice varieties 3729 and 3737 at different dose.

3- Effect of X and Gamma rays on shoot length reduction of 3729 and 3737 varieties

Highly significant interactions ($p < 0.01$) were observed between radiation doses and irradiation type on plant shoot reduction. The assessment of shoot reduction is determined in the third week after sowing. The size of the seedlings of two rice varieties decreases with the dose. Conversely, the 100% reduction rate in this figure Figures 3(A) and (B) means no seedling shoot height reduction was observed compared to the control. When the irradiation dose increases, the shoot length is reduced. At the dose of 75 to 150 Gy, growth reduction was varied 5 to 28% using X ray and 5 to 25% for gamma ray with 3729 variety. This situation was also found in 3737 rice variety at the same doses, shoot length reduction was varied between 3 to 25% using X ray, it was 5 to 25% until 200 Gy with gamma ray. The GR30 was recorded at 192 Gy for X ray and 270 Gy with gamma using 3729 variety. For 3737 variety, it was observed at 235 Gy for gamma and 200 Gy for X ray. The 450 and 600 Gy doses have affected a high shoot reduction of 75 to 90% for 3729 variety with X ray and 55 to 70% with gamma ray. 3737 variety has shown 80 to 90% of shoot reduction using X ray at those same dose of irradiation 450 and 600 Gy, and it was 75 to 85% with gamma ray (Figure 3 (A) and (B)). Regarding GR30, both rice genotypes 3729 and 3737 have shown well-developed shoot growth using gamma irradiation at the doses 75 to 300 Gy compared to those from X-ray. This experiment has demonstrated that low doses of gamma ray below 300 Gy, are favorable for reducing plant size.

This result confirms those of Rakotoarisoa (2001) and Razafinirina (2011) using a radiosensitivity test of some Malagasy rice varieties. For the X ray, the favorable doses for reducing the shoot length of four rice varieties M1 generation are 75 to 150Gy, for the rain fed systems. For the shoot length reduction, a significant difference in plant size of treated rice varieties was observed between gamma and X ray (Photo 3 and 4). Gamma ray promotes shoot length reduction compared to X. The reduction of plant height below 30% is ideal face to various factors limiting production, such as wind, rain, and floods. This situation is similar to that observed by Van Harten (1998). Above 300 Gy with gamma ray and 150 Gy for X ray, the physiological damage about shoot reduction within rice plants is superior to 30%. This hypothesis confirms that mentioned by Song et *al.*, (1988) and Offei et *al.*, (2014). In this study, it was revealed that the rice variety 3737 *indica* ssp can withstand high dose of 450 to 600 Gy treated with gamma radiation than X. This results which have exposed seeds to high doses of irradiation, and have produced dwarf plants with reduced roots, are similar and confirm to those of Khalil *et al.* (1986), who found decreased shoot and root lengths at high doses of gamma ray, this situation is the effect of mitotic activity modification in meristematic tissues and reduced moisture contents in seeds. Also, Borzouei (2010) showed in his study on the two wheat varieties (*Triticum eastivum*) that seedling growth is very low at high rather than low doses. The same, irradiation with gamma ray on wheat seeds reduces shoot and root lengths upon germination.

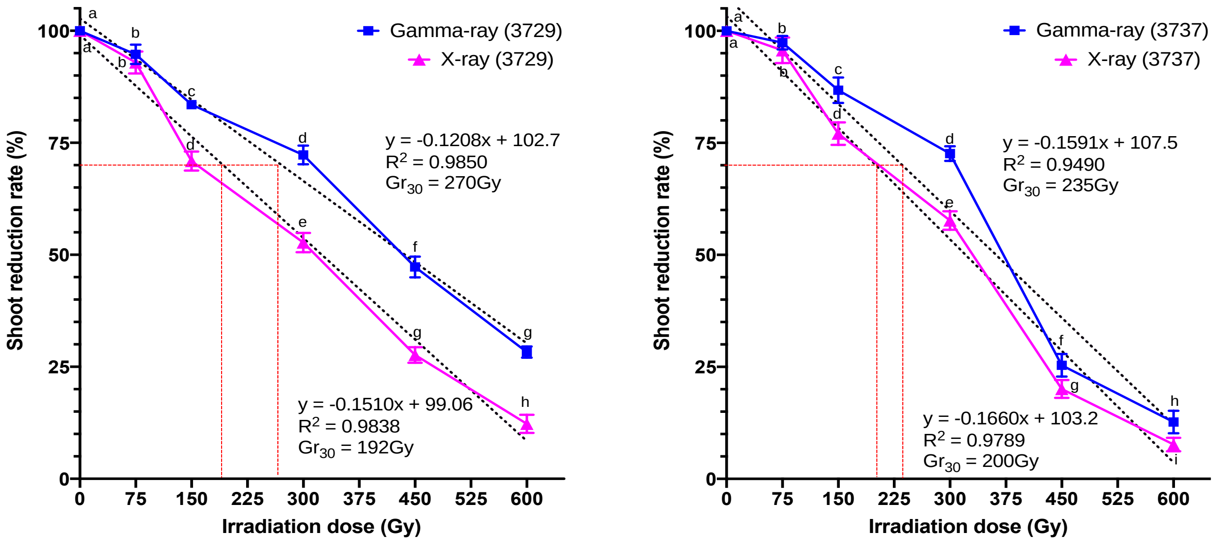

Figure 3: Effect of Gamma and X-rays on shoot reduction rate of two rain fed rice varieties 3729 and 3737.

4-Effect of X and Gamma rays on root length reduction of 3729 and 3737 varieties

The general observation of the two figures 4 (A) and (B) has described that when the irradiation dose increases, the root length is reduced. The analysis of the variance of genotypes has shown a significant difference between the root length of the tested rice between gamma and X-rays and between the irradiated seeds and the control ($p < 0.01$). The difference in root length reduction of rain fed rice is significant. It was ranged from 25 to 100% for the rice variety 3729 irradiated by the gamma ray and from 9.90 to 100% for the rice variety 3729. For the rice variety 3737, this root length reduction has ranged from 5.18 to 100% with the gamma ray irradiation and 3.47 to 100% with the X ray (Figure 4). Based on GR30, using gamma ray at doses of 75 to 300 Gy and 75 to 150 Gy for X rays had a beneficial effect on root growth. For this root reduction rate, the GR30 was observed at 270 Gy for 3729 irradiated with gamma rays and from 192 Gy for 3729 irradiated with X rays. For 3737 irradiated with Gamma ray, it was recorded at 235Gy and 200Gy for X ray (Figure 4 (A) and (B)). The 450 and 600 Gy doses were affected a high shoot reduction (75 to 90%) for 3729 with X ray and 60 to 75% for gamma ray. 3737 variety has presented a high root length reduction 90 to 95% when irradiated with X ray and almost similar from 85 to 90% for gamma ray. Regarding GR30, the doses 75 to 300 Gy using gamma ray were found favorable to maintain few (inferior to 30%) root length reduction, where rice seedling have shown well-developed root growth compared to those from X ray. A significant difference in root length reduction was observed between gamma and X ray on treated rice genotypes. Gamma ray promotes root length reduction compared to X (Photo 5 and 6). At the low doses of 75 to 300Gy, root length reduction was inferior to 30% and the roots of rice plants are more voluminous than those of other rice plants from high doses of 450 and 600Gy. Roots are one of the most important organs for plant development, especially for the uptake of organic and mineral nutrients (Uchida, 2000). According to Thorup-Kristensen and Kirkegaard (2016), improved root systems can help crops use soil resources more efficiently and thereby improve productivity and environmental outcomes. For the doses above 300 Gy, the reduction in root length of the two rice genotypes is evident, more than 50-95%, and roots are significantly reduced using high doses, especially those irradiated with X ray compared to those with gamma ray. Our experiment has demonstrated that the high dose of 600 Gy causes a total inhibition effect on plant growth of rice roots both for 3729 and 3737 genotypes, this result confirms that mentioned by Guha Mallick et *al.* (2022). Similarly, irradiation affects phenotypic changed by stimulating or reducing the root length of rice plants. This is the case of rice root lengths of 3737 and 3729 treated with gamma ray. The effect of gamma and X rays on root length in this experiment differed, and the inhibitor effect is evident when increasing doses of irradiation. Seeds exposed to higher doses produced dwarf plants with reduced roots (Kiani et *al*., 2022).

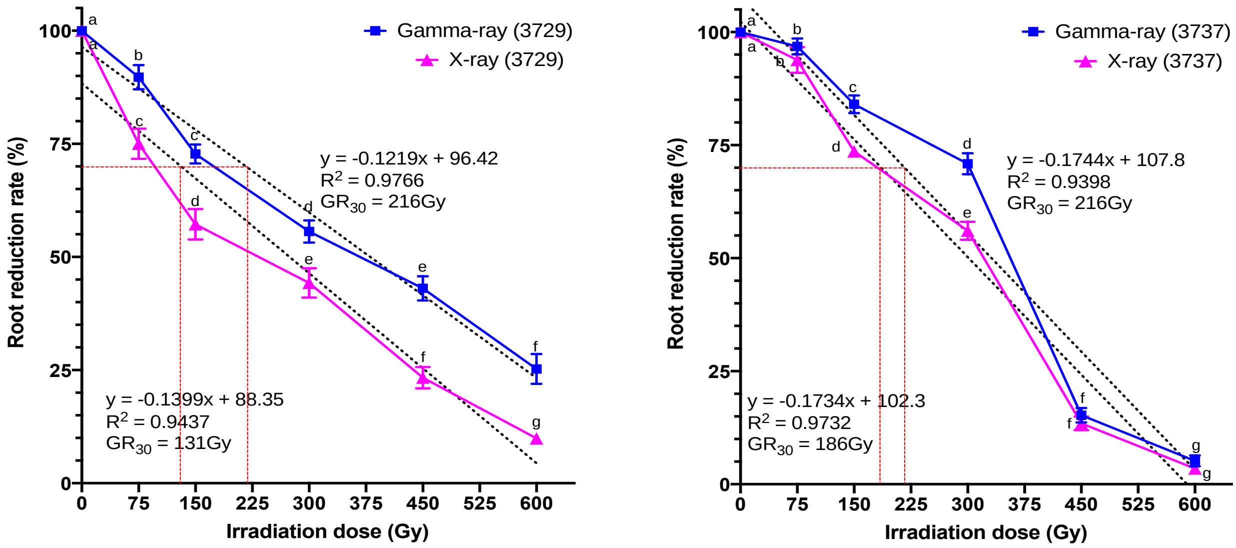

Figure 4: Gamma and X irradiation effects on root length reduction of two rice varieties 3729 and 3737.

CONCLUSION

In conclusion, this study demonstrates the potential of induced mutation through irradiation to improve rice varieties in Madagascar, particularly in addressing challenges posed by climate change and parasitic plants. The optimal irradiation doses for the two *upland rain fed* rice varieties, 3729 (*japonica* subspecies) and 3737 (indica subspecies), were determined to be 75 to 200 Gy for gamma ray and 75 to 100 Gy for X-ray in the case of 3729, and 75 to 200 Gy for gamma ray and 75 to 150 Gy for X-ray in the case of 3737. These doses resulted in germination and survival rates above 50% (LD50) and a reduction in shoot and root length below 30% (GR30). It was observed that gamma ray irradiation was more effective than X-ray irradiation for inducing beneficial effects in the two rice varieties. By applying low doses of gamma rays, it is possible to generate a mutant rice population that is tolerant to the parasitic plant *Striga asiatica* and capable of producing high rice yields. This research contributes to the development of improved rice varieties that can withstand environmental challenges and help ensure food security in Madagascar. Further studies and field trials are recommended to evaluate the performance and agronomic characteristics of the mutant rice populations obtained through irradiation-induced mutation.

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