Gamma radiosensitivity study on MRQ74 and MR269, two elite varieties of rice (*Oryza Sativa* L.)

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Abstract: Gamma ray, a mutagen used in crop and ornamental plant breeding, is expected to induce novel mutations. Therefore, this study aimed to investigate the effect of different doses of gamma-ray (Caesium-137) irradiation on the germination rate, plant height, and appropriate dose for causing boom in two varieties of local rice, namely, MRQ74 and MR269. Dry MRQ74 and MR269 seeds were irradiated at 0, 100, 200, 300, 400, 500, 600, and 700 Gy. Hydroponic germination under controlled conditions followed. The germination rate and the height of the plantlets were investigated as morphological responses. Consequently, compared with the control samples, the plantlets that were given low doses (0 and 100 Gy) showed an increase in height, whereas those given doses ranging from 200 to 500 Gy showed a reduction in germination rate and height. At doses exceeding 500 Gy, physiological damage on the seedling height became severe and none of the varieties survived. Doses between 340 and 360 Gy caused a 40% reduction in seedling height in MR269 and MRQ74. Doses between 351 and 365 Gy caused a LD50 dose in MR269 and MRQ74, respectively.
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Introduction

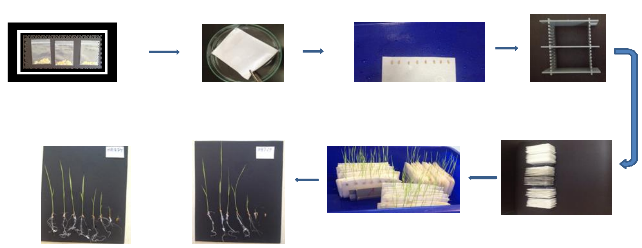
Rice (*Oryza sativa* L.) is considered the most significant cereal yield that has contributed to an essential diet for nearly half of the population worldwide (Adamu et al., 2004; Golestan Hashemi et al., 2013). The recognized crop prospective in rice is mostly obtained by genetically constant diversities with substantial yields. Monocropping of rice dominated through few diversities, causing a narrow genetic base and changing the rice ecosystem that was extremely vulnerable to exterior elements such as radiation stress. Temperature variations from radiation stress on rice not only caused permanent genetic variations but also created the best field residents by severely reducing the germination ability of seeds. Investigating the properties of radiation in plants is considered a broad and complex field. Research has been conducted on a wide range of plants in several geographical zones. Mutation refers to the process of revising the feature and genes that comprise the building blocks and foundation of plant development. Thus, raw ingredients are generated for economic yield and genetic development (Adamu et al., 2004). Mutation origins with dissimilar organizational regulate with DNA, similar to changes in a lone nucleotide basis of a gene (point alteration), changing one nucleotide basis with another, cutting off one or more base pairs in the DNA sequence (frame adaptation change), chromosomal reform, replication, or damage of a chromosomal unit (Poehlman and Sleper, 1995). Gamma (□) ray refers to ionizing radiation that cooperates with molecules in the cells and crops of free radicals that can potentially damage or modify cell features (Minisi et al., 2013). Gamma irradiation is extensively applied as the alteration agent in developing a genetic variety through farming because of its high saturation capability compared with related ionizing radiations (Akshatha et al., 2013). However, the use of gamma irradiation in farming is usually associated with differences in the dose of preservation, resulting in varying harvests and applications (Peri et al., 2011). New investigations indicated that several doses of gamma irradiation on plants may improve tolerance to abiotic stress conditions such as drought and salinity (Moussa, 2011; Song et al., 2012). Radiation usually influences the dimension and mass of plants. In several radiobiological reactions, the influence of the given dose relies on the concentration of radiation or the manner by which the entire dose is fractioned. The biological consequence of gamma rays is usually related to the collaboration with atoms or molecules through the cell and principally water to decrease the free radicals (Kovacs and Keresztes, 2002). Therefore, gamma rays usually affect plant development and improvement by inducing cytological, genetic, biochemical, and physiological changes in cells and tissues (Gunckel and Sparrow, 1961) depending on the amount of radiation of such radicals (Ashraf et al., 2003). The current study attempted to analyze several properties of ionizing radiation by using different doses of Caesium-137 on rice.

Materials and Methods

The sensitivity of two rice varieties to gamma rays was evaluated at the laboratory of the Nuclear Agency Malaysia. Seeds were irradiated using 0, 100, 200, 300, 400, 500, 600, and 700 Gy source of Caesium-137. Germination test was performed using dry seeds of rice varieties with an average moisture content of 13%. The irradiated seeds, along with non-irradiated seeds as controls, were sown in the laboratory. The hydroponic method was employed for seedling cultivation as described by Myhill and Konzak (1967). Seeds were interspersed with the embryo side facing down between two sheets of wet thick filter paper (thickness = 0.75 mm, weight = 400 g/m2, height = 6 cm, and width = 8 cm). After the sheets were rolled up, the resultant seed germination "sandwiches" were held up vertically in a plastic rack placed inside a plastic box (Fig. 1). Subsequently, 10 seeds were located at the top of each filter paper sandwich as the half bottom of the sandwich was submerged in water. The plastic boxes were placed in a growth chamber in darkness at a temperature of 4 °C for 72 h and then subjected to a photoperiod of 16 h light and 8 h darkness at 25 ± 2 °C. The germination rate of the planted seeds was fixed in the first week of the culture. Meanwhile, the height of the plantlets, the length of the roots, and the fresh weight of the plantlets caused a reduction in the seedling length (%); this reduction was recorded in the second week of the culture. The length of the roots and the height of the plantlets were measured using a ruler. Linear regression analysis was performed to assess the optimum LD_{50} doses for rice varieties; the germination rate under laboratory conditions was used as the standard measure of the germination test. The optimum dose for mutagenesis was measured based on a 40% reduction in seedling length (Van Harlen, 1998).

$$\text{Reduction in seedlings length (\%)} = \frac{\text{Mean of seedlings length (control)} - \text{Mean of seedlings length (a given treatment)}}{\text{Mean of seedlings length (control treatment)}} \times 100$$

In this study, the experiments were conducted in 10 replications. ANOVA and Tukey's honestly significant difference (Duncan) test were used to determine the significant differences between the means of each parameter tested in the study. The data were subjected to the analysis of variance using SAS programme (Release 9.1 for Windows, SAS Institute Inc., Cary, NC, USA) software.



Germination (MRQ74&MR269)

Fig. 1. The "sandwiches" of seed germination from MRQ74 and MR269.

Results and Discussion

Germination rate

The effect of gamma irradiation on seed germination of MRQ74 and MR269 was evaluated by identifying the LD_{50} doses of gamma radiation through the particular seeds. The results indicate that for 7 d, doses of gamma irradiation exerted considerable effects on seed germination. Germination rate was reduced by gamma irradiation (Fig. 2). The reduction in seed germination was not directly related to the increment of dosage outcome was not consistent for the nine rice varieties. Our findings were consistent with those of previous studies on rice by Sareen and Koul, 1999; Cheema and Atta, 2003; Harding et al, 2012; and

Pavan Kumar et al., 2013. The present study indicated that after 14 d, LD_{50} of gamma irradiation on the germination rate of the seedlings were estimated to be 365.1061 and 351.3429 for the MRQ74 and MR269 varieties, respectively (Table 1). Moreover, the efficiency of gamma irradiation decreased significantly at 500 Gy and 600 Gy. Beyond 600 Gy, none of the seedlings grew (Figs. 3 and 4).

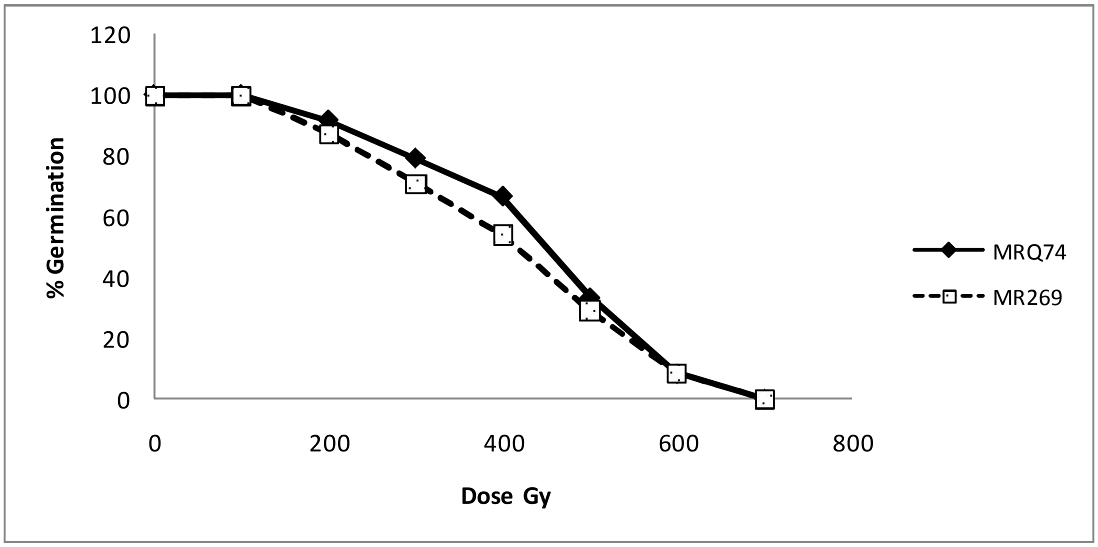


Fig. 2. Effect of Gamma Radiation on the mean Germination rate of two Rice varieties 0-7 days.

Table 1. The Summary of LD_{50} values for two Rice varieties.

Variety	Regression Equation	LD_{50} Dose (Gy)
MRQ74	Y=0.1602x+115.97	365.1061
MR269	Y=0.1607x+112.5	351.3429

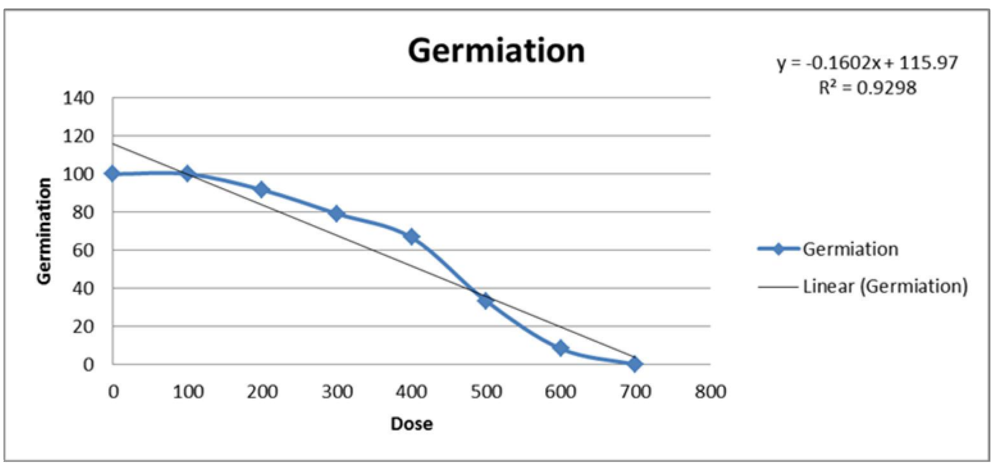


Fig. 3. LD_{50} for MRQ74 = (365.1061)

Height of plantlets

A morphological investigation was conducted for two weeks of culture to compare the heights of irradiated and non-irradiated plantlets. In general, the results suggested that enhancing the degree of gamma irradiation from 0 to 500 Gy caused a decrease in seedling height. However, only plantlets irradiated at 100 Gy of ion dose were 2.56% higher than the control samples (Fig. 5). Doses exceeding 500 Gy severely damaged the seedling elevation and none of the diversities lasted. The Duncan test at $P<0.05$ indicated a significant variation in seedling elevation among all radioactivities in both diversities, that is, 360 Gy for MRQ74 (Fig. 6) and 340 Gy for MR269 (Fig. 7). The results of this study demonstrated that a reduced development in height was markedly observed in the plantlets, as a result of enhancing the ion doses applied on the seeds. The same results were reported by Ling et al. (2013) in a study that indicated that a decrease in plantlet elongation was caused by irradiation of rice seeds through carbon ions. Masuda et al. (2009) also observed a decrease in radical elongation caused by the treatment of tomato seeds with carbon and helium ions. The reduction in the survival fraction with an increase in gamma radiation is consistent with the findings of Harding et al. (2012) on various rice cultivars. They observed a decrease in the survival rate of plants as radiation increased. The negative effect of radiation on plants could be indirectly influenced by metabolic variations over free fundamental development as well as DNA injury of the isolated cells (Jones et al., 2004). Cell separation is considered as the most complex treatment process related to the reduced development of irradiated plantlets (Vazquez-Tello et al., 2005). Irradiation could seriously affect the cell cycle capture at the G2/M stage throughout the somatic cell division or affect the genome, thereby inhibiting development (Preuss and Britt, 2003). Meanwhile, stimulated development was observed on the rice plantlets that developed from the seeds irradiated by a small amount of carbon ions. Such findings were regularly reported on γ-irradiated plant resources (Kim et al., 2004; Ling et al., 2008) and could occur because the hormetic effects of low-dose ionizing radiation on plants resulted in quicker cell proliferation, motivated germination and development, improved stress resistance and enhanced harvest. In several studies, such effects could be considered as the modulation of photosynthesis and antioxidant mechanisms (Kim et al., 2004).

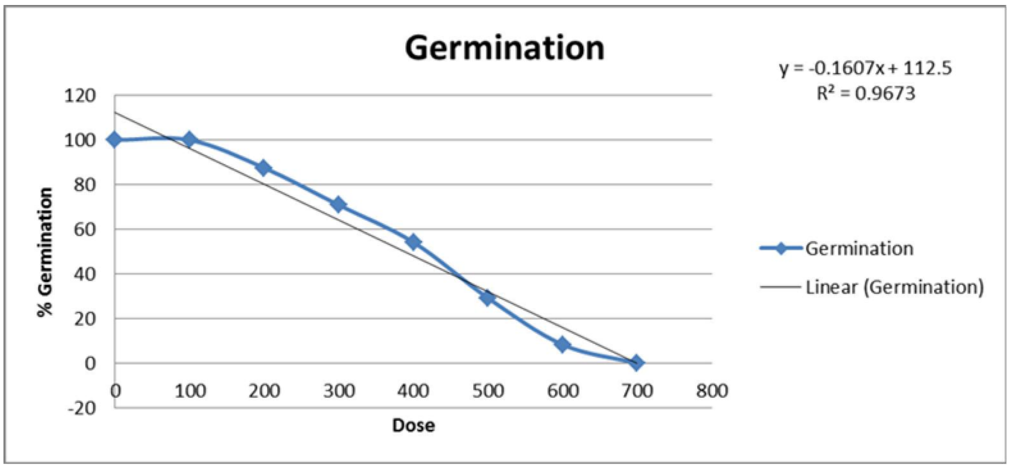


Fig. 4. LD$_{50}$ for MR269 = (351.3429)

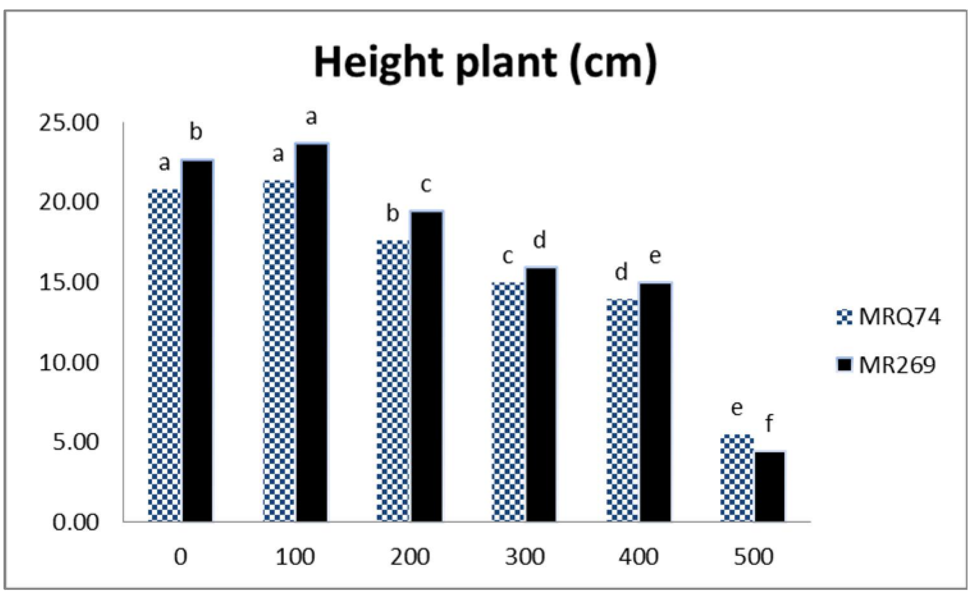


Fig. 5. Effects of ion dose irradiation (Caesium-137) on the height of two rice varieties (MRQ74 & MR269) after two weeks of culture.

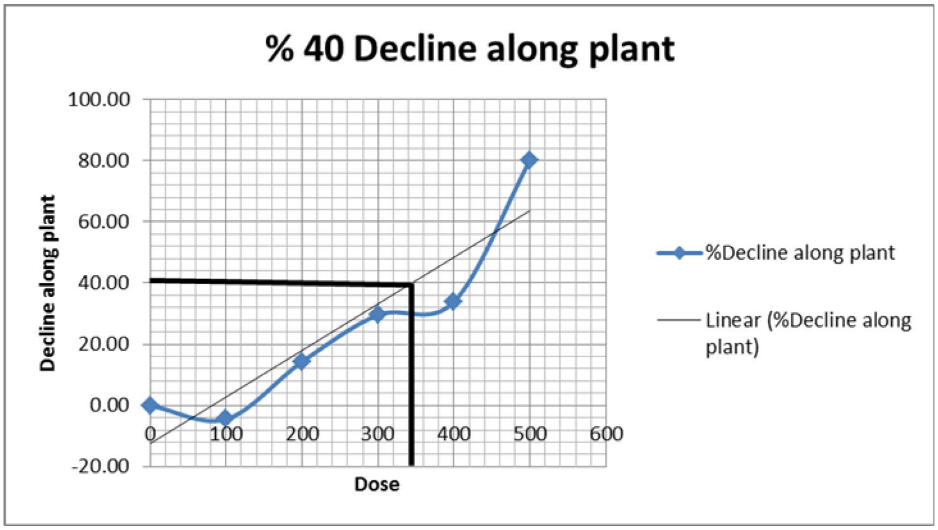


Fig. 6. Reduction in seedlings length (%)for MRQ 74 variety exposed to 360 Gy.

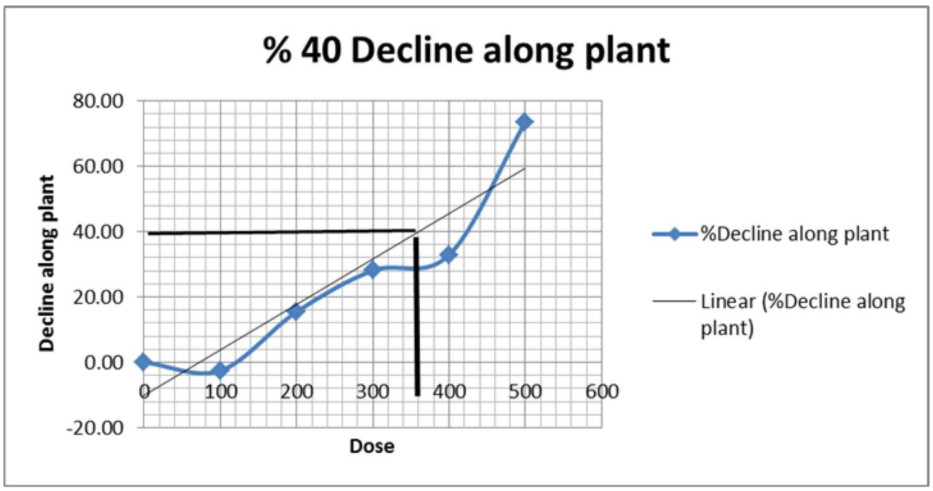


Fig. 7. Reduction in seedlings length (%) for MR269 variety exposed to 340 Gy.

Length of roots

The extent of the roots of the irradiated and non-irradiated plantlets was considered as a factor in investigating the morphological responses of MRQ74 and MR269 exposed to varying amounts of Caesium-137 irradiation. The root length of the plantlets increased in the second week of culture. Plantlets irradiated at 200, 300, 400, and 500 Gy showed considerable changes in height among all doses in both cultivars associated with the control samples (Fig. 8). However, the length of the roots exposed to 100 Gy was equal to that of the control in both cultivars. Therefore, irradiation at high amounts caused plantlets with comparatively short roots. A general reduction in root length was observed with an increasing amount of ion beam (except 100 Gy) because of the important variations among all the actions. Similarly, a reduction in fundamental elongation was observed as a result of the treatment of rice seeds through carbon ions (Ling et al., 2013). The root development of rice vegetable also decreased 3 d after gamma ray irradiation was conducted in a dose-dependent manner (Rakwal et al., 2007). Kalimullah et al. (2003) observed that the elongation of root rice plantlets extracted from the seeds treated with 1 H of heavy ion were approximately 17% less than that of the control plants. The irradiated roots exhibited a fixed growth; however, such growth was usually less than that of the control samples. This finding suggested that irradiation influenced the development of roots. Damage and inactivation of endogenous auxin equilibrium by irradiation could be attributed to the decreased rooting capability of the treated plants (Skoog, 1935; Gordon and Weber, 1953).

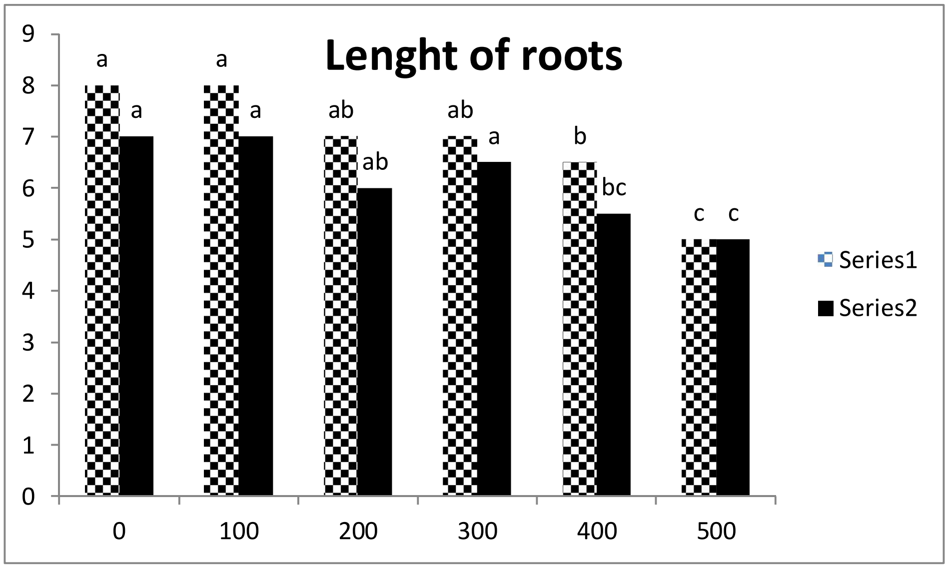


Fig. 8. Effects of ion dose irradiation (Caesium-137) on the Length of roots of the two rice varieties (MRQ74 & MR269) after two weeks of culture.

Conclusion

The germination rate and the heights of MRQ74 and MR269 plantlets were shown to be dose-dependent. Low irradiation at 100 Gy stimulated their growth and rooting ability, whereas irradiation at 200 to 500 Gy resulted in reduction. Consequently, doses of 340 Gy and 360 Gy caused a 40% reduction in the seedling height of MR269 and MRQ74, and doses between 351 and 365 Gy caused a LD50 dose in MR269 and MRQ74, respectively. At doses exceeding 500 Gy, physiological damage occurred, severely affecting the seedling height such that none of the varieties survived. Dose irradiation may not be recognized in M_1 progenies of MRQ74 and MR269 rice varieties because of some mutated characteristics induced by Caesium-137. Further investigations of M_2 and M_3 progenies can help identify the possible mutants induced by irradiation and can help determine whether the effect of such irradiation on these varieties can be transmitted to succeeding generations.

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