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DETERMINATION OF LETHAL DOSE OF RICE (*ORYZA SATIVA* L.) GENOTYPES BY RADIO SENSITIVITY TEST

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ABSTRACT

The purpose of the research was to determine the effects of gamma ray on seed germination, shoot length, root length, and shoot and root fresh weight of Rice (*Oryza sativa* L. spp. *Indica*) to identify the lethal dosage of the radiation. For this research, two rice genotypes viz., Binadhan-17 and Galon were examined for varietal differences in radio sensitivity to gamma radiations. Dry healthy seeds were figured to variable doses of gamma radiations i.e., 50-1000 Gy with 50 Gy intervals using ⁶⁰Co as the radiation source. Highly significant differences among the genotypes (p<0.01) for all traits and treatments were observed. The differences among radiation treatments were highly significant (p<0.01) for germination percentage of seed, shoot and root length, shoot and root fresh weight. The genotype × dose interactions were highly significant for germination percentage, shoot length, root length, shoot fresh weight and root fresh weight indicating no stability of performance for characters across different radiation levels. Mutagenic treatments shifted mean values towards negative direction for almost all traits but not in a definite pattern. In general, genotypes displayed variable response towards gamma radiations and there LD25 and LD50 were different.

Keywords: Radiosensitivity, Lethal Dose, Gamma Ray, Rice Genotypes.

INTRODUCTION

Rice is the most important food habit of the people of Bangladesh as well as the whole world. It is second largest consumed cereal grain. About 90-92% rice is produced in Asia. Production of rice is increasing day by day due to development of rice genotypes and use of modern varieties by the farmers. The land is decreasing day by day due to increase of population and we have to produce more food from the decreasing land that's why the scientists are working for (BBS, 2018). There are many techniques for improvement the rice yield; mutation breeding is one of them. Use of genetic variability for crop improvement is the fundamental research of plant breeder. In plant breeding and genetics researches, ionizing radiation such as Gamma rays used for a longer duration because of its energetic forms of electromagnetic radiation. About 10 kilo electron volts (keV) to several hundred keV energy levels realized for such radiation. Moreover, these radiations are much penetrating than other types of radiation for example alpha and beta rays (Kovacs and Keresztes, 2002). Therefore, for induced mutations the ionizing radiations have been used in plant breeding and classical genetic analysis. Generally Ionizing radiation induces rearrangements and deletions, based on plant genomes (Shikazono *et al.*, 2001). Through, ionizing radiation the mutant species are created. It is very useful in the fields of genetics and mutation breeding. In agriculture for the development of plant few researchers are using nuclear techniques. In cereals, the improvement of those

plants was done through radiation of seeds and here the genetic variability may cause. Then the researcher is able to select new genotypes with better characteristics such as precocity, salinity tolerance, grain yield and quality.

Bangladesh Institute of Nuclear Agriculture (BINA), a research organization of Bangladesh has been working with use of Nuclear power and application of irradiation mutagenesis to development of crops, and maintains gamma-ray irradiation dispensation as a public work. Therefore, it has been strengthening the regional researches should be designed. For crop breeding, the networks need to be embodied to set mutation methods. Insertion, deletions and other chromosomal rearrangements are placed to happen by irradiation with gamma- γ and X-rays. So, few of them has been only executed at the molecular level (Shikazono *et al.*, 2001). So, production of mutants in Rice, two doses of gamma ray (250 and 500 GY) have been exploited to produce a lot of Rice mutants in IR64 (Wu *et al.*, 2005). In chip-based method (in the kilo base range) the size of the genetic bruises actually supposed to be identified (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). The present study was carried out with the objective to determine the response of rice genotypes to mutagenic effects of gamma radiation for different seedling growth, physiological and fertility traits in order to induce genetic

variability in Binadhan-17 and Galon for production of high yielding varieties and these variations will be used in further selection studies for producing high yielding and good premium quality varieties.

MATERIALS AND METHODS

Plant materials

In this research, the seeds of cultivar Galon and Binadhan-17 were chosen for irradiation. Moisture content of the seeds was adjusted at 13% before irradiation.

Gamma irradiation

Paddy seeds of Galon and Binadhan-17 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 Bangladesh Institute of Nuclear Agriculture, Bangladesh.

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°C. 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 trays. 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 three replications and the random block were gamma irradiation (21 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 R software.

RESULTS AND DISCUSSION

Effect of gamma irradiation on seed germination

The high yielding rice variety 'Binadhan-17' and "Galon" another indigenous local rice cultivar of Chattogram Hill Tract of Bangladesh were irradiated with different doses of

Gamma rays (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950 and 1000 Gy). It was found that doses 0-350 Gy have no effects on percentage of germination on Binadhan-17 and Galon. But when the doses increase more than 350 Gy the percentage of seed germination were reduced and in higher dose i.e., 650 Gy, there is no germination of seed (Fig. 1 and Table 1). In field conditions, it can be executed that gamma irradiation caused significant ebb in the germination of seed. Hence, when the gamma dosage was increased, the seed germination was significantly fall. According to Fig. 1 and Table 1, this research results exposed that the after gamma-ray exposure, seed germination was reduced compared to the dosage disclosure. These works were in accordance with the germination test done by Redei and Koncz (1992). So, there was significant difference in germination and survival percentage of irradiated and non-irradiated seeds of rice. Kiong *et al.*, (2008) study showed that the results of survival of plants to maturity rely on the nature and spread of chromosomal injury.

Increasing radiation dose with the increasing frequency of chromosomal damage may be liable for few germ disability and reduction in plant growth and survival. Gamma rays' treatments were imposed to the variation in the germination percentage (Kiong *et al.*, 2008). The promptness of RNA or protein synthesis may be deposited by the excitatory outcome of gamma ray on germination. During the early stage of germination, it may be ensued after the seeds were irradiated (Abdel-Hady *et al.*, 2008). A lower dose i.e., 0.1 kGy the germination percentage was not significantly different from control while, the higher in radiation dose, germination percentage ebbed in addition to root and shoot length (Chaudhuri, 2002). Kiong *et al.*, (2008) reported that plant sensitivity to gamma rays increases with the radiation increases. Due to radiation, the reduced amount of endogenous growth regulators may be caused by this way, particularly the cytokines, as a result of breakdown, or lack of synthesis (Kiong *et al.*, 2008).

Chaomei and Yanlin's (1993) found that treating seeds of wheat (*Triticum aestivum* L.) with high rates of gamma radiation reduced germination with a consisting fall in growth of plants. Hence the seeds after gamma irradiation, the seeds are unable to germinate due to metabolic disorders (Chaudhuri, 2002).

Effect of gamma radiation on shoot length and root length

The average shoot length and root length demonstrate that shoot length decreased in proportion with the increase in the dosage of gamma ray. The average shoots length and root length decreased after all doses of irradiation as compared to non-irradiated control (Figs. 2, 3 and Table1). The average shoot length was measured maximum in 50 Gy on Binadhan-17 and Galon and half in 450 Gy doses

Table 1. Analysis of variance for seedling and physiological traits in rice genotypes

Sources of Vari- ances	D.F.	Germination percentage	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)
Genotype	2	412***	108***	7.9***	1.04***	0.381***
Treatment	20	245382***	8046***	1379.2***	54.64***	24.214***
Interaction	20	1817***	148***	32.2***	2.33***	0.669***
Error	84	141	66	34.1	1.62	0.583

***=Highly significant

Table 2. Mean values of genotypes for different seedlings and physiological traits

Traits	Genotypes	
	Binadhan-17	Galon
Germination percentage	49.25a	52.87a
Shoot length (cm)	8.53a	10.38a
Root length (cm)	4.28a	3.78a
Shoot fresh weight (g)	0.86a	0.68a
Root fresh weight (g)	0.56a	0.45a

Table 3. Treatment means of different seedlings growth and physiological traits

Traits	Treatments							
	0	50	100	150	200	250	300	350
Germination percentage	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a
Shoot length (cm)	18.37ab	19.49a	18.18ab	18.26ab	18.33ab	17.99ab	16.56ab	15.70abc
Root length (cm)	7.84ab	8.11a	7.26ab	7.48ab	7.30ab	7.43ab	6.30abc	6.83ab
Shoot fresh weight (g)	1.52a	1.60a	1.53a	1.47ab	1.54a	1.41abc	1.40abc	1.23abc
Root fresh weight (g)	1.08ab	1.11a	1.10a	0.97abcd	1.01abc	0.98abc	0.85abcde	0.74bcdef

Table 4. Treatment means of different seedlings growth and physiological traits (cont.)

Traits	Treatments												
	400	450	500	550	600	650	700	750	800	850	900	950	1000
Germination percentage	81.96 ab	67.62b	51.96bc	32.88 cd	20.23 de	11.67de	6.00de	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e
Shoot length (cm)	15.24 abc	12.88bcd	10.13cd	10.14 cd	7.28d	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e
Root length (cm)	6.75 ab	5.26abc	5.24bc	5.00 bc	3.87c	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d	0.00d
Shoot fresh weight (g)	1.29 abc	0.84cd	0.91bcd	0.88 bcd	0.46de	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e	0.00e
Root fresh weight (g)	0.72 cdef	0.64def	0.52ef	0.49 f	0.41f	0.00g	0.00g	0.00g	0.00g	0.00g	0.00g	0.00g	0.00g

on Binadhan-17 and 550 Gy on Galon and maximum decreased in 650 Gy. Again, the average root lengths were maximum in 50 Gy on Binadhan-17 and control on Galon and the half in 550 Gy on Binadhan-17 and 575 Gy on Galon and maximum decreased in 650 Gy. In this study, the results showed that the differences between gamma irradiation treatments affect shoot length and root length. In case of seed germination percentage, in 350 Gy this

was 100 % and after that when radiation increases the germination percentage were decreases. Regarding the LD25 and LD50 values on Binadhan-17, based on the growth reduction of shoot length was observed in 350 Gy and 450 Gy respectively. Regarding the LD25 and LD50 values on Galon, based on the growth reduction of shoot length was observed in 475 Gy and 575 Gy, respectively. The biological impact on different physical and chemical

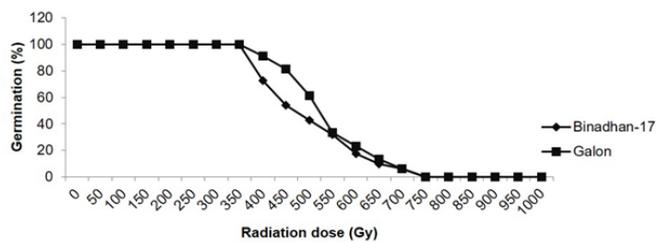


Fig 1. Effect of different doses of gamma irradiation on seed germination (%)

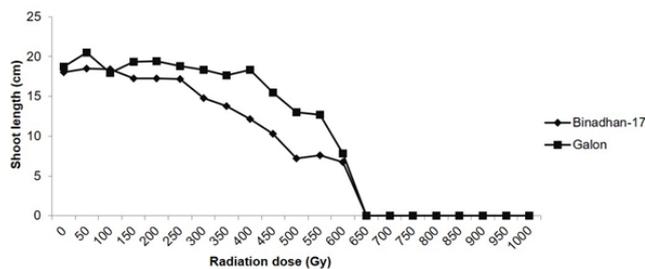


Fig 2. Effect of different doses of gamma irradiation on shoot length (cm)

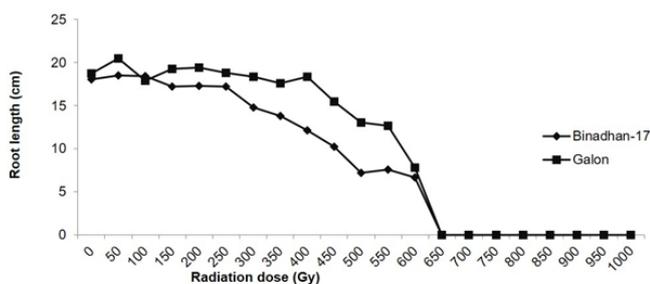


Fig 3. Effect of different doses of gamma irradiation on root length (cm)

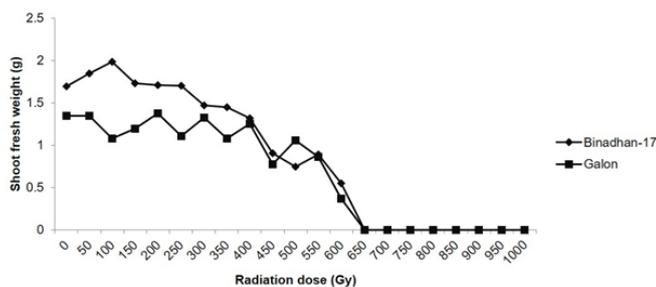


Fig 4. Effect of different doses of gamma irradiation on shoot fresh weight (g)

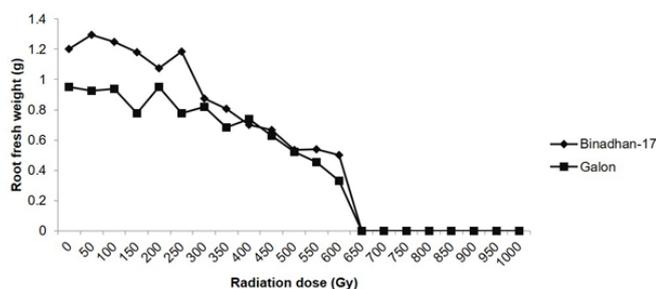


Fig 5. Effect of different doses of gamma irradiation on root fresh weight (g)

mutagens in M_1 is to recognize the gamma radiation effect on root length and shoot length. Seedling height is mostly taken advantage as an exponent (Nadeau and Frankel, 2000). On the dosage of physical and chemical mutagens it has been shown as a linear servitude of seedling height. According to Fig. 2 and Table 1, the results showed that because of increases in gamma-ray doses the seedling height were decreases. It was observed that after irradiation with gamma ray in the rice varieties Binadhan-17 and Galon, the shoot length was significantly $p < 0.01$ decreased as compared to the control Table 1. In rice both Binadhan-17 and Galon had a significant effect $p < 0.01$ of the irradiation dose on the root length (Fig. 3) was observed Table 1.

With each corresponding increase in gamma ray dose the reduction in root length occur which were showed in Fig. 3 and Table 1 in this research. Enhancement or inhibition of germination, seedling growth, and other biological responses symptoms were frequently observed in the low-or high-dose-irradiated plants (Kim *et al.*, 2000; Wi *et al.*, 2007). In contrast, during somatic cell division and/or various harms in the entire genome the high-dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase (Preuss and Britt, 2003). Seedlings irradiated at 200 Gy may have some significant increase in their shoot length Radiation effects on chickpea seeds, but at 400 Gy an obvious depression in shoot length was observed (Toker *et al.*, 2005). The variability as measured by mean values of the root length and seedling height decreased with increase in the radiation dose was observed in the present study. When radiation is sufficient to reduce the rooting percentages, the root lengths do not exceed a few millimeters in length (Chaudhuri, 2002).

Effect of gamma radiation on shoot fresh weight and root fresh weight

Gamma radiation had significant effect on shoot fresh weight and root fresh weight (Figs.4, 5 and Table1). The shoot fresh weight was highest at Binadhan-17 at 100 Gy radiation level and at 200 Gy radiation level at Galon and with the increases of gamma radiation the shoot fresh weight was decreases. The root fresh weight was highest at Binadhan-17 at 50 Gy radiation level and at 200 Gy radiation level at Galon and with the increases of gamma radiation the root fresh weight was decreases. The radiation treatment reduced shoot and root weight but that reduction was not proportional with increases in dose level. Minimum weight was observed at the parameter both shoot and root fresh weight at 600 Gy treatments for both Binadhan-17 and Galon. It was found that gamma radiation had significant effect on shoot fresh weight and root fresh weight (Figs.4, 5 and Table1). Previously, maximum shoot fresh weight was observed in control and at 100 Gy while minimum was observed in at 300 Gy that was also highest in the experiment (Moradi *et*

al., 2009). Our findings are in line with this report as maximum root and shoot weights were observed in 100 Gy at Binadhan-17 and 200 Gy at Galon and minimum at highest radiation dose of 600 Gy. However, in the present study, reduction in these traits was not proportional with increasing dose. In contrast to above mentioned findings, a slight stimulation in shoot fresh weights of rice mutants at 150 and 200 Gy radiation dose has also been reported (Sheeren *et al.*, 2009).

CONCLUSION

In this research, the results showed that the differences between radiation treatments significantly affect $p < 0.01$ shoot and root length. In part of seed germination percentage, shoot and root length, shoot fresh weight and root fresh weight significant differences were observed. Also LD25 and LD50 values on Binadhan-17, based on the growth reduction of shoot length it was observed in 350 Gy and 450 Gy respectively. Regarding the LD25 and LD50 values on Galon, based on the growth reduction of shoot length it was observed in 475 Gy and 575 Gy, respectively.

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