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Optimization of LD_{50}, frequency and spectrum of chlorophyll mutation, efficiency and effectiveness of gamma rays in different cultivars 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, 0.20% 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: Mutation, gamma rays, LD_{50}, 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) [20]. 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, 800 dry seeds (moisture content around 10%) of rice cultivars were irradiated with gamma rays of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy, 300 Gy 350 Gy, 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.

Experimental Results and Discussion

Optimization of LD_{50}

Percent germination (Laboratory experiment)

Effect of different mutagenic treatments on percent germination of rice cultivars was observed under laboratory condition by following Paper Towel method. It was observed that percent germination was reduced as per the increased dose of mutagen. These results are in confirmation with Kumar *et al.* (2013b) Minimum germination percent observed at T_7:350 Gy dose. Percent reduction in germination percentage was increased as increased dose of mutagen among all the gamma rays treatments. Optimized dose of gamma rays estimated on the basis of fifty percent lethality. The dose of 300 Gy gamma rays induced 50% lethality in Chakhao and Bangalya cultivars. While, in case of Ghansal 100 Gy gamma rays dose leads to 50% lethality. Hence these doses are fixed as optimized dose for respective cultivars. Babaei *et al.* (2010) [1] optimized LD_{50} dose of gamma rays 35, 31 and 17 kR for varieties *viz.*, Sange-tarom, Tarom-hashemi and Nemat respectively. Similar results were reported by Kumar *et al.* (2013b), Taher *et al.* (2011) [18].

Seed germination

The effect of mutagen *viz.*, gamma rays on germination of 'Chakhao', 'Bangalya' and 'Ghansal' calculated along with untreated seeds. The is presented in Table 1. Invariably, the maximum percent of germination was recorded for control. The observations revealed that there was a little effect of the lower doses of gamma rays on germination percent, but reduction in germination percent increased with the increase in doses of mutagens (gamma rays) in all the cultivars. Such reduction in germination percent in treated population as compare to control was also reported by Minn *et al.* (2008) [10]. The reduction in percent germination was more pronounced at higher doses of mutagens *i.e.*, 36.89% (T_7: 350 Gy) followed by 49.67% (T_6: 300 Gy) in Chakhao as compared to 98.44% of control. Maximum reduction in germination percent was 43.33% (T_7: 350 Gy) followed by 49.55% (T_6: 300 Gy) in Bangalya as compared to 97.44% of control. Similarly, maximum reduction in germination percent was observed 8.40% at T_7: 350 Gy followed by 12.01% (T_6: 300 Gy) in Ghansal cultivar as compare to control 91.72%. Similar kind of results were given by Sasikala and Kalaiyarasi (2010) [13].

Shoot length reduction

Shoot length also exhibited a decreasing trend in all the cultivars with increase in the doses of gamma rays. Shoot length reduction was measured as reduction in shoot length of seedlings on 14^{th} day after sowing. The observations on reduction in Shoot length as compared to control have been presented in Table 1. Maximum reduction in Shoot length was observed at T_7: 350 Gy (41.64%) followed by T_6: 300 Gy (36.62%) dose of gamma rays in Chakhao cultivar. In Bangalya, maximum reduction in Shoot length was observed at T_7: 350 Gy (60.34%) followed by T_6: 300 Gy (48.47%). Minimum reduction in Shoot length 10.79% was recorded at T_1: 50 Gy dose of gamma rays. Similar trend of Shoot length reduction with increased dose of gamma rays observed in 'Ghansal' cultivar. Maximum Shoot length reduction observed in T_7: 350 Gy (64.36) followed by T_6: 300 Gy (52.34%). The reduction in height as a result of high concentration of mutagen as reported in present investigation has been observed by Minn *et al.* (2008) [10], Sasikala and Kalaiyarasi (2010) [13], Harding *et al.* (2012), Cheema and Atta (2003) [5].

Plant survival at maturity

As evident from Table 1, it was observed that plant survival percent decrease with increase in the doses of gamma rays. Maximum plant survival percent was observed for control (93.25%), (92.27%) and (91.87%) in rice cultivars Chakhao, Bangalya, and Ghansal respectively, followed by 92.20% in Chakhao, 91.82% in bangalya and 85.04% in ghansal at 50 Gy gamma rays dose. Minimum plant survival percent was observed at T_7: 350 Gy dose in all the cultivars under investigation. These results are in confirmation with Maity *et al.* (2005) [9].

Pollen and spikelet sterility (%)

The reduction in pollen and spikelet fertility was observed in all the cultivars as compared to control in the M1 generation (Table 1). The pollen and spikelet sterility percent increased with the increase in the doses of gamma rays mutagen. Maximum pollen sterility in ‘Chakhao’ was observed at T_7: 350 Gy (30.01%) followed by T_6: 300 Gy (29.12%) dose of gamma rays. Maximum spikelet sterility percent was recorded at T_7: 350 Gy (24.12%) followed by T_6: 300 Gy (22.28%) treatments in the same cultivar. In ‘Bangalya’, maximum pollen sterility percent was recorded at T_7: 350 Gy (30.91%) followed by T_6: 300 Gy (28.41%) and maximum spikelet sterility was observed at T_7: 350 Gy (26.21%) followed by T_6: 300 Gy (24.85%) dose of gamma rays. Similarly, in ‘Ghansal’ cultivar Maximum pollen sterility was observed at T_7: 350 Gy (31.57%) followed by T_6: 300 Gy (30.27%) dose of gamma rays while, spikelet sterility percent was observed at T_7: 350 Gy (29.70%) followed by T_6: 300 Gy (27.97%) dose of gamma rays. Such increase in pollen and spikelet sterility in higher doses was observed by Babaei *et al.* (2010) [1], Sasikala and Kalaiyarasi (2010) [13], Bijral *et al.* (1986) [3].

Table 1: Effect of mutagenic treatment (Gamma rays) on biological parameters *viz.* germination (G) (%), Shoot length (SL) (%), shoot length reduction (SLR) (%), plant survival (PSu) (%), pollen sterility (PS) (%) and spikelet sterility (SS) (%) in rice cultivars ‘Chakhao,’ ‘Bangalya’ and ‘Ghansal’.

Teatment	**Chakhao**						**Bangalya**						**Ghansal**					
	G (%)	**SL (cm)**	**SLR (%)**	**PSu (%)**	**PS (%)**	**SS (%)**	**G (%)**	**SL (cm)**	**SLR (%)**	**PSu (%)**	**PS (%)**	**SS (%)**	**G (%)**	**SL (cm)**	**SLR (%)**	**PSu (%)**	**PS (%)**	**SS (%)**
Control	98.44	18.73	-	93.25	13.28	10.66	97.44	12.45	-	92.27	14.96	11.71	91.72	9.34	-	91.87	17.86	14.72
50 Gy γ	95.67	15.59	16.76	92.20	14.81	12.68	93.11	11.45	10.79	91.82	16.93	14.57	74.57	8.49	9.16	85.04	19.60	16.84
100 Gy γ	88.00	15.34	18.09	87.17	16.67	14.37	82.89	10.23	17.88	86.75	19.16	16.02	50.29	7.50	21.04	71.02	22.47	19.25
150 Gy γ	74.78	14.21	24.13	81.58	20.44	16.01	75.11	9.17	27.26	82.07	21.77	17.48	43.00	6.61	30.40	66.13	26.92	22.16
200 Gy γ	70.77	13.71	26.30	75.85	25.49	19.87	64.11	8.07	35.77	73.34	24.94	19.56	40.93	5.36	39.58	62.03	28.86	24.67
250 Gy γ	57.89	12.26	34.54	70.77	26.57	20.95	57.22	7.37	41.43	70.38	27.32	22.18	26.55	5.21	44.18	52.33	29.27	25.56
300 Gy γ	49.67	11.37	36.62	54.24	29.12	22.28	49.55	6.21	48.47	60.86	28.41	24.85	12.01	4.55	52.34	38.83	30.27	27.97
350 Gy γ	36.89	10.93	41.64	50.65	30.01	24.12	43.33	5.09	60.34	46.63	30.91	26.21	8.40	3.83	64.36	36.95	31.57	29.70
Mean	71.51	14.02	28.30	75.71	22.04	17.62	70.35	8.76	34.56	75.52	23.05	19.07	43.43	6.36	37.29	63.03	25.85	22.61
Std. Dev.	1.75	0.48	0.53	1.32	0.52	0.49	1.51	0.36	0.48	1.06	0.62	0.51	1.22	0.17	0.94	1.23	0.68	0.48
CV	2.45	3.44	1.87	1.75	2.36	2.77	2.15	4.13	1.40	1.40	2.69	2.69	2.82	2.73	2.52	1.95	2.63	2.14
SEm±	1.01	0.70	0.30	0.76	0.30	0.28	0.87	0.84	0.28	0.61	0.36	0.30	0.71	0.10	0.54	0.71	0.39	0.28

M_2 generation

Frequency and Spectrum of chlorophyll mutation

Variety of morphological changes were noticed in M_2 generation of all the cultivars of rice *viz.*, Chakhao, Bangalya and Ghansal. They were grouped into chlorophyll and viable mutations. In the seedling stage of M_2 generation of all the cultivars, a number of chlorophyll deficient plants were observed and classified as per classification of chlorophyll mutations given by Venkateswarlu *et.al.* (1988) [21]. The frequency of chlorophyll mutations was calculated as the number of mutations occurring per 100 M_2 seedlings (Table 2). These findings are in confirmation with Singh and Singh (2003) [17], Sellammal and Maheswaran (2013a) [14]. Four different types of chlorophyll mutations were identified which were categorized as follows;

Frequency of chlorophyll mutation

Among the four chlorophyll mutations in ‘Chakhao’ cultivar of rice, the highest mutation frequency per 100 M_2 seedlings was observed at T_7:350 Gy (5.39) dose of gamma rays whereas, the lowest was recorded at T_1: 50 Gy (1.61) dose of gamma rays. Reddi and Suneetha (1992) [12] reported similar kind of results. In ‘Bangalya’, the highest mutation frequency was observed at T_7: 350 Gy (3.52) dose of gamma rays whereas, the lowest was recorded at T_1: 50 Gy (1.02) dose of gamma rays (2.69). Highest mutation frequency was observed at T_7: 350 Gy (4.24) dose of gamma rays whereas, the lowest was recorded at T_1: 50 Gy (1.22) dose of gamma rays in ‘ghansal’ cultivar of rice. Similar trend of chlorophyll mutation frequency observed in all three cultivars. Similar findings were given by Baloch *et al.* (2004) [2].

Spectrum of chlorophyll mutation

i. Albina

The colour of young seedlings was white on germination and they did not survive after a week from germination. chlorophyll or carotenoids was found absent. Albina recorded the highest frequency of chlorophyll mutations as compared to *xantha*, *viridis* and *striata* in all cultivars. Baloch *et al.* (2004) [2] reported that albina type of mutants observed at higher frequency. The frequency of *albina* was more as compared to others reported by Shadakshari *et al.* (2001) [16]. Chakhao cultivar recorded a high frequency 4.10 (19) than the ghansal 3.18 (15) and ‘bangalya’ 2.42 (11) cultivars which indicated that ‘Chakhao’ was more sensitive towards the gamma rays treatments. Their frequencies of mutation for different characters are given in Table 3 for all the cultivars. The frequency of albino mutants was maximum in treatment T_7: 350 Gy followed by treatment T_6: 300 Gy in all cultivars. Similar results obtained by Sellammal and Maheswaran (2013a) [14], Yamaguchi *et al.* (2009) [22].

ii. Xantha

The colour of young seedling was yellow, high pale yellow or orange but they did not survive beyond two weeks. The carotenoids were present but chlorophyll was absent. Total 19, 9 and 15 ‘*xantha*’ mutants were observed in ‘Chakhao’, ‘Bangalya’ and ‘Ghansal’ cultivars respectively in all mutagenic treatments. *Xantha* mutants were observed in all mutagenic treatments except in T_1: 50Gy in Bangalya cultivar. Maximum frequency of *xantha* chlorophyll mutants in Chakhao cultivar was recorded in treatment T_5: 250Gy 4(0.85) and T_6: 300Gy 4(0.85) and minimum in treatment T_1:

50Gy 1(0.20). In case of bangalya cultivar maximum frequency of *xantha* chlorophyll mutants was recorded in treatment T_4: 200Gy 3(0.63). while, in Ghansal cultivar maximum frequency of *xantha* chlorophyll mutants was recorded in treatment T_6: 300Gy 4(0.84) and minimum recorded in treatments T_1: 50Gy, T_2: 100Gy and T_4: 200Gy (0.20) These results are in confirmation with Kumar (1998a) [18], Baloch *et al.* (2004) [2].

iii. Viridis

The colour of seedling was greenish-yellow with whitish tip and started dying within 10-15 days after germination, few survived a little longer without seed setting. Total four *viridis* mutants were recorded in all mutagenic treatments in Chakhao. Eight *viridis* mutants in Bangalya and five *viridis* mutants in Ghansal cultivars of rice. Maximum frequency was observed in treatment T_7:350Gy (0.43) in Chakhao, T_7:300Gy (0.44), T_4:200Gy (0.42) and T_5:250Gy (0.42). Total five *viridis* mutants were recorded in all mutagenic treatments in Ghansal cultivar. Maximum frequency was observed in treatment T_6:300Gy (0.42). Kumar (1998a) [18], Yamaguchi *et al.* (2009) [22] reported similar kind of results.

iv. Striata

They have transverse yellow or white longitudinal bands alternating with green colour. This type of mutants was recorded in mutagenic treatments *viz.* T_2:100Gy T_4:200Gy T_6:300Gy T_7:350Gy in chakhao cultivar of rice. Treatments T_3:150Gy T_5:250Gy T_7:350Gy showed this type of mutants in Bangalya cultivar of rice. While, in Ghansal cultivar treatments T_3:150Gy T_4:200Gy T_5:250Gy T_7:350Gy showed this type of mutants. Vasline (2013a) [19] reported that striata had low frequency than the other type of mutants.

Viable mutations

The characters which are of interest to plant breeders can either be altered or amended by mutation. The frequencies of morphological deviants in respect to duration, leaf size, panicle type and stature are described as viable mutants. In the present investigation, it was observed that the induced viable mutation spectrum varied in all the cultivars *viz.*, Chakhao, Bangalya and Ghansal. (Table 4, 5, 6)

A total of four types of morphological mutations (early maturing, increased tillering, complete spikelet sterility and Reduced plant height) were identified in all these three cultivars. A dose of 150 Gy (2.49) of gamma rays produced higher frequency of viable mutations followed by 250 Gy (2.11) in Chakhao cultivar whereas, in Bangalya viable mutants were produced more at 350 Gy (2.64) followed by 200 Gy (2.33). Ghansal cultivar of rice produced higher frequency of viable mutations at dose 150 Gy (2.47) followed by 250 Gy (2.31). Lower frequencies of viable mutants were produced at 50 Gy (1.01), (0.81) and (1.22) in rice cultivars *viz.*, Chakhao, Bangalya and Ghansal respectively.

Efficiency and Effectiveness of Mutagens

The mutagenic effect of a mutagen, which is an index for the appropriate choice, is evaluated in terms of ‘mutagenic effectiveness and efficiency’ (Konzak *et al.*, 1965) [7]. Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen. Mutagenic efficiency refers to the proportion of mutation in relation to other associated undesirable biological effects such as gross chromosomal aberrations, lethality and sterility induced by the mutagen in question. The utility of a particular mutagen depends not only on its effectiveness but also on its efficiency.

The effectiveness and efficiency of different treatments of the mutagen (gamma rays) on all the rice cultivars were calculated following the methods suggested by Konzak *et al.* (1965) [7]. It is evident from Table 7, 8, 9 that plant survival reduction, pollen sterility percent and spikelet sterility percent increased with the increase in doses of gamma rays. The effectiveness of gamma rays decreased with the increase in mutagenic doses from lower to higher in all the cultivars whereas, the same pattern was not found in case of mutagenic efficiency. The lowest effectiveness was observed at 350 Gy (0.010) dose of gamma rays (Table 8). All these findings are in relation with findings given by Chakraborty and Kole (2009) [4], Baloch *et al.* (2004) [2], Cheema and Atta (2003) [5].

Table 2: Frequency of induced chlorophyll mutations in M_2 in rice cultivars ‘Chakhao, ‘Bangalya’ and ‘Ghansal’.

Chakhao				Bangalya			
Treatment	**M_2 seedlings studied**	**Total of mutants**	**Mutation frequency/100 M_2 seedlings**	**Treatment**	**M_2 seedlings studied**	**Total of mutants**	**Mutation frequency/100 M_2 seedlings**
Control	497	-	-	**Control**	498	-	-
50 Gy γ	494	8	1.61	50 Gy γ	492	5	1.02
100 Gy γ	486	10	2.05	100 Gy γ	488	7	1.43
150 Gy γ	481	15	3.11	150 Gy γ	479	10	2.08
200 Gy γ	478	17	3.55	200 Gy γ	472	11	2.33
250 Gy γ	472	20	4.23	250 Gy γ	468	13	2.77
300 Gy γ	470	22	4.68	300 Gy γ	459	14	3.05
350 Gy γ	463	25	5.39	350 Gy γ	454	16	3.52
Total	3344	117	24.62	Total	3312	76	16.2

Ghansal			
Treatment	**M_2 seedlings studied**	**Total of mutants**	**Mutation frequency/ 100 M_2 seedlings**
Control	499	-	-
50 Gy γ	491	6	1.22
100 Gy γ	487	10	2.05
150 Gy γ	485	12	2.47
200 Gy γ	482	13	2.69
250 Gy γ	476	16	3.36

300 Gy γ	473	18	3.80
350 Gy γ	471	20	4.24
Total	3365	95	19.83

Table 3: Spectrum of induced chlorophyll mutations in M_2 generation of rice cultivars 'Chakhao', 'Bangalya' and 'Ghansal'.

Treatment	'Chakhao'				'Bangalya'			
	Albina	Xantha	Viridis	Striata	Albina	Xantha	Viridis	Striata
Control	-	-	-	-	-	-	-	-
50 Gy γ	6(1.21)	1(0.20)	0(000)	0(000)	4(0.81)	0(000)	1(0.20)	0(000)
100 Gy γ	7(1.44)	2(0.41)	0(000)	1(0.20)	6(1.22)	1(0.20)	0(000)	0(000)
150 Gy γ	12(2.49)	2(0.41)	1(0.20)	0(000)	7(1.46)	1(0.20)	0(000)	2(0.41)
200 Gy γ	13(2.71)	3(0.62)	0(000)	1(0.20)	6(1.27)	3(0.63)	2(0.42)	0(000)
250 Gy γ	16(3.38)	4(0.84)	0(000)	0(000)	9(1.92)	1(0.21)	2(0.42)	1(0.21)
300 Gy γ	15(3.19)	4(0.85)	1(0.21)	2(0.42)	11(2.39)	2(0.43)	1(0.21)	0(000)
350 Gy γ	19(4.10)	3(0.64)	2(0.43)	1(0.21)	11(2.42)	1(0.22)	2(0.44)	2(0.44)
Total	88	19	4	5	54	9	8	5

Treatment	'Ghansal'			
	Albina	*Xantha*	*Viridis*	*Striata*
Control	-	-	-	-
50 Gy γ	5(1.01)	1(0.20)	0(000)	0(000)
100 Gy γ	8(1.64)	1(0.20)	1(0.20)	0(000)
150 Gy γ	9(1.85)	2(0.41)	0(000)	1(0.20)
200 Gy γ	9(1.86)	1(0.20)	2(0.41)	1(0.20)
250 Gy γ	11(2.31)	3(0.63)	0(000)	2(0.42)
300 Gy γ	14(2.95)	4(0.84)	2(0.42)	0(000)
350 Gy γ	15(3.18)	3(0.63)	0(000)	2(0.42)
Total	71	15	5	6

Table 4: Frequency and spectrum of induced viable mutations in M_2 generation of 'Chakhao' cultivar of rice.

Particulars	Gamma rays (Gy)						
	50 Gy γ	100 Gy γ	150 Gy γ	200 Gy γ	250 Gy γ	300 Gy γ	350 Gy γ
Total M_2 plants studied	494	486	481	478	472	470	463
Spectrum of mutations	Frequency of mutations						
Early Maturing	2	1	3	-	2	-	3
Increased tillering	-	4	3	2	3	2	-
Complete spikelet sterility	3	3	4	3	3	4	2
Reduced plant height	-	2	2	3	2	-	1
Total number of mutants	5	10	12	8	10	6	6
Frequency/100 M_2 plants	1.01	2.05	2.49	1.67	2.11	1.27	1.29

Table 5: Frequency and spectrum of induced viable mutations in M_2 generation of 'Bangalya' cultivar of rice.

Particulars	Gamma rays (Gy)						
	50 Gy γ	100 Gy γ	150 Gy γ	200 Gy γ	250 Gy γ	300 Gy γ	350 Gy γ
Total M_2 plants studied	492	488	479	472	468	459	454
Spectrum of mutations	Frequency of mutations						
Early Maturing	1	2	2	3	-	2	3
Increased tillering	1	4	3	3	2		4
Complete spikelet sterility	2	2	2	3	3	3	4
Reduced plant height	-	2	3	2	2	1	1
Total number of mutants	4	10	10	11	7	6	12
Frequency/100 M_2 plants	0.81	2.04	2.08	2.33	1.49	1.30	2.64

Table 6: Frequency and spectrum of induced viable mutations in M_2 generation of 'Ghansal' cultivar of rice.

Particulars	Gamma rays (Gy)						
	50 Gy γ	100 Gy γ	150 Gy γ	200 Gy γ	250 Gy γ	300 Gy γ	350 Gy γ
Total M_2 plants studied	491	487	485	482	476	473	471
Spectrum of mutations	Frequency of mutations						
Early Maturing	1	-	2	1	3	-	2
Increased tillering	3	3	4	-	2	3	2
Complete spikelet sterility	2	2	2	3	3	4	4
Reduced plant height	-	2	4	2	3	2	-
Total number of mutants	6	7	12	6	11	7	8
Frequency/100 M_2 plants	1.22	1.43	2.47	1.24	2.31	1.47	1.69

Table 7: Mutagenic effectiveness and efficiency of gamma rays in 'Chakhao' cultivar of rice.

Mutagenic Treatment	Mutated families% (Msf)	Biological damage			Effectiveness	Efficiency		
		Plant survival reduction% (L)	Pollen Sterility% (PS)	Spikelet Sterility% (SS)	Msf/ dose	Msf/L	Msf/PS	Msf/SS
Control		6.75	13.61	10.91				
50 Gy γ	1.619	7.80	14.92	12.85	0.032	0.207	0.108	0.125
100 Gy γ	2.057	12.83	17.21	14.11	0.020	0.160	0.119	0.145
150 Gy γ	3.118	18.42	20.08	16.01	0.020	0.169	0.155	0.194
200 Gy γ	3.556	24.15	25.84	19.87	0.017	0.147	0.137	0.178
250 Gy γ	4.237	29.23	26.31	20.92	0.016	0.144	0.161	0.202
300 Gy γ	4.680	45.76	29.01	22.81	0.015	0.102	0.161	0.205
350 Gy γ	5.399	49.35	29.95	22.77	0.015	0.109	0.180	0.237

Table 8: Mutagenic effectiveness and efficiency of gamma rays in 'Bangalya' cultivar of rice.

Mutagenic Treatment	Mutated families% (Msf)	Biological damage			Effectiveness	Efficiency		
		Plant survival reduction% (L)	Pollen Sterility% (PS)	Spikelet Sterility% (SS)	Msf/ dose	Msf/L	Msf/PS	Msf/SS
Control		6.57	15.53	12.70	-	-	-	-
50 Gy γ	1.016	7.32	16.93	14.79	0.020	0.138	0.060	0.068
100 Gy γ	1.434	12.65	19.57	16.32	0.014	0.113	0.073	0.087
150 Gy γ	2.087	16.51	22.03	17.88	0.013	0.126	0.094	0.116
200 Gy γ	2.330	25.92	25.17	19.53	0.011	0.089	0.092	0.119
250 Gy γ	2.777	29.44	27.81	22.54	0.011	0.094	0.099	0.123
300 Gy γ	3.050	38.40	29.21	24.59	0.010	0.079	0.104	0.124
350 Gy γ	3.524	54.09	31.05	26.82	0.010	0.065	0.113	0.131

Table 9: Mutagenic effectiveness and efficiency of gamma rays in 'Ghansal' cultivar of rice.

Mutagenic Treatment	Mutated families% (Msf)	Biological damage			Effectiveness	Efficiency		
		Plant survival reduction% (L)	Pollen Sterility% (PS)	Spikelet Sterility% (SS)	Msf/ dose	Msf/L	Msf/PS	Msf/SS
Control		8.96	17.10	14.99				
50 Gy γ	1.221	13.88	19.22	16.81	0.024	0.870	0.063	0.720
100 Gy γ	2.053	28.47	22.50	19.20	0.020	0.072	0.091	0.106
150 Gy γ	2.474	33.60	27.11	22.16	0.016	0.073	0.091	0.111
200 Gy γ	2.697	38.46	29.16	24.33	0.013	0.070	0.092	0.110
250 Gy γ	3.361	48.00	30.30	25.92	0.013	0.070	0.110	0.129
300 Gy γ	3.805	62.50	32.39	28.13	0.012	0.060	0.117	0.135
350 Gy γ	4.246	36.15	34.01	30.11	0.012	0.067	0.124	0.141

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