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Effectiveness and Efficiency of Physical and Chemical Mutagens Inducing Chlorophyll Mutants in Two Rice Genotypes

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Physical and chemical mutagens have been used to raise the genetic variation in most of the crop plants. An experiment was conducted to study the effect of gamma rays and Ethyl Methyl Sulfonate (EMS) in two quality rice genotypes of improved White Ponni and BPT 5204. In this study frequency and spectrum of chlorophyll mutations, mutagenic effectiveness and efficiency and mutation rate were estimated. The result from the study indicated that, *albino* was the predominant chlorophyll mutants that occur in M_2 generations of improved White Ponni and BPT 5204 at lower doses. The mutagenic effectiveness and efficiency were found to be higher at 100Gy of gamma radiation in both the varieties. The mutation rate of gamma rays (0.7131) was recorded high in improved White Ponni whereas, mutation rate of EMS (0.6693) was found to be higher in BPT 5204. In terms of efficiency, the mutation rate of gamma rays was registered to be higher based on injury (6.6587) and sterility (13.8777).

Key words: Rice, Gamma rays, Ethyl Methyl Sulfonate, Mutagenic effectiveness, Mutagenic efficiency and Mutation rate.

Rice (*Oryza sativa* L.) is one of the prime food crops cultivated all over the world and it provides major calorfiic intake to the people in Asian countries. Improvement in rice needs broad genetic variability. Such variability available to the breeder comes from spontaneous or artificially induced mutations. In mutation breeding, the enhancement of the genetic variation is made through the influence of mutagens. Despite the advantages and limitations of this method, it has been applied in the development of numerous improved cultivars, in different crop species. The primary objective of the mutation breeding is to enlarge the frequency and spectrum of mutations and also to increase the incidence of viable mutations, as an approach towards directed mutagenesis (Ando and Mantalvan, 2001).

Artificial induction of mutations is done through the use of physical and/or chemical mutagens, which enlarge the mutation frequency, when compared to the spontaneous occurrence. However, for extensive use of these mutants in plant breeding, high production efficiency is essential. This means that the utility of any mutagen depends not only on its effectiveness (mutation factor / dose), but also on its efficiency. The effectiveness of a mutagen has no practical implications since radiations and chemical mutagens are relatively inexpensive. Mutagenic efficiency is the production of desirable changes which are free from associations with undesirable genetic alterations. This is generally measured by the proportion of the mutation frequency in relation to damages associated to mutagenic treatments such as: height reduction, chromosomes breakages, sterility, lethality, *etc*. (Konzak *et al*., 1965; Gaul *et al*., 1972). The term "effectiveness" is a measure of gene mutation in relation to dose and "efficiency" is an estimate of biological effects induced, such as lethality, sterility and injury. The present investigation deals with the frequency and spectrum of chlorophyll mutations, mutagenic effectiveness and efficiency of both gamma rays (physical mutagen) and Ethyl Methyl Sulfonate (chemical mutagen) in M_2 generations of two popular varieties of rice in India namely Improved White Ponni and BPT 5204.

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Materials and Methods

Source material

The seeds of two varieties namely improved White Ponni and BPT 5204 for the induction of mutation treatment were obtained from Department of Rice, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Coimbatore.

Mutagenesis

Gamma irradiation

Dry dehusked, matured seeds with 10 to 14 per cent moisture content (500 numbers in each treatment) of improved White Ponni and BPT 5204 were irradiated with different doses of gamma rays from Cobalt-60 (^{60}Co) using the Gamma Chamber-Model GC 1200 installed at Centre for Plant Breeding and Genetics, TNAU, Coimbatore. The experiment was conducted during 2011 -2013 at Agricultural College and Research Institute, TNAU, Killikulam. The different doses of gamma rays used for treating the seeds of two varieties are : 100Gy, 200Gy, 300Gy, 400Gy and 500Gy. After the treatment, the irradiated seeds were sown in raised beds on next day morning along with the respective control seeds. The well filled seeds without irradiation treatment were used as control.

Ethyl Methyl Sulfonate (EMS) treatment

Well filled, dehusked seeds with 10 to 14 per cent moisture content (100 seeds per treatment) of mproved White Ponni and BPT 5204 were treated with varied concentrations of EMS solution namely, 100mM, 120mM, 140mM, 160mM and 180 mM. Volume of EMS solution used for soaking is 5 ml for each concentration. Before soaking in EMS solution, the seeds were pre-soaked in double distilled water for 24 hours to enhance the imbibing capacity to absorb the EMS. After pre-soaking, the seeds were treated with EMS of specified concentrations for four hours under controlled conditions with intermittent shaking. After four hours, the EMS solutions was drained and the seeds were thoroughly washed in running tap water for ten times to remove the EMS residues from the seeds and dried for half an hour. Pre-soaked seeds in distilled water for 28 hours were used as control.The seeds were sown in raised beds immediately along with the control seeds.

Observation on chlorophyll mutants

Evaluation of M_1 generation was done for both the varieties treated with gamma rays and EMS. A total of around 174 M_2 families of mproved White Ponni and 279 M_2 families of BPT 5204 treated with gamma radiation were forwarded to M_2 generation. In EMS treatment, around 47 M_2 families of improved White Ponni and 49 M_2 families of BPT 5204 were forwarded to M_2 generation. Usually, chlorophyll mutations occur up to 15 days from the time of seed germination and the primary leaves will be deficient of chlorophyll (Stummann and Henningsen, 1980). In M_2 generation, the chlorophyll mutants were observed in raised nursery beds. The classification and characterization of various chlorophyll mutants was done according to Gustafsson (1940) and Bilxt (1961) and the spectrum was recorded as *xantha, chlorina, viridis and albino*. The different classes of chlorophyll mutations observed in M_2 generation were as follows: *Albino*:- Characterized by entirely white leaves and did not survive 10 days after sowing (DAS) (Fig.1). *Xantha*:- Characterized by yellow to whitish yellow coloured leaves and it also has minimum survival upto 15 to 20 DAS. *Striata*:- Characterized by the presence of white or yellow coloured longitudinal strips on leaves and was viable one.

Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen (kR or Concentration X time). The formulae proposed by Konzak *et al*. (1965) were followed for the calculation of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic frequency}}{\text{Dose or (concentration X time)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutagenic frequency}}{\text{Biological damage}}$$

Mutation rate (MR) was calculated by the following formula

$$\text{Mutation rate} = \frac{\text{Sum of values of efficiency or effectiveness of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

This provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration.

Results and Discussion

Mutation breeding has been very useful in inducing variability and became a potential tool in any plant breeding programme. The use of various mutagens to generate genetic variation in crop plants has a history almost as long as that of conventional breeding. The reliability and success of any mutation breeding depends mainly on the effectiveness and efficiency of the mutagen. Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of mutagen and mutability of the variety.

Relative percentage (frequency) of chlorophyll mutations

The present study on induced mutations with gamma rays and EMS involved in the determination of frequency and spectrum of chlorophyll mutants in M_2 generation of two quality rice varieties of India *viz*., White Ponni and BPT 5204. In this experimental investigation, three different classes of chlorophyll mutants were observed in some of the treatment of gamma rays and EMS in both the genotypes and other treatments does not exhibit any classes of chlorophyll mutants. In general, the frequency and spectrum of chlorophyll mutations were used to determine the mutagenic effectiveness and efficiency of mutagens and this would ultimately provides the information about the dose for inducing mutations in particular genotypes. Chlorophyll mutations though of various types, viable to nonviable, provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in rice (Awan *et al*., 1980).

In the present investigation, the frequency of chlorophyll mutations varied with the genotype as well as mutagen doses in M_2 generation. The frequency and spectrum of induced chlorophyll mutations in White Ponni treated with gamma rays revealed that, among the presence of three classes of chlorophyll mutants, the frequency of *albino* was found to be maximum of 2.04 at 100Gy, 1.79 at 300Gy and 1.64 at 200Gy of gamma irradiation. This result clearly indicated that the maximum frequency of *albino* is higher (5.47) than other two classes of chlorophyll mutants of *xantha* (0.30) and *striata* (0.05) (Table 1). Earlier workers reported the higher frequency of *albino* mutants (Nanda and Misra, 1975; Bhan and Kaul, 1976; Rao and Rao, 1983; Reddi and Rao, 1988; Singh *et al*., 1998; Singh and Singh, 2003) in their studies involving physical and chemical mutagens in rice. Bansal (1969) reported that, among the chlorophyll mutations induced due to the treatments of gamma rays, the frequency of *albino* was found to be more than *xantha*. The total mutagenic frequency was found to be higher in

Table 1. Frequency and spectrum of chlorophyll mutants in M_2 generation of White Ponni

Mutagen	Classes of chlorophyll mutants			Number of chlorophyll mutants	Number of plants observed	Relative percentage (frequency) of chlorophyll mutants			Mutagenic frequency
Gamma rays (Dosage)	*Albino*	*Xantha*	*Striata*			*Albino*	*Xantha*	*Striata*	
Control	0	0	0	0	1000	0	0	0	0.00
100Gy	48	0	0	48	2358	2.04	0	0	2.04
200Gy	31	0	1	32	1889	1.64	0	0.05	1.69
300Gy	56	4	0	60	3125	1.79	0.13	0	1.92
400Gy	0	2	0	2	1156	0	0.17	0	0.17
500Gy	0	0	0	0	1566	0	0	0	0.00
						5.47	**0.30**	**0.05**	**5.82**
EMS (concentration)									
Control	0	0	0	0	1000	0	0	0	0.00
100 mM	0	0	0	0	1132	0	0	0	0.00
120 mM	13	0	0	13	1224	1.06	0	0	1.06
140 mM	7	3	0	7	1322	0.53	0.23	0.00	0.76
160 mM	2	0	0	2	987	0.20	0	0	0.20
180 mM	0	0	0	0	945	0	0	0	0.00
						1.79	**0.23**	**0**	**1.82**

100Gy of gamma rays in White Ponni (2.04) held followed by 300Gy (1.92) when compared to other treatments of gamma radiation (Fig 2a). In EMS treatment of White Ponni, the relative percentage (Frequency) of *albino* was found to be maximum (1.06) at 120mM concentration, which was followed by 140mM (0.53) (Table 1). The total mutagenic frequency was found to be maximum (1.06) at

Table 2. Frequency and spectrum of chlorophyll mutants in M_2 generation of BPT 5204

Mutagen	Classes of chlorophyll mutants			Total Number of chlorophyll mutants	Total Number of plants examined	Relative percentage (frequency) of chlorophyll mutants			Mutagenic frequency
Gamma rays (Dosage)	*Albino*	*Xantha*	*Striata*			*Albino*	*Xantha*	*Striata*	
Control	0	0	0	0	1000	0	0	0	0.00
100Gy	29	0	2	31	1898	1.53	0	0.11	1.63
200Gy	73	0	0	73	4342	1.68	0	0	1.68
300Gy	23	11	0	34	2395	0.96	0.46	0	1.42
400Gy	8	0	0	8	897	0.89	0	0	0.89
500Gy	0	0	0	0	1122	0	0	0	0.00
						5.06	**0.46**	**0.11**	**5.63**
EMS (concentration)									
Control	0	0	0	0	1000	0	0	0	0.00
100 mM	2	0	0	2	867	0.23	0	0	0.23
120 mM	20	0	0	20	1232	1.62	0	0	1.62
140 mM	4	0	0	4	956	0.42	0	0	0.42
160 mM	17	33	0	50	2134	0.80	1.55	0	2.34
180 mM	0	0	0	0	1342	0	0	0	0.00
						3.11	**1.55**	**0**	**4.66**

120mM concentration, followed by 140mM (0.76). This clearly indicated that, the presence of chlorophyll mutants were higher in lower doses of both gamma rays and EMS in White ponni.

The genotype BPT 5204 irradiated with gamma rays and EMS showed high frequency of *albino* mutants at 200Gy (1.68) and 120mM (1.62) (Fig. 2b). The sum total of mutagenic frequency was found to be higher in gamma rays (5.63) when compared to EMS (4.66) (Table 2). The same pattern of mutagenic frequency was also found in White Ponni.

This clearly reported that the frequency of chlorophyll mutations was registered higher in gamma rays for both the varieties and high in White Ponni as compared to BPT 5204. These varietal differences were also reported by Geetha and Vaidyanathan (2000), Paul and Singh (2002).

Table 3. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of White Ponni

Mutagen	seedling height (cm) (I)	% survival reduction (L)	% pollen sterility (S)	mutation frequency (M)	Effectiveness (M x 100) / Gy or C x t	Efficiency (M x 100) / I	(M x 100) / L	(M x 100) / S
Gamma rays (dosage)								
Control	31.25							
100Gy	27.20	11.02	6.56	2.04	2.04	7.48	18.48	31.01
200Gy	25.80	22.60	11.19	1.69	0.85	6.57	7.50	15.13
300Gy	20.50	40.68	9.98	1.92	0.64	9.37	4.72	19.23
400Gy	19.25	73.45	13.63	0.17	0.04	0.90	0.24	1.27
500Gy	18.50	92.66	18.44	0.00	0.00	0.00	0.00	0.00
EMS (concentration)								
Control	28.55							
100 mM	22.45	60.00	5.25	0.00	0.00	0.00	0.00	0.00
120 mM	21.80	68.00	8.67	1.06	0.89	4.87	1.56	12.25
140 mM	20.65	71.00	11.89	0.76	0.54	3.66	1.07	6.36
160 mM	20.20	91.00	15.78	0.20	0.13	1.00	0.22	1.28
180 mM	19.50	96.00	22.29	0.00	0.00	0	0.00	0.00

The increase in mutation frequency has been shown to be accompanied by injuries in M_1 plants (Kawai and Sato, 1966). In general, the chlorophyll mutation frequency increased with increasing dosage upto certain limit, beyond which it exhibited a decline. This shows that a saturation point has been reached at certain dosage. Swaminathan (1961) attributed this decline at higher dose level to the rigor of diplontic and haplontic selections in the irradiated materials.

Table 4. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of BPT 5204

Mutagen	seedling height (cm) (I)	% survival reduction (L)	% pollen sterility (S)	mutation frequency (M)	Effectiveness (M x 100) / Gy or C x t	Efficiency (M x 100) / I	(M x 100) / L	(M x 100) / S
Gamma rays (Dosage)								
Control	25.50							
100Gy	15.80	23.63	4.56	1.63	1.63	10.34	6.91	35.82
200Gy	16.30	35.45	8.73	1.68	0.84	10.31	4.74	19.26
300Gy	19.00	49.67	14.35	1.42	0.47	7.47	2.86	9.89
400Gy	17.25	66.08	20.18	0.89	0.22	5.17	1.35	4.42
500Gy	15.50	92.56	25.3	0.00	0.00	0.00	0.00	0.00
EMS (Concentration)								
Control	26.65							
100 mM	22.56	53.00	6.78	0.23	0.23	1.02	0.44	3.40
120 mM	21.40	61.00	9.56	1.62	1.35	7.59	2.66	16.98
140 mM	19.15	66.00	15.21	0.42	0.30	2.18	0.63	2.75
160 mM	17.25	71.00	19.67	2.34	1.46	13.58	3.30	11.91
180 mM	18.90	74.00	22.3	0.00	0.00	0.00	0.00	0.00

Mutagenic effectiveness and efficiency

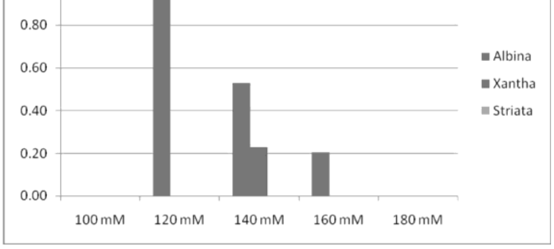
The determination of mutagenic effectiveness was involving the mutagenic frequency and levels of doses. The mutagenic effectiveness was found

Table 5. Mutation rates of mutagen in terms of effectiveness and efficiency in M_2 generation of White Ponni and BPT 5204

Mutagen	Mutation rate in terms of effectiveness		Mutation rate in terms of efficiency					
	White Ponni	BPT 5204	White Ponni			BPT 5204		
			Injury	Lethality	Sterility	Injury	Lethality	Sterility
Gamma rays (kR)	0.7131	0.6340	4.8628	6.1857	13.3298	6.6587	3.1723	13.8777
EMS (mM)	0.3104	0.6693	1.9076	0.5699	3.9792	4.8752	1.4061	7.0091

Fig. 1. Chlorophyll mutants observed in M$_2$ generations

to be higher at gamma rays irradiated with 100Gy in both the varieties of White Ponni (2.04) and BPT 5204 (1.63) whereas, in EMS concentration of 120mM (0.89) in White Ponni and 160mM (1.46) in BPT5204 (Table 3 and 4).The result was in accordance with the result reported by Sellammal and Maheswaran (2013). The mutagenic efficiency was recorded higher in 100Gy of gamma radiation in White Ponni based on lethality (18.48) and sterility



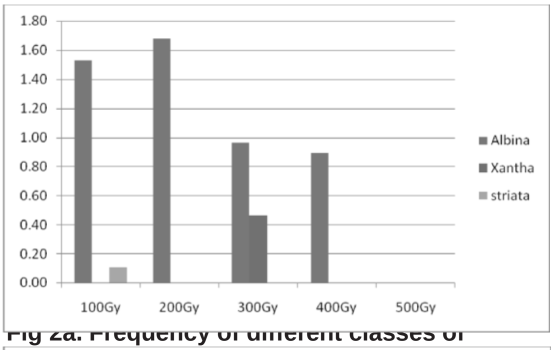
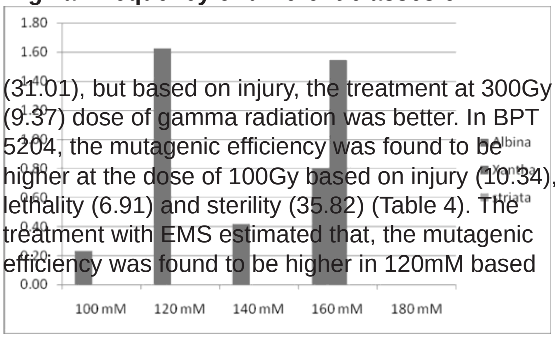


Fig 2a. Frequency of different classes of



(31.01), but based on injury, the treatment at 300Gy (9.37) dose of gamma radiation was better. In BPT 5204, the mutagenic efficiency was found to be higher at the dose of 100Gy based on injury (10.34), lethality (6.91) and sterility (35.82) (Table 4). The treatment with EMS estimated that, the mutagenic efficiency was found to be higher in 120mM based

Fig 2b. Frequency of different classes of chlorophyll mutants in M$_2$ generation of BPT 5204 treated with gamma radiation and EMS

on injury (4.87), lethality (1.56) and sterility (12.25) in White Ponni whereas, in BPT 5204 it was found to be maximum in 160mM based on injury (13.58), 120mM based on lethality (2.66) and sterility (16.98).

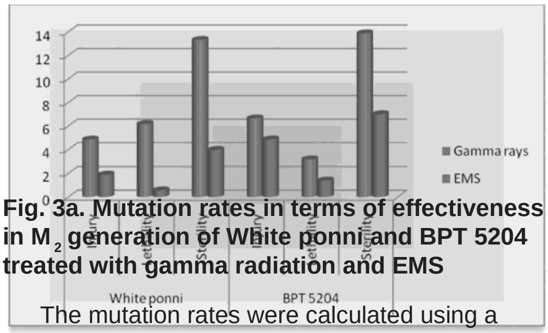


Fig. 3a. Mutation rates in terms of effectiveness in M_2 generation of White ponni and BPT 5204 treated with gamma radiation and EMS

The mutation rates were calculated using a mutagen is useful only if it is effective as well as efficient. Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. In mutation breeding programme, a high mutation rate accompanied by minimal deleterious effects is described. But generally the mutagen that gives the higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Blixt, 1964). In this study, the mutation rate for gamma rays (0.7131) was higher in White Ponni whereas, in BPT 5204 the mutation rate was found

Fig. 3b. Mutation rates in terms of efficiency in M_2 generation of White ponni and BPT 5204 treated with gamma radiation and EMS

to be higher in EMS (0.6693) in terms of effectiveness (Table 5, Fig 3a). When the mutation rates based on efficiency were compared, gamma ray was found to be the most efficient as far as injury, lethality and pollen sterility in both varieties of rice are concerned (Fig 3b). Similar observation has been recorded by Girija and Dhanvel (2009); Sharma *et al*. (2005), Kumar and Ratnam (2010).

Conclusion

This study ultimately concludes that the lower concentrations of the mutagens are more effective for the induction and recovery of mutations for improvement of rice. Mutagenic effectiveness and efficiency plays vital role in any mutation breeding for selecting the optimum dose or dose concentrations while using physical and chemical mutagens.

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