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Appraising LD_{50} dosage for physical mutagen (Gamma rays) in CR1009 and CR1009 *sub1* rice varieties

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Abstract
Present study was aimed to identify the early heading plant types for late maturing rice varieties *viz.*, CR1009 and CR1009 sub1 in order to save resources like irrigation water, fertilizer, labour cost. Mutation breeding was selected as an appropriate breeding methodology to alter the late heading types in CR1009 and CR1009 sub1 rice varieties besides keeping intact of original characteristics. To carry out the successful mutation programme in any of the crop, standardisation of LD_{50} (Lethal Dosage) is the necessary first step. The LD_{50} was estimated using probit analysis based on seedling germination of control and their treated ones (100 Gy, 200 Gy, 300 Gy and 400 Gy). Results revealed that the rice variety CR1009 was sensitive to radioactivity exposure with LD_{50} value 152.52 gray compared to CR1009 sub1 (284.77 Gray). There was a definite relationship for both shoot length and root length in M_1 population in relation to dosage. In the variety CR1009, the dosage 200 gray exhibited highest value for both shoot (18.84cm) and root length (5.68 cm) among other treatments. Similarly, the dosage 400 gray recorded lowest value for shoot (7.42 cm) and root length (2.84 cm) in CR1009. Whereas in CR1009 sub1 variety, the highest and lowest value for shoot and root length was noticed in the dosage of 100 gray and 300 gray respectively.

Keywords: mutation breeding, LD_{50}, rice, early maturing, probit analysis, gamma rays

Introduction
Rice is the most widely cultivated crop and serves as a prominent diet to the people for their calorie requirement. It is consumed by babies, older ones and also by sick people for its laxative nature. Considering the essential needs of rice for world population in day to day life, it is the privilege of plant breeder to further improve the rice crop and also to alter the unwanted traits of them. Mutation breeding is one of the breeding methods to create or increase the genetic variability of the existing germplasm. It helps the breeders to correct the defect trait of concerned variety without affecting its original traits and yielding potentiality. Comparing to other breeding methods, it is simple and does not demand special technical skills. It is widely used in more than 70 countries and out of 3248 crop mutant varieties developed, 820 were rice mutants [1]. Among the physical mutagen, gamma rays are most popular one because of its easy handling and ability of high penetrance into the tissues [2]. Due to its low Linear Energy Transfer (LET) with the range of 0.2-2 keV/µm, wide spread damage can occur in plant DNA. It has the ability to cause transition or transversion and also cause removal ranges from 2 bp to16 bp which occurs once at the nucleotide coverage of 6.2Mb [3]. In order to achieve the high rate of desirable mutants and to maintain the mutant population, fixation of LD_{50} dosage is highly essential [4]. The dosages below LD_{50} are estimated to produce more economically useful mutants [1]. In order to study the influence of gamma rays on M_1 population, seedling germination, shoot and root length were recorded. Out of observations made, seedling germination was used to determine the LD_{50} for the range of gamma rays dosages employed. By using the fixed LD_{50} dosage, screening of early mutant in late maturing variety *viz.* CR1009 and CR1009 sub1 rice variety is possible. The stable variety developed after M_6 –M_7 generation, can be used as direct mutant variety after testing its performance over locations for the trait improved or can be used in breeding programmes [2].

Materials and Methods
Two rice varieties *viz.*, CR1009 and CR1009 sub1 were used as a experimental materials. The objective of the study was to compare the LD_{50} dosage between rice varieties and to standardise the LD_{50} dosage of gamma rays mutagen for both the rice varieties. Genetically pure seeds of these two varieties were collected from Tamil Nadu Agricultural University,

Coimbatore. The dry paddy seeds were used for mutation treatment in order to handle the treated seeds easily [5] with 12-14 % moisture which would enable high mutation rates [6]. The gamma dosages used were 100 gray, 200 gray, 300 gray and 400 gray. To find out the LD_{50} dosage for two varieties, probit analysis was employed [7]. The lethal dosage is determined by using the germination percent of particular variety. And also other seedling parameters like shoot and root length in the field condition, were observed for both the varieties.

The steps involved in probit analysis were described below.

- Initially, the dosages of gamma rays were converted into their respective $\log_{10}$ value
- The lethality in M_1 population at the early stage was evaluated by means of damage occurred for different treatments and converted into percentage in whole numbers.
- Then with the help of Abbott's formula, corrected mortality percentage were calculated and rounded to whole numbers

$$\text{Corrected mortality (\%)} = \frac{M_{observed} - M_{control}}{100 - M_{control}} \times 100$$

- Further, the corrected mortality values were converted into probit values
- Graph was constructed with X axis as log values of gamma dosages and Y axis as probit values of corrected mortality percent of different treatments
- Straight line was drawn such that it passes most of the plotted points of X,Y axis
- Perpendicular line was drawn to y axis from probit value of '5' found at Y axis and already plotted line was intercepted.
- That region was marked and the corresponding log value on X axis was noted.
- Finally the antilog value of it was taken, which is the LD_{50} dosage obtained for the mutagen.

Seedling germination percentage

The seed germination for different treatments as well as for control was taken after one weeks of sowing in field condition.

$$\text{Seedling germination \%} = \frac{\text{No of seeds germinated}}{\text{Total no of seeds used}} \times 100$$

Shoot and root length (cm):

Measurement of shoot and root length of seedling for different treatments and control was taken and recorded after 14 days of sowing.

Result and Discussion:

The widely accepted method to achieve maximum mutation count is "Half Lethal Dosage" among the treated population [6]. One of the important factors to be considered for determining the lethal dosage (LD_{50}) is germination percentage of treated seeds. In case of CR1009 rice variety, the germination percent over control is highest for the treatment of 100 gray with the value of 52.99% and the next higher value (50.14%) was noticed at 200 gray. The percent reduction over control is lesser for the higher value of germination percent and *vice versa.* Hence, the treatment of 100 gray which has highest germination percent had lowest percent reduction over control (47.01%) The lowest value for germination percent over control was observed at 400 gray with the value of 28.21%. And also it has higher percent reduction over control with the value of 71.79 % (Table 1).

Table 1: Effect of gamma ray mutagen on germination percentage of CR1009 and CR1009 *sub1* rice varieties in M_1 generation

Sl. No.	Name of the variety	Dosage of Gamma rays	Germination percentage (%)	Germination percent over control	Percent reduction over control
1.	CR1009	Control	70.20	100.00	-
		100Gy	37.20	52.99	47.01
		200Gy	35.00	50.14	49.86
		300Gy	32.60	46.44	53.56
		400Gy	19.80	28.21	71.79
2.	CR1009 *Sub1*	Control	67.60	100.00	-
		100Gy	57.00	94.06	5.94
		200Gy	52.40	86.47	13.53
		300Gy	32.80	78.22	21.78
		400Gy	18.80	31.02	68.98

For CR1009 *sub1* rice variety, the highest germination percent (94.06 %) over control was observed at 100 gray with lowest percent reduction over control of 5.94. The next higher germination percent (86.47%) over control was recorded at 200 gray with next lower percent reduction over control (13.53). While the 400 gray revealed the lowest germination percent over control (31.02 %) with the highest percent reduction over control (68.98) (Table 1). As a whole there is decrease in seed germination, when there is gradual increase in gamma rays dosage. Similar trend was supported by Rajarajan *et al.*, 2016 [8].

The shoot length of seedling was found to exhibit the effect of mutagen at different dosages in the treated population. It enable the study of mutagen impacts in different varieties in terms of biological aspects [9]. The CR1009 rice variety revealed lower mean shoot length in treatments compared to control. Among the treatments, 200 gray showed the highest shoot length of 18.84 cm with the lowest percent reduction over control (0.32) whereas 400 gray exhibited the lowest shoot length of 7.42 cm with highest percent reduction over control (60.74). The descending order for shoot length according to treatments is 18.84 cm (200Gy)> 17.34 cm (100Gy) > 9.98 cm (300 Gy) > 7.42cm (400 Gy) (Table 2).

Table 2: Effect of gamma rays mutagen on shoot length of CR1009 and CR1009 sub1 in M_1 generation under field condition

Name of the variety	Dosage of Gamma rays	Mean Shoot length (cm)	Percent over control	Percent reduction over control
I. CR1009	Control	18.90	100	-
	100Gy	17.34	91.75	8.25
	200Gy	18.84	99.68	0.32
	300Gy	9.98	52.80	47.20
	400Gy	7.42	39.26	60.74
II. CR1009 Sub1	Control	17.78	100	-
	100Gy	13.50	75.93	24.07
	200Gy	12.82	72.10	27.90
	300Gy	9.38	52.76	47.24
	400Gy	10.28	57.82	42.18

However, the highest shoot length was noticed in CR1009 sub1 at the dosage of 100 Gy with the value of 13.50 cm with lowest percent reduction over control of 24.07. The lowest shoot length was 9.38 cm for the 300 Gy treatment with highest percent reduction over control (47.24). The descending order of shoot length for CR1009 sub1 rice variety is 13.50 cm (100 Gy) > 12.82 cm (200 Gy) > 10.28 cm (400 Gy) > 9.38 cm (300 Gy) (Table 2).

The controls of CR1009 and CR1009 *sub1* rice varieties recorded the root lengths of 6.16 cm and 6.66 cm respectively. Compared to different treatments, controls registered higher value for root length. Among the treatments, 200 gray showed greater root length of 5.68 cm for CR1009 rice variety. Whereas the lowest value of root length was observed at 400 gray with the value of 2.84cm in this variety. Similarly, in CR1009 *sub1* rice variety, 100 gray had exhibited a value of 5.10 cm when compared to all the treatments. Further, the lowest value for root length of 2.84 cm was observed in 300 gray treatment (Table 3).

Table 3: Effect of gamma ray mutagen on root length of CR1009 and CR1009 sub1 in M_1 generation under field condition

Name of the variety	Dosage of Gamma rays	Mean root length (cm)	Percent over control	Percent reduction over control
I. CR1009	Control	6.16	100	-
	100Gy	5.18	84.09	15.91
	200Gy	5.68	92.21	7.79
	300Gy	3.34	54.22	45.78
	400Gy	2.84	46.10	53.90
II. CR1009 Sub1	Control	6.66	100	-
	100Gy	5.10	76.58	23.42
	200Gy	3.50	52.55	47.45
	300Gy	2.84	42.64	57.36
	400Gy	3.32	49.85	50.15

Hence, there is a positive relationship between shoot length and root length among treated rice varieties caused by the gamma rays treatment. And there is relative decrease in root length and shoot length for both varieties while the gamma rays dosage was increased. Similar observations have already been reported by Kadhimi *et al*., 2016 [10].

Most essential initial step to attain desirable mutagenic effect in the developed mutation population is the fixation of Lethal Dosage 50 (LD_{50}) based on the mortality rate of seedling during germination [9]. It was determined using probit analysis. Lethal Dosage 50 is the dosage to which 50% mortality was attained during seedling germination of M_1 population. It is helpful in ensuring increased mutation frequency in M_2 population.

In probit analysis, the range of gamma ray dosages of 100 Gray, 200 Gray, 300 Gray, 400 Gray used for CR1009 and CR1009 sub1 rice varieties, were converted to log value and placed on the X axis *viz.*, 2.00, 2.30, 2.48 and 2.60 (Table 4 and 5) (Fig 1 and 2). And the corresponding improved probit values for CR1009 and CR1009 sub1 rice varieties are 4.84, 5.10, 5.25, 5.37 and 3.76, 4.59, 5.06, 5.40 respectively for different treatments were plotted against the Y axis. (Fig 1 and 2).

Table 4: Calculation of LD_{50} for M_1 population of CR1009 treated with gamma rays using probit analysis

Dosages of gamma rays	Log_{10} of dose	Corrected Mortality percentage	Improved Probit value of mortality	LD_{50} value
100Gy	2.00	47.01	4.84	152.52
200Gy	2.30	50.14	5.10	
300Gy	2.48	53.56	5.25	
400Gy	2.60	71.79	5.37	

Table 5: Calculation of LD_{50} for M_1 population of CR1009 sub1 treated with gamma rays using probit analysis

Dosages of gamma rays	Log_{10} of dose	Corrected Mortality percentage	Improved Probit value of mortality	LD_{50} value
100Gy	2.00	15.68	3.76	284.77
200Gy	2.30	22.49	4.59	
300Gy	2.48	51.48	5.06	
400Gy	2.60	72.19	5.40	

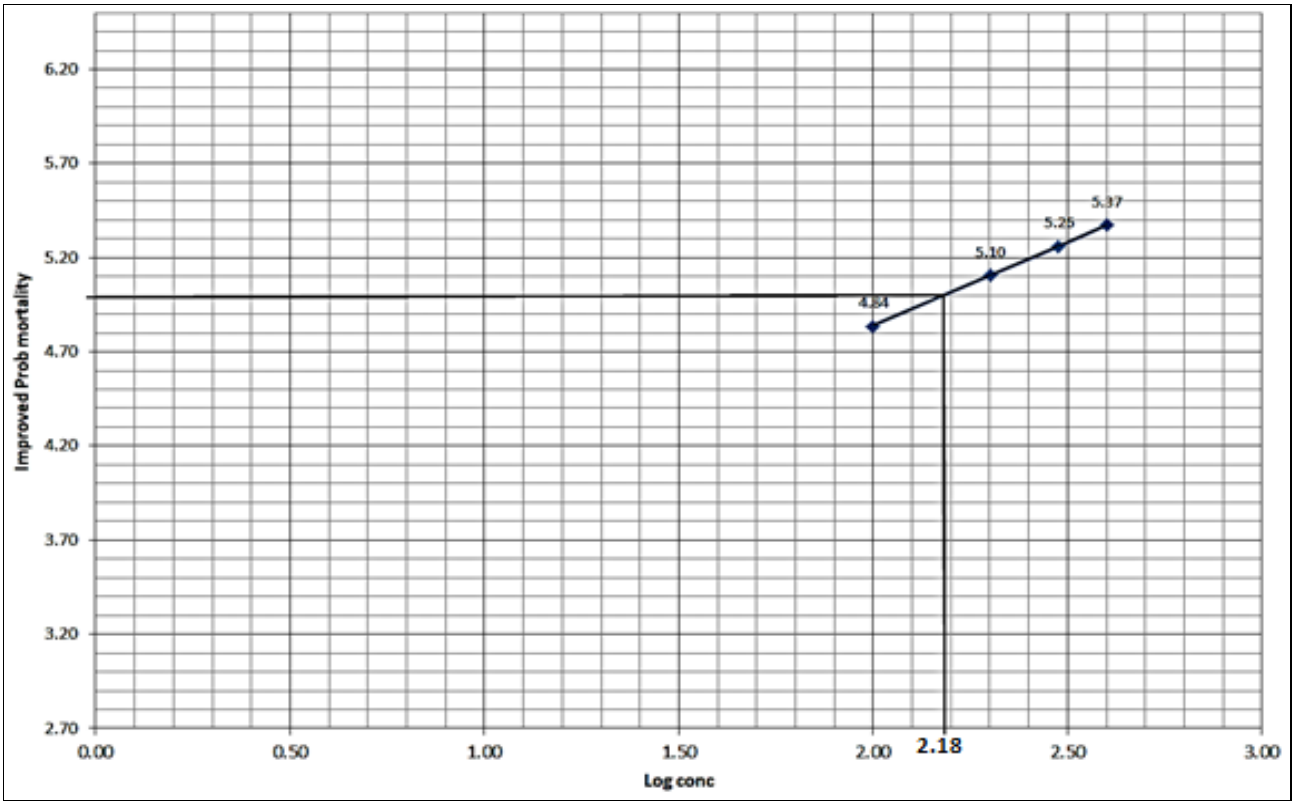

Fig 1: Calculation of LD_{50} using Probit Analysis for CR1009 rice variety

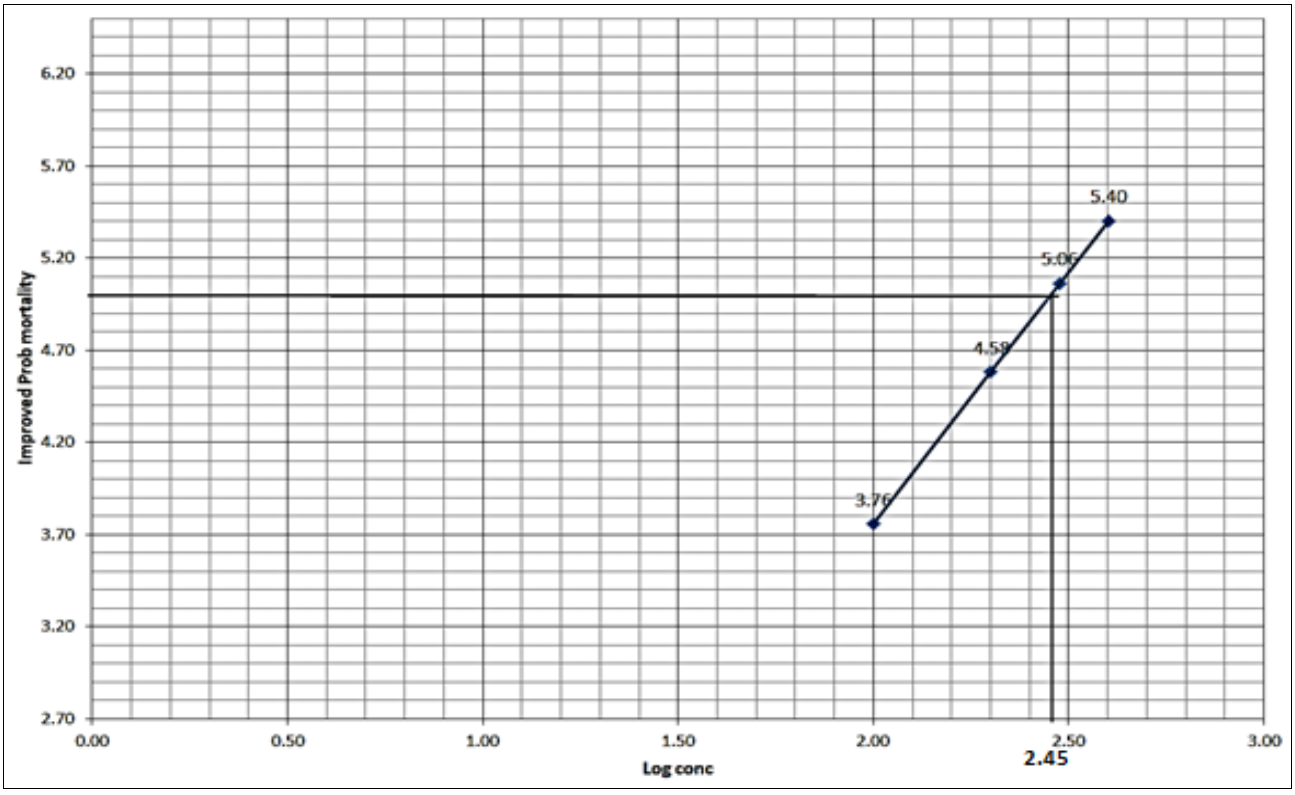

Fig 2: Calculation of LD_{50} using Probit Analysis for CR1009 sub1 rice variety

Based on the results of the study, the LD_{50} value of gamma ray treated CR1009 rice variety was found to be 152.52 gray. And LD_{50} value for the CR1009 sub1 variety was 284.77. The calculated LD_{50} for CR1009 and CR1009 sub1 were within the general range (150 Gy to 350 Gy) of gamma rays reported for *Oryza sativa sp. Indica* [11]. The study also revealed that the rice variety CR1009 was more sensitive to gamma ray exposure than CR1009 sub1. It is also obvious that the different LD_{50} values obtained for different varieties since the factors such as moisture content, hardiness, size, maturity stage and biological content of the seeds tends to modify the range [12]. Therefore, it could be concluded that the dosage ranges which are falling below the LD_{50} could be taken as a standardised range of treatments for these varieties in mutation breeding to alter the traits in order to increase the presence of advantageous mutants in the mutation population [13].

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