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RESEARCH ARTICLE

Irradiating seeds of Rathu Heenati and PTB33 with ¥-rays at various doses and developing mutant populations (M_1 generation) and evaluation

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SUMMARY

Induced mutation has been used to a good extent to create genetic variability in plant species to achieve the desired genetic variability. To attain maximum useful mutation density per unit genome and comparative effectiveness of γ-rays optimal dose for treatment is the key to success. This study focuses on the development and evaluation of mutant population from Rathu Heenati and PTB 33. The data collected on germination percentage, plant height, number of tillers/plant, single plant yield and spikelet sterility and its percentage were analyzed and significance among genotypes, mutagens and levels of mutagens was observed for all traits under study. The probit analysis carried out for determining the LD_{50} values for Rathu Heenati and PTB33 revealed the LD_{50} values of 271.1Gy and 257.2Gy Rathu Heenati and PTB33, respectively. Spikelet sterility showed gradual reduction with increased doses of γ-rays in both the accessions. In Rathu Heenati, the mean percentage of spikelet sterility was 81.05 with gamma ray dose of 350Gy derived plants. Reduction in the yield of single plants was observed with the increasing doses of γ-rays in both the accessions. The mean single plant yield was found to be 18.40 g and 5.160 g in Rathu Heenati and PTB33, respectively at 350Gy of γ-rays.

Key Words : Evaluation, γ-rays, Mutant population, M_1 generation, Rice

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The year 2008 marks the 80th anniversary of mutation induction in plants. The application of mutation techniques, *i.e.* γ-rays and other physical and chemical mutagens, has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies. Improved crop plants in relation to yield and insect resistance are the prime objectives of a typical breeding program (Aslam *et al*., 2013). The widespread use of induced mutants in plant breeding programmes throughout the world has led to the official release of more than 2,700 plant mutant varieties (Liang, 2009). In the context of climate change and variability, mutation induction is a proven way to generate diversity in existing crop varieties, to widen the extent of adaptability and enhance productivity of crop biomass (Lokko, 2011). The application of mutation techniques *i.e.*, γ-rays and other physical and chemical mutagens has generated a vast amount of genetic variability and has played a significant role in plant breeding and genetic studies (Hajos, 2009). Apart from the normal weather conditions, rice production is influenced by the attack of many insect pests and pathogens. Of the insect pests attacking rice, brown planthopper (BPH) [*Nilaparvata lugens* (Stäl.)] is being considered as a most devastating insect pest of rice. Susceptible rice cultivars often showed upto 60 per cent yield loss (Panda and Kush, 1995). Historically, BPH was known as a minor pest of rice and serious BPH outbreaks were reported occasionally before 1960. However, BPH rose from the status of a secondary pest to a major yield constraint beginning in the 1960s in the tropical Asia. Among the several resistant sources identified for BPH resistance in rice, two

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rice accessions *viz*., Rathu Heenati and PTB33 remained with durable and broad-spectrum resistance to BPH across rice growing areas. However, these two rice accessions have not been fully exploited to transfer the genes conferring resistance to cultivated rice varieties due to their photoperiod sensitiveness and tall stature. These two parameters remained as the serious impediments in exploiting the worthiness of Rathu Heenati and PTB33 in using them as potential donors in evolving varieties with resistance to BPH.

MATERIAL AND METHODS

Plant materials :

Two rice varieties *viz*., Rathu Heenati and PTB33 possessing durable resistance to BPH formed the base materials for the present study. Rathu Heenati, a rice accession from Sri Lanka and PTB33 (Pure line selection from Arikkirai), from Pattambi, Kerala are tall growing with photoperiod sensitiveness were used as potential donors for BPH resistance in rice.

¥-rays irradiation :

Seeds of Rathu Heenati and PTB33 obtained from the Department of Rice, Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore were irradiated with different doses of γ-rays from cobalt-60 (^{60}Co) using the Gamma Chamber Model GC 1200 installed at CPBG, TNAU, Coimbatore. The experiment was conducted during 2009-2012. The different doses of γ-rays used for treating the seeds of Rathu Heenati and PTB33 are as follows: 100Gy, 150Gy, 200Gy, 250Gy, 300Gy and 350Gy. A set of 100 well filled and uniform seeds with 12 per cent moisture content of both the varieties were selected for irradiating them with each of the above mentioned doses. The irradiated seeds were sown on the same day in raised bed nursery established at the Paddy Breeding Station, Department of Rice, CPBG, TNAU. The LD_{50} values for both the genotypes were determined based on the Probit analysis (Fienny, 1971, 1978).

Evaluation of M_1 generation :

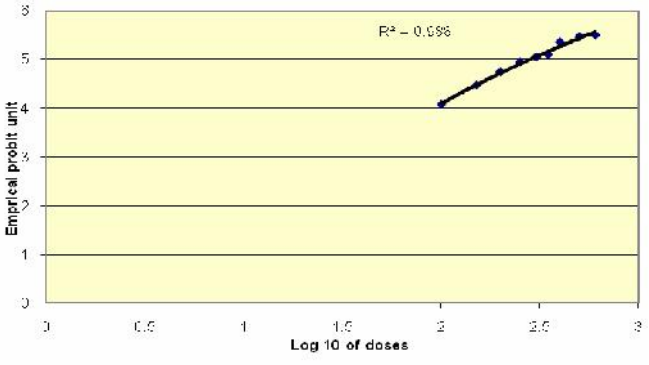
Twenty days old seedlings from each of the treatments of Rathu Heenati and PTB33 were transplanted with the spacing of 20 x 20cm in plots of 2.4 x 1.2m. A spacing of 30cm was maintained between plots. Three replications were maintained for all the treatments including control. Systematic randomization was carried out for all the treatments. Visually recognizable off-type plants, if any, were removed from time to time during the period of experimentation. A total five plants were observed for establishing the effect of different doses of gamma irradiation in each replication.

Data analysis :

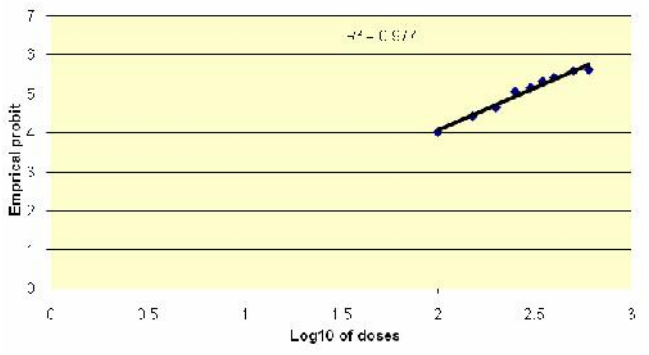
The data from the various traits observed of the above two experiments were analyzed for getting basic statistics and ANOVA using the MINITAB software version 15 (2006).

RESULTS AND DISCUSSION

Brown planthopper was a major threat to rice production in Asia during 1970s and the increase in severity was attributed to newer technologies adopted for rice production (Dyck and Thomas, 1979). Attempts were made to study the nature of resistance to BPH in rice and it is established that the host plant resistance in rice to BPH is simply inherited and biotype specific (Brar *et al*., 2009; Jena and Kim., 2010; Khush, 1979). The first attempt to deviate from the concept of simple inheritance of BPH resistance in rice to complex situation was made by Alam and Cohen (1998) and made them to explore the durability of BPH resistance in rice variety IR64. These attempts made the IRRI scientists to develop a mutant population of IR64 to study the possibilities for exploiting the durability of resistance found in IR64 to various pests and diseases attacking rice. Using IR64 mutant population, Sangha *et al*. (2008) explored the categories of resistance to BPH and reported that the resistance to BPH was simply inherited. In the present investigation, an attempt was made to evaluate the nature of resistance to BPH in Rathu Heenati and PTB33, two durably resistant cultivars of rice and develop mutant populations for studying the categories of resistance in later generations with photoperiod insensitivity and short stature. However, both Rathu Heenati and PTB33 are very tall and photoperiod sensitive making them agronomically inferior to use them regularly in recombination breeding prorammes to exploit their durability of resistance to BPH in rice improvement. Considering these two impediments, an attempt was made to establish mutant populations of Rathu Heenati and PTB33 in line with the mutant population of IR64 to look for short statured photoperiod insensitive mutants with durable resistance as found in Rathu Heenati and PTB33. For mutagenising Rathu Heenati and PTB33, γ-rays were employed in different doses: 100Gy, 150Gy, 200Gy, 250Gy, 300Gy and 350Gy considering its time tested worthiness in inducing mutations in plants and creating new trait variations to complement regular recombination breeding in rice (Babaei *et al*., 2010). In carrying out studies on induced mutations determining the correct doses decide the nature of mutations. The doses can be determined by establishing the LD50 value for the mutagen to be used. Since the LD50 value is genotype dependent, the value has to be decided for each of the genotypes to be mutagenised. Moreover, this value varies with biological materials, nature of treatment and subsequent environmental conditions (Singh, 1994). In the present investigation, the LD50 values for Rathu Heenati and PTB33 were determined based on the survival of seedlings from the seeds treated with different doses of γ-rays adopting probit analysis. The arrived LD50 values for Rathu Heenati and PTB33 were 271.1 and 257.2Gy, respectively. It was observed that the treating of seeds with high doses of γ-rays showed



Rathu Heenati



PTB33

Fig. 1: Graphical representation of dose vs response of ¥-rays on Rathu Heenati and PTB33

reduced germination with a corresponding decline in growth of seedlings. The dose versus response of γ-rays on Rathu Heenati and PTB33 is graphically represented in (Fig.1). No germination was observed when the doses of γ-rays were 600Gy and above. The variation in LD_{50} values for different genotypes of the same species is a common observation in mutation studies depending upon the biological materials, their size, maturity, hardness and moisture content at the time of treatment (Babaei *et al*., 2010; Soriano, 1961; Tabasum *et al*., 2011). The observations recorded from the plants of Rathu Heenati and PTB33 for the traits *viz.* 1) plant height, 2) number of productive tillers, 3) panicle length, 4) number of grains per panicle, 5) spikelet sterility and 6) single plant yield were subjected to statistical analysis to get the basic statistics, the results were presented in (Table 1-6). For plant height, the values in each treatment ranged from 145.00 cm to 176.00 cm in Rathu Heenati. The maximum plant height was recorded in plants derived from 100Gy (165.50 cm). The reduction in plant height was observed with the increase in dose of γ-rays. Since most of the mutations are considered genetically recessive, they cannot be detected in M_1 generation. Even if there are detectable mutations in M_1 generation, their frequency will be very low and detect them one has to have very large size populations. For example, Kiraly and Barabas (1976) screened about 58,000 wheat seedlings from seeds treated with γ-rays for resistance to powdery mildew in the green house in the M_1 generation and found no resistance in the population. More over, the frequency of mutation for the targeted trait would be very low, thus, it requires a large population to discover such a small numbers of mutations. But in most of the induced mutations studies, the mutations do not follow dominant or recessive conditions making the situation very complex to detect incidence of mutations either in M_1 or M_2 generation. Under these circumstances, instead of looking for mutations for the targeted trait some of the prominent associated traits were used as indices to detect the incidence of mutations based on which M_1 selections were made to decide. Comparison of the means and variances of various quantitative traits observed in M_1 and M_2 generations of Rathu Heenati and PTB33 indicated that considerable shift occurred in the mean for all the traits and increased variability was noticed for all the traits in M_2 generation (data not shown). This could be because of recombination happened in the M_1 plants of Rathu Heenati and PTB33. Invariably, the variability increased significantly in both the varieties, irrespective of increase or decrease in the mean values. Similar results were reported by Gupta and Sharma (1994) and Siddiqui and Sanjeeva (2010). Considering mutation and recombination in creating variability, it is expected that recombination creates more genetic variability in all the biological systems (Johnston, 2001). The detection of mutations for a quantitative trait has to be decided based on the progeny values *i.e.* M_2 generation. The mutant population which exhibits high mean coupled with high variance for a trait is the first choice of selection. Usually mutations are detected when there is large phenotypic effect on a particular trait observed in the mutant population. In the present study spikelet sterility was used for selecting a set of M_2 families since such obvious effect was seen only with spikelet sterility (indirectly reflecting on the single plant yield) in the mutant populations of Rathu Heenati and PTB33. The percentage of spikelet sterility was found to be increasing with increased doses of γ-rays. In the M_1 generation of Rathu Heenati, the spikelet sterility was found to be low (36.23%) with 100Gy and high (81.05%) with 350Gy of γ-rays (Table 5). In Rathu Heenati, out 415 M_1 plants 246 showed more than 80 per cent spikelet sterility whereas in PTB33, 223 plants showed more than 80 per cent spikelet sterility out of 440 M_1 plants. Across the M_1 plants of PTB33, the spikelet sterility was 25.88 per cent with 100Gy and 71.90 per cent with 350Gy of γ-rays. This situation is in corroboration with the findings of Woo *et al.* (1971), Avan and Bari (1979), Sanjeev *et al.* (1998) and Fu *et al.* (2008). The M_1 plants showing 80 per cent and above spikelet sterility from all the doses of γ-rays irradiation with Rathu Heenati and PTB33 were selected for further advancement. Advanced generation studies will help to select the lines having photoperiod insensitive with BPH resistance. So further studies will reveal the nature of resistance and pave the way for selecting lines having good agronomic traits with

Table 1: Effect of ¥Jrays on plant height in M_1 generations of Rathu Heenati and PTB33

Treatments	Range	Mean	S.D.	Variance
Rathu Heenati				
Control	160.0-175.0	166.00	0.91	0.95
100Gy	155.4-173.0	165.50	3.21	1.79
150Gy	145.0-172.0	161.30	2.47	1.57
200Gy	150.0-176.0	163.10	2.47	1.57
250Gy	150.0-175.0	162.40	2.76	1.66
300Gy	145.0-165.0	158.20	4.02	2.00
350Gy	145.0-170.0	155.30	2.39	1.55
PTB33				
Control	148.0-170.0	162.40	5.30	2.30
100Gy	145.0-170.0	158.90	6.31	2.51
150Gy	145.0-170.0	162.80	3.75	1.94
200Gy	145.0-172.0	159.20	8.66	2.94
250Gy	145.0-170.0	157.80	8.81	2.97
300Gy	140.0-170.0	155.50	7.58	2.75
350Gy	140.0-170.0	153.80	2.04	1.43

Table 2 : Effect of ¥Jrays on number of productive tillers per plant in M_1 generations of Rathu Heenati and PTB33

Treatments	Range	Mean	S.D.	Variance
Rathu Heenati				
Control	7.00-20.00	14.20	0.80	0.89
100Gy	9.00-20.00	14.73	1.14	1.07
150Gy	8.00-18.00	11.67	0.50	0.71
200Gy	9.00-16.00	11.87	0.81	0.89
250Gy	7.00-18.00	13.00	1.40	1.18
300Gy	7.00-19.00	11.67	0.70	0.84
350Gy	9.00-16.00	15.20	1.94	1.39
PTB33				
Control	12.00-29.00	19.33	4.04	2.01
100Gy	10.00-21.00	15.20	0.60	0.78
150Gy	11.00-20.00	15.80	0.72	0.85
200Gy	10.00-21.00	14.73	1.70	1.30
250Gy	11.00-23.00	16.93	2.05	1.43
300Gy	10.00-23.00	14.60	2.40	1.55
350Gy	10.00-20.00	16.67	1.20	1.09

Table 3: Effect of ¥Jrays on panicle length in M_1 generations of Rathu Heenati and PTB33

Treatments	Range	Mean	SD	Variance
Rathu Heenati				
Control	27.00-32.10	29.07	1.27	1.13
100Gy	28.00-33.00	30.87	1.10	1.05
150Gy	24.00-32.00	29.40	0.54	0.73
200Gy	28.00-32.00	30.24	0.46	0.68
250Gy	23.00-31.50	26.65	3.67	1.92
300Gy	10.00-20.00	14.60	2.40	1.55
350Gy	10.00-20.00	15.20	1.20	1.09
PTB33				
Control	19.00-25.00	21.94	0.56	0.75
100Gy	20.00-25.00	22.78	0.19	0.44
150Gy	19.50-25.00	22.31	0.28	0.53
200Gy	19.20-25.00	21.89	0.26	0.51
250Gy	20.20-25.00	22.13	0.45	0.67
300Gy	19.00-23.50	21.59	0.48	0.69
350Gy	19.00-23.00	21.15	0.14	0.37

Table 4 : Effect of ¥Jrays on number of grains per panicle in M_1 generations of Rathu Heenati and PTB33

Treatments	Range	Mean	S.D.	Variance
Rathu Heenati				
Control	70.00-280.00	123.90	13.80	3.72
100Gy	11.00-217.00	117.60	49.12	7.01
150Gy	14.00-254.00	108.50	46.86	6.85
200Gy	15.00-260.00	85.70	45.86	6.77
250Gy	0.00-217.50	90.40	67.81	8.23
300Gy	0.00-204.00	46.08	55.77	7.47
350Gy	0.00-182.00	76.65	56.24	7.50
PTB33				
Control	60.00-150.00	46.93	8.53	2.92
100Gy	20.00-120.00	54.43	21.27	4.61
150Gy	13.00-116.00	61.15	23.33	4.83
200Gy	2.00- 122.00	50.48	24.67	4.97
250Gy	3.00- 85.00	44.48	20.93	4.58
300Gy	0.00-148.00	39.23	30.45	5.52
350Gy	0.00-104.00	29.43	24.24	4.92

Table 5 : Effect of ¥Jrays on spikelet sterility in M_1 generations of Rathu Heenati and PTB33

Treatments	Range	Mean	S.D.	Variance
Rathu Heenati				
Control	30.00-46.00	37.55	2.34	1.53
100Gy	21.00-58.20	36.23	1.16	1.08
150Gy	23.00-65.10	42.81	4.58	2.14
200Gy	31.00-78.00	54.95	1.84	1.36
250Gy	45.00-99.00	65.39	6.96	2.64
300Gy	59.20-86.00	77.97	2.37	1.54
350Gy	67.90-99.10	81.05	3.93	1.98
PTB33				
Control	11.00-20.00	13.86	1.40	1.18
100Gy	15.00-51.20	25.88	1.23	1.11
150Gy	23.30-62.50	44.71	0.74	0.86
200Gy	42.00-68.00	55.84	1.52	1.23
250Gy	40.00-67.00	47.17	0.89	0.94
300Gy	43.00-71.00	56.08	2.14	1.46
350Gy	64.00-84.00	71.90	1.36	1.17

Table 6 : Effect of ¥Jrays on single plant yield in M_1 generations of Rathu Heenati and PTB33

Treatments	Range	Mean	S.D.	Variance
Rathu Heenati				
Control	20.00-49.00	30.60	2.84	1.68
100Gy	18.00-44.00	30.13	1.03	1.01
150Gy	16.00-47.00	30.33	5.61	2.37
200Gy	9.00-33.00	19.47	4.84	2.20
250Gy	5.00-41.00	17.87	3.88	1.97
300Gy	7.00-40.00	17.87	2.40	1.55
350Gy	9.00-27.00	18.40	1.73	1.32
PTB33				
Control	12.50-21.00	16.35	0.65	0.81
100Gy	12.00-20.00	16.23	0.42	0.65
150Gy	10.00-25.00	15.55	0.38	0.61
200Gy	8.00-17.00	12.93	0.49	0.70
250Gy	4.50-13.00	7.18	1.36	1.17
300Gy	2.20-8.600	5.13	0.67	0.82
350Gy	4.00-7.500	5.16	0.20	0.44

BPH resistance.

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