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RADIOSENSITIVITY RESPONSE TO ACUTE GAMMA IRRADIATION IN A MALAYSIAN RICE VARIETY, MR284

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ABSTRACT

Rice is an important staple food and provides 20% of the world's dietary energy supply. Presently, mutation-based breeding is extensively used in rice to induce genetic variation by improving yield and agronomic traits as well as increasing tolerance to pest and diseases. Our aim in this study is to determine the radiosensitivity of a Malaysian rice (Oryza sativa L.) variety, MR284 to acute gamma irradiation. Dry MR284 seeds were exposed to acute gamma irradiation (high dose rate radiation) using Biobeam GM8000 with Caesium-137 as a source at doses of 0, 100, 200, 300, 400, 600, 800 and 1000 Gy at Malaysian Nuclear Agency. Effects of irradiation were measured in terms of shoot and root length. Study showed that irradiated seedlings demonstrated a reduction in plant height with increasing doses of gamma irradiation. Based on the survival curve, the 50% shoot length (SD_{50}) for MR284 was 390.74 Gy. At doses above 800 Gy, physiological damage on the seedling in terms of plant height and root length became more severe and none of the seedlings were survived. Therefore, the study concludes that the SD_{50} for MR284 rice was in the range of 300 to 400 Gy and provides evidence that MR284 rice genotype displayed variable response towards gamma radiations after germination.

Keywords: Acute gamma irradiation, MR284, *Oryza sativa* L., radiosensivity, shoot reduction (SD_{50})

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple crop in many countries and is ranked the second most consumed cereal grain. Asia dominates rice production and consumption with 92% of the world's rice. In recent years, yields of rice have fallen due to global climate change, including higher temperatures, severe droughts, floods, salinity and diseases, coupled with more erratic rainfall. Thus, identification and creation of genetic variation is of utmost importance for genetic improvement of crops (Raina et al., 2018).

Mutation induction is a powerful technique for plant improvement by creating new genetic variations, enhancing the agronomical and physiological characters such as pest and disease resistance, improve yield and yield component traits in crops. Induced mutation using physical mutagen like gamma rays is known to be the most popular mutagen because of their relatively simple use, high penetration, high reproducibility, high mutation frequency, and less disposal problems (Li et al., 2019). Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanuts, and beans, which are seed propagated (Ahloowalia and Maluszynski, 2001). Ionizing radiations have been successful in inducing genetic variability in rice (Tabasum et al., 2011). Many attempts in the field of mutation research have been made by different scientists to get desirable traits in cultivated rice and in the determining the most effective mutagenic treatment (Oladosu et al., 2016). Some of the rice mutants that have been developed through induced mutation in Malaysia are NMR151 and NMR152; both are high yield and adaptable to minimal water condition (Kamaruddin et al., 2018).

In Malaysia, rice is a high demand and important crop for calories sources. However, local rice varieties are susceptible to pest and disease which leads to a decrease in yield production (Chukwu et al., 2019). Therefore, it is very important to have varieties that are tolerant to these diseases and one way to generate new varieties is through induced mutation by gamma irradiation. In order for the induced mutation technique to work out efficiently, a prior radiosensitivity study needs carried out to determine the sensitivity of the plants to ionizing radiations and to select the optimum dose for subsequent irradiation activities. Hence, this research was carried out to determine the SD_{50} of MR284 rice seeds and to study the effect of gamma radiation at different doses on its growth.

MR284 is a modern rice variety developed by MARDI. It was officially released in 1986. It is preferred by most local consumers and farmers for its good characteristics including a short maturation period (122 days), fairly tall but strong culm and producing high yields (Elixon et al., 2017). MR284 at one time was the most widely planted rice variety in irrigated areas under direct seeding conditions before it was devastated by bacterial leaf blight disease (Saad et al., 2012). Currently, there is no information available on MR284 improvement by radiation induced mutagenesis. Therefore, there is a need to develop a radiosensitivity data for this variety for future mutation breeding work.

MATERIALS AND METHODS

Plant Material

The study was conducted using MR284 rice seed. The seed were obtained from Malaysian Agricultural Research and Development Institute (MARDI).

Gamma Irradiation

Irradiation of rice seeds was accomplished using Biobeam GM8000 (Germany) with Caesium-137 as a source at the Malaysia Nuclear Agency. Rice seeds were treated with 100, 200, 300, 400, 600, 700, 800, and 1000 Gy gamma rays at dose rate 4.640 Gy/min. The non-treated (0 Gy) seeds of rice were considered as control.

Radiosensitivity Test

After irradiation, 10 seeds were sown in four replications per treatment on blotting paper using sandwich blotter technique as described by Myhill and Konzak (1967). Seeds were placed between two wet blotters, which were supported vertically between slots in Poly Vinyl Chloride (PVC) racks. The racks were placed in plastic trays containing enough water to immerse the lower edge of the filter papers. Germination percentage were recorded 7 days after sowing, meanwhile shoot length and root length were measured 14 days after sowing. The experiment was laid out in completely randomized design with three replications

Statistical Analysis

An analysis of variance (ANOVA) of all traits was calculated using SAS program version 9.3 to determine the variation among the Gamma-ray doses. Additionally, the mean, range and standard deviation were calculated for each trait. Mean comparisons were performed using Least Significant Difference (LSD). Determination of SD_{20} and SD_{50} were done using Curve Expert Software 1.4 based on reduction of shoot length as compared with control.

RESULTS AND DISCUSSION

In this study, the effect of different doses of gamma irradiation on MR284 rice variety was analyzed based on seed germination ability at day 7 after irradiation as well as root and shoot length of 14 days old irradiated seedlings. The character of the seeds that were exposed to eight different doses (100, 200, 300, 400, 600, 700, 800, and 1000 Gy) were compared with the unirradiated rice seeds (control). Table 1 shows the germination percentage of MR284 after 7 days of irradiation treatment.

Table 1: Germination percentage of MR284 seeds after 7 days of irradiation process

Dose (Gy)	Percentage Germination (%)
0	100^{a}
100	100^{a}
200	100^{a}
300	100^{a}
400	100^{a}
600	93.3^{a}
800	0^{b}
1000	0^{b}

Mean within a column followed by the same letter are not significantly different ($p < 0.05$)

The seed germination test after gamma irradiation (100 - 1000 Gy) revealed that irradiated seedlings remained almost unaffected by increasing gamma radiation doses until it reached 600 Gy. A 100% germination rate was recorded at all doses below 400 Gy, and then slightly reduced to 93.3% at 600 Gy before sharply dropped to 0% at 800 and 1000 Gy. These findings match with a previous report by Sasikala and Kalaiyarasi (2010) depicting that germination percentage did not reduce greatly on exposure to lower dose (200, 250 and 300 Gy) of gamma radiations. Such little or no effect of gamma radiation on germination of genotypes under study depicts that these genotypes may be successfully used for the creation of genetic variability through induced mutations in further breeding experiments. However, a sharp reduce on germination rate with increasing radiation dose has also been reported (Cheema and Atta, 2003). High exposure in gamma radiation can cause blockage in the cellular DNA which caused plants to growth slower, stop or eventually die.

This study also revealed that the unirradiated rice seeds (control plant) exhibited the highest shoot and root length of rice seedlings. The irradiated seeds also developed into shorter seedlings as compared to the control plant with increasing doses of irradiation (Figure 1). These results indicated that application of gamma radiation has a tremendous effect on the growth performance on rice seedlings.

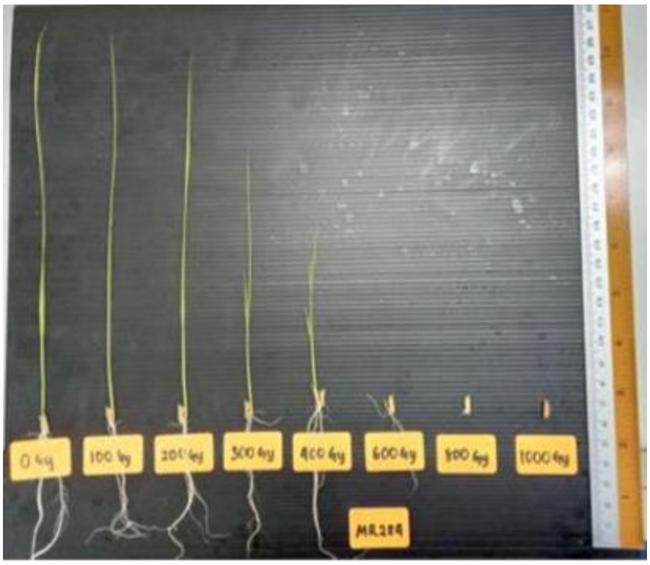

Figure 1: Rice seedlings on 14th day after exposed to acute gamma irradiation

Seedling height is the primary index in M_1 generation in any mutation studies (Konzak et al., 1972). The result of present study in MR284 rice showed that seedling height significantly decreased with increasing doses of gamma irradiation. The gamma ray dose of 400 Gy caused 50% seedling height reduction in MR284 rice (Table 2). Furthermore, at higher doses of gamma irradiation (600-1000 Gy), the seedlings were basically not growing at all. The reduction in the plant height may be attributed to damage to the process of cell division and cell elongation as a result of mutagenic treatment. Similar results have been reported by Cheema and Atta (2003). Linear dependency between seedling height and radiation dose have been reported by Tabasum et al. (2003).

Table 2: Germination percentage of MR284 seeds after 7 days of irradiation process

Dose (Gy)	**Shoot Length (cm)**	**Root Length (cm)**
0	18.23 ± 0.73^{a}	10.23 ± 0.73^{a}
100	17.96 ± 1.05^{b}	9.60 ± 1.29^{b}
200	16.54 ± 0.65^{c}	8.86 ± 0.60^{c}
300	13.29 ± 13.29^{d}	8.55 ± 8.55^{d}
400	9.64 ± 0.28^{e}	9.62 ± 0.25^{e}
600	0^{f}	2.66 ± 0.09^{f}
800	0^{f}	0^{g}
1000	0^{f}	0^{g}

Data are the means of three replicates ± SD [Standard Deviation]; Different letter within a column are significantly different ($p < 0 .05$)

Study on root length variation also showed that there was a significantly difference in the length of roots with the increase of radiation dose (Table 2). The inhibition of root development was higher in dose of 600 Gy in MR284 rice variety. Similar results have been reported by Cheema and Atta

(2003), in which the root length was reduced in seedlings irradiated at higher dose. At higher dose of 600 Gy root length is very much affected in the MR284 rice variety (50% reduction). Furthermore, all of the tested seedlings were not grown at the doses of above 800 Gy. This indicates that the tested variety, MR284 was more sensitive towards gamma irradiation.

Retarded growth in the surviving fraction became a constraint in obtaining the expected number of mutants. The reduction of seedlings after the mutagenic treatment were due to physiological and biochemical disturbances, fluctuation in ascorbic acid content, and destruction of auxin (Thirumeni et al., 2017). Therefore, it is better to consider the growth reduction doses, GR_{20} and GR_{50}, which respectively signifies the 20 and 50 percent reduction in growth parameters being considered, over respective control. In the present study, growth reduction in GR_{20} and GR_{50} groups was calculated for the shoot length traits and evaluated after the gamma irradiation. The estimation of these values was based on the formula for the linear regression lines obtained in a plot of dose versus shoot length characters (Figure 2). The SD_{20} and SD_{50} values ranged from 257.46 Gy and 390 Gy, respectively. This finding was also aligned with the study reported by Chauhan et al. (2019) who considered the SD_{50} value for phenotypic traits as a reliable parameter for extrapolating mutagenesis experiments to actual field conditions.

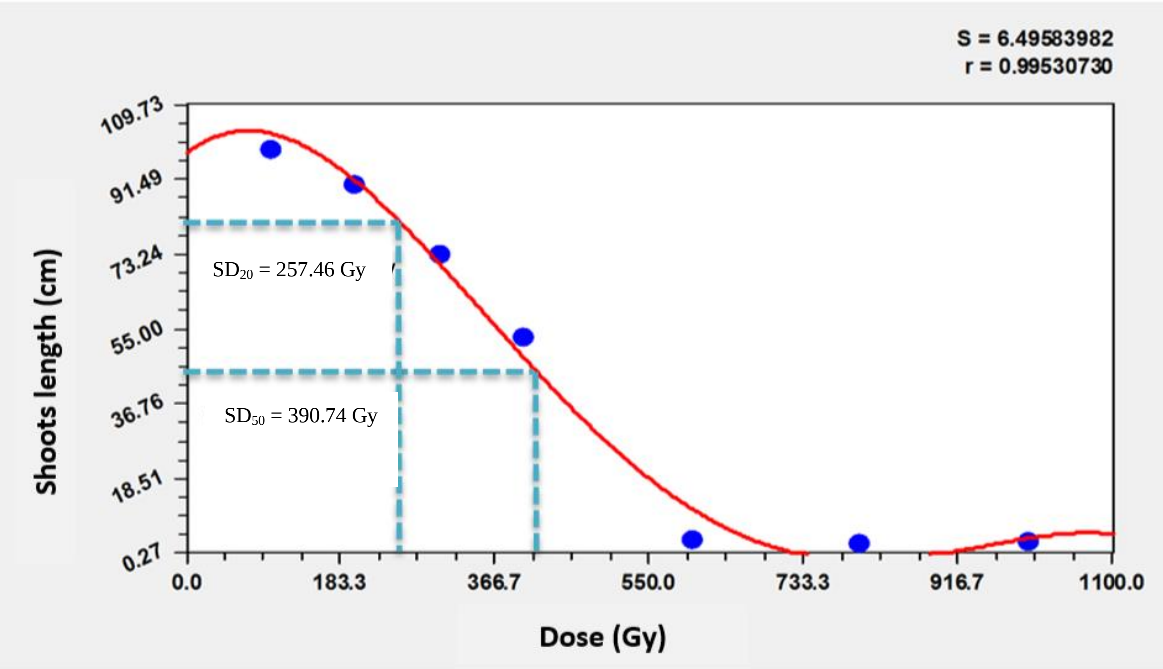

Figure 2: SD_{20} and SD_{50} determination from a plot between shoot length and values of dose (Gy) for gamma irradiation of MR284 variety using Curve Expert Software 1.4

CONCLUSIONS

From the radiosensitivity study for MR284 rice variety, the SD_{20} for shoot length of acute gamma irradiation was 257.46 Gy and SD_{50} was 390.74 Gy. Seedling height and root length were decreased with increasing doses of gamma irradiation. The findings in this study were very useful for subsequent mutation breeding programs using gamma irradiation for rice especially for MR284 variety.

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REFERENCES

Ahloowalia, B. and Maluszynski, M. (2001). Induced mutations - A new paradigm in plant breeding, *Euphytica* 118: 167-173.

Chauhan, A.V., Kumar, R., Preethi, I., Gautam, V., Nair, J.P., Surendran, P., Sparrow, H., Gupta, A.K., Shitre, A.S., Shinde, A.K. and Kunkerkar, R.L. (2019). Effect of proton ion beam irradiation on survival and seedling growth parameters of Indian rice (*Oryza sativa* L.) variety 'Indira Barani Dhan 1', *J. Plant. Breed.* 10(2): 490-499.

Cheema, A.A. and Atta, B.M. (2003). Radiosensitivity studies in basmati rice, *Pak. J. Bot*. 35: 197-207.

Chukwu, S.C., Rafii, M.Y., Ramlee, S.I., Ismail, S.I., Hasan, M.M., Oladosu, Y.A., Magaji, U.G., Akos, I. and Olalekan, K.K. (2019). Bacterial leaf blight resistance in rice: A review of conventional breeding to molecular approach, *Mol. Biol. Rep.* 46(1): 1519-1532.

Elixon, S., Asfaliza, R., Othman, O., Siti Norsuha, M., Maisarah, M.S., Allicia, J.and Shahida, H. (2017). Evaluation on yield, yield component and physico-chemicals of advanced rice lines, *J. Trop. Agric. Food Sci.* 45(2): 131-143.

Kamarudin, Z.S., Yusop, M.R., Tengku Muda Mohamed, M., Ismail, M.R. and Harun, A.R. (2018). Growth performance and antioxidant enzyme activities of advanced mutant rice genotypes under drought stress condition, *Agronomy* 8: 279- 294.

Konzak, C.F., Wiekham, I.M. and De Kock, M.J. (1972). Advances in methods of mutagen treatment. In Induced mutations and plant improvement, IAEA, Vienna, 95-119.

Li, F., Akemi, S., Takeshi, N., Nobuhiro, T. and Hiroshi, K. (2019). Comparison and characterization of mutations induced by gamma-ray and carbon-ion irradiation in rice (*Oryza sativa* L.) using whole-genome resequencing, *Genes, Genomes, Genetics* 9(11): 3743-3751.

Myhill, R.R. and Konzak, C.F. (1967). A new technique for culturing and measuring barley seedlings, *Crop Sci.* 7(3): 275-276.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli,A., Rahim, H.A. and Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: A review, *Biotechnol. Biotec. Eq*. 30(1): 1-16.

Raina, A.L., Roshan, J., Rafiul, A.L. and Ruhul, A. (2018). Mutation breeding for crop improvement. In introduction to challenges and strategies to improve crop productivity in changing environment, Enriched Publications, PVT, lTD, 303-317.

Saad, A. Yahya, H. Nik Mohd Noor, N.S. Azmi, M. Badrulhadza, A. Siti Norsuha, M. Azimah, A.K. and Sivapragasam, A. (2012). Pengurusan perosak bersepadu: Prinsip, konsep dan amalan. Dalam: Pengurusan perosak bersepadu tanaman padi kearah pengeluaran lestari, *MARDI*. 2 – 46.

Sasikala, R. and Kalaiyarasi, R. (2010). Sensitivity of rice varieties to gamma irradiation, *Electron. J. Plant Breed.* 1(4): 885-889.

Tabasum, A., Cheema, A.A., Hameed, A., Rashid, M. and Ashraf, M. (2011). Radiosensitivity of rice genotypes to gamma radiations based on seedlings traits and physiological indices, *Pak. J. Bot*. 43(2): 1211-1222.

Thirumeni, S. Seetharam, K., Paramasivam, K. and Souframaien, J. (2017). Induced mutagenesis in rice (*Oryza sativa* L.) for improving salt tolerance, *Oryza* 53(4): 385-390.