Agro-morphological response of rice (Oryza sativa L.) (cv MR 284) to chronic gamma irradiation

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Abstract. An experiment to study the effect of cobalt 60 gamma irradiation on agronomic traits of Malaysian rice variety, MR284, was performed by using randomized complete block design (RCBD) with three replications in Gamma Green House (GGH) located at Malaysia Nuclear Agency. The GGH facility can produce a low dose rate of chronic gamma-ray which causes less damage to the plant cell and eliminate undesirable traits as well as produce wide mutation spectrum. Seedlings in pots at the age of 14 days were exposed to gamma rays in GGH at different dose rate from 0.03 Gy/hour to 0.66 Gy/hour for 77 days (flowering stage). Data on survival rate and growth performance such as plant height and tiller number were recorded every 7th day. The result showed that, chronically irradiated MR284 plant exhibited various significant responses of agronomic characteristic against different doses of chronic gamma irradiation. Based on the survival curve, growth reduction (GD50) for MR284 was recorded at 318.63 Gy based on filled grain per plant trait. At cumulative doses of 68.87 Gy and 167.25 Gy (0.07 and 0.17 Gy/hour, respectively), the rice seedlings demonstrated the highest plant height with maximum tillering and highest number of filled grains. Those rates are stimulating to the growth and development of the plants. This study helps explicate the dose-response on local rice varieties through chronic radiation in a Gamma Green House facility in Malaysia.

1. Introduction
Rice (Oryza sativa L.) is the staple crop and ranked the second most consumed cereal grain globally. Asia is dominant in rice production and consumption with 92% of the world's rice. Rice yields have declined in recent years due to shifting weather trends including higher temperatures, extreme droughts, flooding, salinity and diseases. The identification and creation of genetic variation are thus important for crop genetic improvement [1]. Among the different breeding methods, mutagenesis has been applied to produce new mutant varieties of rice, to enhance the agronomical and physiological characters such as pest and diseases resistance, and to improve yield and yield component traits in crops. Compared to conventional breeding, mutagenesis is more useful and effective in producing many new varieties in a shorter time period [2]. Mutagenesis has been commonly studied in terms of the application dimension using actual mutagen (radiation) due to its easy use, high penetration, reproducibility, high mutation frequency and low-disposal problems [3]. Mutations induced were used...
to improve important crops such as *Triticum* sp., *Oryza sativa*, *Hordeum vulgare*, *Gossypium* sp, *Glycine max* and *Phaseolus vulgaris*, which are seed propagated [4]. There are an estimated 800 rice varieties that have been registered under the Mutant Varieties Database from the International Atomic Energy Agency, IAEA [5]. The first rice mutant cultivar popularly known Reimei, a short-straw mutant (semi-dwarf) from cv. Fujiminori was released by Futsuhara in Japan in 1966 [6]. Dee-geo-woo-gen (DGWG), a spontaneous mutant discovered by Chinese scientists, has given rise (after crossing) to the important cv. Taichung Native-1, and after a cross with the tall cv. Peta from Indonesia, to the even more famous cv. IR 8 from the International Rice Research Institute (IRRI) in the Philippines.

The Gamma Green House located at Malaysia Nuclear Agency uses chronic gamma irradiation to produce a low dose rate of gamma ray. Chronic gamma irradiation is a successful technique for developing crop varieties by creating somaclonal variation which affects essential structural genes for useful expression without altering other important traits. Since 2009-2016, a few plant species have been irradiated at Gamma Greenhouse facility including *Oryza sativa*, *Hibiscus cannabinus* L., *Stevia rebaudiana* (Bertoni), *Capsicum sp.*, *Hibiscus rosa sinensis*, *Arachis sp.*, *Amarillis sp.*, *Alpinia luteocarpa*, *Chrysanthemum sp.*, *Musa sp.*, *Ananas sp.*, *Canna sp.*, *Turnera sp.*, *Jathropa sp.*, *Curcuma alismatifolia*, *Piper nigrum*, *Eurycoma longifolia*, *Sansevieria sp.* and *Tradescanthia pallida* [7]. Despite the advantage of chronic mutation and the facilities' availability, studies conducted on Malaysian rice varieties to improve agronomic characteristics through induced mutation are lacking. 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 sound characteristics, including an intermediate maturation period (122 days), fairly tall but strong culm and high yields [8]. MR284 at one time was the most widely planted rice variety in irrigated areas under direct seeding conditions before it was devasted by bacterial leaf blight disease [9]. Currently, there is no information available on MR284 improvement by radiation-induced mutagenesis. Therefore, the present study was conducted to investigate the response of MR284 variety to chronic gamma irradiation at GGH facility.

2. Materials and Methods

2.1. Plant material

MR284 seeds variety (*O. sativa*) were obtained from the Malaysian Agricultural Research and Development Institute (MARDI) GeneBank germplasm. The germination experiments were carried out on ten seeds from each of the three replications. Surface sterilization of seeds was performed using fungicide and the seeds were incubated overnight in the dark at room temperature followed by rinsing in distilled water. Sterilized seeds were then spread out on wet Whatman filter paper no. 1 (15cm × 15cm dimension, folded with each fold measuring 3 cm in width) and were placed in germination rack. The seeds were placed underneath with their embryonic portion facing. The rack was positioned in rack stands with 3 - 4 cm of water in it. Plant growth chamber (Sanyo, Japan) was used to keep the rack stand with a daily cycle of a 14 h light/10 hours (hr) dark [10]. The seed moisture content was measured using a moisture analyser (AND MX-50).

2.2. Irradiation method

Irradiation of the plant materials was carried out at Gamma Green House (GGH) of the Malaysian Nuclear Agency using Caesium-137 source in GGH. The source of gamma rays was Cobalt 60. Fricke dosimeter was used for dose mapping for each ring in the gamma greenhouse. Fourteen day seedlings were transferred into the pot at six-point (also known as ring) distances from GGH and chronically irradiated at the gamma greenhouse. The growing plants received different dose rates ranging from 0.03 Gy/day (ring 10, 10 meters from the radioactive source) to 0.66 Gy/day (ring 2, 2 meters from the radioactive source) for 20 hours every day. Control plants were grown outside the gamma house under conditions very similar to those of the irradiated plants.
2.3. Data analysis
The experiment was constructed with three replications in Completely Randomised Design (CRD). For each dose, 10 seedings were treated. The total radiation doses received by the rice plants were from 20 to 600 Gy for 77 days. Growth reduction (GD50) was calculated. After 77 days (flowering stage), the plants were removed from GGH and transferred to a glasshouse until maturity. Water was manually applied to maintain the soil moisture at field capacity and manually controlled weeds. There was no application of pesticide and herbicide during the analysis to avoid any intervention. Recommended practices to elevate a healthy crop including the NPK fertilizer were pursued. At maturity (122 days), the number of filled grains per panicle, number of tillers, number of panicles and weight of 100 grains were recorded. Analysis of variance test was performed using Statistical Analysis Software (SPSS) Version 16 to determine the effect of gamma irradiation. The mean differences between the doses were further tested using Tukey’s method at 5%.

3. Results and Discussion

3.1. Survival rate
The effect of chronically exposed 14 days seedlings grown at different doses of chronic gamma rays is summarized in Table 1. The susceptibility to radiation of the MR284 rice variety was measured by evaluating irradiated plants; survival (percentage) at 77 days (flowering stage) after irradiation. The plant survival rate showed no significant differences with increasing irradiation dosage. The plant located closest to the radiation source (ring 2) was found growing after two weeks of chronically being exposed at GGH (Figure 1). This might result from the damage caused after irradiating embryos is considered low. According to [11] high exposure of gamma irradiation is attributed to the blockage in the cellular DNA which cause the plant growth to slow or stop and eventually death. Thus, cell will repair any DNA breakage that could causes fatality of the organisms undergoing mutation process. However, in this study, this effect was not observed. The sensitivity of plant material depends on the genetic composition, the dose-employed, the volume of DNA, the quality of moisture and the stage of development and genotype [12]. The choice of the dose to be administered for the highest rescue of mutants is always left to the breeder’s familiarity with the actual plant material, its genetics, and its physiology. A lethal dose of 50% deaths ($L_{50}$) could not be extrapolated since the survival rate for control and irradiated plants was 100%. Therefore, it is easier to consider growth reduction doses, $GR_{50}$, which means that growth parameters are assumed to be decreased by 50 percent over the respective power. In the present study, growth reduction in $GR_{50}$ groups was calculated for the filled grain per panicle traits and evaluated after the gamma irradiation. The estimation of these values was based on the linear regression line formula obtained in a dose plot versus the shoot length characters (Figure 2). The values recorded for $GR_{50}$ were 318.63 Gy.

3.2. Plant height
Plant height exhibited significant variation against treatments of gamma rays. The tallest plants were recorded from the treated plants (167.25 Gy) with 106.6 cm and 659.16 Gy irradiated plants produced the lowest plant height (55.0 cm) (Fig. 3). These results are in agreement with the findings of Azliana et al. [13] on traditional variety and Yasmein et al. [14] on three modern varieties. Reduction of plant height and dwarfism may be due to conflict with normal mitosis and regular incidence of mitotic aberrations, inhibition of assimilation rates, and consequent changes in plant nutrient levels [165]. In addition, mutagenic effects such as inhibition of phytohormones or the synthesis of nucleic acids that are essential for the process of cell division can cause growth reductions and thus cause cell division to stop [16].
**Figure 1.** The plant closest to the source of radiation (Ring 2, 2m from source) were found to be stunted, with narrow leaves while the plant further from the radiation source (Ring 10, 10m from source) were found to be tall with broad leaves after two weeks in GGH.

**Figure 2.** GR\(_{50}\) determination from a plot between spikelet fertility (%) and values of dose (Gy) for gamma irradiation of MR284 variety using Curve Expert Software 1.4.

**Figure 3.** Effect of chronic gamma irradiation towards plant height and length of panicle.

**Figure 4.** Effect of chronic gamma irradiation towards number of tillers and panicles.
3.3. Length of panicle
Cultivated rice varieties influence the length of rice plant panicle that will affect the amount of seeds per panicle [17]. A panicle is very important in rice plants as a place for grain growth with a maximum length of panicle is preferable. Based on Figure 3, the lengthiest panicle was found in rice plant irradiation of 167.25 Gy with panicle length of 30.87 cm. The panicle’s length aspect is a significant supporting factor in yield efficiency. The maximum length of the panicle bears more rice grain, which resulted from the higher grain content [18]. Results of the panicle length in this study showed not significantly differences with increasing gamma doses. It can be summarized that the irradiated dose contributes insignificantly to the length of the panicle. This is contrary to Tabasum et al. [19]’s opinion, since the existence of mutant plants or plants with positive properties was not determined by the average value of the panicle length but by each plant that could potentially become mutant.

3.4. Number of tillers and panicles
Based on the result, the number of tiller and panicles produced per plants was significantly influenced by the dose received. A reduction in the number of tiller and panicles was observed at 295.15 and 659.16 Gy of irradiation dose. However, plants exposed to 167.25 Gy produced the highest number of tillers and panicles compared to control, respectively (Figure 4). Tillering or lateral branch production is an important agronomic trait that determines the architecture of the shoot. Panicles can emerge from paddy with many tillers, thus increasing grain production in grasses. It can be shown that a positive correlation occurs in plants exposed to 167.25 Gy with a maximum number of the tiller and filled grains per panicles.

3.5. Filled grains per plant
Filled grains per plant is a parameter affecting potential yield [20]. Filled grains per plant data in Figure 4 shows the significant differences between unirradiated plants and chronically irradiated plant of various doses. Seeds for unirradiated plants were 189 per plant. Treatment of 167.25 Gy resulted in the maximum filled grains per plant showing as many as 272 amounts of filled grains per plant (Figure 5). Furthermore, the filled grains per plant is correlate with the grain content; which the high grain content the high weight of seed. According to Haris et al. [21] irradiation causes mutations to occur in the plant leading to an increase in grain weight diversity per tillers in the rice plant. Arifin [22] also stated that the number of grains filled and the weight of 100 irradiated gamma-ray seeds with a dose of approximately 200 Gy have a more significant effect on yield and productivity. However, at a higher dose of gamma rays, plants produced a lower number of filled grains. According to Sparrow [23], the consequences of induced mutation include aborted pollen grains or embryos and decreased accumulation of food in developing seeds.

![Figure 5. Effect of chronic gamma irradiation towards filled grains per plant.](image1)

![Figure 6. Effect of chronic gamma irradiation towards 100-grains weight.](image2)
3.6. 100-grains weight
The grain weight per plant is determined by the number of tillers, the amount of grains and the percentage of filled grains [24]. If these components are of high value, the weight of the grains will most probably also be high. As reported by Akhi et al. [25] there is a positive correlation between number of grains per plant and 100 grain weight. The weight of rice grain is mainly affected by the nutrient supply or other environment factors and genotype. These factors alter the effectiveness of mutagens in rice cells and their effectiveness. From the study, the highest 100 grain weight was recorded at 167.25 Gy (Figure 6). This finding also agrees with Azliana et al. [13] study in which ring 4 demonstrated the highest number of 100-grain weight. However, the result was not significantly different. According to Yoshida [26], 100-grain weight is a stable varietal character and it does not represent individual grain.

3.7. Spikelet fertility
The fertility rate (%) decreased significantly with increasing irradiation dosage (Figure 7). The growth plant treated with 659.16 Gy was less sensitive than other plants to gamma irradiation (64.8%). The 167.25 Gy irradiated plant showed the highest fertility rate (83.9%). A similar result was observed in Purusothaman [27] Awan and Bari [28] and Wang et al. [29]. The higher the dose received the lower the grain fertility. The marked reduction in seed yield per plant caused by mutagens can be due to high seed sterility due to physiological and biochemical disruptions in seed production [27].

![Figure 7. Effect of chronic gamma irradiation towards spikelet fertility.](image)

4. Conclusion
Chronic gamma irradiation has radically affected the plant height, number of tiller and number of grains in rice plant. The study revealed that at cumulative doses of 68.87 Gy and 167.25 Gy (0.07 and 0.17 Gy/hr, respectively), the rice seedlings showed the tallest plant with maximum tillering and highest number of filled grains. Growth reduction (GD₅₀) was recorded at 318.63 Gy for filled grain per plant trait. Finding in this study could be successfully used in next generation to observe the spectrum and frequency mutation and future for its improvement.

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