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EFFECTIVENESS AND EFFICIENCY OF GAMMA RAY AND EMS INDUCED CHLOROPHYLL MUTANTS IN RICE ADT(R) 47

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Abstract

A field experiment was conducted to study the effect of gamma rays and Ethyl Methyl Sulfonate (EMS) in rice variety ADT (R) 47. The frequency and spectrum of chlorophyll mutations, mutagenic effectiveness and efficiency, mutation rate of physical mutagen over chemical mutagen were estimated at different levels to study the nature and effect of mutagens in rice. The result from the study indicated that, *albino* was the predominant class of chlorophyll mutants that occurred in M_2 generations at lower doses. The mutagenic effectiveness and efficiency were found to be higher at 200Gy of gamma irradiation and 120mM of EMS. The mutation rate of gamma rays (0.57) was higher in terms of effectiveness than that of EMS (0.15). In terms of efficiency, the mutation rate of gamma rays was higher based on injury (4.84), lethality (2.55) and sterility (6.29). It could also be observed that increase in dose or concentration of the mutagen did not increase the relative frequency of chlorophyll mutants.

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

1. Introduction

The variability available to the breeder is considered to be the essential criteria for crop improvement which comes from spontaneous (or) artificially induced mutation and other methods. Among different methods, induced mutagenesis serves as an important tool for correcting a particular defect or creating usable genetic variation. Artificial induction of mutations is done through the use of physical/chemical mutagens which increase the mutation frequency, when compared to the occurrence of mutants, through spontaneous means. Before the start of any sound breeding programme, knowledge of relative biological effectiveness and efficiency of various mutagens and their selection is essential to recover high frequency of desirable mutations (Smith, 1972; Kumar and Mani, 1997).

The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. The term "mutagenic effectiveness" is a rate of mutations produced by the mutagen in relation to its dose (or) it is an index of the response of a genotype to the increasing does of the mutagen. The "mutagenic efficiency" is an estimate of mutation rate in relation to the damage (Konzak *et al.*, 1965). It is not necessary that an effective mutagen should be an efficient one also (Gaikward and Kothekar, 2004). Both of these though are two different properties, the usefulness of any mutagen in a plant breeding programme depends on both of them. Chlorophyll mutation frequency is useful in assessing the potency of a mutagen. Hence, scoring of chlorophyll mutations has proved to be a much more dependable index for evaluating the genetic effects of mutagenic treatments. 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 ADT (R) 47 rice cultivar.

2. Materials and Methods

The seeds of rice variety ADT (R) 47 for the induction of mutation were obtained from Tamil Nadu Rice Research Institute, Aduthurai, Tamil Nadu Agricultural University (TNAU).

2.1 Mutagenesis

2.2 Gamma Irradiation

Dry and matured seeds with 12 per cent moisture content (500 numbers for each dose) of the cultivar was irradiated with different doses of gamma rays 150Gy, 200Gy, 250Gy, 300Gy and 350Gy *viz.*, from Cobalt-60 (^{60}Co) using the Gamma Chamber – Model GC 1200 installed at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University (TNAU), Coimbatore. The treatments were undertaken in the evening, one day before sowing. The experiment was conducted during 2012 - 2014 at South farm of Tamil Nadu Rice Research Institute, Aduthurai. After the treatment, the irradiated seeds were sown in raised nursery beds the next day morning along with the control (untreated) seeds.

2.3 Ethyl Methyl Sulfonate (EMS) treatment

Well filled seeds with 12 per cent moisture content (200 seeds per treatment) of ADT (R) 47 was treated with different concentrations of an alkylating agent Ethyl Methyl Sulfonate solution *viz.*, 80mM, 100mM, 120mM, 140mM and 160mM for six hours under controlled conditions with intermittent shaking. Uniform volume of 20ml EMS solution was used for treatment soaking with various concentrations. The treatment was performed at room temperature of 22 ± 2^0C at early morning hours. Before soaking of seeds in EMS solution, the seeds were pre-soaked in double distilled water for 12 hours to enhance the imbibing capacity of seeds to absorb the EMS. After the completion of soaking time, the EMS solutions were drained and the seeds were thoroughly washed with running tap water for half an hour to remove the residues of chemical. The excess moisture in seed coat was removed by using folds of blotting paper. Pre-soaked seeds in distilled water for 18 hours were used as the control. The seeds were sown in raised nursery beds in the field immediately after the treatment along with the control seeds.

2.4 Observation of chlorophyll mutants

The M_1 generation of both gamma rays and EMS treated seeds were raised and evaluated during 2012. The biological damage was measured in terms of injury (reducing height), lethality (% survival) at 30 DAS in the nursery and pollen sterility (s) at flowering stage. The M_1 plants were separately harvested. In both EMS and gamma treatments, a total of 250 M_1 families (50 families/each treatment) were forwarded to M_2 generation. In M_2 generation, the occurrence of chlorophyll mutants were observed in raised nursery beds. The classification and characterization of various chlorophyll mutants was done according to Gustafsson (1940) and Blixt (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). *Xantha* **-** Characterized by yellow to whitish - yellow coloured leaves and it also has minimum survival up to 15 to 20 DAS. *Striata* **-** Characterized by the presence of white or yellow coloured longitudinal strips on leaves and was viable one. *Chlorina* **-** Characterized by the presence of light green coloured seedlings and did not survive 15 days after sowing.

The formula proposed by Konzak *et al*. (1965) was followed for the calculations of mutagenic effectiveness and efficiency by incorporating the mutation frequency values recorded for each mutagenic treatment.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutagenic Frequency}}{\text{Dose or (concentration (c) x time (t))}}$$

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

Mutation rate (MR) which provides the knowledge of mutations induced by a particular mutagen irrespective of dose or concentration was calculated as follows.

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

3. Results and Discussion

Mutation breeding is a quick method for rectification of defects in varieties besides inducing polygenic variations and development of different ideotypes. Several mutants were isolated in different crops and utilised for crop breeding programme. Basic information on the type and dose of mutagens, relative effectiveness and efficiency of mutagens are necessary in utilising mutation breeding techniques for the improvement of any crop including rice. Chlorophyll mutations are considered as the most dependable indices for evaluating the effectiveness and efficiency of different mutagens and mutability of a cultivar.

3.1 Relative frequency of chlorophyll mutations

Usually, chlorophyll mutations occur up to 15 days from the time of seed germination and in them, the primary leaves will be deficient of chlorophyll (Stummann and Henningsen, 1980). In general, the frequency and spectrum of chlorophyll mutations have been used to determine the mutagenic effectiveness and efficiency of mutagens which would ultimately provide the information about the dose for inducing mutations in particular genotype. 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 mutagen as well as with different doses in M_2 generation. The frequency and spectrum of induced chlorophyll mutations in ADT (R) 47 with gamma rays revealed the absence of *Striata* type mutation. Among the other three classes of chlorophyll mutants, **(Table 1.)** the frequency of albino was found to be higher (4.87) than the other two classes *viz., Xantha* (0.91) and *Chlorina* (0.14) the frequency of albino was maximum (2.03) at 200 Gy followed by a gradual reduction at increasing doses **(Fig 1.)**. At 300 & 350 Gy, only *albino* class of chlorophyll mutant was present. Earlier workers also reported a higher frequency of albino mutants (Swaminathan *et al*., 1970; Kawai and Sato, 1966; Nanda and Misra, 1975; Bhan and Kaul, 1976; Kaul and Basu, 1977; Awan and Bari, 1979; Rao and Rao, 1983; Bural *et al*., 1986; Singh and Singh, 2003). However Awan *et al*. (1980), Reddi and Rao (1988) and Reddi and Suneetha (1992) observed a higher frequency of *viridis* than *albino* or *xantha* in their studies in rice by involving physical and/or chemical mutagens. The total mutagenic frequency was found to be higher in 200Gy of gamma rays (2.74) followed by 150Gy (1.12) when compared to other doses of gamma irradiation. Singh *et al*. (1998) observed highest frequency of chlorophyll mutations (2.04%) at 20 kR dose of gamma radiation in rice variety Lanjhi. Reddi and Rao, 1988; Singh *et al.*, 1998 observed that the frequency of chlorophyll mutants was found independent of mutagenic doses of gamma rays.

In EMS induced mutations, all the four classes of chlorophyll mutations were present in the relative frequency order of *albino* (3.94), *xantha* (0.60), *striata* (0.41) & *chlorina* (0.23). The relative percentage (frequency) of *albino* was found to be maximum (1.21) at 120mM concentration which was followed by 100mM (1.12) as inferred from **Fig 2.** The total mutagenic frequency was found to be maximum (1.55) at 120mM concentration followed by 100mM (1.38). 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. This study has revealed that the total frequency of chlorophyll mutations was higher in gamma rays (5.93) when compared to EMS (5.18).

3.2 Mutagenic effectiveness and efficiency

Among the different doses of gamma rays irradiated, the mutagenic effectiveness was maximum (1.37) at 200 Gy followed by 0.75 at 150 Gy **(Table 2.)**. In EMS, the mutagenic effectiveness was only 0.23 at 100 mM closely followed by 0.22 at 120 mM. Hence it could be concluded that gamma rays have higher mutagenic effectiveness compared to EMS (**Fig 3.**). These results are in line with the results of (Bansal *et al.*, 1990; Pillai *et al.*, 1993). On the contrary to the present study, Rao and Rao (1983), Reddi and Rao (1988) pointed out that chemical mutagens have been found to be more efficient and effective than physical mutagens in the production of chlorophyll mutants in rice. Siddiq and Swaminathan (1968) found that EMS was most efficient mutagen followed by gamma rays and Nitroso-Guanidine.

On the contrary to the reports of Gautam *et al.* (1992) and Ratnam and Rao *et al*. (1993) that mutagenic efficiency increased with an increase in the dose of mutagens, in the present study it could be also observed that increase in dose or concentration of the mutagen did not increase the relative frequency of chlorophyll mutants, rather a decreasing trend was observed at higher doses. This could either be due to the death of the mutated plants leading to their consequent elimination or due to a selection within the plant, i.e., the diplontic selection (Bekendam, 1961; Yamaguchi, 1962; Gaul, 1961; 1964) since two or more primordial cells are involved in the ontogeny of a spike in cereals Mackay (1954). Also, Nilan and Konzak (1961), Konzak *et al.*, (1965) opined that higher efficiency at the lower concentration of an agent is due to the fact that biological damage (lethality and sterility) increased within dose at a faster than the mutations.

Among the five different doses, the mutagenic efficiency was found to be higher in 200 Gy of gamma irradiation based on injury (10.58), lethality (6.53) and sterility (15.16) and there is a clear cut expression at this dose compared to other doses. Whereas in EMS, the efficiency is expressed at two different concentration *viz.*, 120 mM, for injury (6.33) and 100 mM for lethality (3.87) and sterility (10.29). Reddi and Suneetha (1992) also inferred that lower concentrations were most effective in all three rice cultivars taken in their study when compared to higher concentrations.

The mutation rates 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 desired. But generally the mutagen that gives the higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects. In this study, the mutation rate for gamma rays (0.57) was higher than EMS rays (0.15) in terms of effectiveness (**Fig 3**). When the mutation rates based on efficiency were compared, gamma rays were found to be most efficient **(Fig 4.)** as far as injury (4.84), lethality (2.55) and pollen sterility (6.29). Such variations were noticed in rice by Sharma *et al.* (2005), Girija and Dhanvel (2009) and Kumar and Ratnam (2010).

4. Conclusion

It can be inferred from the present study that the lower concentrations of the mutagens are more effective than higher concentrations. The study also reveals the potential of gamma rays when compared to EMS in inducing mutations in rice cultivar ADT(R) 47. Since chlorophyll mutants indicate genetic change in the progeny and structure of rice plant being chimeric (Nanda *et al*., 1974; Chaudhary *et al*., 1978), selection may be done in progenies showing any type of chlorophyll mutants in order to isolate agronomically useful mutants in M_2 generation.

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Table 1. Frequency and spectrum of chlorophyll mutants in M_2 generation of ADT (R) 47 rice

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*	*Chlorina*			*Albino*	*Xantha*	*Striata*	*Chlorina*	
Control	0	0	0	0	0	1000	0.0	0.0	0.0	0.00	0.0
150Gy	49	17	0	0	66	5905	0.83	0.29	0.0	0.00	1.12
200Gy	126	35	0	9	170	6213	2.03	0.56	0.0	0.14	2.74
250Gy	69	4	0	0	73	6912	1.00	0.06	0.0	0.00	1.06
300Gy	51	0	0	0	51	7139	0.71	0.00	0.0	0.00	0.71
350Gy	19	0	0	0	19	6249	0.30	0.00	0.0	0.00	0.30
							4.87	**0.91**	**0.0**	**0.14**	**5.93**
EMS (Concentration)											
Control	0	0	0	0	0	1000	0.0	0.0	0.00	0.00	0.0
80 mM	23	0	0	2	25	5341	0.43	0.00	0.00	0.04	0.47
100 mM	56	5	7	1	69	4984	1.12	0.10	0.14	0.02	1.38
120 mM	77	14	1	7	99	6379	1.21	0.22	0.02	0.11	1.55
140 mM	41	5	9	1	56	5840	0.70	0.09	0.15	0.02	0.96
160 mM	29	12	6	3	50	6126	0.47	0.20	0.10	0.05	0.82
							3.94	**0.60**	**0.41**	**0.23**	**5.18**

Table 2. Mutagenic effectiveness and efficiency based on chlorophyll mutations in M_2 generation of ADT (R) 47 rice

Mutagen	seedling height in M_1 (cm) (I)	% survival reduction in M_1 (L)	% pollen sterility in M_1 (S)	Mutation frequency (M)	Effectiveness (M X 100) / Gy or cxt	Efficiency (M x 100) / I	Efficiency (M x 100) / L	Efficiency (M x 100) / S
Gamma rays (Dosage)								
Control	29.10							
150Gy	26.40	37.42	13.76	1.12	0.75	4.24	2.99	8.14
200Gy	25.90	41.94	18.07	2.74	1.37	10.58	6.53	15.16
250Gy	23.20	56.77	22.16	1.06	0.42	4.57	1.87	4.78
300Gy	21.40	73.76	28.58	0.71	0.24	3.32	0.96	2.48
350Gy	20.10	80.43	34.00	0.30	0.09	1.49	0.37	0.88
				5.93	**2.86**	**24.20**	**12.73**	**31.45**
EMS (Concentration)								
Control	30.00							
80 mM	26.70	33.40	11.58	0.47	0.10	1.75	1.40	4.04
100 mM	26.00	35.76	13.45	1.38	0.23	5.32	3.87	10.29
120 mM	24.50	52.89	16.03	1.55	0.22	6.33	2.93	9.68
140 mM	23.50	62.10	22.23	0.96	0.11	4.08	1.54	4.31
160 mM	22.50	67.67	27.28	0.82	0.09	3.63	2.21	2.99
				5.18	**0.74**	**21.12**	**10.96**	**31.32**

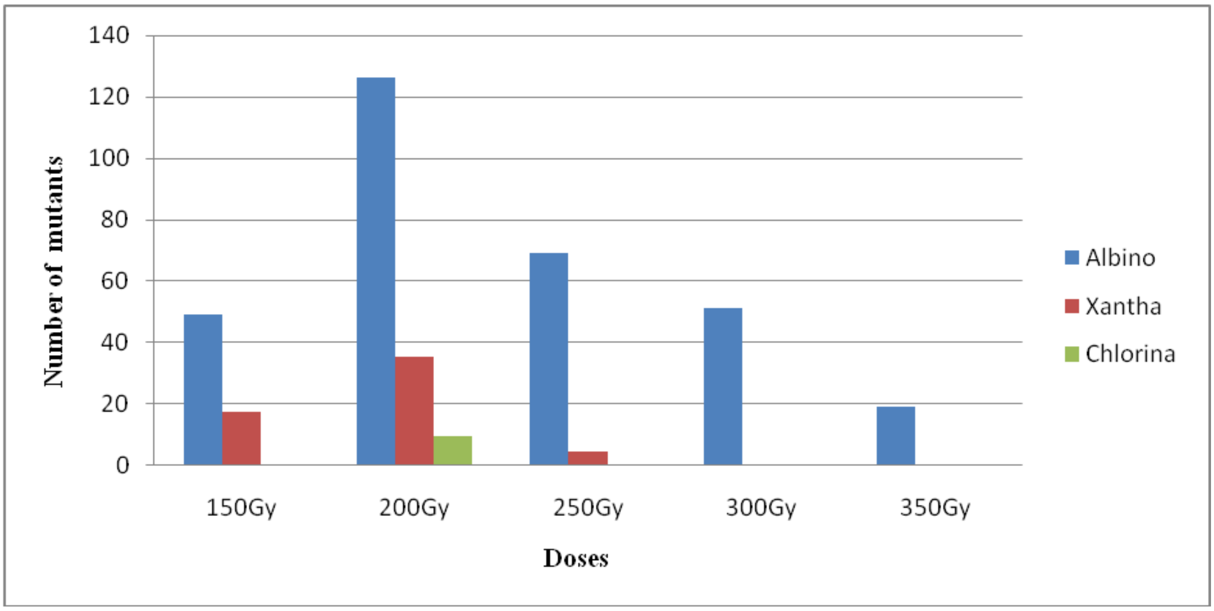

Fig 1. Frequency of different classes of chlorophyll mutants in M_2 generation of ADT (R) 47 rice treated with gamma radiation

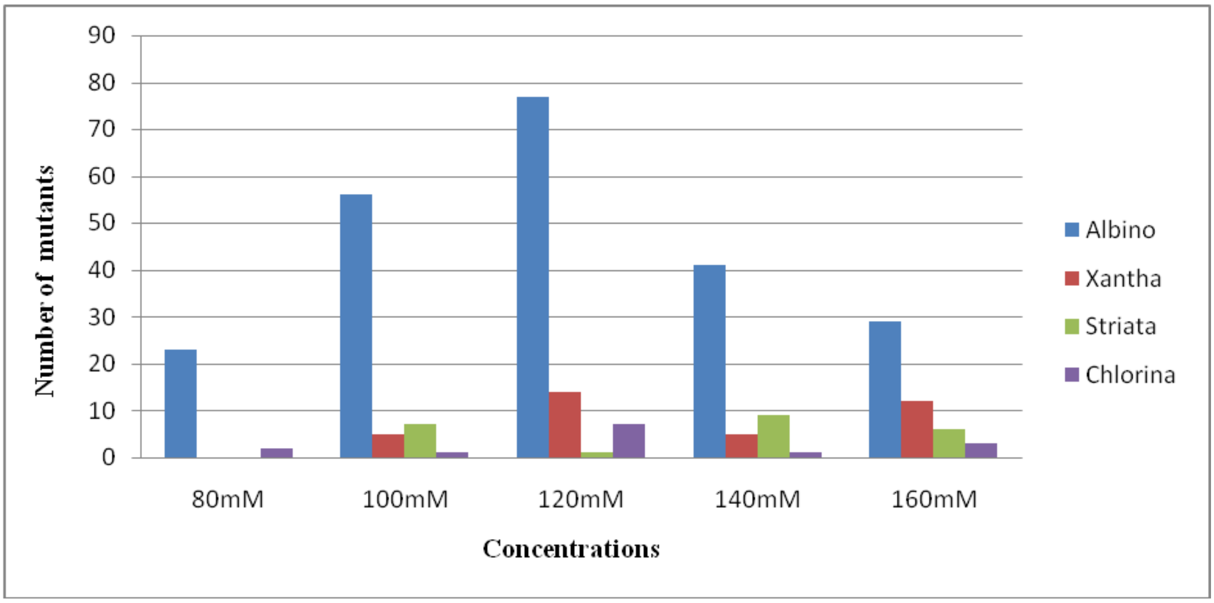

Fig 2. Frequency of different classes of chlorophyll mutants in M_2 generation of ADT (R) 47 rice treated with EMS

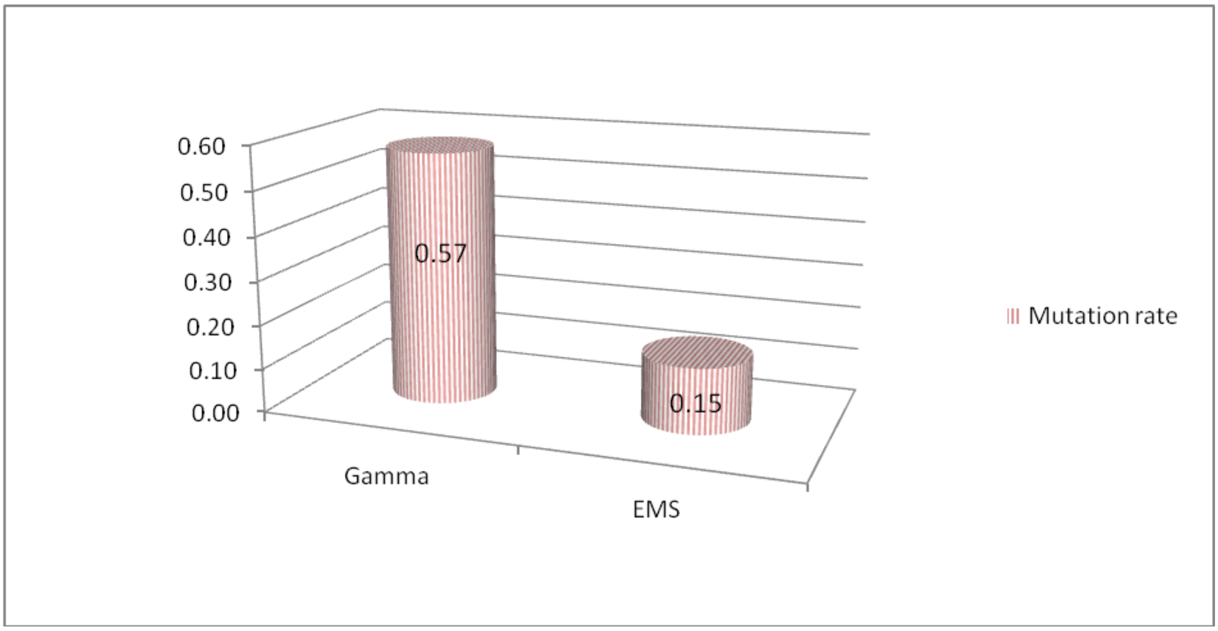

Fig 3. Mutation rates in terms of efficiency in M_2 generation of ADT (R) 47 rice treated with gamma radiation and EMS

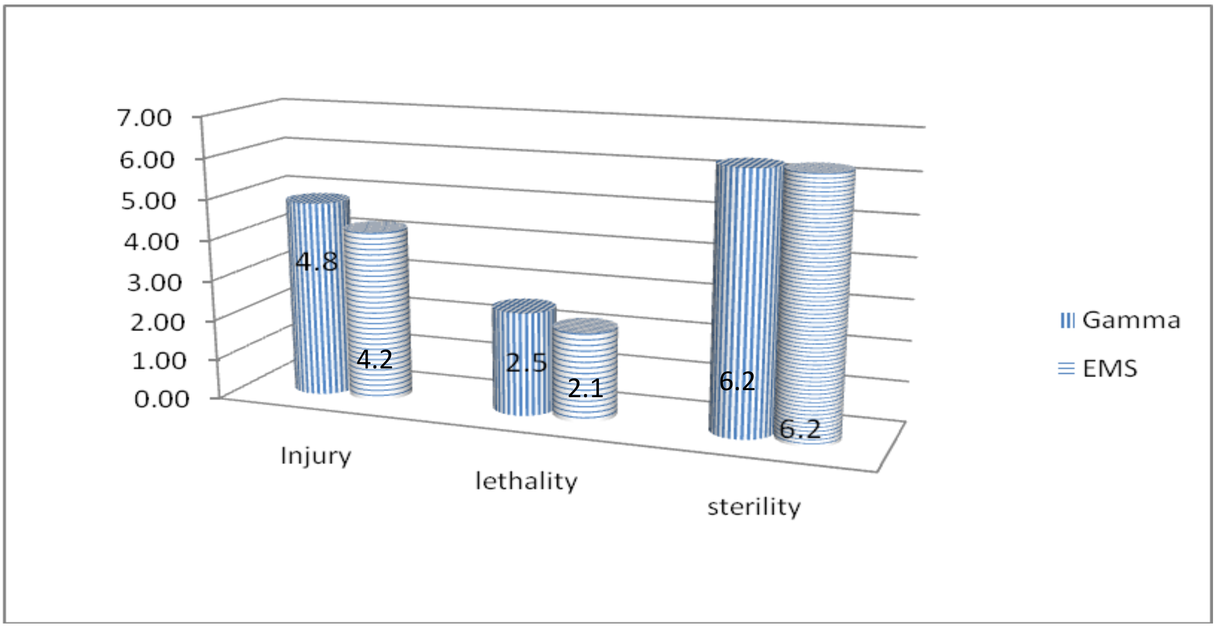

Fig 4. Mutation rates in terms of efficiency in M_2 generation of ADT (R) 47 rice treated with gamma radiation and EMS