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Radiosensitivity Study for Identifying the Lethal Dose in MR219 *(Oryza sativa L. spp. Indica cv. MR219)*

Ali Benjavad Talebi[1]*; Amin Benjavad Talebi[2]

[1] Department of Plant Biotechnology, National institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran *Email: benjavadtalebi.ali@gmail.com
[2] Department of Computer Sciences, Faculty of Science and Information Technology, National University of Malaysia(UKM), Bangi, Malaysia

Abstract

The purpose of the research was determine the effects of gamma ray on seed germination, seedling height and root length of Rice (*Oryza sativa L. spp. Indica cv. MR219)* to identify the Lethal Dosage of the radiation. Paddy seeds of MR219 were exposed to different doses of gamma radiations viz 50,100,150,200,250,300,350,400,450,500,600,700,800,900 and 1000 Gy using 60^{Co} as the radiation source. The effect of radiation was determined by measuring the Seed germination, seedling height, root length and emergence under the conditions of the M_1 generation. The research results showed that the differences among radiation treatments significantly affect ($p<0.01$) seedling height and root length. In part of seed germination percentage, insignificant differences were observed. Also the LD_{25} and LD_{50} values observed based on the growth reduction of seedlings after treatments occurred during 250 and 450GY of exposure for the variety *Oryza sativa L. spp. Indica cv. MR219*. **[Talebi and Talebi. Radiosensitivity Study for Identifying the Lethal Dose in MR219 *(Oryza sativa L. spp. Indica cv. MR219)*. International Journal of Agricultural Science, Research and Technology, 2012; 2(2):63-67].**

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1. Introduction

In plant breeding and genetics researches, Gamma rays have been used as ionizing radiation for a long time because it is one of the most energetic forms of electromagnetic radiation. This energy level perceived approximately from 10 kilo electron volts (keV) to several hundred keV. Therefore they are more penetrating than other types of radiation such as alpha and beta rays (Kovacs and Keresztes, 2002).

Moreover, ionizing radiations have been frequently utilized to induce mutations in plant breeding and classical genetic analysis. There are a few organisms have been analyzed in-depth at the molecular level. Based on the plant genomes, ionizing radiation usually induces rearrangements and deletions (Shikazono et al., 2001). It has been demonstrated that the mutants in crop species created by ionizing radiation. They are valuable in the two fields of genetics and mutational breeding.

In agriculture field there are some usages of nuclear techniques. In plant improvement, the irradiation of seeds may cause genetic variability. It can enable plant breeders to select new genotypes with improved characteristics such as precocity, salinity tolerance, grain yield and quality (Ashraf et al., 2003).

The International Atomic Energy Agency has strongly supported the application of irradiation mutagenesis to improve crops, and carrying on gamma-ray irradiation provision as a public service. Moreover, it has been emphasized the regional researches should be planned. The networks need to be organized to employ mutation methods for crop breeding. Deletions and other chromosomal rearrangements are identified to occur by irradiation with gamma-(γ) and X-rays. So, small number of them has been only observed at the molecular level (Shikazono et al., 2001). Regarding to produce mutants in Rice (Wu et al., 2005), two doses of gamma ray (250 and 500 GY) have been utilized to make a big collection of Rice mutants in IR64 (Wu et al., 2005).

The size of the genetic lesions actually seemed to be identified in chip-based Method (in the kilo base range) (Bhat et al., 2007).

The ionizing radiations are employed to sterilize some agricultural products due to increase their conservation time or to reduce pathogen propagation when dealing these products within the same country or from country to country (Melki and Marouani, 2010).

An amount of radiobiological parameters are frequently used in early assessment of effectiveness of radiation to induce mutations. According to physiological changes such as inhibition of seed germination and shoot and root, methods have been reported elongation for detection of irradiated cereal grains and legumes.

(Chaudhuri, 2002) and (Kiong et al., 2008) reported that the irradiation of wheat seeds reduced shoot and root lengths upon germination. Gamma radiation can be useful for the alteration of physiological characters (Chaudhuri, 2002), (Kiong et al., 2008). The biological effect of gamma-rays is due to the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovacs and Keresztes, 2002).These radicals can damage or change important components of plant cells. They have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf et al., 2003).

As a result, the selection of a mutagen should be based on its effectiveness and specificity to induce mutations such the final mutant library has a manageable size. It is important for the mutagenesis procedure to be as simple as possible.

Therefore, it is quite significant to find out the main type of mutation induced by a special mutagen since the screening strategy to be employed. This mutation depends on the predominant kind of mutation it produces (Liu et al., 1999). Consequently this scheme has led to an increasing interest in applying irradiation mutagenesis in model organisms in the domain of functional genomics studies (Nambara et al., 1994).This present research was conducted to determine effects of gamma ray on seed germination, seedling height, root length for identify the Lethal Dose in Rice variety MR219.

2. Materials and methods

Plant materials:

In this research, the seeds of cultivar MR219 (*Oryza sativa L. spp. Indica cv. MR219*) were chosen for irradiation. Moisture content of the seeds was adjusted at 13% before irradiation.

Gamma irradiation:

Paddy seeds of MR219 were irradiated with 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 and 1000 Gy of gamma rays. Gamma irradiation was conducted using 60 Co gamma source at a dose rate of 0.862 kGy/h at The Department of Nuclear Science, National University of Malaysia (UKM), Bangi, Malaysia.

Study of germination and seedling height and root length:

Based on the radiation, forty seeds were sown for each treatment beside un-irradiated control on filter paper in Petri dishes by the use 5ml of distillate water. Petri dishes were placed in an incubator for 7 days at 25^{oC}. After seven days number of germination was recorded. The grown seeds from each irradiated dose with non-irradiated control were transferred and planted in the rice field soil prepared in plastic pots. Also the plants were watered with just distillate water in the green house. The seedling height and root length of the plants were measured after two weeks.

In this present experiment, quantitative determinations were applied as a regular procedure. The related data about seedling height, root length and percent of germination were collected and recorded. Variable means were calculated for each treatment.

Statistical analysis:

This current experiment was organized based on a completely randomized Block design with four replications and the random block were gamma irradiation (15 levels). Least Significant Different (LSD) test ($P<0.01$) was used to investigate the differences in average of all tested parameters between irradiated and non-irradiated plantlets. Therefore statistical analysis was carried out by using S.A.S software.

3. Results and discussion

Effect of gamma irradiation on germination

Data analysis on number of seed germination showed that the germination and emergence declined after gamma irradiation as the dosage increases.

According to Fig 1 and Table 1, the research results showed that the seed germination decreased following gamma irradiation. The decline was neither proportional to the increase in dosage nor to the special pattern found in this rice variety.

As Table 1, irradiated rice seeds kept their germination capacity compared to the control.

Effect of gamma radiation on root length and seedling height

Analysis of the average of roots length and seedling height showed the Gamma ray imposed a significant impact on the seedling height. According to research results, Figure 2 and Table 1 demonstrate that seedling height decreased in proportion with the increase in the dosage of gamma ray $P<0.01$.

Root length decreased after all doses of irradiation as compared to non-irradiated control Table 1. Maximum reduction in root length was observed after 200 Gy dose in MR219. Figure 3 and Table 1 in this research, showed that the reduction in root length occurred with each corresponding increase in gamma ray dose $P<0.01$.

The highest length of seedling 31.7cm was observed at 50 Gy. Seedling height reduced during subsequent doses of gamma radiation. Based on Table 1, the maximum reduce in Seedling height was observed, when rice MR219 was exposed to gamma ray dose higher than 400 Gy. Also, Maximum root length was measured for control 96.4 mm, while root length of 250 Gy seedlings height 25.59 cm was in statistically similar group. It is a minimum length of the root was found in 500 Gy.

We could not get results of root length and seedling height after irradiation with doses above 500Gy in this genotype.

Discussion

Effect of gamma irradiation on germination

It can be observed in field of conditions, gamma irradiation caused insignificant reduction in the germination. Therefore, when the gamma dosage was increased, the seed germination was no significantly declined.

According to Fig 1 and Table 1, the current research results showed that the after gamma-ray exposure, seed germination was nonscientific decreased compared to the dosage exposure.

These results were in accordance with the germination test done by (Redei and Koncz, 1992).

So, there was no significant difference in germination and survival percentage of irradiated and non-irradiated seeds of rice.

According to (Kiong et al., 2008) study, results have shown that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for less germ inability and reduction in plant growth and survival. Changes in the germination percentage were attributed to gamma rays treatments (Kiong et al., 2008).

The stimulating effect of gamma ray on germination may be credited to the activation of RNA or protein synthesis. It may be occurred during the early stage of germination after the seeds were irradiated (Abdel-Hady et al., 2008). Based on (Chaudhuri’s 2002) report the higher radiation dose, germination percentage reduced in addition to root and shoot length, while, at a lower dose i.e., 0.1 kGy the germination percentage was not significantly different from control (Chaudhuri, 2002).

In another study by (Kiong et al., 2008) found that radiation increases plant sensitivity to gamma rays. This way may be caused by the reduced amount of endogenous growth regulators, particularly the cytokines, as a result of breakdown, or lack of synthesis, due to radiation (Kiong et al., 2008).

Regarding to (Chaomei and Yanlin’s 1993) findings on wheat Triticum aestivum L. these results are shown in agreement. They noticed that treating seeds with high rates of gamma radiation reduced germination with a corresponding decline

in growth of plants (Chaomei and Yanlin, 1993).

Effect of gamma radiation on root length and seedling height to identify the biological influences of different physical and chemical mutagens in M1, Seedling height is mostly utilized as an index (Nadeau and Frankel, 2000). It has been shown as a linear dependency of seedling height on the dosage of physical and chemical mutagens.

According to Fig2 and table 1, the results proved that the decreases in seedling height were because of the increase in gamma-ray doses.

The results showed that after irradiation with gamma ray in the rice variety MR219, the seedling height were significantly $p<0.01$ decreased as compared to the control Table 1.

In rice MR219 a significant effect $p<0.01$ of the irradiation dose on the root length was observed Table 1. Figure 3 and Table 1 in this research, showed that the reduction in root length occurred with each corresponding increase in gamma ray dose. The symptoms frequently observed in the low-or high- dose-irradiated plants are enhancement or inhibition of germination, seedling growth, and other biological responses (Kim et al., 2000; Wi et al., 2007). Although, no certain explanations for the Stimulatory effects of low-dose gamma radiation are available until now. The results are in accordance to the results obtained by Wi et al., 2007 that there is a hypothesis in low dose irradiation will induce growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti-oxidative capacity of the cells. It can easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth condition (Wi et al., 2007).

In contrast, the high-dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome (Preuss and Britt, 2003). radiation effects on chickpea seeds by (Toker et al., 2005) seedlings irradiated at 200 Gy may have some significant increase in their shoot length, but at 400 Gy an obvious depression in shoot length was observed (Tokeret et al., 2005).In this present study, the variability as measured by mean values of the root length and seedling height decreased with increase in the radiation dose. According to Chaudhuri’s report when radiation is sufficient to reduce the rooting percentages, the root lengths do not exceed a few millimeters in length.

Table1. Mean Value of Germination, Seedling Height, Root length and Emergence Following Gamma Irradiation

Treatment (GY)	Germination		Seedling Height (Cm)		Root Length (mm)	
	Actual	% of control	Actual	% of control	Actual	% of control
control	38	100	32.93*	100	96.4*	100
50	38	100	31.70*	97.08	93.4*	96.57
100	36	94.73	31.24*	94.86	92.5*	95.53
150	37	97.36	30.72*	93.28	89.5*	92.84
200	35	92.10	30.13*	91.49	80.4*	83.40
250	38	100	25.59*	77.71	72*	74.68
300	35	92.10	21.38*	64.92	59.5*	61.17
350	35	92.10	21.17*	64.28	48.9*	50.41
400	35	92.10	21.03*	63.86	44.2*	45.85
450	36	94.73	17.11*	51.95	40.8*	42.32
500	36	94.73	13.28*	40.32	34.5*	35.78
600	37	97.36	0	0	0	0
700	36	94.73	0	0	0	0
800	37	97.36	0	0	0	0
900	37	97.36	0	0	0	0
1000	34	89.47	0	0	0	0
LSD%	1.9674		2.2425		3.0445	
C.V%	2.01410		2.04227		2.04227	

* Significant at 1% level, the values are mean of four replicates.

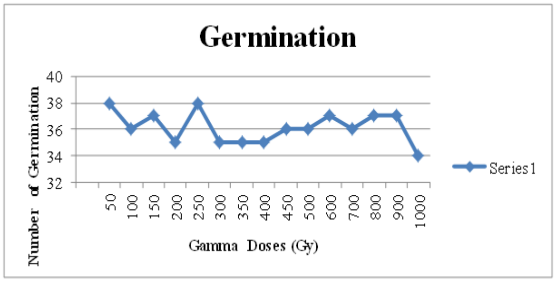

Fig1. Effect of Different Doses Gamma Irradiation on Seed Germination

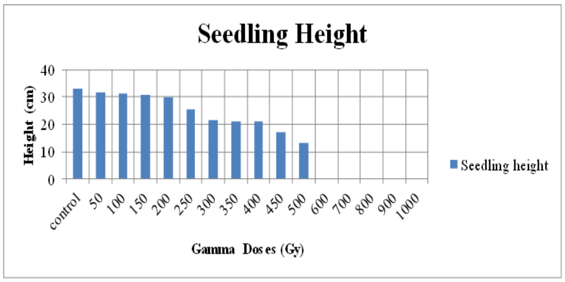

Fig2. Effect of Different Doses Gamma Irradiation on Seedling Height

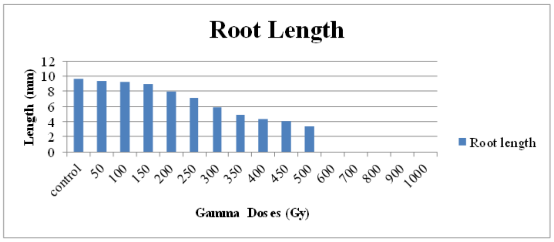

Fig 3. Effect of Different Doses Gamma Irradiation on Root Length

Hence due to metabolic disorders in the seeds after gamma irradiation, the seeds are unable to germinate (Chaudhuri, 2002).

4. Conclusion

In this research, the results showed that the differences between radiation treatments significantly affect p<0.01 seedling height and root length. In part of seed germination percentage, insignificant differences were observed. Also the LD25 and LD50 values observed based on the growth reduction of seedlings after treatments occurred during 250 and 450GY of exposure for the variety *(Oryza sativa L. spp. Indica cv. MR219)*.

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