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Assessment of genetic variability in gamma rays induced mutants of rice (*Oryza sativa* L.)

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Abstract
The present investigation entitled "Induction of genetic variability through gamma rays and assessment of variants by molecular markers in rice (*Oryza sativa* L.)" was carried with aim to create genetic variability by using different mutagenic treatments of gamma rays in rice cultivars Chakhao, Bangalya and Ghansal. The trials were carried out during *kharif* 2021 and *Summer* 2022 at Botany farm, Department of Agricultural Botany, College of Agriculture and molecular analysis was carried out in the laboratory of Plant Biotechnology Centre, Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli.

Decreasing trend was observed for percent germination in all mutagenic treatments with increased dose of mutagen under laboratory as well as under field condition. Similar trend was also recorded in germination percent, shoot length, pollen fertility and spikelet fertility. Considering laboratory and field observation on percent germination and other related parameters, LD_{50} dose was optimized. For chakhao and bangalya cultivars mutagenic treatment 300Gy and in case of ghansal cultivar 100 Gy, doses were optimized as a LD_{50} dose.

During M_1 generation, early flowering, late flowering, sterile spikelet mutants were recorded. Four types of chlorophyll mutations *viz*. Albina, Xantha, Chlorina and Striata, were noticed in all mutagenic treatments. Mutation frequency, mutagenic efficiency and mutagenic effectiveness were reduced as per the increased dose of mutagen.

Wide range of variation was observed in quantitative characters during M_2 generation *viz.*, days to fifty percent of flowering, maturity duration, plant height, number of tillers per plant, number of spikelets per panicle and grain yield per plant.

Keywords: variability, Mutation, gamma rays, efficiency, cultivars, dose

Introduction
India has a long history of rice cultivation. Rice occupies a pivotal place in Indian agriculture as it is a staple food of India. Globally, it stands first in rice area and second in rice production, after China. It contributes 21.5 percent of global rice production. Within the country, rice occupies one quarter of the total cropped area, contributes about 40 to 43 percent of total food grain production and continues to play a vital role in the national food and livelihood security system. India is one of the leading exporter of rice. Mutation is a change in the DNA at a particular locus in an organism. Mutation is a weak force for changing allele frequencies, but is a strong force for introducing new alleles. It is also the source of new alleles that create new genotypes. Small populations have fewer alleles due to genetic drift and also because fewer mutations are generated in a small population. Mutation plays an important role in evolution. The ultimate source of all genetic variation is mutation. Mutation is important as the first step of evolution because it creates a new DNA sequence for a particular gene, creating a new allele. Recombination also can create a new DNA sequence (a new allele) for a specific gene through intragenic recombination. Mutation acting as an evolutionary force by itself has the potential to cause significant changes in allele frequencies over very long periods of time.

Rice has been a popular subject to mutagenesis because it is the world's leading food crop. Being diploid species, maximum genetic variability is available for selection in M_2 generation. According to Novak and Brunner (1992) [11], induced mutagenesis is one of the powerful tools for creation of genetic variability in plants and other living organisms. Mutations can create novel and unique variations when natural variability is not capable of providing the gene for desired traits (Velmurugan *et al.*, 2010) [17]. Mutation breeding is an established method for affecting genes either by treating seeds or other plant parts through chemical and / or physical mutagens.

The high selection pressure applied in rice breeding since its domestication thousands of years ago has resulted in narrowing in its genetic variability. Obtaining new rice cultivars therefore becomes a major challenge for breeders and developing strategies to increase the genetic variability has demanded the attention of several research groups. Understanding mutations and their applications have paved the way for advances in the elucidation of a genetic, physiological, and biochemical basis of rice traits. Creating variability through mutations has therefore grown to be among the most important tools to improve rice. The small genome size of rice has enabled a faster release of higher quality sequence drafts as compared to other crops. The move from structural to functional genomics is possible due to an array of mutant data- bases, highlighting mutagenesis as an important player in this progress. Successful gene modifications have been obtained by random and targeted mutations.

Material and Methods

The present investigation, "Induction of genetic variability through gamma rays and assessment of variants by molecular markers in rice (*Oryza sativa* L.)" was carried out during *Kharif*-2021 and *Summer*-2022 at Botany farm, Department of Agricultural Botany, College of Agriculture, Dapoli and molecular analysis was carried out in the laboratory of Plant Biotechnology Centre, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli- 415712, Maharashtra state, India (altitude of 243.84 m between 17° 47' 32" N latitude and 73° 11' 8" E longitude). The material and methods used in this investigation are summarized in this chapter.

Seeds were treated with gamma rays, seven sets of each cultivar i.e., chakho, bangalya and ghansal. 800 dry seeds (moisture content around 10%) of rice cultivars were irradiated with gamma rays of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250Gy, 300 Gy 350Gy, doses respectively at Bhabha Atomic Research Centre, Mumbai.

The treated seeds were sown to raise M_1 generation. The single plant of M_1 was used to raise M_2 generation. Seeds of each M_1 survived plant harvested separately for raising M_2 generation. 200 plants per treatment from M_1 generation were selected on the basis of variants for qualitative and quantitative characters. Sixty seedlings of each selected plant from M_1 generation were transplanted following plant to row method. Each observational plant including variants scored was harvested separately to future use. The spectrum of chlorophyll and morphological mutation was scored treatment wise to study mutagenic effectiveness and efficiency of each treatment. Observations were recorded for different characters on 50 plants for each treatment.

Observations recorded

Days to 50% flowering

Number of days required for first flowering from the date of sowing was
recorded.

Plant height (cm)

Plant height was taken from the ground level to the tip of the plant and was recorded in centimetre.

Days to maturity

Days to maturity was recorded from date of sowing to the date when more than 95 percent panicles were matured and the plant was ready to harvest.

Number of tillers per plant

The total number of tillers per plant was counted at maturity

Panicle length (cm)

It was measured in cm from collar to the tip of panicle excluding awns if any.

Number of grains panicle per plant

The total number of filled grains per panicle was counted at maturity.

Spikelet sterility (%)

Spikelet sterility was obtained by using following formula

$$\text{Spikelet sterility (\%)} = \frac{\text{No. of empty spikelets}}{\text{No. of total spikelets}} \times 100$$

Test weight (g)

A representative sample of grains was taken from the total produce of each net plot and 1000 grains were counted and weight was recorded as pre the treatments.

Grain yield per plant (g)

Grains of ten randomly selected plants were hand threshed, sun dried, weighed and finally obtained on per plant basis.

Straw yield (g)

The straw yield was obtained by weighing air dried straw which remained after threshing from each plant.

Harvest index (%)

Harvest index of both rice and field bean was calculated by using the formula by Donald (1962).

$$\text{Harvest index (\%)} = \frac{\text{Total grain yield}}{\text{Biological yield}} \times 100$$

Amylose content (%)

Amylose contents of the milled rice samples were estimated by the method suggested by Juliano (1971) [6] involving the spectrophotometer.

Determination of amylose Content

Materials: (1) Balance (mg); (2) Water bath; (3) Tri pod; (4) Gas burner; (5) 100 ml volumetric flask; (6) Pipettes (1 ml, 5 ml); (7) 1 ml and 10 ml automatic dispensing pipettes; (8) Absolute ethanol; (9) Methonol; (10) 1.0 N Sodium Hydroxide; (11) 1.0 Acetic acid; (12) Stock iodine solution (0.2% KI); (13) Amlysoe (Purified Potato, Sigma); (14) Wig-L-Amalgamator as UD Cyclone mill.

Alkali spreading value (ASV)

The spread of 6 milled rice kernels in 1.7% KOH solution for a period of 23 hours was rated as per standard evaluation systems for rice (IRRI, 1996). Six milled kernels were placed in 10 ml of 1.7% KOH solution in Petri dishes and arranged in such a manner that they did not touch each other and was

allowed to stand for 23 hours at 30^0C to score spreading of rice kernels on 1 – 7 scale.

Water uptake (g/g)

Water uptake ratio of milled rice samples were estimated by the method suggested by Bhattacharya and sowbhagya (1971)

Protein content (%)

The protein content in grain of rice was calculated by multiplying the nitrogen percentage with factor 6.25 (Subbaiah and Asija, 1956).

Percent protein = N percentage × 6.25

Statistical analysis

The range, mean, S.E., variance, C.V. and C. D. were calculated by using following formulas and 't' test was applied for testing significance, following Panse and Sukhatme (1967) [12].

Experimental Results and Discussion

Wide range of variation for all the characters under study in each cultivar studied under present investigation. Characters Days to 50 percent flowering, Days to maturity, Plant height (cm), Number of tillers per plant, Panicle length (cm), Number of grains per panicle, Spikelet fertility (%), Test weight (g), Grain yield per plant (g), Straw yield per plant (g), Harvest Index (%), protein content (%), amylose content (%), Water uptake (g/g), Alkali Spreading Value were studied in this study. among all the characters some important characters are presented described in this section

Among all the gamma rays treatments given to chakhao cultivar, earliest 50% flowering plant was observed in treatments T_7: 350 Gy (99 days) which exhibited about 13-15 days earlier flowering than the control (Table.1). These plants showed approximately 11-13 days earlier 50% flowering. Similar results were reported by Labrada *et al.,* 2001 [9]. Mutants observed in bangalya showed about 20-24 days earlier flowering (Table.2). These plants were observed in treatments which containing higher dose of gamma radiation. Chakravarti *et al.* (2013) [3] reported the similar kind of results. Aapproximately about 14-18 days earlier 50% flowering as compare to control in ghansal mutants (Table.3). All treatments showed negative shift in mean and indicates in desirable direction. Almost all the treatments displayed increased variance over the control. It indicates that more variability present in gamma rays irradiated treatments the control.

It is observed that treatment of higher dose of gamma radiation leads to developed of some plants with minimum days to plant maturity. All treatments exhibited significantly reduced mean values for days to maturity as compared to control. Percent coefficient of variance was increased in all treatments over the control. Among all the gamma rays treatments, most dwarf plant observed in treatment T_7: 350 Gy which is followed by treatment T_6:300 Gy in mutants from chakhao cultivar (Table.4). These plants showed about 20-28 cm reduced height over the control. Negative shift in mean was recorded by all the treatments indicates in desirable direction. Increase in coefficient of variance was noticed in all the mutagenic treatments over the control (Table.4). Kole *et al.* (2012) [7] reported increased coefficient of variance for the character plant height. Baloch *et al*. (1999) [1] and Sellammal and Maheswaran (2013b) [13] reported similar findings in rice.

Range of variation for plant height was observed from 101 cm (T_7: 350 Gy) to 147 cm (T_1: 50 Gy and T_2: 100 Gy) in treated population as compared to control. These plants showed about 40-47 cm reduced height over the control (Table.5). Increase in coefficient of variance was noticed in treatments T_1: 50 Gy, T_3: 150 Gy, T_5: 250 Gy, T_6: 300 Gy over the control Increase in coefficient of variance was noticed in treatments T_1: 50 Gy, T_3: 150 Gy, T_5: 250 Gy, T_6: 300 Gy over the control (Table.6). Range of variation for plant height was observed from 98 cm to 148 cm in treated population as compared to control (140-149 cm) in Ghansal cultivar of rice (Table.6). Among all the gamma rays treatments, dwarf plants had showed about 42 cm reduction in height (Table.6). Singh *et al.* (1989) also reported similar kind of results. Siddiqui and Singh (2010) [14] also reported that mutagen treatment had wider values of mean, range, CV over the control.

All the treatments exhibited reduced number of tillers per plant as compared to control. All treatments showed negative shift in mean. Maximum and minimum number of tillers per plant were noticed in treatment T_7: 350 Gy. Increased number of tillers in higher doses of gamma radiation also reported by Singh (2006) [15] and Kulkarni and Gangaram (1998) [8]. Increase in variance was noted in all treatments over control except treatment T1: 50 Gy in ghansal cultivar. revealed that the range of variation for panicle length was found widened in treated population as compared to control. Among all the gamma rays treatments, shortest panicle was observed in treatments T7: 350 Gy and longest panicle was found in T1: 50 Gy. Promising mutants with increased panicle length were reported by Kole and Chakraborty (2012) [7].

Increased variability in mutagenic treated population as compared to control also reported by Kole and Chakraborty (2012) [7] Among the gamma rays treatments, treatment T7: 50 Gy exhibited plants with minimum upto (126) and T1: 50 Gy showed maximum (164) number of grains per panicle. It is observed that the mean number of grains per panicle was decreased in all treatments as compared to control. In bangalya cultivar treatment T7: 50 Gy exhibited plants with minimum (109) and T1: 50 Gy with maximum (151) number of grains per panicle. Decrease in number of grains per panicle in mutagenic population reported by Sellammal and Maheswaran (2013b) [13] It is observed that spikelet fertility decreases with increased dose of gamma radiation in all three cultivars under the study. It has been observed that increased variance in all mutagenic treatments over the control in each cultivar. Some panicles from higher doses of gamma radiation observed completely sterile. Presence of complete or partial sterile panicles in treated population was reported by Singh (2006) [15] Increase in coefficient of variance was exhibited by all the treatments over the control.

There was no significant variation found in the character test weight as all these treatments showed less than one shift in mean over the control. All the mutagenic treatments in each variety showed variance less than one. But some plants showed increased or decreased test weight than the control. Similar findings were given by Bughio *et al.* (2007) [2], El Degwy (2013) [5]. Among all the mutagenic treatments, treatment mean for the character grain yield per plant does not show much more variation. Some plants show increased yield as compared control. Such increased yield in some plants was also reported by Domingo *et al.* (2007) [4]. Highest coefficient of variance was exhibited by treatment T_7: 350 Gy followed by T_5: 250 Gy. It indicates that these treatments shows more

variation than the control. These results are in confirmation with Mohamed *et al.* (2006) [10]. Among all the mutagenic treatments, treatment mean for the character straw yield per plant decreased as gamma radiation dose increased.

Increased harvest index observed in mutagenic treatments. Increase in variance was noticed in all mutagenic treatments over control. These variations in the characters harvest index may be due to variation plant height, number of tillers, number of grains per panicle, spikelet fertility. Similar results were given by Kole and Chakraborty (2012) [7]. There was no significant variation in protein content, amylose content, ASV and water uptake.

Table 1: Variation for 50% flowering in M_2 generation of 'Chakhao' cultivar of rice.

Treatment	Range	Mean	S.E.	Variance	CV	C.D. at 5%	Shift in		Observational plants (%)	
							Mean	Variance	Above µ	Below µ
Control	112-115	113.82	0.35	1.13	0.31	1.42			54.00	46.00
T_1-50 Gy γ	112-116	113.62	0.16	1.22	0.97	0.63	-0.2	0.09	48.00	52.00
T_2-100 Gy γ	111-117	113.12	0.16	1.36	1.03	0.66	-0.7	0.23	46.00	54.00
T_3-150 Gy γ	110-115	112.86	0.13	1.58	0.84	0.54	-0.96	-0.23	56.00	44.00
T_4-200 Gy γ	109-116	112.23	0.36	1.65	2.28	1.47	-1.59	5.52	38.00	62.00
T_5-250 Gy γ	108-115	111.48	0.16	2.23	0.98	0.63	-2.34	0.1	48.00	52.00
T_6-300 Gy γ	101-115	111.30	0.25	3.03	1.55	0.99	-2.52	1.9	52.00	48.00
T_7-350 Gy γ	99-115	110.26	0.29	4.07	1.79	0.60	-3.56	2.94	56.00	44.00
Average		112.12							49.75	50.25
Range	99-117	110.26-113.62		1.22-4.07	0.97-2.28					

Table 2: Variation for 50% flowering in M_2 generation of 'Bangalya' cultivar of rice.

Treatment	Range	Mean	S.E.	Variance	CV	C.D. at 5%	Shift in		Observational plants (%)	
							Mean	Variance	Above µ	Below µ
Control	114-118	115.40	0.25	3.02	1.51	0.99			46	54
50 Gy γ	113-118	115.35	0.14	3.32	1.56	0.55	-0.05	0.3	56	44
100 Gy γ	113-116	114.3	0.26	3.40	1.61	1.16	-1.10	0.38	42	58
150 Gy γ	113-117	115.1	0.29	4.26	1.79	1.17	-0.30	1.24	42	58
200 Gy γ	111-117	114.2	0.34	5.67	2.09	1.35	-1.20	2.65	46	54
250 Gy γ	108-116	114.94	0.33	5.53	2.05	1.34	-0.46	2.51	52	48
300 Gy γ	95-117	112.82	0.58	5.76	3.63	2.33	-2.58	2.74	56	44
350 Gy γ	94-115	112.74	0.60	6.11	3.78	2.42	-2.66	3.09	46	54
Average		114.20							48.57	51.43
Range	94-118	112.74-115.35		3.32-6.11	1.56-3.78					

Table 3: Variation for 50% flowering in M_2 generation of 'Ghansal' cultivar of rice.

Treatment	Range	Mean	S.E.	Variance	CV	C.D. at 5%	Shift in		Observational plants (%)	
							Mean	Variance	Above µ	Below µ
Control	111-114	112.74	0.13	0.85	0.82	0.52			46	54
T_1-50 Gy γ	111-114	112.40	0.12	0.69	0.74	0.47	-0.34	-0.16	52	48
T_2-100 Gy γ	109-114	111.72	0.16	1.31	1.02	0.65	-1.02	0.46	44	56
T_3-150 Gy γ	105-113	110.82	0.28	4.03	1.81	1.14	-1.92	3.18	50	50
T_4-200 Gy γ	101-112	110.42	0.19	1.80	1.21	0.76	-2.32	0.95	48	52
T_5-250 Gy γ	101-115	110.54	0.26	3.31	1.65	1.03	-2.2	2.46	56	44
T_6-300 Gy γ	105-112	109.84	0.21	2.30	1.38	0.86	-2.9	1.45	48	52
T_7-350 Gy γ	97-111	108.32	0.34	5.94	2.25	1.38	-4.42	5.09	54	46
Average		110.74							49.75	50.25
Range	97-115	108.32-112.40		0.69-5.94	0.74-2.25					

Table 4: Variation for Plant height in M_2 generation of 'Chakhao' cultivar of rice.

Treatment	Range	Mean	S.E.	Variance	CV	C.D. at 5%	Shift in		Observational plants (%)	
							Mean	Variance	Above µ	Below µ
Control	148-156	151.84	0.29	4.42	1.38	1.19			48	52
T_1-50 Gy γ	146-155	151.60	0.31	4.89	1.45	1.25	-0.24	0.47	56	44
T_2-100 Gy γ	145-156	150.52	0.36	6.49	1.69	1.44	-1.32	2.07	50	50
T_3-150 Gy γ	142-154	149.92	0.38	7.75	1.83	1.56	-1.92	3.33	54	46
T_4-200 Gy γ	140-153	148.08	0.43	9.42	2.06	1.74	-3.76	5.00	42	58
T_5-250 Gy γ	138-154	147.92	0.48	11.87	2.32	1.95	-3.92	7.45	46	54
T_6-300 Gy γ	134-154	147.14	0.57	16.49	2.75	2.30	-4.70	12.07	54	46
T_7-350 Gy γ	128-152	146.06	0.67	22.09	3.21	2.67	-5.78	17.67	58	42
Average		148.74							51	49
Range	128-156	146.06-151.60		4.89-22.09	1.45-3.21					

Table 5: Variation for Plant height in M_2 generation of 'Bangalya' cultivar of rice.

Treatment	Range	Mean	S.E.	Variance	CV	C.D. at 5%	Shift in		Observational plants (%)	
							Mean	Variance	Above µ	Below µ
Control	141-148	144.96	0.77	29.92	4.02	3.11			48	52
T_1-50 Gy γ	137-147	143.82	0.90	40.31	4.61	3.61	-1.14	10.39	54	46
T_2-100 Gy γ	126-147	142.24	0.66	21.49	3.40	2.64	-2.72	-8.43	46	54
T_3-150 Gy γ	125-146	140.14	0.93	43.63	4.85	3.75	-4.82	13.71	56	44
T_4-200 Gy γ	121-144	139.48	0.58	16.58	3.01	2.31	-5.48	-13.34	50	50
T_5-250 Gy γ	109-145	139.11	1.02	52.29	5.37	4.11	-5.85	22.37	46	54
T_6-300 Gy γ	103-144	137.64	0.88	38.52	4.64	3.53	-7.32	8.6	40	60
T_7-350 Gy γ	101-145	137.24	0.64	20.42	3.33	2.57	-7.64	-9.5	52	48
Average		139.95							49.00	51.00
Range	101-147	137.24-143.82		16.58-52.29	3.01-5.37					

Table 5: Variation for Plant height in M_2 generation of 'Bangalya' cultivar of rice.

Treatment	Range	Mean	S.E.	Variance	CV	C.D. at 5%	Shift in		Observational plants (%)	
							Mean	Variance	Above µ	Below µ
Control	140-149	143.52	0.29	4.21	1.43	1.17			52	48
T_1-50 Gy γ	139-147	143.06	0.27	3.57	1.32	1.07	-0.46	-0.64	46	54
T_2-100 Gy γ	136-148	141.40	0.24	2.86	1.20	0.96	-2.12	-1.35	56	44
T_3-150 Gy γ	131-143	140.54	0.26	3.36	1.30	1.04	-2.98	-0.85	54	46
T_4-200 Gy γ	132-146	140.52	0.25	3.19	1.27	1.02	-3.00	-1.02	46	54
T_5-250 Gy γ	132-143	138.66	0.36	4.51	4.59	3.62	-4.86	0.3	48	52
T_6-300 Gy γ	114-146	139.58	0.40	8.13	2.04	1.62	-3.94	3.92	52	48
T_7-350 Gy γ	98-142	135.84	0.76	8.63	3.94	3.04	-7.68	4.42	54	46
Average		142.39							51	49
Range	98-148	140.40-143.06		2.86-8.63	1.20-4.59					

Conclusion

From the result obtained in the present investigation, it is concluded that the different mutagenic treatments are highly effective in inducing genetic variability with significant alteration in yield contributing parameters. Higher doses of gamma rays are not suitable for rice as few treated seeds were germinated. The results obtained decisively demonstrate the usefulness and effective potential of the induced mutational approach in genetic improvement of rice (Oryza sativa (L.)) for recovering superior mutant plant types having enhanced yield.

The present study, with respect to isolation of 'viable mutants', particularly plant stature, earliness and higher yield, have been remarkably successful. Some of these macro mutants have been found to be superior to their parent cultivars in several respects (Yield contributing traits). Some of the mutants isolated in the present investigation were exhibiting negative selection value in respect to the day to 50% flowering, plant height and days to maturity these might be useful only to the plant breeder in hybridization programmes.

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