

Field Emergence and Seed Vigor Sensitivity of Special Purpose Rice (*Oryza sativa* L.) Irradiated with Gamma Rays

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Abstract

Special purpose rice (SPR) are varieties with notable features such as aroma, grain color and eating quality. Lower yield is one of the challenges in the production of SPR which this study ultimately aimed to address by developing mutant varieties with higher yields and good eating quality. In the process of obtaining desirable mutants through gamma irradiation, different doses were applied which could negatively affect the germination and seed vigor of the irradiated seeds. Thus, the responses of four newly-developed special purpose rice varieties to different irradiation doses were evaluated in terms of field emergence and seed vigor. Five irradiation doses of gamma rays were used, namely: 0 Gy (control), 150 Gy, 200 Gy, 250 Gy and 300 Gy using the Ob-Servo Sanguis Co-60 Irradiation facility at the Philippine Nuclear Research Institute (PNRI). The study was laid out in the open field following the Complete Randomized Design arrangement with four replications. Results indicated that irradiation doses of 150 to 300 Gy resulted to significantly lower field emergence and seed vigor when compared to the untreated seeds but the reduced levels of field emergence ranging from 89.60 to 92.97% were still within the acceptable minimum level of germination percentage which is 80%. Among the varieties/line tested and within the range of doses studied, it appeared that CLS 4, with waxy endosperm, was relatively more sensitive to irradiation treatment in terms of field emergence and seed vigor.

1. Introduction

Special purpose rice has unique characteristics such as aroma, pericarp color, excellent eating quality and higher nutrient content [1]-[4]. As such, it is priced higher [5]. However, only few farmers plant special purpose rice varieties because they have relatively lower yields [6]-[8]. Although the lower yield can be compensated by higher price of the harvested paddy, it will be advantageous if current yield levels could be further increased without sacrificing grain color, aroma and other sensory attributes.

One of the initial steps of breeding programs that can be used to genetically improve productivity and/or quality of crops is induction of mutations in seeds. Mutant lines of aromatic rice had been

successfully produced through mutation breeding. Special purpose rice *Sinar 2* is an example of a mutant variety produced and registered in 2020 by Indonesia which was produced by irradiating its parent variety using 0.1 kGy or 100 Gy. The mutant variety is aromatic and resistant to diseases [9]. Thailand has also produced mutant aromatic varieties, namely, RD6 and RD15, which were derived by irradiating KDML 105 by 20 and 15 kilorad (200 and 150 Gy), respectively [10].

The Central Luzon State University (CLSU) through its Crops and Resources Research and Development Center (CRRDC) has developed SPR varieties that are pigmented and with excellent eating qualities [11]. Central Luzon Selection 1 or CLS 1 and CLS 2 are among the SPR rice varieties which are aromatic with long and slender grains and potential yield of 4-5 t ha⁻¹ and 3-4 t ha⁻¹, respectively. These varieties were developed through conventional breeding that took almost ten years. Aside from the aromatic qualities, two of these varieties are pigmented. Induced mutation breeding on pigmented rice is limited. To cut down the time of developing new and improved varieties of special purpose rice, CLSU is undertaking a parallel project on varietal development through mutation breeding. This paper reports on the study which aimed to determine the effects of different doses of gamma rays on the field emergence and seed vigor of four SPR varieties using five levels of gamma irradiation doses. Information generated from this study will serve as guide for future mutation breeding work on similar types of rice.

2. Materials

2.1 Characteristics of Special Purpose Rice

Table 1 and Figure 1 show the grain qualities of the special purpose rice that were irradiated with gamma rays with the aim of bringing about improvement in terms of higher yield and characteristics such as increase in aroma. These special purpose rice varieties were developed at CLSU and have been registered and protected for 20 years under the Philippine Plant Variety Protection (PPVP) Law. CLS 1, CLS 2 and CLS 3 are all aromatic varieties with non-waxy endosperm. CLS 2 and CLS 3 are pigmented. On the other hand, CLS 4 is opaque white and has waxy endosperm.

Table 1 Grain qualities of the SPR varieties under investigation

Variety	Description
CLS 1	Long-grain, slender, white seed coat, non-waxy endosperm, aromatic; intermediate maturing variety
CLS 2	Medium grain, dark purple pericarp, non-waxy endosperm, aromatic; intermediate maturing variety
CLS 3	Long-grain, slender, red pericarp, non-waxy endosperm, aromatic; intermediate maturing variety
CLS 4	Long grain, slender, opaque white, waxy endosperm; late maturing variety



Fig. 1 SPR varieties subjected to gamma irradiation

2.2 Experimental Procedures

2.2.1 Irradiation of Samples

Figure 2 shows the flowchart of the procedure. One thousand dry seeds each of the four promising special purpose rice, were subjected to gamma irradiation doses of 0 (control), 150 Gy, 200 Gy, 250 Gy and 300 Gy to induce mutation. The seeds which were approximately 14 percent moisture content were packaged in brown envelop and irradiated at the Philippine Nuclear Research Institute (PNRI) using the Ob-Servo Sanguis Co-60 Irradiation model (dose rate: 0.266 k Gy h⁻¹). The periods of exposure of the seed samples were determined by the PNRI based on the weight of the seeds submitted and the radiation dose required.

2.2.2 Field emergence

To test whether the irradiation doses used in this experiment had negative effects on the varieties of seeds used, field emergence (FE) was evaluated. This was done to ensure that enough healthy seedlings would be available for further evaluation and which will serve as additional information for similar irradiation activities in the future using similar types of rice seeds. High levels of radiation could render the seeds ungerminable [12]. The irradiated seeds were soaked in distilled water for 24 hr and incubated for 24-48 hr after which they were sown in plastic trays filled with clay loam type of soil. Each treatment combination had four replications, each replication consisting of 128 seeds. Replications were done to build confidence in the scientific merit of results. The plastic trays were laid out in the open field at the CLSU Experimental Station in January 2021 following Complete Randomized Design (CRD). The plastic trays were watered using sprinkler every morning and afternoon or when the soil surface had dried down.

Field emergence was observed and counted on the 6th, 9th and 12th days after sowing (DAS). FE was computed as the number of healthy or normal, 3-cm seedlings that emerged 12 DAS and was expressed as a percentage of the total seeds sown. Observations were terminated after 12 days and the seedlings were allowed to grow until maturity.

2.2.3 Seed Vigor

The ability of the treated seeds to survive and vigorously emerge when irradiated with different doses of gamma rays was also determined along with the field emergence test. The speed of emergence is used as an index of seed vigor [13]. The data were taken from the same set up used in determining field emergence. The number of seedlings emerging on the 6th, 9th and 12th DAS were counted. The seed vigor index (SVI) is computed using the formula:

$$SVI = \frac{N_6}{6} + \frac{N_9}{9} + \frac{N_{12}}{12}$$

Where, N₆, N₉ and N₁₂ are the number of healthy seedlings that emerged 6, 9 and 12 DAS, respectively. This was replicated four times with 128 seeds per replication. Seed lot with high SVI was considered more vigorous.

2.2.4 Statistical Analysis

Results are presented as means ± standard deviation (sd). All the data collected were subjected to analysis of variance (ANOVA) using the Statistical Package for Social Research software program version 21 (SPSS, Inc., Chicago, IL, USA). The test of differences on the mean effects of irradiation doses on the FE and SVI across varieties were done using the Dunnett's t-test, comparing means of the doses of gamma irradiation to that of the untreated control. Separate analyses on the individual effects of radiation doses on each parent variety were also done using the Fisher's Least Significant Difference (LSD). Statistical significance was set at p ≤ 0.05.

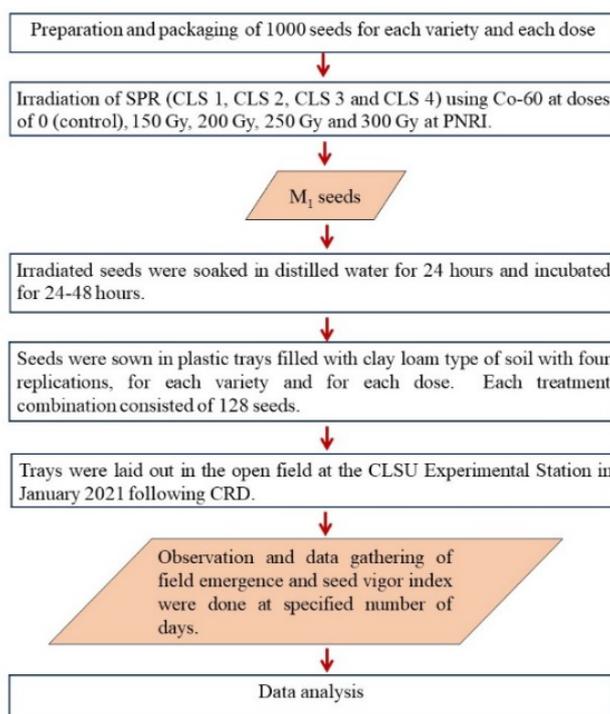


Fig. 2 Flowchart of research implementation of irradiated SPR

3. Results and Discussion

Gamma irradiation significantly affected FE and SVI (Table 2). Generally, there was a downward trend in FE and SVI as the irradiation dose increased from 0 (control) to 300 Gy. FE and SVI of the seeds irradiated with 150 and 200 Gy were not significantly different to the untreated control but the higher dose of 250 and 300 Gy had a significantly lower FE and SVI. On the average, the untreated control had 96.63% FE and 20.33 SVI which were comparable to those irradiated at 150 to 200 Gy with FE of 92.97 and 92.87%, respectively and SVI of 19.55 and 19.58, respectively. In a similar study conducted on rice in Sierra Leone, similar results were found [14], where an increase in radiation above 300 Gy caused a significant reduction in percentage survival. The decrease in germination did not follow a definite pattern for all the thirteen rice varieties, indicating that different rice varieties reacted differently to different doses of irradiation. Rice seeds exposed to doses of 0 to 600 Gy showed that germination rate decreased to below 87% and seedling survival rate to below 43% at 400 Gy [14].

The irradiation dose of 250 to 300 Gy apparently weakened or damaged the seeds resulting to lower FE and SVI compared to the untreated control. Across the four varieties evaluated, 250 and 300 Gy reduced the FE by 6.17 and 7.28%, respectively, when compared to the control. However, the resulting FE level was still higher than the acceptable minimum germination percentage of 80% set by the Seed Certification Standard of the Philippines. Working on seeds with relatively high initial germination percentage, potential reduction in FE and SVI that could be brought about by the use of irradiation doses of 250 and 300 Gy will still produce M₁ seeds that are within the acceptable minimum limits of germination and seed vigor.

Table 2 Field emergence and seed vigor index of treated seeds resulting from gamma irradiation across four parent varieties

Gamma irradiation dose (Gy)	Field emergence (%)	Seed vigor index
0 (Control)	96.63±3.26 a	20.33±0.63 a
150	92.97±4.61 a	19.55±1.08 a
200	92.87±6.36 a	19.58±1.56 a
250	90.67±6.53 b	19.04±1.56 b
300	89.60±6.70 b	18.78±1.57 b

Note: Means within column followed by the same letter are not significantly different compared with the control using Dunnett's t test at p≤0.05

3.1 Field Emergence and Seed Vigor of Irradiated Seeds of each Parent Varieties

The effects of doses of gamma irradiation on treated seeds of each variety were further evaluated to have additional information on specific effects of irradiation treatments on each variety. Figures 3 and 4 show the FE and SVI, respectively, of the different varieties as affected by the doses of gamma rays. FE and SVI had similar trend in their responses to irradiation dose.

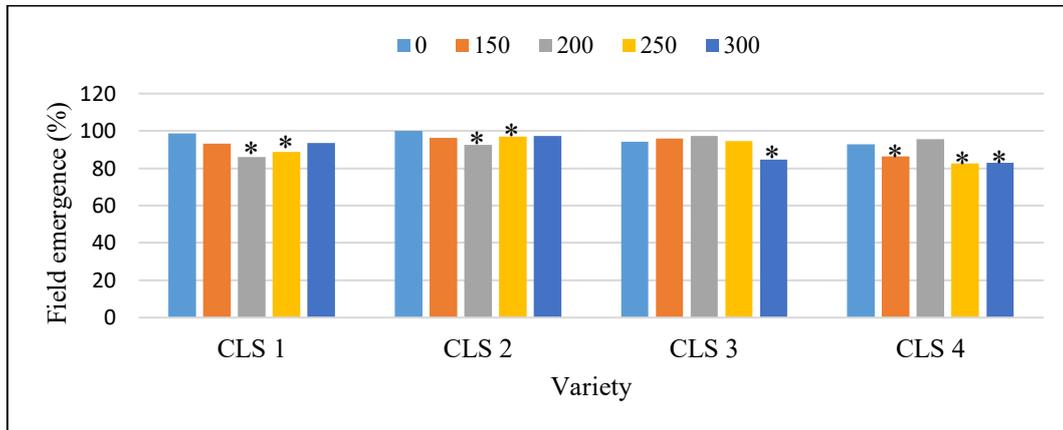


Fig. 3 Field emergence of seeds from different varieties irradiated by gamma rays. Within a variety, bars with asterisk are significantly different with the control (0 Gy) using LSD at $p \leq 0.05$

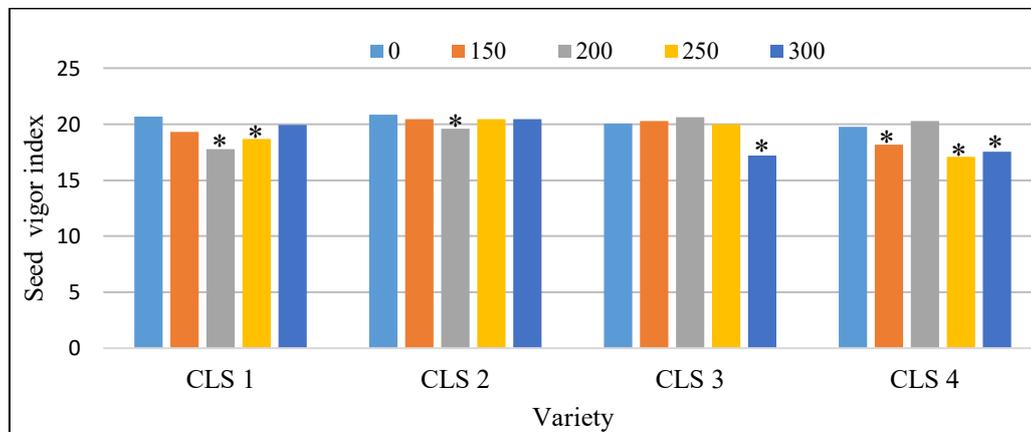


Fig. 4 Seed vigor index of seeds from different varieties irradiated by gamma rays. Within a variety, bars with asterisk are significantly different with the untreated (0 Gy) using LSD at $p \leq 0.05$

CLS 1 and CLS 2 had similar responses to irradiation doses of 200 and 250 Gy in terms of FE. The FE and SVI of CLS 1 seeds irradiated with 200 and 250 Gy were significantly lower than the control while those irradiated with 150 and 300 Gy were comparable. While similar response was exhibited by CLS 2 in terms of FE, only the seeds irradiated at 250 Gy had significantly lower SVI than the control. On the other hand, CLS 3 was sensitive to higher irradiation dose of 300 Gy with significantly lower FE and SVI. The seeds of CLS 4 irradiated at doses of 150, 250 and 300 Gy exhibited negative responses in term of lower FE and SVI. Among the varieties evaluated, CLS 4 had a waxy endosperm while the other three varieties were classified as aromatic and non-waxy. Except for CLS 1 which has white endosperm, both CLS 2 and CLS 3 are pigmented. Marcu et al. [15] mentioned that high-dose irradiation could inhibit growth which could be attributed to cell cycle arrest in the G_2/M phase during somatic cell division and/or to a variety of damages in the entire genome. Chromosomal damage with increasing dosage may be responsible for less germinability of seeds and reduction of plant survival. Plant survival until maturity depends on the nature and extent of chromosomal damage. Additionally, physiological and biochemical disturbances could induce the inhibition of plant germination and development [15]. Metabolic disorders after high doses of gamma radiation could reduce viability of seeds.

All the levels of FE ranging from 82.4 to 100% were more than the acceptable minimum germination percentage set by the Seed Certification Standard of the Philippines [11] which is 80% indicating that the irradiation dose of 150 to 300 Gy could still be used for inducing mutations in similar types of rice.

4. Conclusion

Irradiation using five levels of gamma rays (0 (control), 150, 200, 250, and 300 Gy) were applied to four newly-developed rice varieties (*Oryza sativa* L.) of CLSU to hasten the development of rice mutants with desired agronomic characteristics. Although there was an overall reduction in seedling performance of the irradiated seeds, the resulting FE was still within the standard acceptable limit of 80% germination level. This finding imply that gamma irradiation when applied to paddy seeds with similar characteristics using 150 to 300 Gy could still produce enough seedlings that may have the characteristics desired by the breeder. Among those evaluated, the line with opaque, white, and waxy endosperm appeared to be more sensitive to irradiation treatment in terms of initial seedling performance as more levels of Gy resulted to lower seedling emergence than the untreated seeds.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: **study conception and design:** Leah S.M. Dela Cruz; **data collection:** Elmer P. Natividad Jr.; **analysis and interpretation of results:** Leah S.M. Dela Cruz, Paulina J. Alvaran; **draft manuscript preparation:** Leah S.M. Dela Cruz. All authors reviewed the results and approved the final version of the manuscript.

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