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Identify the Lethal Dose of EMS and Gamma Radiation Mutagenesis in Rice MR219

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Abstract. Chemical and physical mutagenesis has been used to raise the genetic variation in crop plants. More than 501 new varieties have been obtained from Rice mutants by applying different mutagenic agents in Rice (*Oryza sativa* L.). Chemical mutagens Such as EMS, DEB and sodium azide and irradiation (Gamma rays, X-rays and fast neutrons) have been broadly used to cause a majority of practical variations in Rice and others crops. In this research, it attempted to find out the effects of gamma ray and EMS on seed germination, seedling height and root length of Rice MR219 to identify the Lethal Dosage (LD). The Seeds of potential genotype of the favorite variety, (*Oryza sativa* L. spp. Indica cv. MR219) exposed to different doses of gamma radiations with 50,100,150,200,250,300,350,400,450,500,600,700,800,900 and 1000 Gy using 60 Co as the radiation source. Also the seeds treated using EMS with concentration of 0.25%, 0.50%, 0.75%, 1%, 1.25%, 1.5% and 2%. The increase in concentration of EMS, a decrease in germination, seedling height, root length and emergence under field conditions was observed. In addition, Seedling height and Root length decreased with the increase in Gamma Dose and an abnormal decrease in germination was observed. The Lethal Dose (LD) was determined by different measurements on the M_1 generation. The LD_{25} and LD_{50} values observed based on the growth reduction of seedlings after treatment that was 0.25% and 0.50% in the EMS Mutagenesis, Also the LD_{25} and LD_{50} values occurred during 250 and 450GY of exposure for the variety Oryza sativa L. spp. Indica cv. MR219.

Keywords: Lethal Dose, Chemical Mutagenesis, Ethylmethane Sulfonate (EMS), Ionizing Radiation, Gamma Ray, *Oryza sativa*, Indica cv. MR219.

1. Introduction

Chemical mutagens and ionizing radiation have been used for long time as the plant mutagens in breeding research and genetic studies [5]. So far, throughout such mutagenesis, 2,428 crop varieties have been released and among them 501 rice varieties [2]. Production of mutants by chemical or irradiation mutagenesis is fairly economical. Any genotype can be mutagenized and the distribution of mutations is maybe accidental in the genome. Genome-wide saturation mutagenesis can be achieved using a quite small mutant population because of the high concentration of mutations [6]. Moreover, it provides a large allelic series as an accompaniment of the knockout mutants created by inserted mutagenesis or transformation methods (over- and under–expression). Chemical and irradiation forms of mutagenesis do not depend on transformation. In spite of these advantages, the use of chemical and irradiation- induced mutants as gene identification means has been restricted. The reason for this is that the molecular isolation of mutated gene(s) needs significant efforts as the

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mutations are not physically tagged. However, advances in high performance genotyping have considerably improved the efficiency for detection of point mutations or deletions [2]. As a result, there has been growing interest in using chemical and irradiation mutagenesis in model organisms for functional genomics research [7]. Chemicals cause mainly point mutations, therefore are perfect for production of missense and nonsense mutations, which would result a series of change-of function mutations. However, ionizing radiations normally cause chromosomal rearrangements and deletions. Then the selection of a mutagen should be based on its efficiency and specificity to cause mutations, such that the resulting mutant library is of manageable size. At the same time, the mutagenesis process should be as simple as possible. In addition, it is important to know the main type of mutation induced by a specific mutagen, as the screening approach to be used will depend on the predominant type of mutation it creates [2]. Rice could be a food crop and a model system for scientific research [8]. MR219 is the most broadly grown Malaysian rice. It possessed many positive agronomic characteristics that make it a perfect genotype for identification of mutational changes in features of agronomic value.

2. Material and Methods

In this research, the seeds of cultivar MR219 (*Oryza sativa* L. spp. Indica cv. MR219) have been selected to induce mutagenesis. The seeds of MR219 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 60Co gamma source at a dose rate of 0.862 kGy/h at The Department of Nuclear Science, National University of Malaysia (UKM), Bangi, Malaysia. Also for EMS Mutagenesis, seeds of MR219 have been placed in a 100 ml flask and distillate water was added to about 2 cm above the seeds (~80 ml).Seeds were soaked overnight at room temperature for 18 hours. Water was decanted and 25 ml of 0.25%, 0.50%, 0.75%, 1%, 1.25%, 1.5% and 2%. EMS (v/v) in water was added. Seeds were incubated for 12 hours at room temperature followed by decanting of the EMS and rinsing with 50 ml of distillate water (5 times, 3 minutes each) and 80 ml of distillate water (5 times, 10 minutes each). Seeds were then rinsed under running tap water for 5 hours before planting in flats. Based on the Gamma radiation and EMS mutagenesis, forty seeds were sown for each treatment beside un-treatment 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^{o}C. After seven days number of germination was recorded. And the grown seeds from each irradiated dose and Ems concentration with non-treatment 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. Lethal Dose experiment was organized based on a Completely Randomized Block Design (RCBD) with four replications and the random block were gamma irradiation (15 levels) and EMS Mutagenesis (7 levels) Least Significant Different (LSD) test ($P<0.01$) was used to investigate the differences in average of all tested parameters between treatment and non-treatment plants. Therefore statistical analysis was carried out by using S.A.S.

3. Results

3.1. Impact of gamma Irradiation and EMS Mutagenesis on Germination

There was an abnormal reduction in the germination in M_1 generation with the raise of Gamma dose. Conversely, there was an increase above the non-treatment control (Table.1) and (Fig.1).In addition, with the increase in concentration of EMS, the decrease in germination was observed in M_1 generation as well as the increase above the non-treatment control (Table.2) and (Fig.2).

3.2. Impact of Gamma Radiation and EMS Mutagenesis on Root Length and Seedling Height

Table 1, Figure 3 and Figure 4 demonstrated seedling height and Root length decreased with the increase in Gamma Dose on approximately linear mode. Besides that, seedling height and Root length decreased within the increase in EMS mutagenesis on approximately linear mode (Table 2, Figure 5 & Figure 6).

4. Discussion

The results showed that the differences among radiation treatments considerably influence ($p<0.01$) seedling height and root length. In the germination test, significant differences were not observed, there were a significant decrease in the level of germination, seedling height, root length and the plant growth under field condition with the increase of the concentration of EMS comparing to non -treatment control. Moreover, with the raise in the amount of EMS mutagenesis, plant height and root length approximately decreased as a liner chart. In this survey, the results of gamma irradiation were confirmed with the findings of germination test has done by Borzuei et al., 2010[3]. The stimulating effect of gamma ray on germination maybe attributed to the activation of RNA or protein synthesis. It may be occurred during the early stage of germination after the seeds were irradiated [1]. According to Chaudhuri's report (2002), the critical dose that prevented the root elongation varied from 0.1 to 0.5 kGy. In higher radiation dose, there were the reduction in the germination proportion as well as root and shoot length whereas there were no significant difference in lower dose comparing to non -treatment control [4]. To recognize the biological impact of different physical and chemical mutagens in M_1, Seedling height was mostly used as an index [2]. The results of radio sensitivity study were confirmed by the results obtained by Wi et al., 2007. Besides that, 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 conquer daily stress factors such as fluctuations of light intensity and temperature in the growth conditions [9].

5. Conclusion

In this research, lethal Dose was determined by measuring the Seed germination, seedling height, root length and emergence under the Field conditions of the M_1 generation. In addition, quantitative determinations applied as a regular process. The data regarding to seedling height, root length and percentage of germination collected and recorded and also variable means considered.

In addition, The LD_{25} and LD_{50} values observed according to the growth reduction of seedlings after treatment that was 0.25% and 0.50% in the EMS Mutagenesis, Also the LD_{25} and LD_{50} values occurred during 250 GY and 450 GY of exposure for the variety Oryza sativa L. spp. Indica cv. MR219.

6. References

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Table.1: 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
C.V%	2.01410		2.04227		2.04227	

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

Table.2: Mean Value of Germination, Seedling Height, Root length and Emergence Following EMS Mutagenesis

Treatment (GY)	Germination		Seedling Height (Cm)		Root Length (mm)	
	Actual	% of control	Actual	% of control	Actual	% of control
control	39	100	33.04*	100	96.6*	100
0.25	28	71.79	24.58*	74.39	69.80*	72.25
0.50	19	48.71	16.16*	48.91	45.15*	46.73
0.75	11	28.20	8.40*	25.42	22.37*	23.15
1	7	17.94	0	0	0	0
1.25	2	5.12	0	0	0	0
1.5	0	0	0	0	0	0
2	0	0	0	0	0	0
LSD%	3.78		5.07		4.44	
C.V%	2.13		2.26		2.26	

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

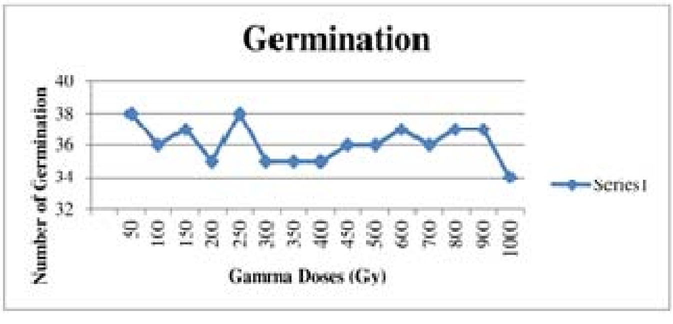

Fig. 1: Effect of Different Doses Gamma Irradiation on Seed Germination

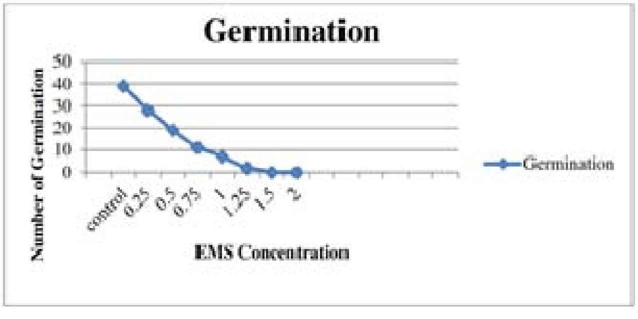

Fig. 2: Effect of Different concentration of EMS mutagenesis on Seed Germination

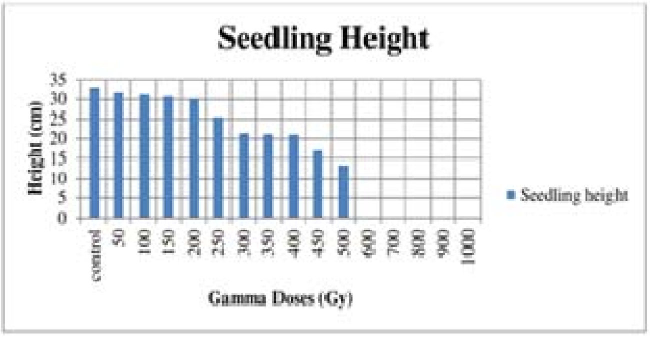

Fig. 3: Effect of Different Doses Gamma Irradiation on Seedling Height

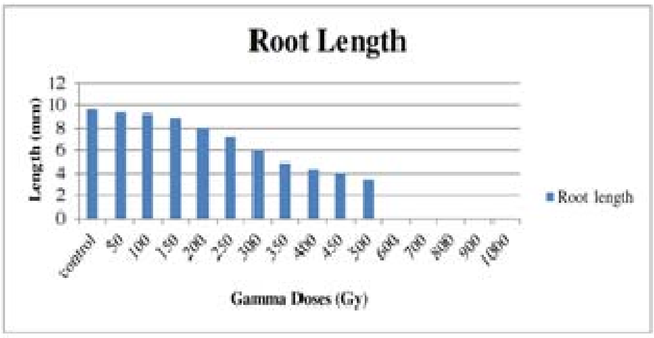

Fig. 4: Effect of Different Doses Gamma Irradiation on Root Length

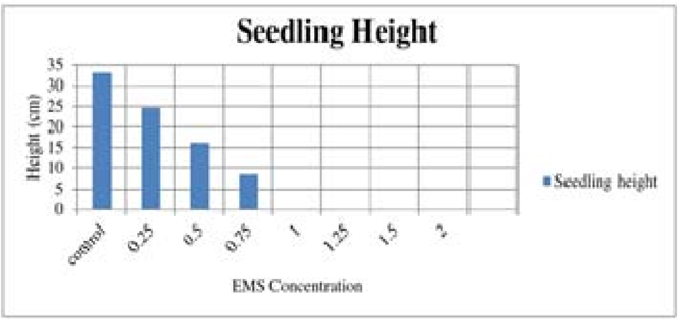

Fig. 5: Effect of Different Concentration of EMS On Seedling Height

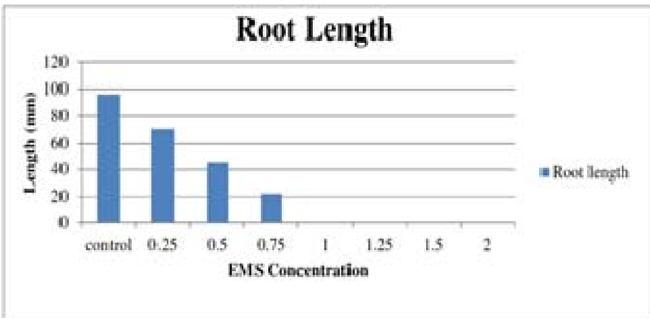

Fig. 6: Effect of Different Concentration on Root Length