

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.901.300>

Mutagenic Effects of Gamma Rays and EMS in M_1 and M_2 Generations in Rice (*Oryza sativa* L.)

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ABSTRACT

Keywords

Chlorophyll, EMS,
gamma rays,
mutation, rice

Article Info

Accepted:
20 December 2019
Available Online:
20 January 2020

Mutation breeding programme(s) aims to enlarge the frequency and spectrum of mutations and to increase the frequency of viable mutations. The cultivar BPT-5204 revealed maximum reduction in germination (69.00%), seedling height reduction (52.54%), plant survival at maturity (62.50%), pollen sterility (32.82%) and spikelet sterility (29.80%) and Adamchini exhibited maximum reduction in germination (62.33%), seedling height reduction (65.71%), plant survival at maturity (50.90%), pollen sterility (50.86%) and spikelet sterility (34.80%) at a combined dose of 30 kR + 0.02 M EMS. The maximum and minimum frequency of mutation per 100 M_2 seedlings was recorded for a combined dose of 30 kR + 0.02 M EMS and 10 kR doses of gamma rays in BPT-5204 and Adamchini respectively. The spectrum of chlorophyll mutations consisted of only four mutant types, viz., albina, xantha, viridis and striata. In the present investigation, albina mutant occurred at higher frequencies and Adamchini recorded the highest frequency of chlorophyll mutations indicating varietal responses.

Introduction

The prime objective of any mutation breeding programme(s) is to develop varieties that would be high yielding coupled with short stature, early maturing and disease resistant. Selection of efficient and effective mutagen(s) is the first priority to produce a high frequency of desirable mutations in mutation breeding programme(s). Variability already present in nature has been the basis of crop improvement through selection and or/recombination approaches but mutation breeding is relatively a quicker method for the

improvement of crops (Kharkwal *et al.*, 2004). Induced mutations are the plant breeder's hope for freedom from complete dependence on nature as only source of genetic variants necessary in plant improvement (Hayes *et al.*, 1955).

These mutations typically occur at much higher frequencies than spontaneous mutations do (Satoh *et al.*, 1982). Induced mutations have great potential and serves as a complimentary approach in genetic improvement of crops (Mehandjiev *et al.*, 2001). Many physical and chemical mutagens

have been used for the induction of useful mutations in rice (Singh and Singh, 2003).

Induced mutations act as valuable supplements to naturally existing variability, though their phenomenon may be the same they contribute directly or indirectly towards required variability by plant breeders. In a mutation breeding programme(s), choice of mutagen is the most important criterion and various methods have been developed to ascertain the most efficient and effective mutagen(s) for attaining the desirable characters in cultivated crops. The present investigation deals with the effects of two mutagens (gamma rays and EMS) applied singly and in combinations on the biological parameters *viz.*, seed germination, seedling height reduction, plant survival at maturity and pollen and spikelet sterility in M₁ generation calculated as percent of control and appraisal of chlorophyll mutations in M₂ generation.

Materials and Methods

The present investigation was carried out at the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi, India in two consecutive seasons *Kharif* 2011 and *Kharif* 2012. Two rice cultivars *viz.*, BPT-5204 (also known as 'Samba Mahsuri', high yielding, semi-dwarf, non-lodging, medium slender grain, white rice, long maturity duration, excellent consumer preference) and Adamchini (popular aromatic variety, grown in parts of Eastern Uttar Pradesh, tall plant height, long maturity duration, short bold grain, white rice and excellent cooking quality) were used to induce mutations.

Three doses of gamma rays (⁶⁰Co source) *viz.*, 10 kR, 20 kR and 30 kR and one concentration (conc.) of ethyl methane sulphonate (EMS) *viz.*, 0.02 M (approx. 1.49%) were used as single doses. For

combination treatments, three doses of gamma rays along with 0.02 M conc. of EMS as 10 kR + 0.02 M EMS, 20 kR + 0.02 M EMS and 30 kR + 0.02 M EMS were used. One thousand and seventy five pure, uniform, healthy and dry (12% moisture) seeds for each treatment of two rice varieties were irradiated at National Botanical Research Institute, Lucknow, India. Seventy five seeds were kept for laboratory observations. EMS solution with one conc., *i.e.*, 0.02 M was prepared by mixing appropriate volume of EMS and phosphate buffer (pH 7.0).

One thousand seeds were subjected to presoaking in distilled water for 6 hrs at room temperature. The soaked seeds were then transferred to EMS solution and kept for 6 hrs; the seeds were given intermittent shaking throughout the period of treatment to maintain uniformity and then the mutagen solution was drained out. The treated seeds were then washed in running tap water for another 6 hrs to remove the residual chemical from the seeds. For combination treatments, gamma ray treated seeds were soaked in distilled water for six hours and then treated with EMS solution of 0.02 M conc. followed by washing in running tap water for six hours. The treated seeds were then immediately sown in nursery beds of 3 m length × 1 m breadth accommodating 30 rows to assure enough seedling population to be grown in the M₁ generation. The remaining 75 seeds were used in laboratory for observing germination (after 7th day of sowing) and seedling height reduction (after 14th day of sowing).

Looking to the mortality percent in nursery and further to maintain uniform plant population in each treatment, 420 survived and healthy seedlings were selected from each treatment for transplanting in the field. All the 21 days old seedlings were transplanted in a well prepared puddled field at a distance of 20 cm × 15 cm between row to row and plant to

plant in seven rows plot accommodating 140 seedlings per replication (total of 420 seedlings over replication) in a Randomized Complete Block Design (RCBD) with three replications. Each plot consisted of 3 m length \times 1.4 m breadth. Recommended dose of fertilizers and intercultural operations were carried out to assure a good stand and healthy crop. The M_1 plants were studied critically and carefully. Any deviations in the characters of treated plants from the control (parent) plants were screened and marked. The morphological variations, if any, were noticed and recorded. The data on pollen and spikelet sterility (%) was recorded on twenty randomly selected plants from each treatment. For pollen sterility study, five spikelets were plucked from main panicle which emerged first of the tagged plants were collected during morning hours, one anther from each spikelet was taken and pollen grains were stained in 1% Iodine Potassium Iodide (IKI) solution and examined under light microscope. Unstained, half stained, shriveled and empty pollen grains were classified as sterile while well filled stained and round pollen grains as fertile. The percentage of pollen sterility was calculated by dividing the sterile pollen grains by the total number of pollen grains. Similarly, the percentage of spikelet sterility was determined by estimating the proportion of unfilled spikelets out of the total number of spikelets of plants used earlier for studying pollen sterility.

Based on pollen / spikelet fertility % in M_1 generation, 20 plants showing more than 60% pollen fertility and above were selected and the remaining plants were bulked together. The 20 rows of selected plants (plant to progeny rows) of each treatment were grown in a Randomized Complete Block Design with three replications and 10 rows of bulked seed (plants whose fertility % being less than 60) of each treatment were also grown at the Agricultural Research Farm during *Kharif*,

2012 as per layout mentioned earlier for raising M_1 generation.

The mutant population obtained from bulked seed was screened for macro-mutations *i.e.*, chlorophyll and viable mutations. The viable mutations included various morphological as well as physiological mutants whereas; the chlorophyll mutations were the plants with various degrees of deficiency in pigmentation of leaves that emerged at seedling stage. The frequency of induced chlorophyll mutations in M_2 generation has been considered as a reliable index for noting the potency of mutagens due to greater accuracy in their scoring (Gustaffson, 1940; Mackey, 1951). The chlorophyll mutation serves not only as a measure for evaluating effectiveness and efficiency of mutagens, but also as indicators to predict the size of vital factor mutations. Chlorophyll mutations were classified on the basis of criterion given by Venkateswarlu *et al.*, (1988) and the mutation frequency per 100 M_2 seedlings was calculated following Gaul (1957, 1960).

Results and Discussion

The effect of two mutagens *viz.*, gamma rays and EMS applied singly and in combinations on germination of 'BPT-5204' and 'Adamchini', calculated as percent of control is depicted 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, EMS and their combinations on germination percent, but reduction in germination percent increased with the increase in doses of mutagens (gamma rays, gamma rays + EMS) in both the varieties. The reduction in germination percent was more pronounced at higher doses of combined treatment of both the mutagens *i.e.*, 69.00% at 30 kR + 0.02 M EMS followed by 74.67% at 20 kR + 0.02 M EMS in BPT-

5204 as compared to 94.33% of control. Similarly, the reduction in germination percent was 62.33% at 30 kR + 0.02 M EMS followed by 69.00% at 20 kR + 0.02 M EMS in Adamchini as compared to 91.33% of control. The combination treatments exhibited maximum reduction in germination percent as compared to single doses of both the mutagens.

A reduction in germination percent in the M₁ generation due to mutagenic treatments has been reported in rice by (Awan *et al.*, 1980). The diminution in seed germination percent has been elucidated to the interruption of physiological and biological processes indispensable for seed germination including enzymatic activity (Kurobane *et al.*, 1979).

Seedling height also exhibited a decreasing trend in both the cultivars with increase in the doses of gamma rays and combined treatments. Seedling height was measured as reduction in shoot length of seedlings on 14th day after sowing (Table 1). Maximum reduction in seedling height was observed for 30 kR + 0.02 M EMS (52.54%) followed by 30 kR of gamma rays (45.59%) and 20 kR + 0.02 M EMS (38.43%) in BPT-5204. In Adamchini, maximum reduction in seedling height was observed for 30 kR + 0.02 M EMS (65.71%) followed by 20 kR + 0.02 M EMS (44.61%) and 30 kR of gamma rays (43.51%). Minimum reduction in seedling height percent was recorded for 10 kR dose of gamma rays in BPT-5204 and Adamchini. The M₁ seedling growth is widely used as an index in determining the biological effects of various physical and chemical mutagens (Konzak *et al.*, 1972). Mutagens differ in their mechanism and mode of action in the biological system. Hence, the extent of reduction in growth is related to the mechanism of action for a given mutagen. The reduced growth of seedlings has been explained on the basis of auxin destruction,

changes in ascorbic acid content and physiological and biochemical disturbances (Gunckel and Sparrow, 1954; Gordons, 1957; Singh, 1974; Usuf and Nair, 1974).

As evident from Table 1, it was observed that plant survival percent decreased with increase in the doses of gamma rays and combined treatments of both the mutagens. Maximum plant survival percent was observed for control (90.20%) followed by 10 kR (85.32%) and 0.02 M dose of EMS (84.70%) in BPT-5204. Minimum plant survival percent was observed for 30 kR + 0.02 M EMS dose (62.50%) in BPT-5204. Similarly, maximum plant survival percent was recorded for control treatment (88.40%) followed by 0.02 M dose of EMS (82.90%). The minimum plant survival percent was observed for 30 kR + 0.02 M (50.90%) dose of combined treatment in Adamchini. The survival percentage of M₁ plants decreased considerably in the mutagenic treatments in both the varieties. Physiological damage, chromosomal and point mutations may be attributed for the cause of reduction in germination and plant survival percentage due to mutagenic treatments in M₁ generation (Gaul *et al.*, 1972). According to Swaminathan *et al.*, (1962), the decrease in survival of plants at maturity is due to rapid infusion of mutagens and their ability to produce chromosomal aberrations. Qurainy and Khan (2009), Sasikala and Kalaiyarasi (2010), Taher *et al.*, (2011), Wattoo *et al.*, (2012) and Vasline (2013) have also reported decrease in biological parameters in their investigations in rice following mutagenic treatments.

The reduction in pollen and spikelet fertility was observed in the present investigation in both the cultivars as compared to control in the M₁ generation (Table 1). The pollen and spikelet sterility percent increased with the increase in doses of gamma rays and

combined treatments of both the mutagens. Maximum pollen sterility in BPT-5204 was observed for 30 kR + 0.02 M EMS (32.82%) followed by 20 kR + 0.02 M EMS (30.66%) and 30 kR (28.70%) dose of gamma rays.

Maximum spikelet sterility percent was recorded for 30 kR + 0.02 M EMS (29.80%) followed by 20 kR + 0.02 M EMS (25.40%) and 10 kR + 0.02 M EMS (20.80%) of combined treatments in the same.



(Albina)



(Xantha)



(Striata)

Table.1 Effect of mutagenic treatments (gamma rays, EMS and their combinations) on biological parameters *viz.*, germination (%), seedling height reduction (%), plant survival (%), pollen sterility and spikelet sterility (%) in rice varieties ‘BPT-5204’ and ‘Adamchini’

Treatment	BPT-5204 (Samba Mahsuri)						Adamchini					
	G (%)	SHR (%)		PSu (%)	PS (%)	SS (%)	G (%)	SHR (%)		PSu (%)	PS (%)	SS (%)
		(cm)	(%)					(cm)	(%)			
Control	94.33	14.52	0.00	90.20	14.34	12.00	91.33	15.40	0.00	88.40	20.80	17.60
10 kR	90.33	12.67	12.74	85.32	18.90	16.20	88.33	13.94	9.48	82.30	26.45	20.42
20 kR	86.67	9.45	34.92	82.70	24.92	19.68	82.00	11.27	26.81	75.36	30.78	23.56
30 kR	82.00	7.90	45.59	75.16	28.70	20.46	75.33	8.70	43.51	70.34	32.90	27.78
EMS (0.02 M)	90.00	12.80	11.85	84.70	20.45	18.67	87.33	12.89	16.30	82.90	30.45	26.68
10 kR + EMS	81.67	10.48	27.82	78.80	24.60	20.80	73.33	10.20	33.77	69.90	35.56	25.90
20 kR + EMS	74.67	8.94	38.43	70.80	30.66	25.40	69.00	8.53	44.61	60.78	46.60	27.84
30 kR + EMS	69.00	6.89	52.54	62.50	32.82	29.80	62.33	5.28	65.71	50.90	50.86	34.80
Mean	83.58	10.46		78.77	24.42	20.38	78.62	10.78		72.61	34.30	25.57
Std. Dev.	8.54	2.66		8.99	6.28	5.41	10.28	3.29		12.42	10.00	5.22
CV	10.21	25.42		11.41	25.71	26.57	13.08	30.58		17.11	29.16	20.41
SEm±	3.02	0.94		3.18	2.22	1.91	3.63	1.16		4.39	3.54	1.85

Table.2 Frequency of induced chlorophyll mutations in M₂ generation in two rice varieties ‘BPT-5204’ and ‘Adamchini

Treatment	BPT – 5204 (Samba Mahsuri)			Adamchini		
	M ₂ seedlings studied	Total number of mutants	Mutation frequency per 100 M ₂ seedlings	M ₂ seedlings studied	Total number of mutants	Mutation frequency per 100 M ₂ seedlings
Control	1000	-	-	1000	-	-
10 kR	980	19	1.94	965	26	2.69
20 kR	926	25	2.70	897	32	3.57
30 kR	892	32	3.59	874	37	4.23
EMS (0.02 M)	970	21	2.16	930	28	3.01
10 kR + EMS (0.02 M)	915	23	2.51	890	29	3.26
20 kR + EMS (0.02 M)	880	26	2.95	858	34	3.96
30 kR + EMS (0.02 M)	825	32	3.88	802	41	5.11
Total	6388*			6216*		

*does not includes control

Table.3 Spectrum of induced chlorophyll mutations in M₂ (based on per 100 M₂ seedlings) in rice varieties ‘BPT-5204’ and ‘Adamchini’

Treatment	Chlorophyll mutations - BPT – 5204 (Samba Mahsuri)				Chlorophyll mutations - Adamchini			
	<i>Albino</i>	<i>Xantha</i>	<i>Viridis</i>	<i>Striata</i>	<i>Albino</i>	<i>Xantha</i>	<i>Viridis</i>	<i>Striata</i>
Control	-	-	-	-	-	-	-	-
10 kR	15 (1.53)	4 (0.41)	0 (0.00)	0 (0.00)	20 (2.07)	3 (0.31)	3 (0.31)	0 (0.00)
20 kR	20 (2.16)	5 (0.54)	0 (0.00)	0 (0.00)	22 (2.45)	10 (1.11)	0 (0.00)	0 (0.00)
30 kR	19 (2.13)	11 (1.23)	0 (0.00)	2 (0.22)	28 (3.20)	9 (1.03)	0 (0.00)	0 (0.00)
EMS (0.02)	18 (1.86)	3 (0.31)	0 (0.00)	0 (0.00)	25 (2.69)	2 (0.22)	0 (0.00)	1 (0.11)
10 kR + 0.02 M EMS	17 (1.86)	5 (0.55)	1 (0.11)	0 (0.00)	24 (2.70)	5 (0.56)	0 (0.00)	0 (0.00)
20 kR + 0.02 M EMS	22 (2.50)	4 (0.45)	0 (0.00)	0 (0.00)	30 (3.50)	4 (0.47)	0 (0.00)	0 (0.00)
30 kR + 0.02 M EMS	25 (3.03)	5 (0.61)	2 (0.24)	0 (0.00)	35 (4.36)	3 (0.37)	1 (0.12)	2 (0.25)
Total	136	37	3	2	184	36	4	3

In Adamchini, maximum pollen sterility percent was recorded for 30 kR + 0.02 M EMS (50.86%) followed by 20 kR + 0.02 M EMS (46.60%) and 10 kR + 0.02 M EMS (35.56%) of combined treatments and maximum spikelet sterility percent was observed for 30 kR + 0.02 M EMS (34.80%) followed by 20 kR + 0.02 M EMS (27.84%) and 30 kR dose of gamma rays (27.78%). The combined treatments caused more decline in pollen and spikelet fertility as compared to single doses of gamma ray and EMS. It is obvious from the experimental results that mutagenic treatments enhanced pollen and spikelet sterility in both the cultivars in M₁ generation as compared with their respective controls. The higher degree of pollen and spikelet sterility was reported to be linked with meiotic abnormalities (Khan and Wani, 2005; Muthusamy and Jayabalan, 2002) because meiosis is more prone to conceivable type of turbulences (Khan and Goyal, 2009). In the present investigation, the increase in pollen and spikelet sterility as a consequence of mutagenesis is corroborated with the findings of (Cheema and Atta, 2003; Singh and Singh 2003; Kole and Chakraborty, 2012; El-Degwy, 2013; Kumar *et al.*, 2013) in rice.

The cultivar Adamchini was found to be more sensitive towards mutagenic treatments as biological damage caused was more in terms of germination, seedling height, plant survival, pollen and spikelet sterility as compared to BPT-5204. This may be due to the differential responses of different varieties towards different mutagens and doses.

Varietal differences were also reported earlier with respect to mutagen sensitivity (Irfaq and Nawab, 2003). The sensitivity of an organism depends upon the type of mutagen employed and its genetic makeup (Kaul, 1988), amount of DNA and its replication time in the initial stages (Varughese and Swaminathan, 1968) besides physiological factors such as pH,

moisture, oxygen and temperature (Konzak *et al.*, 1965). Genetic differences even though very small can induce significant changes in mutagen sensitivity, which in turn influences various plant characters (Khan and Goyal, 2009).

The frequencies of chlorophyll mutations computed on the basis of M₂ seedlings in cultivars, BPT-5204 and Adamchini have been presented in Table 2 and 3. The highest and lowest frequency of chlorophyll mutations in BPT-5204 and Adamchini were obtained at a combined dose of 30 kR + 0.02 M EMS and 10 kR respectively. In general, the frequency of chlorophyll mutations was higher in both the varieties at higher doses. The chlorophyll mutants, such as, *albina* – white leaves without chlorophyll (lethal), *xantha* – light yellow to complete yellow colour of leaves (lethal), *viridis* – uniform light yellow green colour of leaves (viable) and *striata* – transverse white or yellow bands alternating with green colour were scored in M₂ generation at the seedling stage.

The *albina* and *xantha* mutants did not survive (lethal mutants) whereas *viridis* and *striata* were observed as viable mutants (survived till maturity of the crop). Chlorophyll mutants were induced by all the doses of mutagens alone and in combinations at fair frequencies in both the varieties in M₂ generation. *Albina* was the highest in frequency followed by *xantha*, *viridis* and *striata* which is in conformity with the findings of Chakravarti *et al.*, (2013) who observed *albina* mutant to be the most frequent in both the genotypes of aromatic rice which he investigated. The frequency of chlorophyll mutations increased with the increase in doses of gamma rays and combined treatments of both the mutagens which is in consonance with the reports of Chakraborty and Kole (2009), Yamaguchi *et al.*, (2009), Mishra and Chaudhary (2011),

Sharma *et al.*, (2011), Vasline and Sabesan (2011) in rice.

Chlorophyll mutations provide one of the most dependable indices for evaluation of genetic effects of mutagenic treatments and have been reported in rice by several workers (Singh, 2006; Sellammal and Maheswaran (2013). The frequency of chlorophyll mutations was higher in Adamchini as compared to BPT-5204. Such varietal differences were also reported earlier by Sharma *et al.*, (2011) and Sellammal and Maheswaran (2013) in rice who elucidated that frequency of chlorophyll mutations were more in some varieties as compared to the others. The frequency of mutations generated was found to be independent of the doses of gamma rays (Bekendam, 1961; Reddi and Rao, 1988). The segregation ratio did not show dose dependency with gamma rays, EMS and their combinations.

Acknowledgements

The first author is grateful to the University Grants Commission for providing fellowship and BHU for providing necessary funds and resources for carrying out this research work.

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How to cite this article:

Amita Sharma, S. K. Singh, Rajesh Singh, P. K. Bhati and Meena M. K. 2020. Mutagenic Effects of Gamma Rays and EMS in M₁ and M₂ Generations in Rice (*Oryza sativa* L.). *Int.J.Curr.Microbiol.App.Sci*. 9(01): 2645-2655. doi: <https://doi.org/10.20546/ijcmas.2020.901.300>